Neonatal Amygdala Lesions and Stress Responsivity in Rats

Relevance to schizophrenia

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Neonatale Amygdala Lesies en Stress Responsiviteit in ratten
Relevantie voor schizofrenie
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de Rector Magnificus, prof. dr. W.H. Gispen,
ingevolge het besluit van het College voor Promoties
in het openbaar te verdedigen
op dinsdag 1 juni 2004
des middags te 12.45 uur

door

Jeroen Terpstra

geboren te Heemskerk op 19 januari 1974

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layout and cover: R.J. van Rijn (www.debrigade.nl)
printing: Ponsen & Looijen BV
photo cover backside: F.T.A. Pijlman

ISBN: 90-393-3700-4

I maintain there is much more wonder in science than in pseudoscience. And in addition, to whatever measure this term has any meaning, science has the additional virtue, and it is not an inconsiderable one, of being true.
Carl Sagan (1934 - 1996)

Aan mijn ouders

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chapter 1

General introduction

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1.1. The amygdala lesion model: tool of investigation

1.1.1. The amygdala

The amygdala (fig. 1), which has recieved its name from its close resemblance to the shape of an almond, is a structure in the medial temporal lobe of all vertebrates. The amygdala, not a nucleus in the strictest sense, is a collection of distinct, yet interconnected nuclei and cortical areas. In the rat, the amygdala complex consists of seven major nuclei: the lateral, basolateral, basomedial, central, medial, cortical, and intercalated nucleus (Paxinos and Watson, 1998).

The amygdala complex is highly connected and shares reciprocal connections with various cerebral structures: i.e. basal forebrain, cingulate cortex, frontal cortex, hypothalamus, hippocampus, insular cortex, occipital cortex, olfactory cortex, orbital cortex,

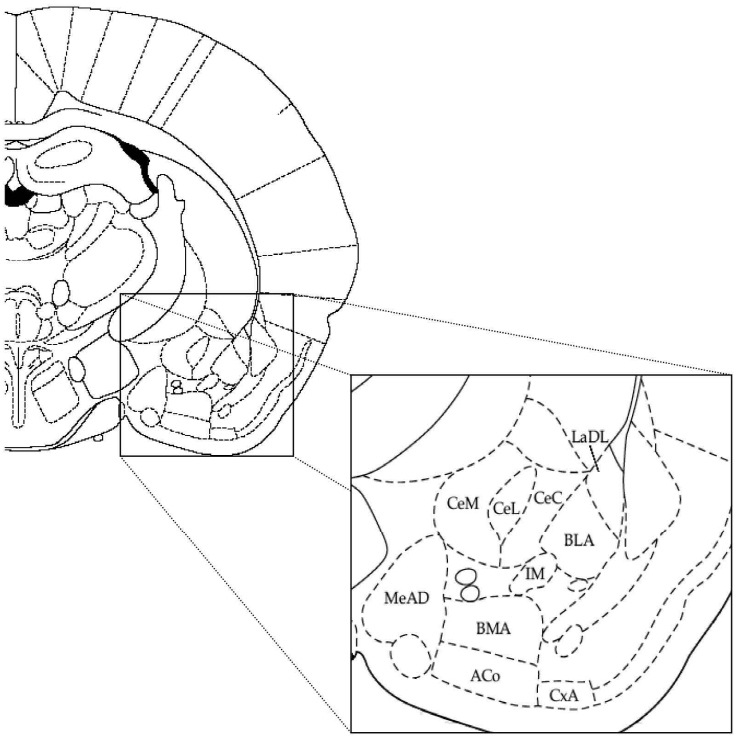

Fig. 1. The amygdaloid complex and its relative location inside the rat brain (figure derived from Paxinos and Watson, 1998). Anterior cortical amygdaloid nucleus (ACo); basolateral amydaloid nucleus, anterior part (BLA); basomedial amygdaloid nucleus, anterior part (BMA); central amydaloid nucleus, capsular part, lateral division, medial division (CeC, CeL, CeM); cortex-amygdala transition zone (CxA); intercalated nuclei of the amygdala (IM); lateral amygdaliod nucleus, dorsolateral part (LaDL); medial amygdaliod nucleus, anterodorsal part (MeAD).

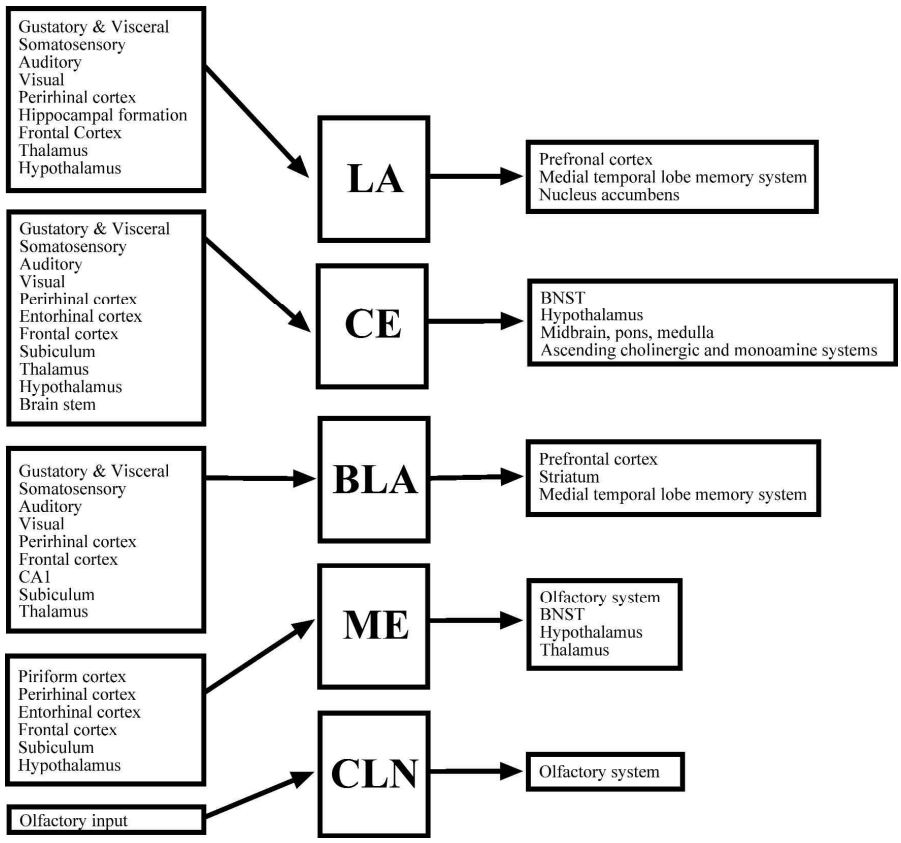

Fig. 2. Schematic representation of the amygdaloid nuclei and their connections (for extensive review see Sah et al., 2003). LA: lateral amygdaloid nuclei, CE: central amygdaloid nuclei, BLA: basolateral amygdaloid nuclei, ME: medial amygdaloid nuclei, CLN: cortex-like amygdaloid nuclei.

striatum, temporal cortex, and thalamus (Amaral and Cowan, 1980; Amaral et al., 1982; Amaral and Price, 1984; Amaral, 1986; Amaral and Insausti, 1992; Amaral et al., 2003; Sah et al., 2003) (fig. 2).

1.1.2. The amygdala during development

Because the amygdala is such a highly interconnected structure, it has been postulated that this structure is important for the development of its projection areas (Wolterink et al., 2001; Bouwmeester et al., 2002). Indeed neonatal lesions in the basolateral amygdala likely result in morphological changes in the medial prefrontal cortex (mPFC) in adult life, and likely not in the nucleus accumbens (Acb) nor the mediodor-

sal thalamic nucleus (MDT) (Bouwmeester et al., 2002). Thus, the basolateral amygdala is critically important, but selective, for the development of its target structures.

1.1.3. The amygdala as center for stress and emotion

The amygdala is the neural structure most intimately tied to emotion. It has long been known that damage to the amygdala produces profound disturbances in emotional behavior, in particular when negative emotions related to fear and anxiety are involved (Davis, 1992). It also mediates the impact of emotion on learning an memory (Kesner et al., 1992). With the development of functional imaging techniques, this role of the amygdala in mediating the response to fear related stimuli and the regulation and interpretation of emotions has been made more visible in humans (Birbaumer et al., 1998; Posse et al., 2003; Sander et al., 2003). Studies support the consensus role of the amygdala, i.e. connecting internal and external stimuli and appraise them in their environmental context. Anatomically, the amygdala is directly and indirectly connected to other brain structures that cope with stress, among them the hypothalamus (Wallace et al., 1992). The amygdala may therefore act as a feedforward device that generates responses in anticipation of stress, f.i. the sympathetic and endocrine stress response.

1.1.4. The amygdala in relation to developmental psychopathological disorders: schizophrenia

Amygdalar dysfunction has been postulated to be related to behavioral abnormalities in several psychopathologies, among which schizophrenia (for a detailed discription of schizophrenia, see box 1). Behavioral disturbances in schizophrenia, i.e. blunted/inappropriate affect, social withdrawal, difficulty identifying the emotional status of people (Bellack et al., 1992; Cramer et al., 1992), and paranoid delusions (Epstein et al., 1999), have all been connected, in some way, to limbic (amygdaloid) disturbance. Due to its size and location in the human brain, it has been difficult to assess the amygdala of schizophrenic patients using in vivo imaging techniques (Lawrie et al., 1999). Postmortem studies in schizophrenic patients failed to find significant differences in amygdalar volume (Chance et al., 2002). Yet, with advancing techniques, amygdalar volume reduction has been found in schizophrenia (Gur et al., 2000; Hulshoff Pol et al., 2001; Anderson et al., 2002).

However, as important as anatomical data is evidence based on functional data. derived from pharmacological and lesion studies linked to the amygdala, that may mimick part of the schizophrenic syndrome.

Box 1: Schizophrenia

In 1896 Emile Kraepelin (1856-1926) was the first to describe a specific set of symptoms, consisting of long-term cognitive and emotional deterioration, combined with hallucinations and delusions. He called this illness, which seemed to affect young people in the prime of their life, 'dementia praecox' as opposed to the 'late onset' dementia described by the contemporary neurologist Alois Alzheimer (1864–1915). The term 'schizophrenia' was first used by Eugen Bleuler (1857-1939) in 1908. He used this term to replace 'dementia preacox' because of the striking schism he saw between rational thought, emotion and behaviour in these patients.
Since 1952, when the American Psychiatric Association's Committee on Nomenclature and Statistics published the first edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM), the criteria to diagnose schizophrenia have been consistently refined. The syndrome is currently defined as a chronic psychiatric disorder that is characterized by psychotic episodes with hallucinations and/or delusions, and a marked decline in social and occupational functioning. The criteria for the diagnosis of schizophrenia are listed in the DSM-IV (APA, 1994). The core symptoms for schizophrenia are divided into three distinct categories: positive, negative, and cognitive symptoms (Carpenter and Buchanan, 1994). The positive symptoms constitute florid psychotic symptoms, like hallucinations and delusions, whilst the negative symptoms constitute the absence or diminuation of behavior, like social withdrawal, poverty of speech/psychomotor activity, and affective flattening. The cognitive symptoms consist of formal thought disorder, memory impairments, and impaired executive functioning (McKenna et al., 1990; Saykin et al., 1991).
One of the most intriguing aspects of the disease is its epidemiologic profile. It occurs only after puberty (except for a rare form of 'childhood schizophrenia' (Nicolson et al., 2000)), with males being at the highest risk for developing the disease between the age of 15-25, and females being at the highest risk between the age of 20-35 (Hafner et al., 1998). The late onset of the disease symptoms is of interest to our studies, for the profile suggests a developmental/maturational component (Walker and Bollini, 2002). This is also an essential part of the postnatal day 7 amygdala lesion model used in this thesis.

Etiology

Neuronanatomical abnormalities have been found from early on in the development of the disease (Schulz et al., 1983), suggesting a neurodevelopmental origin of

the syndrome. At the same time, the clinical symptoms and concurrent neuropathology seem to be progressive (Gur et al., 1998), supporting the neurodegenerative hypothesis of schizophrenia first formulated by Emile Kraepelin. Based on this neuroanatomical evidence it is believed that schizophrenia is a progressive neurodevelopmental disorder (Weinberger, 1987; Woods, 1998), with elements of both neurodevelopment and neurodegeneration contributing to the syndrome (for review, see Shenton et al., 2001).
The lifetime prevalence of schizophrenia is approximately 1% for the general population, but the risk for developing the disease increases with the degree of kinship (monozygotic twins have a concordance rate of 50%). Genetic research into schizophrenia suggests multiple genes to be involved in its etiology rather than a single gene (McGuffin et al., 1994). Also environmental components seem to contribute to the risk of developing schizophrenia. For instance, infection or nutritional deficiency during pregnancy, and obstetric complications during birth increase the risk of the infant to develop schizophrenia in later life (O'Callaghan et al., 1992; Sham et al., 1992; Susser and Lin, 1992). Also, the existence of life events may have their impact on the development of the disease, as is expressed in the 'vulnerability-stress model' (Yank et al., 1993; Nuechterlein et al., 1994). It describes schizophrenia as a disease originating from a combination of both a strong biological basis and environmental influences. In this model, individuals 'at risk' have a certain vulnerability to develop the disease, yet stressful environmental factors contribute to the development of the actual symptoms of the disease. Although evidence suggests the vulnerability-stress model to have merit, it remains a hypothesis that is difficult to prove, since in prospect no determinants for vulnerability can be identified as yet.

1.2. Animal models of schizophrenia

Various animal models for schizophrenia have been put forward, but all of them have been met with skepticism because of the gap between animal physiology and behavior, and complex human mental disorders such as schizophrenia (Lipska and Weinberger, 2000). There have been many pharmacological approaches, mainly focussing on either the dopamine (Gambill and Kornetsky, 1976; Nielsen et al., 1983; Weiner et al., 1984; Swerdlow et al., 1998; Suzuki et al., 2002) or the glutamate system (Javitt and Zukin, 1991; Noda et al., 1995; Linn et al., 1999). Also morphological approaches have been put forth, trying to mimic structural deficits seen in schizophrenia, predominanly using lesions of the prefontal cortex (Lipska et al., 1998; Brake et al., 2000; Lacroix et al., 2000), hippocampus (Lipska et al., 1993; Lipska et al., 1995; Lipska and

Weinberger, 1995; Chambers et al., 1996; Lipska et al., 2001; Goto and O'Donnell, 2002; Lipska et al., 2002*a*), and recently the amygdala (Wolterink et al., 1998; Daenen et al., 2001; Wolterink et al., 2001; Daenen et al., 2002*a*; Daenen et al., 2003*a*). Although also lesions of other limbic structures, e.g. the nucleus basalis magnocellularis, have been investigated in relation to schizophrenia (Schauz and Koch, 1999), other approaches have been either genetic, using receptor knockout mice (Clifford et al., 2000; Gainetdinov et al., 2001), or environmental, using maternal deprivation as a model of aspects of schizophrenia (Geyer et al., 1993; Ellenbroek et al., 1998).

1.2.1. Pharmacological models

1.2.1.1. Dopaminergic agonists

The involvement of the neurotransmitter dopamine (DA) in schizophrenia was based on the observation that dopamine agonists, e.g. amphetamine and apomorphine, are able to elicit positive schizophrenic symptoms (Kapit, 1977), and that these symptoms are treated succesfully with DA antagonists, e.g. haloperidol (Creese et al., 1976). It was therefore hypothesized that schizophrenic patients suffer from an excess of dopaminergic activity (Meltzer and Stahl, 1976). Currently the DA hypothesis postulates that hyperactivity of dopaminergic neurons in the ventral tegmental area (VTA) contributes to the emergence of psychotic symptoms, and hypoactivity of mesocortical dopaminergic neurons contributes to the cortical hypofunction and the negative symptoms.

There are two types of DA receptors: D_1-like (D_1, D_5) and D_2-like (D_2, D_3, and D_4) (Civelli et al., 1993; Gingrich and Caron, 1993). Research has shown that the antipsychotic actions of neuroleptic drugs are mediated via the mesolimbic D_2 receptors, and that the extrapyramidal side effects of those drugs are related to blockade of the nigrostriatal DA system (Deutch, 1993). To which extent other DA receptor subtypes are involved in schizophrenia remains to be seen, due to the lack of highly selective ligands. However, recent data suggest that at least the D_3 receptor is potentially interesting for schizophrenia (Schwartz et al., 2000; Joyce, 2001), since atypical neuroleptic drugs have affinity for this receptor subtype. Typical neuroleptc drugs, like haloperidol, mainly bind to D_2 receptors, whilst atypical neuroleptic drugs, like clozapine, show a lower occupancy of striatal D_2 receptors, despite their high affinity for this receptor (Altar et al., 1986). The binding profiles of the atypical neuroleptic drugs show that they also have high affinity for other than DA receptors, e.g. serotonin (5-HT) and noradrenalin (NA) receptors) (Altar et al., 1986; Kahn et al., 1993; Glavin and Hall, 1994; Kahn et al., 1994; Kalkman and Loetscher, 2003), which demonstrates that the DA hypothesis is not complete in this respect.

Although the pathophysiology of schizophrenia proved to be seriously more complex than the DA hypothesis postulated, the use of aspecific dopaminergic agonists have remained a valid disease model, despite the fact that other than the positive symptoms of the disease can not be elicited via this route. Moreover, dopaminergic agents can be used to validate other (non-pharmacological) models.

1.2.1.2. Glutamatergic antagonists

Phencyclidine (PCP) was developed as an anesthetic drug in the 1950's. It soon became a drug of abuse known as 'angel-dust'. The PCP model for schizophrenia was formulated in the 1970's when a syndrome emerged, often (mis)diagnosed as schizophrenia, that could be attributed to PCP. PCP-induced psychosis strongly resembles acute paranoid schizophrenia with classic symptoms like bizarre behavior, catatonia, paranoid delusions, delusions of being controlled by others, grandiosity and violence (Allen and Young, 1978). Important in this respect is that symptoms can persist for days to several weeks, suggesting prolonged alteration of the neurotransmitter systems involved. PCP is one of various glutamate receptor antagonists. There are three different subtypes of glutamatergic receptors each named after their preferred agonist: N-Methyl-D-Aspartate (NMDA), kainate, and quisqualate (α-amino-3-hydroxy-5-methyl-4-isoxalepropionic acid (AMPA)). NMDA-receptor antagonists (PCP, ketamine, dextrorphan) mimick schizophrenic symptoms in healthy human volunteers (Allen and Young, 1978; Krystal et al., 1994; Oranje et al., 2002). In animals, PCP induces prefrontal cortical dopaminergic and cognitive deficits (Jentsch et al., 1997; Sams-Dodd, 1997) and therefore constitutes an appealing animal-model of schizophrenia. NMDA-receptor antagonists induce the following symptoms: 1) hyperlocomotion in reaction to novelty-stress, the stress of a saline injection or amphetamine administration, 2) prefrontal cognitive dysfunctions, and 3) prepulse-inhibition (PPI) deficits to an acoustic-startle-response.

1.2.2. Lesion models

1.2.2.1. Prefrontal lesions

Because patients suffering from schizophrenia display cognitive deficits attributed to dysfunction of prefrontal cortical areas (Berman et al., 1988; Andreasen et al., 1992; Castellon et al., 1994; Knable and Weinberger, 1995), lesions of the prefrontal cortex in animals have been studied with great interest. Cortical lesions in non-human primates and in rats result in cognitive dysfunctions pertaining to planning, strategy, and goal-directed behaviors (Robbins, 1990; Joel et al., 1997; Collins et al., 1998), similar to those seen in schizophrenia. Neonatal cortical lesions in rats result in post-pubertal

increased locomotor activity to a novelty stressor or a dopaminergic agonist (Flores et al., 1996), and an enhanced DA release in the accumbens nucleus (Acb) after stress (Brake et al., 2000). All in all, lesions in the prefrontal cortex are able to mimic some important defects seen in schizophrenia.

1.2.2.2. Lesions in limbic structures (hippocampus and amygdala)

Based on evidence from functional, pharmacological, imaging and lesion studies limbic structures have been implicated in schizophrenia. For example, imaging studies in schizophrenic patients have shown volume reductions in several limbic structures (e.g. hippocampus, amygdala) (Bogerts et al., 1985; Rossi et al., 1994; Hulshoff Pol et al., 2001). Together with the evidence from functional and pharmacological studies, lesioning limbic structures (i.e. nucleus accumbens, amygdala, hippocampus, and nucleus basalis magnocellularis) has been an approach that has received much attention.

In rats these lesions result in a grossly similar syndrome, mimicking several aspects of schizophrenia (Swerdlow et al., 1986; Lipska et al., 1992; Lipska et al., 1993; Lipska and Weinberger, 1993; Wolterink et al., 1998; Schauz and Koch, 1999; Hanlon and Sutherland, 2000; Daenen et al., 2001; Wolterink et al., 2001; Daenen et al., 2002*a*; Lipska et al., 2002*b*; Lipska and Weinberger, 2002; Daenen et al., 2003*a*). These include, but are not limited to: disturbed PPI, changed mesolimbic dopamine function, enhanced sensitivity to dopaminergic agonists, disturbed social behavior, and disturbed behavioral performance in place navigation and spatial tasks.

a. Hippocampal lesions

Structural pathology in schizophrenia is most reliably observed in the hippocampal region (Bogerts et al., 1985; Jakob and Beckmann, 1986; Hulshoff Pol et al., 2001). The hippocampal formation is thought to participate in the regulation of the mesolimbic DA system, which is affected in schizophrenia (Yang and Mogenson, 1985; Groenewegen et al., 1987; Csernansky et al., 1988; Sesack and Pickel, 1990). Lesions in the hippocampus are therefore thought to constitute a dysregulation of the mesolimbic DA system. Several lesion studies in animals have indeed shown symptoms bearing resemblance to positive and negative symptoms in schizophrenia (Lipska et al., 1992; Lipska et al., 1993; Lipska et al., 1995; Sams-Dodd et al., 1997; Wood et al., 1997; Lipska et al., 2002*b*; O'Donnell et al., 2002).

Lesions in the ventral hippocampus of neonatal rats have shown some remarkable similarities to symptomatology in schizophrenia (Chambers et al., 1996). The symptoms observed are: hyperresponsivity to the stimulant effects of amphetamine,

impaired grooming, disrupted social interactions, and impaired spatial learning and memory. Moreover these symptoms emerge during adolescence, which is also a pattern seen in schizophrenia (Hafner et al., 1998).

b. Amygdala lesions

On the basis of lesion studies it has recently been suggested that lesions in and adjacent to the amygdala may have a role in schizophrenia (Fudge et al., 1998). Whether disturbances in brain structures or function found in patients are of developmental nature or due to the presence of the disease itself, remains subject of ongoing debate (Shenton et al., 2001). To mimic a neurodevelopmental process in the animal model, chemical lesions in the rat amygdala where made on postnatal day 7 (D7 AMX) and postnatal day 21 (D21 AMX). The lesions on postnatal day 7, but not postnatal day 21, are associated with symptoms that become manifest after puberty and bear resemblance to symptoms found in schizophrenia: i.e. impaired PPI, impaired behavioral response to stress, impaired social behavior, and increased sensitivity to PCP (Wolterink et al., 1998; Daenen et al., 2001; Wolterink et al., 2001; Daenen et al., 2002*a*; Daenen et al., 2003*a*; Daenen et al., 2003*b*).

1.2.3. Other models

Various other models have been investigated including environmental models where rats have either been reared in isolation, or maternally deprived (Geyer et al., 1993; Varty and Higgins, 1995; Domeney and Feldon, 1998; Ellenbroek et al., 1998). These models have shown disturbances in PPI, albeit not consistently (Lehmann et al., 2000). Another model showing disturbed PPI constitutes rats genetically selected for their locomotor hyper/hypo responsiveness to apomorphine (Ellenbroek et al., 1995). Genetic approaches consist mainly of knockout mice showing behavioral deficits with relevance to schizophrenia. Examples are mice lacking the MAO-A/B gene (Shih and Chen, 1999), the cathechol-o-methyltransferase gene (Gogos et al., 1998), the dopamine transporter gene (Gainetdinov et al., 2001), dopamine receptors (Clifford et al., 2000), or NMDA receptor subunits (Miyamoto et al., 2001).

Based on the observation that schizophrenia may be related to aberrant neurodevelopment during pregnancy (DeLisi et al., 1988; Sham et al., 1992; Davis et al., 1995), in utero exposure to pharmacological agents has been investigated in rats (Talamini et al., 1998; Fiore et al., 1999; Talamini et al., 1999). These studies revealed morphological changes similar to those found in post mortem studies in schizophrenia, and several aberrant behaviors suggesting abnormal reactivity to external stimuli.

1.3. Stress responsivity

1.3.1. What constitutes stress?

One of the most common but vague definitions of stress was made in 1914 by Walter B. Cannon (1871–1945). He introduced the term homeostasis and postulated that significant aversive emotional events, which he called stress, would be able to disturb this biophysiological balance (Cannon, 1929; Cannon, 1935) He observed that when this balance was threatened various physiological systems are activated, i.e. the sympathetic-adrenomedullary system and the hypothalamic-pituitary-adrenal axis, to preserve this balance. In 1936 the concept of stress was extended by Hans Selye (1907–1982), who discovered a nonspecific reaction to noxious stimuli which he called the 'General Adaptation Syndrome' (Selye, 1936). Later, Selye modified his view on stress, and stated that both occurence and size of the inherently nonspecific stress response are dependent on characteristics of the stimulus and of the subject. Although a clear-cut definition has not been made, recent views on stress include notion that stress is not necessarily harmfull, but serves to trigger an organism to adapt itself to new environments (Huether, 1996). The term allostasis, the 'ability to achieve stability through change', was introduced to describe the plasticity of this adaptational process: the ability of an organism to re-set his/her homeostatic set point to adapt to a novel situation (McEwen, 1998). Whatever the term used, recent views on stress all include the concepts of adaptation, learning and habituation. Research has shown that the more controllable a stimulus is, the less stressful it is appraised (Dess et al., 1983; Gannon and Pardie, 1989; Kushner et al., 1992).

Therefore, a stimulus is a stressor only when the subject appraises it as (a) uncontrollable, and (b) emotionally and/or physically aversive. Besides the behavioral response, the stress response has traditionally included two read-out systems (Huether, 1996): the sympathetic-adrenomedullary system (SAM), and the hypothalamic-pituitary-adrenal system (HPA). Heart rate, blood pressure and peripheral (nor)adrenalin secretion are often taken as an index of SAM activity, and ACTH and cortisol secretion as an index of HPA-axis activity.

1.3.2. Substrates

For a short discription of neurotransmitters related to stress responsivity see box 2.

1.3.2.1. The prefrontal cortex

The prefrontal cortex and the mesocortical dopaminergic system play a pivotal role in the interpretation of and response to stressful stimuli. Planning and strategy manage-

Box 2: Neurotransmitters related to stress

Dopamine (DA): the involvement of the DA system in stress responsivity has been established by various lines of research (Thierry et al., 1976; Deutch et al., 1990; Puri et al., 1994; Cabib et al., 1998; Giardino et al., 1998; Sasaki et al., 1998). Specifically, the meso-cortical and meso-limbic DA systems contribute to the heightening of attention in response to a stressful stimulus (Thierry et al., 1976; Sawaguchi et al., 1990).
Serotonin (5-HT): the 5-HT system seems to reduce fear and inhibit the heightening of attention in response to a stressful stimulus, thereby having a modulating role (Aston-Jones et al., 1991; Engberg, 1992; Graeff et al., 1996; Carrasco and Van de Kar, 2003).
Noradrenalin (NA): the noradrenalin system seems to serve the purpose of raising the level of vigilance or attention to environmental stimuli (Foote et al., 1983; Aston-Jones, 1985; Jacobs et al., 1991; Cole and Robbins, 1992). It has an excitatory effect on both the DA and 5-HT systems, and is inhibited by activity from 5-HT, GABA and opioid neurons.
Glutamate: the glutamate system has been implicated in the regulation of stress induced dopamine release (Enrico et al., 1998; Feenstra et al., 1998; Kulagina et al., 2001). Glutamate is one of the major excitatory neurotransmitters in the CNS (Collingridge and Lester, 1989; Dori et al., 1989). Glutamatergic neurons have an important role in cognition, learning and memory (Morris et al., 1986; Collingridge, 1987), neural plasticity of synaptic connections (Kaczmarek et al., 1997), pain perception (Klepstad et al., 1990), information processing (Daw et al., 1993) and the regulation of neuroendocrine secretion (Brann and Mahesh, 1994).

ment are among the main functions, as has been shown in animals (Thierry et al., 1976; Herman et al., 1982). In patients, it has been suggested that failure of prefrontal cortical activation during a cognitive challenge, as measured by in vivo imaging, is the result of dopaminergic dysfunction in that area (Weinberger et al., 1988; Daniel et al., 1989; Daniel et al., 1991; Dolan et al., 1995; Weinberger and Berman, 1996). To further support the central role of the mesocortical dopamine system in stress responsiveness, there is evidence that in animals, dopamine dysfunction in this area leads to limbic hypersensitivity to stress (Carter and Pycock, 1980; Leccese and Lyness, 1987; Haroutunian et al., 1988; Deutch et al., 1990).

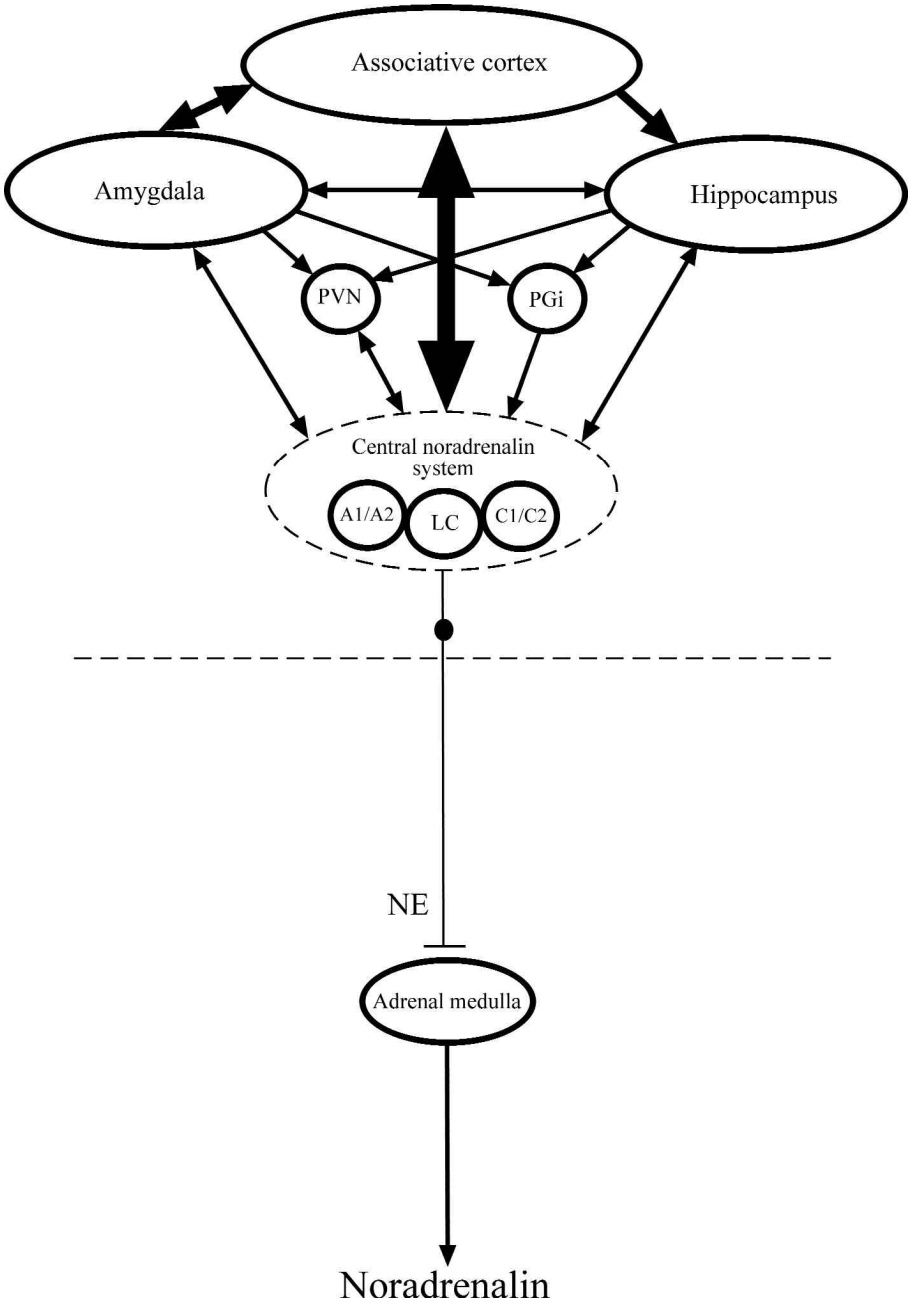

Fig. 3. Schematic representation of the sympathetic-adrenomedullary (SAM) axis. LC: locus coeruleus, PGi: nucleus paragigantocellularis (for details see text).

1.3.2.2. The limbic system

Arousal through fear or anxiety, a prerequisite for the activation of the general stress response (Mason, 1971; Burchfield, 1979; Huether, 1996), leads to an activation of the limbic system. This system consists of a number of circumscribed brain regions: the hypothalamus, septum, hippocampus, accumbens, ventral striatum and amygdala. The amygdala plays a pivotal role in the arousal of fear and anxiety by integrating the input of its numerous afferents, and hence in the activation of the neuroendocrine stress response (Sarter and Markowitsch, 1985; Davis, 1992). The amygdala is thought to be concerned with the attribution of affective qualities to the relevant stimuli. The septum and the nucleus accumbens are thought to be important for the inhibition or selection of behavioral response (Albert and Walsh, 1984; Huether, 1996).

1.3.2.3. The sympathetic-adrenomedullary (SAM) axis

The central noradrenergic system, comprising the locus coeruleus (LC) and several medullary nuclei (A1/A2 and C1/C2) (fig. 3), plays a pivotal role in the initiation and prolongation of the stress response (Huether, 1996). It has a potentiating and reinforcing effect on both the generation of fear and anxiety, and the activation of the HPA axis. The central noradrenergic system recieves input from the associative (prefrontal) cortex, amygdala, hippocampus, paraventricular nucleus of the hypothalamus (PVN), and nucleus paragigantocellularis (Aston-Jones et al., 1986; Ennis and Aston-Jones, 1988; Buchanan et al., 1994*b*; Van Bockstaele et al., 2001). The LC is widely regarded as the center of both central and periferal noradrenergic ativity. In reaction to a stressful stimulus, afferents to the LC provoke noradrenalin release. Centrally this release exerts a reinforcing effect of the afferent CRH input into the LC, especially from the amygdala, thus constituting a positive feedback loop (Huether, 1996). Peripherally, noradrenalin is released from postganglionic neurons to their target organs. The central and peripheral release of noradrenaline leads to arousal and peripheral adaptation of bodily functions to prepare the subject for the altered demands of the environment.

1.3.2.4. The hypothalamic-pituitary-adrenal (HPA) axis

The PVN plays a central role in the regulation and activation of the HPA axis (fig. 4). It receives afferent information from various systems in the brain (e.g. visual, somatic/special sensory, circumventricular, intrahypothalamic and limbic (Huether, 1996), and functions as a regulator of pituitary ACTH secretion (Gaillet et al., 1991). It does so by secreting CRH which can be potentiated by the secretion of other peptides (e.g. arginin-vasopressin (AVP), oxytocin, vasoactive-intestinal-polypetide (VIP), angiotensin II), that by themselves are not strong secretagogues of ACTH (Rivier et al., 1984; Antoni, 1986; Plotsky, 1987; Familari et al., 1989; Scaccianoce et al., 1991). Both

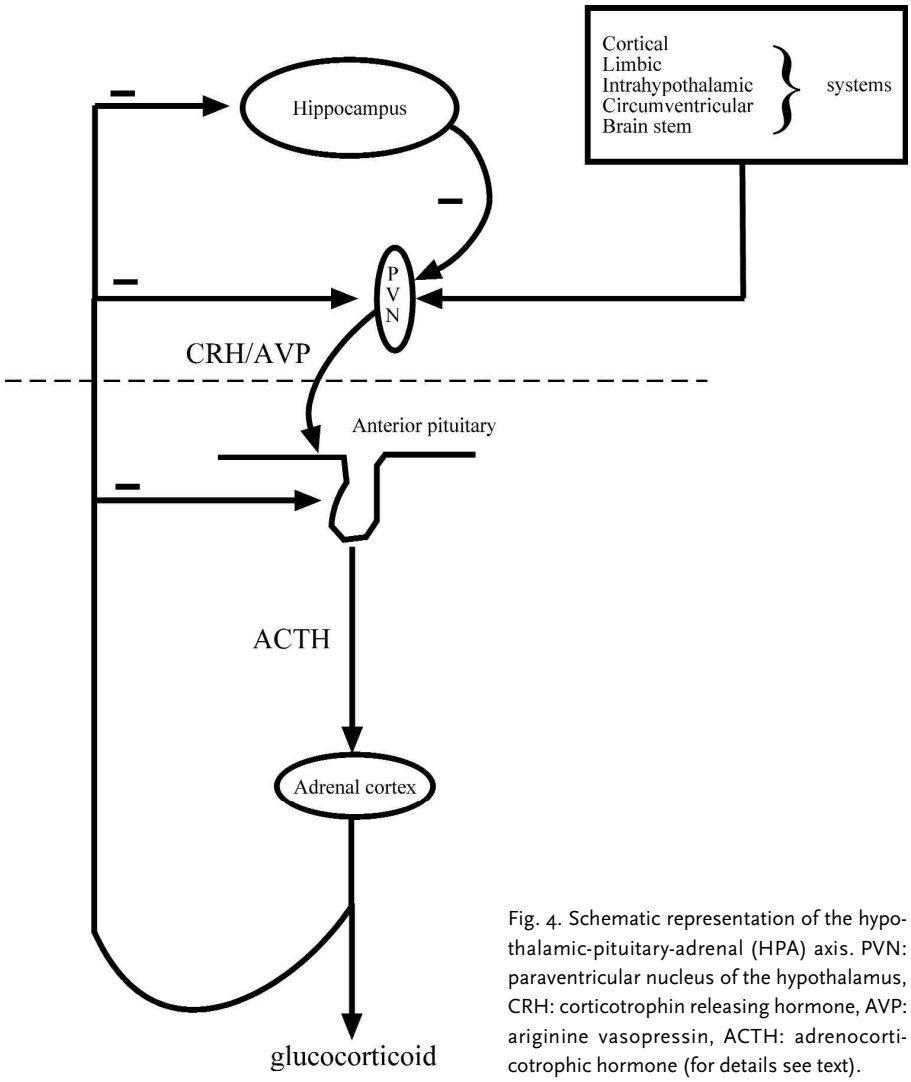

Fig. 4. Schematic representation of the hypothalamic-pituitary-adrenal (HPA) axis. PVN: paraventricular nucleus of the hypothalamus, CRH: corticotrophin releasing hormone, AVP: ariginine vasopressin, ACTH: adrenocorticotrophic hormone (for details see text).

in animals and in humans, this activation of the HPA-axis is stimulus dependent (Romero and Sapolsky, 1996). ACTH subsequently stimulates the synthesis and release of glucocorticoids from the adrenals. Glucocorticoids reach their target organs and tissues via the systemic circulation. Because prolonged exposure to high levels of glucocorticoids is detrimental to many processes, negative feedback inhibition of corticosteroid release is effectuated at the level of the adrenal, pituitary, hypothalamus

(PVN) and hippocampus. The role of the HPA stress response is to restore homeostasis and turn-off those processes that have been set in motion by the primary response systems, such as the sympathethic- and immune systems.

1.3.3. Stress responsivity in schizophrenia

There is evidence that schizophrenic patients are more vulnerable to stress than healthy subjects (Yank et al., 1993). Stressful life events and 'expressed emotions', i.e. the 'propensity of the family to become critical or over-involved with the patient at times of trouble', increase the risk of a psychotic relapse (Brown and Birley, 1968; Birley and Brown, 1970; Rabkin, 1980; Lukoff et al., 1984; Day et al., 1987; Nuechterlein et al., 1992; Norman and Malla, 1993; Nuechterlein et al., 1994; Doering et al., 1998). Even the number of daily stressors has been implicated in the number of schizophrenic symptoms (Norman and Malla, 1994).

The impact of stress on disease is a very well known concept, but the mechanisms underlying this phenomenon remain enigmatic. It has been shown that schizophrenic patients have a diminished HPA response to a cold pressor stimulus (Albus et al., 1982), the anticipation of lumbar puncture (Breier et al., 1988), a surgical procedure (Kudoh et al., 1997), and a public speaking stressor (Jansen et al., 1998; Jansen et al., 2000*a*). This suggests that stress responsivity is impaired in schizophrenia. This is the case predominantly for (psycho)social stimuli, since the response to a physical or metabolic stimulus appears unaltered from that of controls (Kathol et al., 1992; Jansen et al., 2000*a*). Although it has been suggested that neuroleptic treatment might hamper the stress response in schizophrenic patients via its activity at the hypothalamic level (Keim and Sigg, 1977; Meador-Woodruff et al., 1990), medication-naive first-episode schizophrenic patients already exhibit a stress response deficit (Van Venrooij et al., In Preparation). It thus appears that impairments in the stress response may be intrinsic to the (progression of the) disease. The explanation has been sought in the fact that schizophrenic patients cope differently with their environment, have an altered cognition and may not interpret stimuli as healthy indivuals do, and are thus not well adapted. Part of these defects also run in families of schizophrenic patients (Van den Bosch et al., 1992; Jansen et al., 2000*a*; Malaspina et al., 2000; Johnson et al., 2003). Both the prefrontal cortex and the limbic system are thought to be involved in these cognitive deficits (Beatty et al., 1993).

1.4. Aim and outline of this thesis

The aim of this thesis is to investigate the stress response in an animal model that has relevance to schizophrenia, and to hypothesize on the role of the amygdala in the

process of stress responsivity, given its crucial role in mediating the response 'stressfull/negative' stimuli.

In chapter 2 we therefore investigated the stress response in the amygdala lesion model, using rats lesioned on postnatal day 7 or 21, and their sham operated controls. We speculated that results might shed light on the developmental nature of lesion effects on the stress response, and on the role of the amygdala and its developmental target structures in this respect.

To probe the mechanisms undelying the HPA hyporesponsiveness found in chapter 2, we further investigated the phasic and tonic regulation of the HPA axis in the neonatally (day 7) lesioned animal in chapter 3. The sensitivity of the pituitary for CRH (feedforward) was tested, as well as the sensitivity of the pituitary to glucocorticoid feedback, and the circadian secretion of corticosterone.

For its role in the regulation of HPA axis activity, and the notion that noradrenergic transmission in the brain is altered in the amygdala lesion model (Bouwmeester, 2002), we further investigated the central noradrenergic system in chapter 4. To that end, we used in vitro autoradiography of the α_1- and α_2-adrenoreceptors in brain areas involved in the stress response; namely the medial prefrontal cortex (mPFC), accumbens nucleus (Acb), caudate putamen (CPu), paraventricular nucleus of the hypothalamus (PVN), and locus coeruleus (LC).

For its relevance to the schizophrenic syndrome in humans, we investigated in chapter 5 whether the impaired stress response found in chapter 2, could be influenced by chronic neuroleptic treatment. Based on the knowledge that neuroleptic treatment in schizophrenia is of paramount importance to treat the symptoms, but also to prevent relapse, the atypical neuroleptics (e.g. clozapine) being preferential over the typical neuroleptics (e.g. haloperidol), we hypothesized that clozapine, but not haloperidol, would exert its protective advantage through alteration of cerebral systems involved in stress processing

chapter 2

Attenuated stress responsiveness in an animal model for neurodevelopmental psychopathological disorders

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Abstract

Day 7 amygdala-lesioned (D7 AMX) rats have been proposed as a model for neurodevelopmental psychopathological disorders, such as schizophrenia. Patients with schizophrenia are sensitive to stress and show an impaired hypothalamic-pituitary-adrenal response to certain stressful stimuli. Therefore, we investigated neuroendocrine and behavioral stress responses in the D7 AMX lesion model. Plasma concentrations of ACTH, corticosterone, and catecholamines were measured in response to foot shock and novelty in D7 and D21 lesioned (AMX) and non-lesioned (SHAM) animals. Behavior was recorded and analyzed afterwards. D7 AMX rats, unlike other rats, had a reduced ACTH response to foot shock and showed less active behavior in response to novelty. Neurodevelopmental dysfunction of target structures of the amygdala is associated with disturbed endocrine and behavioral responses to stress. These data accord with the notion that the D7 amygdala-lesioned rat can function as a neurodevelopmental model with relevance to schizophrenia.

Introduction

A number of animal models have been described that are believed to mimic deficits seen in schizophrenia (Lipska et al., 1993; Hanlon and Sutherland, 2000). Recently, it was found that rats that had received an amygdala lesion on postnatal day 7 (D7 AMX) showed a number of deficits that are also seen in schizophrenia, for example, an impaired prepulse inhibition, impairments in social behavior, stereotyped hyperactivity, and an increased sensitivity to phencyclidine and apomorphine (Wolterink et al., 1998; Daenen, 1999; Wolterink et al., 2001). These deficits were not observed in sham-operated rats (D7 SHAM) or in rats in which the amygdala was lesioned later, on postnatal day 21 (D21 AMX). Rats bearing an amygdala lesion made on day 7 have therefore been proposed as a model for neurodevelopmental psychopathological disorders such as schizophrenia (Wolterink et al., 2001). Patients with schizophrenia seem to be particularly vulnerable to stressful stimuli, because the severity of symptoms in these patients is related to the amount of perceived stress (Nuechterlein et al., 1992; Norman and Malla, 1994). There is also evidence that the response of the hypothalamus-pituitary-adrenal (HPA) axis, a system crucially involved in stress adaptation, to various types of stressful stimuli is impaired in schizophrenia (Breier et al., 1988; Kudoh et al., 1997; Jansen et al., 1998; Kudoh et al., 1999; Jansen et al., 2000*a*). Moreover, morphological abnormalities in brain areas that are involved in stress processing, for example a reduced amygdala volume, have been observed in the brains of schizophrenic patients (Wright et al., 2000; Hulshoff Pol et al., 2001).

Abnormalities in HPA axis responsiveness have been described in amygdala-lesioned rats (Beaulieu et al., 1986; Beaulieu et al., 1987; Roozendaal et al., 1991*a*; Prewitt and Herman, 1994; Marcilhac and Siaud, 1996; Dayas et al., 1999), and it is thought that the central and medial nuclei of the amygdala play a role in the potentiation of the HPA axis and the sympatho-adrenal response to stress (Roozendaal et al., 1991*a*). Data available so far, however, only concern the (sub)acute effects of lesions in adult rats. To our knowledge, the effects of amygdala lesions made early in life on responsiveness to stress later in life have not been studied.

In view of the above, we set out to investigate the responsiveness to stress in the amygdala lesion model. Two stress paradigms were used: exposure to foot shocks and to novelty. The levels of ACTH and corticosterone in plasma were measured as indices of HPA axis function, and those of adrenaline and noradrenaline as indices of sympatho-adrenal function. Attention, rearing, and grooming were monitored as indices of active behavior, and immobility as an index of passive behavior. The following two hypotheses were tested: (1) D7 AMX rats respond to stress differently from D21 AMX rats, and (2) D7 AMX rats show a diminished HPA axis response to stress compared to D7 SHAM rats.

Experimental procedures

All procedures were performed in accordance with the guidelines established by the Committee for the Ethical Treatment of Animals Utrecht (DEC Utrecht), The Netherlands, and the Society for Neuroscience Policy on the Use of Animals in Research.

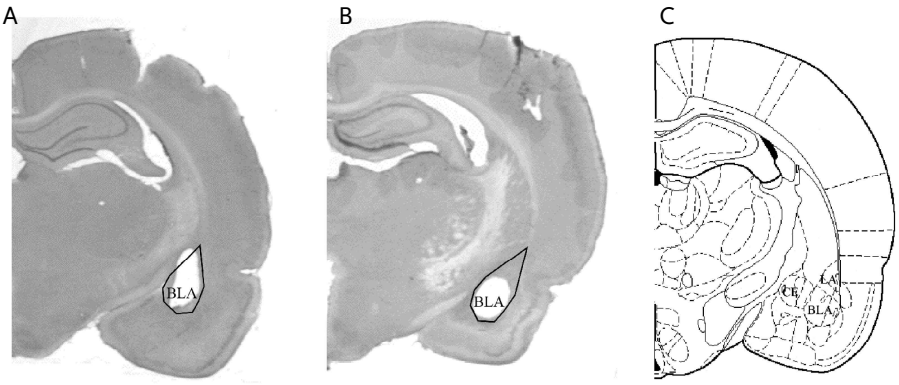
Animals and housing

Pregnant female Wistar rats [U:WU] were obtained at day 18 of gestation and were housed individually in Macrolon® Type III cages with sawdust bedding. After delivery, male and female pups were separated; male pups were randomized and returned to a nest. All nests contained at least two female pups and up to seven male pups. Male pups were operated on on postnatal day 7 or 21 and were weaned at 21 days. After weaning, all operated rats were housed socially (3 to 4 rats per cage) in Macrolon® Type III cages, with sawdust bedding, until they reached adulthood (approximately 13 weeks, average weight 369.7 ± 40.6 g). Thereafter all rats received a chronic jugular vein cannula and were allowed to recover for two weeks in individual transparent Perspex™ cages measuring 25 × 25 × 34 cm (l × w × h) with sawdust bedding. During this recovery period, the cannulas were flushed regularly and the rats were habituated to the blood sampling procedure. The rats were given standard chow (Hope Farms®, standard laboratory animal food) and water ad libitum. They were weighed daily after surgery until they reached their pre-surgical weight and were then weighed weekly. A 12-hour light/dark cycle was used, with lights on from 07.00 to 19.00. Ambient temperature was held constant at 21 ± 1 °C with humidity at 50 ± 10%.

Surgical procedures and histology

The lesioning procedure (fig. 1) was performed according to the technique described by Wolterink et al. (2001). Male Wistar rats were anesthetized with fentanyl® (4 µg/rat, s.c. for D7) or Hypnorm® (0.1 µg/kg, i.m. for D21). The heads of the D7 rat pups were placed in a specially constructed head mould to enable stable fixation of the head. The coordinates for the positioning of the needles for the amygdala lesions were 3.8 mm lateral to the midline, 1.0 mm posterior to bregma, and 6.0 mm below the surface of the skull, at an angle of 4°. The tip of the needles was aimed at the basolateral nucleus of the amygdala (BLA). The heads of the D21 rat pups were secured in a David Kopf® stereotaxic apparatus with the incisor bar at horizontal zero. The coordinates for the positioning of the needles for the amygdala lesions were 4.0 mm lateral to the midline, 2 mm posterior to bregma, 6.8 mm below the surface of the skull, at an angle of 0°. Lesions were made by bilateral infusion of ibotenic acid (3 µg/0.3 µl phosphate-buffered saline [PBS] for D7 or 4 µg/0.3 µl PBS for D21) over two minutes, using an

Fig. 1. Micrographs of representative amygdala lesions. Highlighted, after histological evaluation, are the relative locations of the basolateral amygdala. A: D7, B: D21. C: anatomical drawing of the important amygdala nuclei, BLA: basolateral nucleus of the amygdala, LA: lateral nuclei of the amygdala, CE: central nuclei of the amygdala (figure derived from Paxinos and Watson, 1997)



infusion pump. After infusion, the needles were left in place for 4 minutes. SHAM-lesioned rats received the corresponding amount of vehicle.

Within two weeks after the end of the experiments, all rats were killed with an overdose of intravenous pentobarbital. After decapitation, the brains were collected individually in formaldehyde-containing vials and were then sectioned (50 μm) and stained with hematoxylin and eosin. Histological evaluation showed that AMX lesions in fourteen rats (approximately 15% of the lesions) were either too big or entirely outside the BLA (Paxinos and Watson, 1986), and data from these animals were discarded (table 1).

Table 1. Number of rats per group after histological analysis

	AMX		SHAM	
	footshock	novelty	footshock	novelty
day 7	13	15	17	15
day 21	6	4	10	10

Stress test-procedure and radioimmunoassays (RIAs)

All animals were attached, in their home cage, to the sampling cannula 90 minutes before stress exposure (t= - 90 minutes). A basal blood sample was taken at t = - 10 minutes, and the animals were transferred from their home cage to the novel test cage at t = 0 minutes. The test started at t = 0, and consisted of a 10-minute period during

which foot shocks or no foot shocks (novelty) were given. Blood samples were taken at t = 5, 10, 20, 30 and 60 minutes. A total of ten individual foot shocks with a current of 0.25 mA and lasting 1 second were given at 1:00, 1:40, 2:40, 4:00, 4:40, 5:40, 7:00, 7:40, 8:40, and 9:30 minutes after the start of the test. Behavior was videotaped for later analysis. At the end of the test, a blood sample was collected in heparin/EDTA-containing vials and the animals were put back into their home cage. Samples were kept on ice and were centrifuged within four hours of sampling, and plasma was stored at - 80 °C. Corticosterone was determined using an ImmuChem® ^{125}I RIA kit (ICN Biomedicals, Inc.), and ACTH with an in-house ^{125}I RIA using the S-5 230676 antibody as described previously (Van Oers and Tilders, 1991). Catecholamines were determined using an in-house method with high-performance liquid chromatography separation and electrochemical detection (Nijsen et al., 1998).

Behavioral analysis

Behavior was analyzed with Observer® software (Noldus Information Technology, Wageningen, The Netherlands). The ethogram consisted of four behavioral parameters: attention (sitting while head and whiskers are moving), locomotion, rearing (front paws are of the ground, head lifted up), immobility (includes freezing), and grooming (cleaning head or body with tongue or paws).

Experiments

Two experiments were performed. To test the first hypothesis, 47 male pups were operated on postnatal day 7 (D7): 25 received an amygdala lesion (AMX) and 22 were SHAM (SHAM) operated. A split-litter design was used and AMX and SHAM pups were evenly distributed over the litters. At 21 days of age, the pups were weaned and housed socially, with AMX and SHAM rats being evenly distributed. After a 3-month maturation period, a jugular vein cannula was implanted in all rats. Several rats were lost during or after surgery, or due to problems with the patency of the cannula. After a two-week recovery period, the remaining thirty rats were tested and blood samples were obtained.

In the second experiment, we sought to replicate the results of the first D7 experiment and test whether D7 AMX rats differ from D21 AMX rats in responsiveness to stress (second hypothesis). For this study, 91 male pups underwent surgery. The groups consisted of 27 D7 AMX, 20 D7 SHAM, 30 D21 AMX, and 23 D21 SHAM pups. At 21 days, the pups were weaned and housed socially (3 to 4 per cage), with AMX and SHAM rats being evenly distributed. After a three-month maturation period, a jugular vein cannula was implanted in all rats. Several rats were lost during or after surgery, or due to

problems with patency of the cannula. After a two-week recovery period, the remaining 74 animals were tested and blood samples were obtained.

Statistical analysis

Statistical analysis (ANOVA, repeated measures) revealed that the data for D7 AMX and D7 SHAM groups from the first and second experiments were not statistically different. All results are therefore presented as for a single experiment (table 1). No difference was found in pretest weight between the AMX and SHAM groups; however, the 21-day-old rats were significantly heavier than the 7-day-old rats ($p = 0.002$). Endocrine data were analyzed using repeated measures analysis of variance with weight as covariate (ANCOVA, SPSS 10.0 for Macintosh®). The analysis included the within subjects factor 'time' (the endocrine response over time; six levels: t= −10, 5, 10, 20, 30, and 60 minutes) and the between subjects factors 'day' (two levels: D7 and D21) and 'lesion' (two levels: AMX and SHAM). When relevant, post-hoc analyses were performed using an ANCOVA repeated measures. Behavioral data were analyzed using multivariate analysis of variance (MANOVA, SPSS 10.0 for Macintosh®). Significance was reached when $p \leq 0.05$. Results are given as means ± SEM.

Results

Histological evaluation showed that the size and location of the AMX lesions (fig. 1) were not different from those previously described by Wolterink et al. (2001).

Responses to foot shock stress

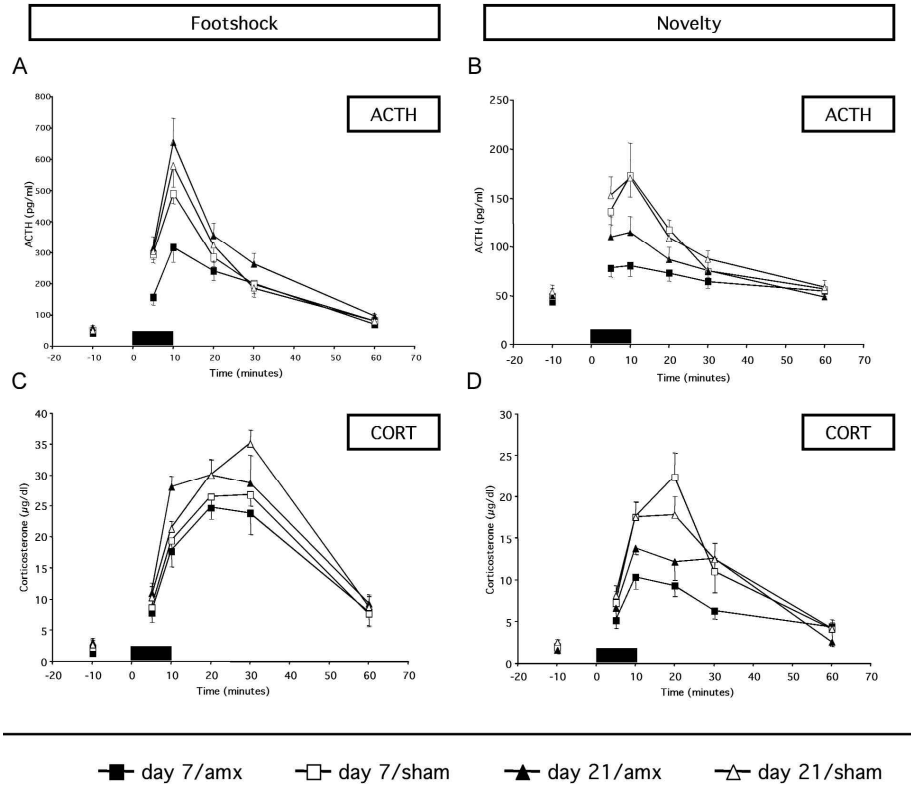
Plasma ACTH (fig. 2A)

An interaction was found between lesion × day × time (table 2). Post hoc analysis revealed a difference between the D7 AMX and D21 AMX subgroups ($F_{(1,17)} = 14.406$, $p < 0.001$), and between the D7 AMX and D7 SHAM subgroups ($F_{(1,28)} = 6.690$, $p < 0.001$). However, there was no difference between the D7 SHAM and D21 SHAM subgroups, or between the D21 AMX and D21 SHAM subgroups. These data indicate that D7 AMX rats had an attenuated ACTH response to foot shock compared with that of the other experimental groups.

Plasma corticosterone (fig. 2C)

There was no difference over time between the D7 and D21 groups, or between the AMX and SHAM groups. The between subjects analysis revealed a difference between the D7 and D21 rats (table 2): the total amount of corticosterone released was lower in the D7 subgroups than in the D21 subgroups.

Fig. 2. Hypothalamus-pituitary-adrenal axis response to a 10-minute foot shock session (black bar), A: ACTH to foot shock, B: ACTH to novelty, C: CORT to foot shock, D: CORT to novelty.



	footshock							
within subjects analysis	ACTH		CORT		NA		A	
	$F_{(5,210)}$	p	$F_{(5,210)}$	p	$F_{(5,135)}$	p	$F_{(5,135)}$	p
time	153.15	**0.000**	157.74	**0.000**	300.18	**0.000**	254.87	**0.000**
day × time	8.21	**0.000**	1.95	0.087	0.94	0.460	0.43	0.827
lesion × time	1.96	0.086	1.84	0.107	2.65	**0.026**	1.03	0.400
day × lesion × time	2.61	**0.026**	1.19	0.316	0.83	0.529	0.48	0.793
between subjects analysis								
	$F_{(1,41)}$	p	$F_{(1,41)}$	p	$F_{(1,26)}$	p	$F_{(1,26)}$	p
day	6.31	**0.016**	13.05	**0.001**	10.08	**0.004**	9.98	**0.004**
lesion	0.31	0.576	0.07	0.793	0.34	0.565	0.60	0.445
day × lesion	4.40	**0.042**	0.82	0.371	0.00	0.987	2.75	0.109

Plasma catecholamines(table 3)
An interaction was found between lesion × time for the noradrenaline, but not the adrenaline, response (table 2): the AMX subgroups released less noradrenaline in response to foot shock than did the SHAM subgroups. Between subjects analysis revealed a difference between D7 and D21 groups in both the noradrenaline response and the adrenaline response (table 2): the D7 subgroups released less catecholamines in response to foot shock than did the D21 subgroups.

Behavior (table 4)
There was a main effect of lesion ($F_{(10, 32)} = 2.252$, $p = 0.040$). Contrasts (table 5) revealed effects on attention, locomotion, and immobility. AMX rats showed more active behaviors and less immobility in response to foot shock than did SHAM animals.

Response to novelty stress

Plasma ACTH (fig. 2B):
An interaction was found between lesion × time (table 2). The lesioned groups (D7 AMX and D21 AMX) had a lower ACTH response to novelty than did the SHAM groups.

Table 2: Statistical analysis of the endocrine data (ANCOVA repeated measures). time = endocrine response over time, lesion = AMX vs SHAM, day = day 7 vs day 21, NA= Noradrenaline, A = Adrenaline

				novelty				
ACTH		CORT		NA		A		within subjects analysis
$F_{(5,200)}$	p	$F_{(5,200)}$	p	$F_{(5,125)}$	p	$F_{(5,125)}$	p	
34.37	**0.000**	48.80	**0.000**	112.16	**0.000**	73.07	**0.000**	time
0.64	0.668	1.27	0.277	0.48	0.790	0.42	0.835	day x time
5.59	**0.000**	4.58	**0.001**	2.53	**0.032**	1.73	0.132	lesion x time
0.50	0.773	1.26	0.284	0.46	0.808	0.45	0.814	day x lesion x time
								between subjects analysis
$F_{(1,39)}$	p	$F_{(1,39)}$	p	$F_{(1,24)}$	p	$F_{(1,24)}$	p	
0.74	0.394	0.42	0.52	1.03	0.320	0.00	0.946	day
7.38	**0.008**	5.96	**0.019**	1.89	0.182	0.83	0.371	lesion
0.07	0.79	0.67	0.417	0.05	0.819	0.41	0.529	day x lesion

Table 3: Mean values ± SEM of the catecholamine response per timepoint.

					adrenaline (pg/ml)			
	group		-10 min	5 min	10 min	20 min	30 min	60 min
footshock	D7	AMX	52 ± 3	118 ± 13	227 ± 25	419 ± 60	266 ± 45	123 ± 18
	D7	SHAM	63 ± 4	133 ± 11	289 ± 11	455 ± 18	317 ± 19	125 ± 15
	D21	AMX	72 ± 4	141 ± 17	299 ± 21	466 ± 24	311 ± 25	148 ± 14
	D21	SHAM	63 ± 5	161 ± 10	294 ± 16	472 ± 26	352 ± 27	137 ± 9
novelty	D7	AMX	57 ± 5	111 ± 15	207 ± 31	255 ± 45	183 ± 40	125 ± 26
	D7	SHAM	64 ± 4	151 ± 18	275 ± 28	321 ± 45	186 ± 19	104 ± 7
	D21	AMX	59 ± 4	125 ± 9	210 ± 16	259 ± 39	197 ± 24	114 ± 8
	D21	SHAM	63 ± 3	126 ± 12	219 ± 21	292 ± 35	193 ± 18	101 ± 7
					noradrenaline (pg/ml)			
	group		-10 min	5 min	10 min	20 min	30 min	60 min
footshock	D7	AMX	77 ± 4	140 ± 18	284 ± 34	417 ± 28	253 ± 28	117 ± 16
	D7	SHAM	78 ± 3	146 ± 7	270 ± 18	416 ± 18	281 ± 17	135 ± 10
	D21	AMX	80 ± 4	160 ± 17	315 ± 21	442 ± 42	269 ± 25	149 ± 14
	D21	SHAM	83 ± 7	148 ± 10	288 ± 13	469 ± 9	348 ± 13	154 ± 11
novelty	D7	AMX	75 ± 3	125 ± 13	215 ± 26	276 ± 45	184 ± 29	120 ± 22
	D7	SHAM	77 ± 4	153 ± 9	249 ± 15	326 ± 35	172 ± 14	93 ± 8
	D21	AMX	77 ± 1	136 ± 16	211 ± 16	275 ± 28	199 ± 6	110 ± 11
	D21	SHAM	83 ± 3	139 ± 12	256 ± 21	339 ± 35	215 ± 18	118 ± 7

Table 4: Mean values ± SEM of the behavioral response to the 10 minute stress test.
FR = frequency (per 10 minutes), DR = duration (in seconds).

	footshock			
behavior	D7 AMX	D7 SHAM	D21 AMX	D21 SHAM
attention FR	49.9 ± 4.2	40.1 ± 3.0	68.3 ± 5.7	54.3 ± 5.5
attention DR	265.5 ± 34.3	145.3 ± 13.7	311.7 ± 47.5	250.0 ± 43.3
locomotion FR	41.9 ± 5.6	27.6 ± 2.8	42.5 ± 5.2	29.8 ± 2.8
locomotion DR	82.5 ± 18.8	49.6 ± 10.5	46.8 ± 3.5	34.0 ± 4.7
rearing FR	20.8 ± 2.4	21.1 ± 2.8	28.0 ± 5.3	22.9 ± 5.0
rearing DR	110.7 ± 31.8	157.8 ± 23.9	144.7 ± 32.2	124.5 ± 27.4
immobility FR	19.7 ± 4.7	18. ± 2.0	16.7 ± 3.7	12.3 ± 2.5
immobility DR	97.0 ± 18.6	239.1 ± 24.7	96.6 ± 29.9	188.1 ± 50.5
grooming FR	1.4 ± 0.5	0 ± 0	0.3 ± 0.3	0.6 ± 0.4
grooming DR	8.3 ± 3.9	0 ± 0	0.3 ± 0.3	3.5 ± 2.6

Plasma corticosterone (fig. 2D)
As for ACTH, an interaction was found between lesion × time (table 2). The lesioned groups (D7 AMX and D21 AMX) had a lower corticosterone response to novelty than did the SHAM groups.

Plasma catecholamines (table 3)
An interaction was found between lesion × time for noradrenaline, but not for adrenaline (table 2). The lesioned groups (D7 AMX and D21 AMX) had a lower noradrenaline response to novelty than did the SHAM groups (data not shown).

Behavior (table 4)
A day × lesion interaction was found ($F_{(10, 29)} = 2.512$, $p = 0.026$). Contrasts (table 5) revealed an effect on some, but not all, active behaviors measured. Attention (frequency) and rearing (frequency and duration) were attenuated in D7 AMX rats as compared to D21 AMX rats, while these behaviors were increased in D21 AMX versus D21 SHAM rats.

Discussion
Our results show that day 7 amygdala-lesioned rats (D7 AMX) differ from D21 AMX rats in that they have a significantly diminished HPA axis response to foot shock. Behaviorally, they can be distinguished from D21 AMX rats in that they exhibit more active behavior in response to foot shock and more passive behavior in response to novelty. D7 AMX rats had a smaller HPA axis response to both foot shock and novelty

novelty				
D7 AMX	D7 SHAM	D21 AMX	D21 SHAM	behavior
52.1 ± 4.0	67.0 ± 2.8	74.0 ± 9.1	63.4 ± 3.1	attention FR
363.5 ± 25.5	341.6 ± 20.5	391.3 ± 32.0	409.5 ± 15.0	attention DR
37.5 ± 4.5	43.5 ± 2.7	47.3 ± 3.5	38.9 ± 3.5	locomotion FR
66.0 ± 14.2	56.3 ± 5.2	43.4 ± 7.7	36.2 ± 4.5	locomotion DR
15.8 ± 2.5	28.5 ± 1.7	28.0 ± 5.2	22.4 ± 2.2	rearing FR
46.6 ± 9.2	108.2 ± 12.0	118.8 ± 22.8	68.4 ± 8.7	rearing DR
11.1 ± 3.1	10.1 ± 2.0	6.8 ± 2.7	6.9 ± 1.5	immobility FR
82.0 ± 23.3	45.6 ± 7.4	36.1 ± 12.6	54.9 ± 13.5	immobility DR
3.8 ± 0.5	4.9 ± 0.9	1.8 ± 0.9	3.9 ± 0.5	grooming FR
41.8 ± 6.3	43.7 ± 9.9	10.5 ± 5.1	31.0 ± 4.9	grooming DR

	footshock lesion		novelty day × lesion	
	$F_{(1,41)}$	p	$F_{(1,38)}$	p
attention FR	6.75	**0.013**	0.23	**0.008**
attention DR	7.51	**0.009**	0.00	0.475
locomotion FR	8.70	**0.005**	0.07	0.117
locomotion DR	2.38	0.131	0.48	0.918
rearing FR	0.40	0.533	1.55	**0.003**
rearing DR	0.18	0.673	0.16	**0.000**
immobility FR	0365	0.425	0.02	0.842
immobility DR	12.56	**0.001**	0.19	0.182
grooming FR	2.37	0.132	3.66	0.533
grooming DR	0.93	0.341	1.30	0.348

Table 5: Statistical analysis of the behavioral data (Multivariate Analysis). Lesion = AMX vs SHAM, day = day 7 vs day 21, FR = frequency (per 10 minutes), DR = duration (in seconds)

than day 7 sham-operated rats (D7 SHAM). However, they displayed more active behavior during foot shock stress and more passive behavior in response to novelty than D7 sham-operated rats.

The difference in the ACTH response to stress between D7 AMX and D21 AMX rats suggests that the absence of normal amygdala function is not the most critical factor in the stress response, because this structure was lesioned in both groups. Rather, the results suggest that different structures are impaired in D7 AMX rats and in D21 AMX rats and contribute to the difference in HPA axis function. These differences between the D7 AMX and D21 AMX rats provide support for the neurodevelopmental hypothesis that has been proposed for this animal lesion model (Wolterink et al., 1998; Wolterink et al., 2001). This hypothesis proposes that at a certain stage in development an intact amygdala is required for trophic stimulation of other developing brain areas (e.g. prefrontal cortex, striatal areas, and thalamus (Bouwmeester et al., 2002)). Damage to the amygdala during this period may lead to maldevelopment of those target areas. When the target areas have matured beyond a certain point (D21), trophic stimulation is no longer required for their normal development, and amygdala damage then results in a different type of deficit (a lesion of the amygdala accompanied by a developmental disorder (D7 AMX) versus solely a lesion of the amygdala (D21 AMX)).

The finding that lesioning of the amygdala lowered the noradrenaline response to both foot shock and novelty stress is consistent with previously published data, and sup-

ports the notion that the amygdala is important for the interpretation of environmental stimuli (Roozendaal et al., 1991*b*). Thus, the amygdala-lesioned animals could be less aroused, and therefore have an attenuated noradrenaline response to stress. On the basis of this finding and the observation that the catecholamine response of both lesioned groups (D7 AMX and D21 AMX) was similar, we assume that the stimuli were experienced similarly by both lesioned groups. Thus the two groups of lesioned animals can be distinguished by their endocrine and behavioral responses to stress, and not by their catecholamine response to stress. The discrepancy between the ACTH and corticosterone responses to foot shock can be explained in terms of the corticosterone response already being maximal at about 300 pg/ml ACTH in our rats (Nijsen et al., 2000).

The HPA axis is considered to mediate the adaptation of an organism to changing environmental circumstances, and therefore its response to stress (Huether, 1996). As patients with schizophrenia have a more passive coping style than healthy individuals, patients with schizophrenia may be less able to adapt to changing environmental circumstances (Gispen-de Wied, 2000; Jansen et al., 2000*a*). In our experiment, D7 AMX rats showed an attenuated response to stress not unlike the response seen in schizophrenic patients. D7 AMX rats also displayed a decrease in habituation and adaptation of locomotor activity to an open-field stimulus compared to D7 SHAM rats (Daenen, 1999), suggesting an inability to cope with a new environment.

The present data support the idea that the amygdala is not only critical in stress processes per se but is also vital for trophic input to its target structures (e.g. prefrontal cortex, striatal areas, and thalamus) in early life. Furthermore, the data highlight the similarity in deficits found (an attenuated stress response) in this animal model and in schizophrenic patients. These data therefore accord with the notion that the day 7 amygdala lesion in rats might be a neurodevelopmental model with relevance to schizophrenia. Further research should focus on the possible relationship between amygdala function in schizophrenic patients, their responsiveness to stress, and the effect of treatment on the responsiveness of the HPA axis, for which this animal model might offer a novel approach.

Acknowledgments

We thank Inge Wolterink-Donselaar and Annemarie van der Linden for expert surgical assistance, Henk Spierenburg for performing the catecholamine assay, and dr H.J.A. Wijnne for statistical support.

chapter 3

Reduced pituitary-adrenal response to CRH and elevated nocturnal plasma cortisol in adult rats with neonatally lesioned amygdala

Jeroen Terpstra, Christine C. Gispen-De Wied, Maarten van der Meer, Mark H. Broekhoven, Henk Spierenburg, Jan M. van Ree, Victor M. Wiegant

Abstract

Adult rats with amygdala lesions made on postnatal day 7 (D7 AMX rats) have been proposed as a model for neurodevelopmental psychopathological disorders such as schizophrenia. Recently, we found that the responsivity of the hypothalamus-pituitary-adrenal (HPA) axis to stress is impaired in these rats (Terpstra et al., 2003). Here we investigated the mechanism underlying this deficit, particularly the involvement of reduced sensitivity to CRH and of increased sensitivity to negative feedback actions of glucocorticoids. To that end, we determined (a) the plasma corticosterone (CORT) response following i.v. injection of graded doses of ovine CRH (oCRH: 0, 0.3 or 0.9 µg/kg), (b) the effect of pretreatment with a low dose of CORT on the plasma CORT response induced by novelty stress, and (c) the basal circadian plasma CORT profile. As compared with D7 SHAM rats, D7 AMX rats displayed a reduced CORT response to 0.9 µg/kg oCRH. The inhibitory effect of CORT pretreatment on the plasma CORT response induced by novelty stress was similar in D7 AMX and SHAM rats, suggesting that glucocorticoid feedback mechanisms were unaltered in D7 AMX rats. These data suggest that hyporesponsiveness to CRH of the pituitary corticotrophs underlies the previously observed deficit of D7 AMX rats in HPA responding to stress. In addition, D7 AMX rats displayed elevated basal plasma CORT levels, particularly during the nocturnal phase of the circadian cycle, that is when rats are awake and active. In absence of changes in glucorticoid feedback, this may suggest increased arousal. The present data add to evidence that amygdala lesions early in life may lead to developmental deficits in stress responsive systems in the rat. These deficits may be of relevance for neurodevelopmental disorders such as schizophrenia.

Introduction

Adult rats with amygdala lesions made on postnatal day 7 after birth (D7 AMX rats) have been proposed as a model for neurodevelopmental psychopathological disorders such as schizophrenia (Daenen et al., 2001; Wolterink et al., 2001; Daenen et al., 2002*a*). In contrast to rats with amygdala lesions made on day 21 after birth, D7 AMX rats show a number of deficits that are also seen in schizophrenia, for instance impaired pre-pulse inhibition, impaired social behavior, stereotyped hyperactivity, and increased sensitivity to phencyclidine and apomorphine (Daenen, 1999; Daenen et al., 2002*b*; Daenen et al., 2003*a*; Daenen et al., 2003*b*). Recently we found that the responsiveness of the HPA axis to stress is impaired in D7 AMX rats (Terpstra et al., 2003), thus adding another feature shared by the amygdala lesion model with schizophrenic patients. Indeed, it has been reported that schizophrenic patients display a blunted response to surgical stress (Kudoh et al., 1997; Kudoh et al., 1999), lumbar puncture stress (Breier et al., 1988), and psychosocial stress (Jansen et al., 1998; Jansen et al., 2000*a*).

In addition to aberrant stress responses, the tonic regulation of the HPA axis also appears to be affected in schizophrenia. Although a clear phase shift in the circadian rhythm of cortisol thusfar has not been established (Van Cauter et al., 1991; Rao et al., 1995; Lee et al., 2001), there is evidence that chronic schizophrenic patients display an irregular diurnal cortisol profile (Kaneko et al., 1992) and higher overall circulating cortisol levels during the nocturnal phase of the circadian cycle (Van Cauter et al., 1991; Monteleone et al., 1992). This evidence suggests a role of the stress-adaptive HPA system in schizophrenia, and underscores the relevance of further exploration of the D7 AMX model to elucidate the mechanisms underlying these deficits.

To investigate the mechanism underlying the blunted HPA axis response to stress of D7 AMX versus D7 SHAM rats, we assessed in these animals (1) the sensitivity of the pituitary-adrenal system to corticotrophin-releasing hormone (CRH) by testing the corticosterone (CORT) response following i.v. injection of synthetic ovine CRH (feed-forward test), and (2) the sensitivity of the HPA axis to glucocorticoid feedback by testing the inhibitory effect of CORT pretreatment on the novelty-stress induced CORT response (feedback test). In addition, we determined a complete circadian plasma CORT profile in D7 AMX and D7 SHAM rats under resting conditions to establish possible changes in the tonic regulation as a consequence of the amygdala lesions.

Experimental procedures

All procedures were performed in accordance with the guidelines established by the Committee for the Ethical Treatment of Animals of Utrecht University and of the Society for Neuroscience Policy on the Use of Animals in Research.

Animals and housing

Pregnant female Wistar rats (GDL, Utrecht, The Netherlands [U:WU]) were obtained at either day 17 or day 18 of gestation and were housed individually in Macrolon® Type III cages with sawdust bedding. After delivery, male and female pups were separated, and male pups were randomized among nests. All nests were made to consist of nine pups and contained at least two female pups and up to seven male pups. Male pups were lesioned on postnatal day 7 and were weaned at 21 days of age. Thereafter they were housed socially (2 to 3 rats per cage) in Macrolon® Type III cages with sawdust bedding. At the age of approximately thirteen weeks a chronic jugular vein cannula was surgically implanted and the rats were individually housed in transparent Perspex™ cages measuring 25 × 25 × 34 cm (l × w × h) with sawdust bedding, and were allowed to recover for two weeks. During this recovery period, the cannulas were flushed regularly and the rats were habituated to the blood sampling procedure. The rats were weighed daily after surgery until they reached their pre-surgical weight and were then weighed weekly. They were given standard chow (Hope Farms BV, Woerden, The Netherlands) and water ad libitum. A 12-hour light/dark cycle was used, with lights on from 07.00h to 19.00h. Ambient temperature was kept constant at 21 ca. 1 ˚C, humidity at 50 ca. 10%.

Surgical procedures and histology

In total, sixty male pups were lesioned as described previously (Wolterink et al., 2001). The pups were anesthetized with fentanyl (0.3 mg/kg, s.c.), and placed with their head in a specially constructed head mould to enable stable fixation. The coordinates for positioning of the needles for the bilateral amygdala lesions were 3.8 mm lateral to the midline, 1.0 mm posterior to bregma, and 6.0 mm below the surface of the skull. Needles were placed at an angle of 4˚ with the tip aimed at the basolateral nucleus of the amygdala (BLA). Lesions were made by infusion of ibotenic acid (a total of 3 µg/0.3 µl infused over a two minute period), using a microinfusion pump (Harvard apparatus 22). After infusion, the needles were left in place for 4 minutes. SHAM-lesioned rats received the corresponding volume of vehicle (0.1 M phosphate-buffered saline (PBS), pH 7.4). Three months after the operation all animals were sugically provided with a permanent jugular vein cannula, to facilitate repeated blood sampling. Surgery was performed under 0.1 ml/100 g i.m. fentanyl/fluanisone anaesthesia (Hypnorm®,

Janssen Parmaceutica, Beerse, Belgium). Within two weeks after the end of the experiments rats were killed with an overdose of intravenous pentobarbital. Brains were collected in formaldehyde-containing vials, sectioned (50 µm) and stained with hematoxylin and eosin. Histological evaluation showed that AMX lesions in five rats (approximately 13% of the lesions) were either too large or outside the BLA (Paxinos and Watson, 1997), and data from these animals were discarded.

Experimental Design

Feedforward test

To investigate the pituitary-adrenal response to corticotrophin releasing hormone (CRH), groups of D7 AMX and D7 SHAM rats were injected (i.v.) with ovine CRH (oCRH: American Peptide Comp., Sunnyvale, CA, USA; dissolved in a sterile saline solution) or a similar volume of vehicle (Veh). Two doses of oCRH were used: 0.3 mg/kg and 0.9 mg/kg (Buwalda et al., 1999; Kamphuis et al., 2002). After the two-week recovery period following the jugular vein cannulation, the rats were divided among six groups: AMX/0.3 µg/kg CRH (n = 4), AMX/0.9 µg/kg CRH (n = 6), AMX/Veh (n = 6), SHAM/0.3 µg/kg CRH (n = 7), SHAM/0.9 µg/kg CRH (n = 5), SHAM/Veh (n = 5). All rats were tested in the home cage under resting conditions, and hooked up to the blood-sampling cannula 90 minutes prior to the first blood sample. At t = -5 minutes (before start of the experiment) a blood sample (ca. 150 µl) was taken through the jugular vein cannula to assess basal HPA axis activity. At t = 0 minutes oCRH or vehicle was injected (i.v.), using a separate long line (rinsed with CRH solution before use) hooked up to the jugular vein cannula. After the infusion, the blood-sampling line was reconnected and blood samples (ca. 150 µl) were taken on t = 5, 10, 20 and 30 minutes to monitor the pituitary-adrenal axis.

Feedback test

To investigate the sensitivity of the HPA axis to glucocorticoid feedback, groups of D7 AMX and D7 SHAM rats were pretreated with a subcutaneous injection of 0.1 mg/kg corticosterone (CORT: Sigma, St Louis, Mo, USA; dissolved in ethanol:saline solution 1:9) or an equal volume of vehicle (Veh), using a previously published protocol (Kamphuis et al., 2002).

Two weeks after the Feedforward (FF) test the animals were divided among four groups: AMX/CORT (n = 7), AMX/Veh (n = 5), SHAM/CORT (n = 8), SHAM/Veh (n = 9). Animals received an 0.25 ml injection (s.c.) of either CORT or vehicle 3 hours before the start of the test. All rats were hooked up to the blood-sampling cannula 90 minutes prior to the first blood sample. At t = -5 minutes (before start of the test) a

blood sample (ca. 150 µl) was taken through the jugular vein cannula to assess HPA axis activity prior to the test. On t = 0 minutes the animals were transported from the home cage to the test cage (a novel box with grid-floor measuring 32 × 30 × 38 cm) where they remained for the duration of the experiment. Blood samples (ca. 150 µl) were taken at t = 10, 20 and 30 minutes to monitor the HPA axis response to the novelty stress.

Circadian corticosterone profile

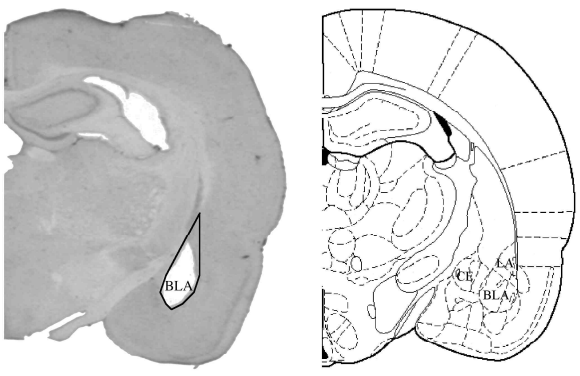
Two weeks after the feedback (FB) test the animals (7 AMX rats, 10 SHAM rats) were submitted to scheduled bloodsampling every 2 hours for one 24 hour cycle. All rats were hooked up to the blood-sampling cannula 90 minutes prior to the first blood sample. Blood samples (ca. 50 µl) were taken via the jugular vein cannula under resting conditions in the home cage.

Analysis of corticosterone

Blood samples were collected in heparin/EDTA-containing vials and kept on ice, centrifuged, and plasma was stored at -80˚C until determination of hormones. Corticosterone was determined using an ImmuChem® ^{125}I RIA kit (ICN Biomedicals, Inc.).

Statistical analysis

The data were analyzed using repeated measures analysis of variance (ANOVA, SPSS 10.0.8 for Macintosh®). The analysis of the feedforward (FF) data included the within subjects factor 'time' (the CORT response over time; five levels: t= –5, 5, 10, 20 and 30 minutes), 'treatment' (three levels: 0.3 µg/kg oCRH, 0.9 µg/kg oCRH and vehicle) and



A B

Fig. 1.
Panel A: Micrograph of a brain section from a D7 AMX rat showing a representative amygdala lesion. Highlighted, after histological evaluation, is the location of the basolateral amygdala.
Panel B: Anatomical map of several amygdala nuclei. BLA: basolateral nucleus of the amygdala, LA: lateral nuclei of the amygdala, CE: central nuclei of the amygdala (figure derived from (Paxinos and Watson, 1997).

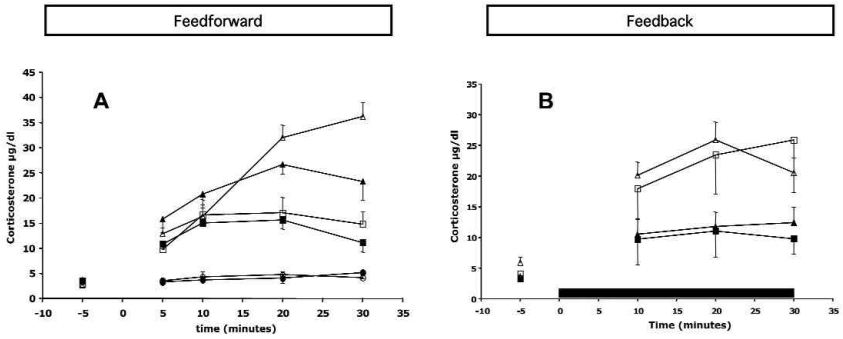


Fig. 2. Panel A: Feedforward test. Plasma CORT response (µg/dl) induced by i.v. injection of oCRH (0.3 µg/kg, 0.9 µg/kg) or vehicle (Veh). Open symbols represent SHAM animals, closed symbols AMX animals. Squares represent 0.3 µg/kg CRH, triangles 0.9 µg/kg CRH, and circles Veh.
Panel B: Feedback test. Plasma CORT response (µg/dl) induced by novelty stress (black bar) three hours following pretreatment with CORT. Open figures represent CORT pretreated, closed figures Veh pretreated rats. Squares represent AMX, triangles SHAM animals.

'lesion' (two levels: AMX and SHAM). The analysis of the feedback (FB) data included the within subjects factor 'time' (the CORT response over time; four levels: t = −5, 10, 20 and 30 minutes), 'treatment' (two levels: 0.1 mg/kg CORT and vehicle) and 'lesion' (two levels: AMX and SHAM). The analysis of the circadian data included the within subjects factor 'time' (the circadian profile over time; thirteen levels: t = 16:00, 18:00, 20:00, 22:00, 24:00, 02:00, 04:00, 06:00, 08:00, 10:00, 12:00, 14:00 and 16:00(2) hours), and 'lesion' (two levels: AMX and SHAM). When relevant, post-hoc analyses were performed using an ANOVA repeated measures. Significance was reached when $p \leq 0.05$.

Results

Histological evaluation showed that the size and location of the amygdala lesions (fig. 1) were similar to those previously described (Wolterink et al., 2001).

Feedforward test (fig. 2)

The within subjects analysis of the CORT response in the feedforward test revealed an effect of 'time', and 'lesion × time', 'treatment × time', and 'lesion' × 'treatment' × 'time' interactions (table 1). D7 AMX rats showed a less pronounced response to the highest dose of oCRH (0.9 µg/kg) compared to D7 SHAM rats. The between subjects analysis revealed an effect of 'treatment', indicating that the oCRH-induced CORT response was dose dependent.

feedforward test		
within subjects effects	CORT	
	$F_{(4,108)}$	p
time	71.391	**0.000**
lesion × time	4.618	**0.002**
	$F_{(8,108)}$	p
treatment × time	24.520	**0.000**
lesion × treatment × time	3.139	**0.003**
between subjects effects		
	$F_{(1,27)}$	p
lesion	0.831	0.370
	$F_{(2,27)}$	p
treatment	54.734	**0.000**
lesion × treatment	0.191	0.827

Table 1: Statistical analysis of the corticosterone (CORT) response induced in D7 AMX and D7 SHAM rats by i.v. injection of oCRH (ANOVA repeated measures). Time = endocrine response over time, lesion = AMX vs. Sham, and treatment = 0.3 µg/kg oCRH vs. 0.9 µg/kg oCRH vs. Saline.

feedback test		
within subjects effects	CORT	
	$F_{(3,75)}$	p
time	16.667	**0.000**
lesion × time	0.924	0.433
treatment × time	2.107	0.106
lesion × treatment × time	0.135	0.939
between subjects effects		
	$F_{(1,25)}$	p
lesion	0.001	0.980
treatment	7.881	**0.010**
lesion × treatment	0.154	0.698

Table 2: Statistical analysis of the corticosterone (CORT) response induced in D7 AMX and D7 SHAM rats by novelty stress following pretreatment with CORT or vehicle (Veh) (ANOVA repeated measures). Time = endocrine response over time, Lesion = AMX vs. Sham, and Treatment = 0.1 mg/kg CORT vs. Veh.

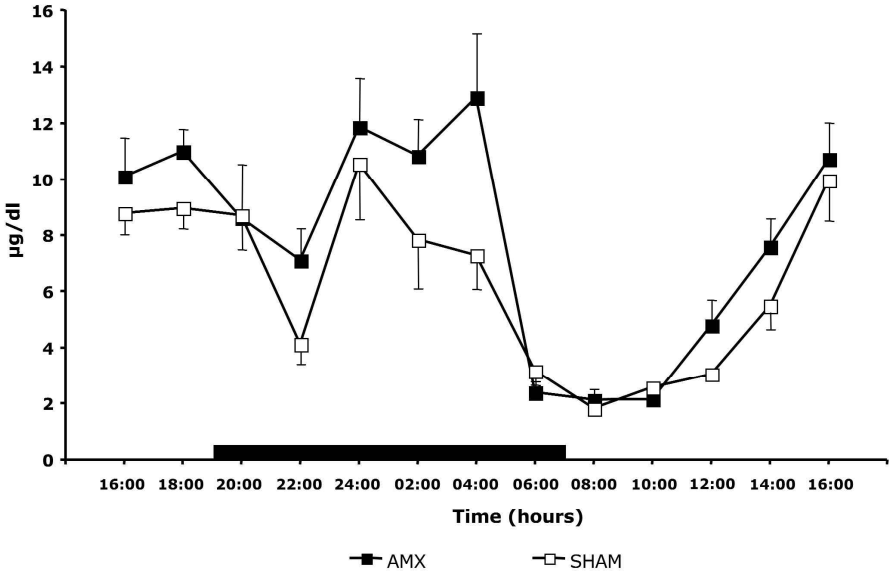
circadian rhythm		
within subjects effects	CORT	
	$F_{(12,180)}$	p
time	17.007	**0.000**
lesion × time	1.099	0.364
between subjects effects		
	$F_{(1,15)}$	p
lesion	8.038	**0.013**

Table 3: Statistical analysis of the circadian profile of basal plasma corticosterone (CORT) concentrations in D7 AMX and D7 SHAM rats (ANOVA repeated measures). Time = circadian rhythm, Lesion = AMX vs. Sham.

Feedback test (fig. 2)
The within subjects analysis of the CORT results from the feedback test (table 2) revealed an effect of 'time', indicating that the novelty stress induced a CORT response. The between subjects analysis revealed an effect of 'treatment', indicating that the CORT pretreatment led to a lower novelty-induced CORT response.

Circadian corticosterone profile (fig. 3)
The within subjects analysis of the basal circadian CORT profile revealed an effect of 'time' (table 3), indicating a biphasic circadian rhythm. The between subjects analysis revealed an effect of 'lesion', indicating higher basal levels of CORT in D7 AMX than in SHAM rats. Post-hoc between subjects analysis of the nocturnal part of the circadian curve (from 20:00 until 06:00) revealed an effect of 'lesion' ($F_{(1,15)} = 5.231$, $p = 0.037$), indicating that basal CORT levels are higher in D7 AMX than in SHAM rats during the nocturnal phase of the circadian cycle.

Fig. 3. Circadian profile of basal plasma corticosterone (CORT) concentrations (µg/dl). Open symbols: D7 SHAM rats. Closed symbols: D7 AMX rats. Black bar represents the nocturnal period (lights off from 19:00 to 7:00).



Discussion

We have shown previously that D7 AMX rats display a reduced HPA response to stress as compared to D7 SHAM rats. Here, we investigated the possible involvement in this phenomenon of reduced responsivity of the pituitary-adrenocortical axis to CRH and enhanced negative glucocorticoid feedback.

D7 AMX rats showed a lower CORT response after i.v. injection of oCRH than D7 SHAM rats, suggesting reduced signalling at the level of CRH receptors. It has been shown that peripherally adminstered oCRH does not cross the blood brain barrier (Martins et al., 1996), but can easily reach the pituitary corticotrophs, the main targets of hypothalamic CRH in regulating pituitary-adrenocortical activity. In addition, it can reach CRH receptors located at other peripheral sites that might be relevant in the present context. There is evidence, for instance, that stimulation of CRH receptors in the adrenal cortex, adrenal medulla and sympathetic ganglia can enhance adrenocortical sensitivity to ACTH (Udelsman et al., 1986; Willenberg et al., 2000), thereby altering the CORT/ACTH ratio. However, using the data on the ACTH and CORT responses to novelty from our previous study (Terpstra et al., 2003; Chapter 2), we found no difference in the CORT/ACTH ratio between D7 AMX and D7 SHAM rats (fig. 4), indicating normal adrenocortical sensitivity for corticotropin in D7 AMX rats. The present data therefore suggest that the mechanism underlying the reduced responsivity of the HPA axis to stress in D7 AMX rats likely involves reduced responsivity of the pituitary corticotrophs to CRH.

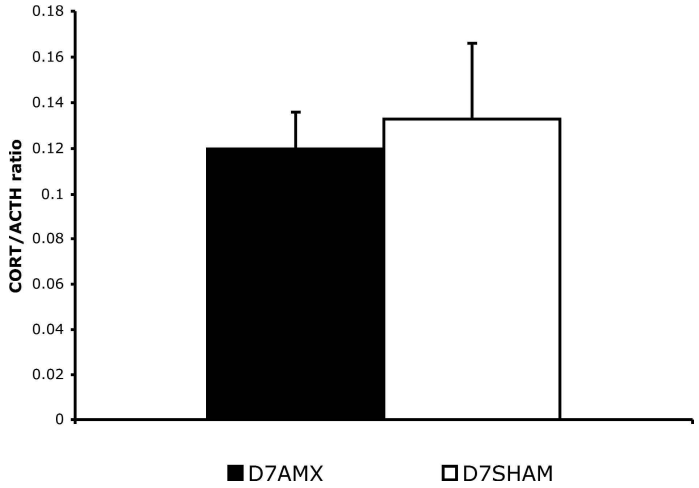


Fig. 4. CORT/ACTH ratio calculated from the area under the curve (AUC) of the plasma ACTH and CORT response to novelty (data from (Terpstra et al., 2003); Chapter 2). Statistical analysis (univariate ANOVA) of the CORT/ACTH ratio on the fixed factor 'lesion' (AMX vs SHAM) showed no effect ($F_{(1,16)} = 0.155$, $p = 0.699$).

The possibility that the reduced HPA response of D7 AMX rats to novelty stress involves increased sensitivity to glucocorticoid feedback was tested using rats pretreated with a dose of CORT that has previously been established in our lab as threshold-dose for inhibition of the novelty induced HPA response in rats of the same Wistar strain as used in the present study (Kamphuis et al., 2002). CORT-pretreated D7 AMX and D7 SHAM rats showed similar relative reductions of the stress induced plasma CORT response (AMX: 53+/-15%, SHAM: 49+/-10%), suggesting that negative feedback mechanisms were not affected by the neonatal amygdala lesion.

The CORT responses of vehicle pretreated D7 AMX and D7 SHAM rats following novelty stress were also similar, which seems to be at variance with our previous observations (Terpstra et al. 2003; Chapter 2). It should be noted, however, that the rats used in the present feedback experiment were not naïve, as were the ones in our previous study (Terpstra et al., 2003), but had been subjected to extensive handling and testing in the feedforward experiment. It has been reported (Daenen et al., 2001) that D7 AMX rats do not habituate to environmental stimuli to the same extend as D7 SHAM rats. The experimental procedure used in the feedback experiment may therefore have represented a stronger stimulus for the AMX than for the SHAM rats, thus masking the deficit in HPA responding to stress of D7 AMX rats.

Finally, we determined the basal plasma CORT concentration in D7 SHAM and D7 AMX rats throughout a complete circadian cycle. Plasma CORT levels were found to be higher in D7 AMX than D7 SHAM rats during the peak, but not during the trough of the circadian cycle. Circulating CORT controls HPA axis activity by negative feedback action on brain and pituitary via interaction with mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) located along the axis, in the hippocampus (MR, GR), hypothalamus (GR) and anterior pituitary (GR). MR possess high affinity for CORT and are almost saturated even during the circadian trough, while GR have relatively low affinity for CORT and only become operational at higher CORT levels, e.g. during the circadian peak and during stress (Reul and de Kloet, 1985; de Kloet et al., 2000; Sapolsky et al., 2000). It is unlikely, however, that the elevated nocturnal CORT levels in D7 AMX relate to a deficit in either of these mechanisms, since we found normal CORT levels in D7 AMX rats during the circadian trough, and intact negative feedback when CORT levels were high due to novelty stress.

Rats are nocturnal animals, and the increased plasma CORT levels of D7 AMX rats were found only during the phase of the circadian cycle when rats are awake and active. It has been shown that D7 AMX rats are more active than D7 SHAM rats in open field tests, and do not habituate to novelty when tested repeatedly (Daenen et al., 2001, 2002*a*), suggesting a deficit in processing of and adaptation to environmental stimuli. We therefore speculate that the increased nocturnal plasma CORT levels of D7 AMX

rats reflect a state of increased arousal during wakefulness, although data concerning spontaneous home cage behaviour of D7 AMX rats during the dark phase of the circadian cycle are lacking.

D7 AMX rats have been proposed as an animal model of schizophrenia, since they share a number of deficits with the disease that include impaired HPA responses to stress. Interestingly, there are literature data suggesting that basal glucocorticoid levels may be increased in unmedicated schizophrenic patients in a similar vein as in D7 AMX rats. Thus, Lee and coworkers found higher cortisol levels compared to healthy controls during the waking stage but not during sleep (Lee et al., 2001). Others found normal cortisol levels during wakefulness, but elevated levels during the first four hours after sleep onset, suggesting a relationship with the abnormally long sleep latencies of the patients (Van Cauter et al., 1991).

In conclusion, our present data indicate that the impaired HPA response to stress of D7 AMX rats are, at least in part, due to hyporesponsiveness of the pituitary corticotrophs to CRH. In addition, the elevated nocturnal basal CORT levels might reflect hyperarousal of D7 AMX rats during the active phase of the circadian cycle.

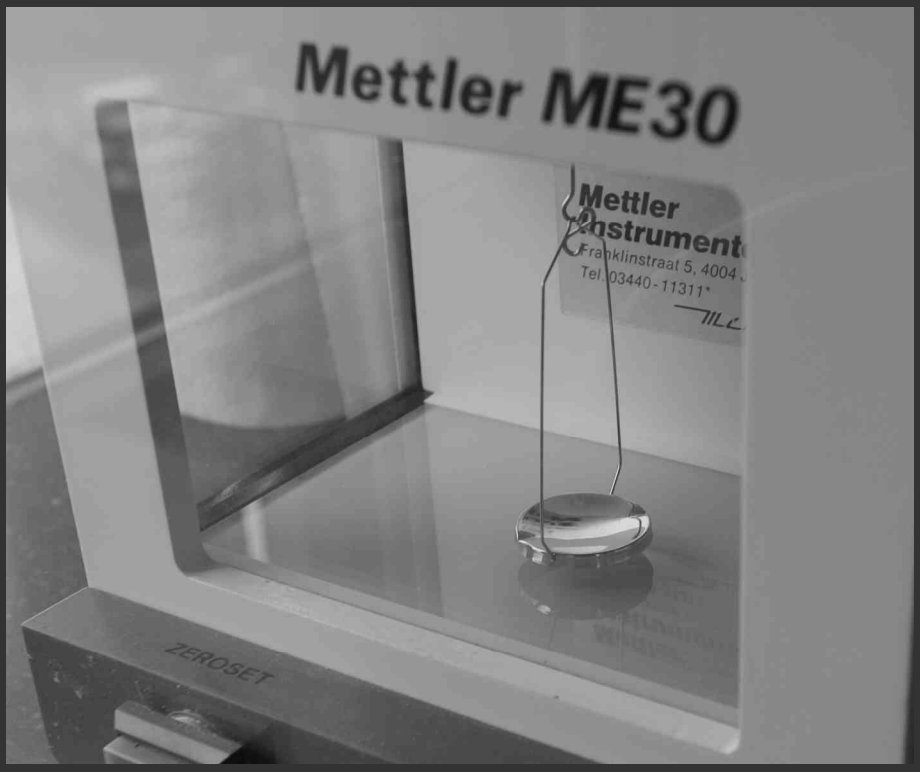
Acknowledgments

We thank Inge Wolterink-Donselaar and Annemarie van der Linden for their expert surgical assistance.

chapter 4

α_1- and α_2-adrenoceptor balance in brain regions of adult rats with neonatally lesioned amygdala

Jeroen Terpstra, Christine C. Gispen-De Wied, Maarten van der Meer, Mark H. Broekhoven, Henk Spierenburg, Jan M. van Ree, Victor M. Wiegant



Abstract

In an animal model for neurodevelopmental disorders, in which the effects of an early neonatal (postnatal day 7 (D7)) basolateral amygdala lesion are compared to the effects of a lesion later in life (D21), it was observed that the noradrenalin (NA) transmission in particular brain regions of adult rats was reduced. To further investigate the disturbances in brain NA systems, the present study focused on NA receptor density in the model. Animals were lesioned in the basolateral amygdala at D7 or D21, and compared to sham operated animals. Autoradiography was performed with [^{3}H]Prazosin and [^{3}H]UK14.304, ligands specific for α_1- and α_2-adrenoceptors respectively, on coronal cryosections of adult rat brains, using saturating radioligand concentrations to estimate B_{max}. As brain regions of interest were analyzed the medial prefrontal cortex (mPFC), the nucleus accumbens (Acb), the caudate putamen (CPu), the paraventricular nucleus of the hypothalamus (PVN), and the locus coeruleus (LC).

No difference in specific α_1- and α_2-adrenoceptor binding between the experimental groups was observed, indicating there was no difference in adrenoceptor density. The α_1/α_2-receptor balance was: $\alpha_1 > \alpha_2$ in the mPFC and CPu, $\alpha_1 = \alpha_2$ in the Acb, and $\alpha_1 < \alpha_2$ in the PVN and LC. Although the NA receptor density and the NA transmission were apparently not changed simultaneously, further investigations are needed addressing dynamic changes over time and the various adrenoceptive subtypes, before definite conclusions can be drawn about functional changes in brain NA systems as related to the observed behavioural and endocrine deficits in this model.

Introduction

Neonatal excitotoxic amygdala lesions in rats have been put forward as a neurodevelopmental animal model of some aspects of schizophrenia (Wolterink et al., 2001). Adult rats, with basolateral amygdala lesions made on postnatal day 7 (D7 AMX) show a number of deficits that are also seen in schizophrenia, for instance an impaired prepulse inhibition, impaired social behaviour, stereotyped hyperactivity, increased sensitivity to phencyclidine and apomorphine, and a blunted HPA axis response to stressful stimuli (Daenen et al., 2002*b*; Daenen et al., 2003*a*; Daenen et al., 2003*b*). These deficits, except the impaired social behaviour, were not observed when similar lesions were made on postnatal day 21 (D21 AMX), indicating that neurodevelopmental disturbances with functional implications may underly the observed deficits. Neuroanatomical studies have revealed that the adult-like reciprocal connections between the basolateral amygdala and the medial prefrontal cortex occurred around D9 / D11. Thus, the lesions made on D7 may have interfered with the physiological development of the medial prefrontal cortex.

Recently it was found that dopamine metabolism and dopamine receptor levels in meso(cortico)limbic projection regions of adult rats were affected following neonatal amygdala lesions (Bouwmeester, 2002). The dopamine (DA) turnover was increased, and the density of D_1- and D_2-like receptors decreased in the mesolimbic, but not in the nigrastriatal DA system. In addition, decreased concentrations of noradrenalin (NA) and its metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) were found in the medial prefrontal cortex (mPFC), the nucleus accumbens (Acb), and the caudate putamen (CPu) indicating a reduced NA neurotransmission in these brain structures. To further investigate the disturbances in brain NA systems, the present study focusing on NA receptor density was performed.

The cerebral NA system utilizes several receptor subtypes (α_1, α_2, β_1, and β_2), of which the α_1- and α_2-adrenoceptors are the most abundant. Although several studies have focused on specific adrenoreceptor subtypes and their distribution in various brain regions (Jones et al., 1985; Nicholas et al., 1993; Pieribone et al., 1994; Scheinin et al., 1994), the distribution of both α_1- and α_2-adrenoceptors in a single study has hardly been measured (Young and Kuhar, 1980; Chamba et al., 1991). This approach could be important, since α_1- and α_2-adrenoceptors have been shown to mediate opposite effects in mPFC (Birnbaum et al., 1999; Li et al., 1999), Acb (Nurse et al., 1984; Tuinstra and Cools, 2000), and paraventricular nucleus of the hypothalamus (PVN) (Brooks et al., 1986), suggesting that the balance between the two receptors may be of functional importance. The locus coeruleus (LC), known to regulate its own NA activity via α_2 autoreceptors (Washburn and Moises, 1989), has been shown to contain also

α_1-adrenoceptors (Jones et al., 1985; Chamba et al., 1991), although their cellular localization and specific function remains largely unknown (Chamba et al., 1991).
Based on the reported alterations in NA neurotransmission in the mPFC, Acb and CPu, and the attenuated plasma NA response to stress in D7 AMX rats (Bouwmeester, 2002; Terpstra et al., 2003), we hypothesized a change in the α_1/α_2-adrenoreceptor balance to be involved in the deficits observed at the endocrine and behavioral levels in the neonatal amygdala lesioned animals. As regions of interest were selected the mPFC, Acb, CPU and, in addition, the PVN and the LC. The PVN is pivotal in the regulation of the HPA axis (Gaillet et al., 1993) and its NA innervation, originating from a small group of neurons located in the brainstem (A1/A2 nuclei), plays an important role in the control of the PVN (Day et al., 1985; Cunningham and Sawchenko, 1988; Day, 1989; Plotsky et al., 1989). The locus coeruleus (LC) was included, since it is the origin of the vast majority of NA projections in the central nervous system including those to the mPFC, Acb, and CPu (Dahlstrom and Fuxe, 1964).
Quantitative receptor autoradiography of the α_1- and α_2-adrenoreceptor, utilizing [^{3}H]Prazosin and [^{3}H]UK14.304 respectively, has shown to be a highly sensitive method for discerning quantitative differences in receptor binding, whilst providing information on anatomical localization (Morien et al., 1999; Diaz-Cabiale et al., 2000). Therefore, specific [^{3}H]Prazosin and [^{3}H]UK14.304 binding was determined on coronal cryosections of adult animals (sham) lesioned in the basolateral amygdala at postnatal day 7 or 21, using saturating radioligand concentrations to estimate B_{max} in the brain regions of interest.

Materials and methods

All procedures were performed in accordance with the guidelines established by the Committee for the Ethical Treatment of Animals Utrecht (DEC Utrecht), The Netherlands, and the Society for Neuroscience Policy on the Use of Animals in Research.

Animals and housing

Pregnant female Wistar rats [Harlan, HsdCpb:WU] were obtained at either day 17 or day 18 of gestation and housed individually in Macrolon® Type III cages with sawdust bedding. After delivery, male and female pups were separated, and male pups were randomized among nests. All nests contained nine pups (at least two female pups and up to seven male pups). Male pups were lesioned on postnatal day 7 or 21 and weaned at 21 days of age. Thereafter they were housed socially (2 to 3 rats per cage) in Macrolon® Type III cages with sawdust bedding, until they reached adulthood (approximately 13 weeks). They were given standard chow (Hope Farms BV, Woerden, The

Netherlands) and water ad libitum. A 12-hour light/dark cycle was used, with lights on from 07.00 to 19.00. Ambient temperature was kept constant at 21 ± 1 ˚C and humidity at 50 ± 10%.

Surgical procedures and histology

In total, forty male pups randomly assigned to day of surgery and to actual or sham lesion, were lesioned as described previously (Wolterink et al., 2001). The pups were anesthetized with fentanyl (0.3 mg/kg, s.c. (Janssen Pharmaceutica, Beerse, Belgium)). D7 pups were placed with their head in a specially constructed head mould to enable stable fixation. The coordinates for positioning of the needles for the bilateral amygdala lesions were 3.8 mm lateral to the midline, 1.0 mm posterior to bregma, and 6.0 mm below the surface of the skull. Needles were placed at an angle of 4˚ with the tip aimed at the basolateral nucleus of the amygdala (BLA). D21 pups were placed with their head in a David Kopf® stereotaxic apparatus with the incisor bar at horizontal zero. The coordinates for positioning of the needles for the bilateral amygdala lesions were 4.0 mm lateral to the midline, 2 mm posterior to bregma, and 6.8 mm below the surface of the skull. Needles were placed at an angle of 0˚ with the tip aimed at the basolateral nucleus of the amygdala (BLA). Lesions were made by constant infusion of ibotenic acid (with an infusion rate of 0.15 µl/min over a two minute period, constituting a total of 3 µg ibotenic acid/0.3 µl phosphate-buffered saline (PBS) for D7, or 4 µg ibotenic acid/0.3 µl PBS for D21), using a micro infusion pump (Harvard apparatus 22). After infusion, the needles were left in place for 4 minutes. SHAM-lesioned rats received the corresponding volume of vehicle (0.1 M PBS, pH 7.4). Three months after the AMX procedure all animals were killed by decapitation. Brains were rapidly removed from the skull, and immediately frozen at - 40˚C in isopenthane and stored at - 80 ˚C until sectioning. Coronal sections (20 µm thick), starting from the prefrontal cortex and terminating after the cerebellum, were made on a cryostat, and thaw-mounted on gelatin-coated slides in series of fourteen consecutive slides. Sections were stored at - 80 ˚C until autoradiography. Per rat, one series of sections containing the amygdala was stained with hematoxylin and eosin for histological evaluation of the lesions. In one rat the lesions were too large and largely outside the BLA (Paxinos and Watson, 1997), and data from this animal were discarded.

Quantitative receptor autoradiography

The autoradiographic procedures were based on previously published protocols (Kitchen et al., 1997; Morien et al., 1999; Diaz-Cabiale et al., 2000; Lesscher et al., 2003). Briefly, sections were preincubated for 30 minutes at room temperature in a 50 mM Tris HCl buffer (pH 7.4) containing 0.9% NaCl. Subsequently the sections were

incubated for 60 minutes at room temperature in a 50 mM Tris HCl buffer (pH 7.4) containing either 5 nM [^{3}H]Prazosin (KD = 0.28 nM, B_{max} ≈ 2-4 nM (Greengrass and Bremner, 1979); Amersham, Buckinghamshire, UK), or 5 nM [^{3}H]UK14.304 (KD ≈ 3 nM, B_{max} ≈ 5-10 nM (Diaz-Cabiale et al., 2000); Perkin-Elmer, Boston, MA, USA). Saturating ligand concentrations were chosen to assess B_{max}, in order to obtain an indication of receptor density. Non-specific binding was determined on adjacent sections, and defined as radioligand binding in the presence of excess unlabelled Phentolamine (Sigma-Aldrich, Steinheim, Germany). The excess concentration of Phentolamine was 100 µM for non-specific [^{3}H]Prazosin binding, and 10 µM for [^{3}H]UK14.304. After incubation, the sections were washed three times for five minutes in an ice-cold 50 mM Tris HCl rinse buffer, pH 7.4, and air-dried. The dried sections were stored in an airtight container filled with anhydrous calcium sulphate drying agent (Fluka Chemie, Buchs, Germany). For autoradiography, the sections were apposed to tritium-sensitive BAS-IP-TR2040 phosphor-imager plates (Fuji Photo Film Co., Japan) for approximately 72 hours. The highly sensitive plates were shielded from environmental background radiation with a lead casing (3 mm thick).

Computer-assisted image analysis

After exposure, the BAS-IP-TR2040 plates were scanned in a Fuji film FLA-5000 scanner with a 635 nm laser, at 8 bit (256) grey depth and 25 µm resolution. The digital files produced were converted to 8 bit TIFF images using the Advanced Image Data Analyzer (AIDA) version 3.11.002 (Raytest Isotopenmeßgeräte, Germany). Brain regions of interest (medial prefrontal cortex (mPFC), accumbens nucleus (Acb), caudate-putamen (CPu), paraventricular nucleus of the hypothalamus (PVN), and locus coeruleus (LC)) were analyzed using the ImageJ image analysis program version 1.29 (by Wayne Rasband , Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA). ^{3}H-labeled polymer microscale strips (Amersham microscale, UK), were included with each plate, and were used to calculate standard curves and convert grey values into fmol/mg binding values. For each brain region, two to four quantified measures were taken from both hemispheres, yielding a total of four to eight measurements (freehand drawing). Brain regions were identified according to The Rat Brain Atlas (Paxinos and Watson, 1997).

Statistical analysis

For statistical analysis of the data SPSS 11.0 for Macintosh® was used. The α_1 and α_2-adrenoceptor data were analyzed separately by multivariate analysis of variance (MANOVA), with 'day' (D7 vs. D21) and 'lesion' (AMX vs. SHAM) as fixed factors. Since we used saturating radioligand concentrations, specific binding approached

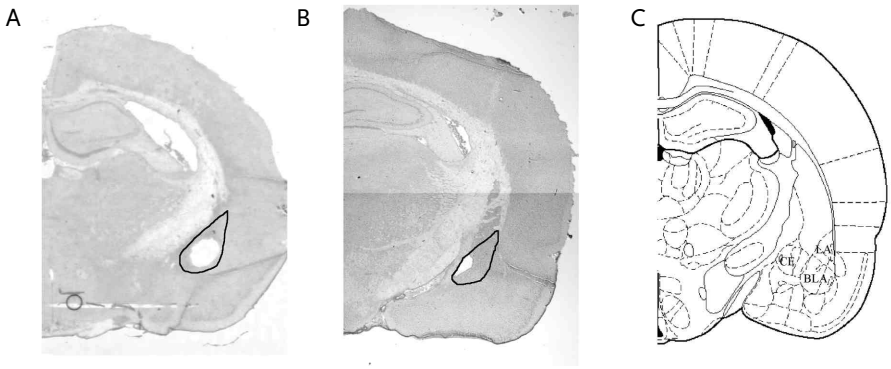


Fig. 1. Micrograph of a representative amygdala lesion. Highlighted, after histological evaluation, is the relative location of the basolateral amygdala. A: D7 AMX, B: D21 AMX, and C: anatomical drawing of several amygdala nuclei, BLA: basolateral nucleus of the amygdala, LA: lateral nuclei of the amygdala, CE: central nuclei of the amygdala (figure derived from Paxinos and Watson, 1997).

B_{max}, and could be used as an indication of receptor density. The regional α_1- and α_2-adrenoceptor balance was post-hocanalyzed using a multivariate analysis of variance (MANOVA), with 'receptor' (α_1 vs. α_2) as a fixed factor. Significance was accepted at $p \leq 0.05$. Data are presented as mean ± SEM.

Results

Histological evaluation showed that the size and location of the AMX lesions were similar to those previously described (Wolterink et al., 2001) (fig. 1.). The α_1- and α_2-ligand binding resulted in a high foreground and low background (fig. 2).

α_1-adrenoceptor autoradiography: specific [^{3}H]Prazosin binding

Quantitative analysis of α_1-radioligand binding in the mPFC, CPu, Acb, PVN and LC did not reveal effects of 'lesion' (mPFC: $F_{(1,28)} = 3.144$, $p = 0.087$; CPu: $F_{(1,28)} = 1.011$, $p = 0.323$; Acb: $F_{(1,28)} = 1.834$, $p = 0.186$; PVN: $F_{(1,28)} = 0.773$, $p = 0.387$) or 'day' (mPFC: $F_{(1,28)} = 0.545$, $p = 0.466$; CPu: $F_{(1,28)} = 1.550$, $p = 0.223$; Acb: $F_{(1,28)} = 0.469$, $p = 0.499$; PVN: $F_{(1,28)} = 0.870$, $p = 0.359$), indicating that there were no differences between the experimental groups in α_1-adrenoceptor density in these brain regions (fig. 3A).

α_2-adrenoceptor autoradiography: specific [^{3}H]UK14.304 *binding*

Quantitative analysis of α_2-radioligand binding in the mPFC, CPu, Acb, PVN and LC did not reveal effects of 'lesion' (mPFC: $F_{(1,27)} = 1.751$, $p = 0.197$; CPu: $F_{(1,27)} = 0.165$, $p = 0.688$; Acb: $F_{(1,27)} = 0.549$, $p = 0.465$; PVN: $F_{(1,27)} = 1.590$, $p = 0.218$) or 'day' (mPFC: $F_{(1,27)} = 0.619$, $p = 0.438$; CPu: $F_{(1,27)} = 0.185$, $p = 0.670$; Acb: $F_{(1,27)} = 0.211$, $p = 0.650$; PVN: $F_{(1,27)} = 0.527$,

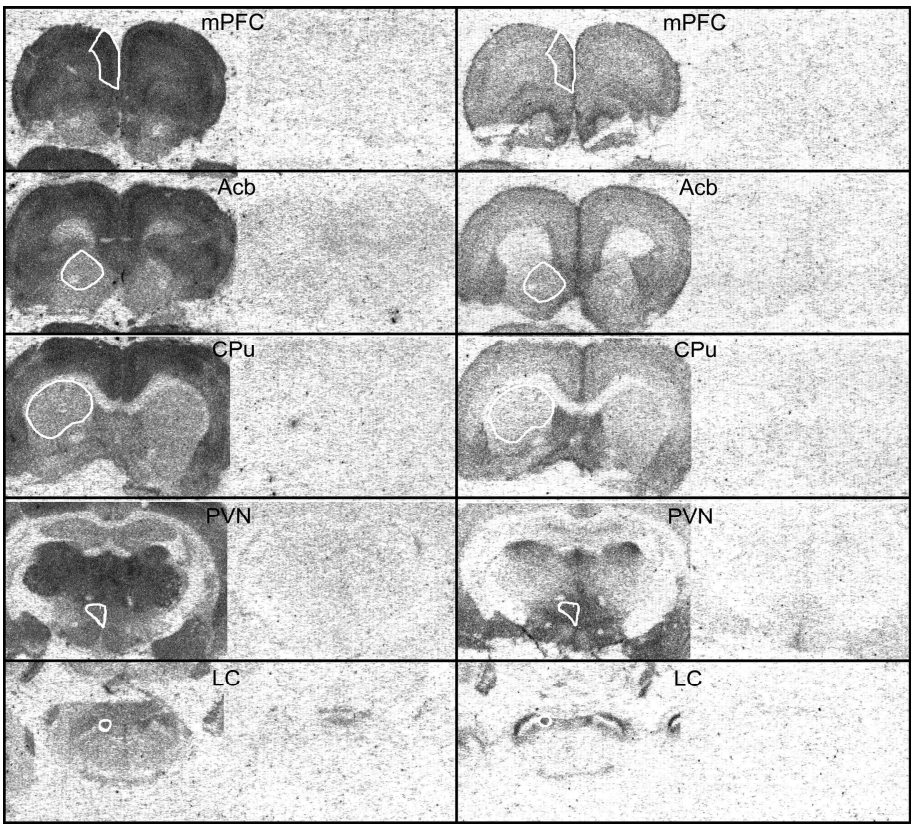


Fig. 2. Representative autoradiographs.
Column A: left hand side: α_1 [^{3}H]Prazosin binding; right hand side: corresponding background staining.
Column B: left hand side: α_2 [^{3}H]UK14.304 binding; right hand side: corresponding background staining.

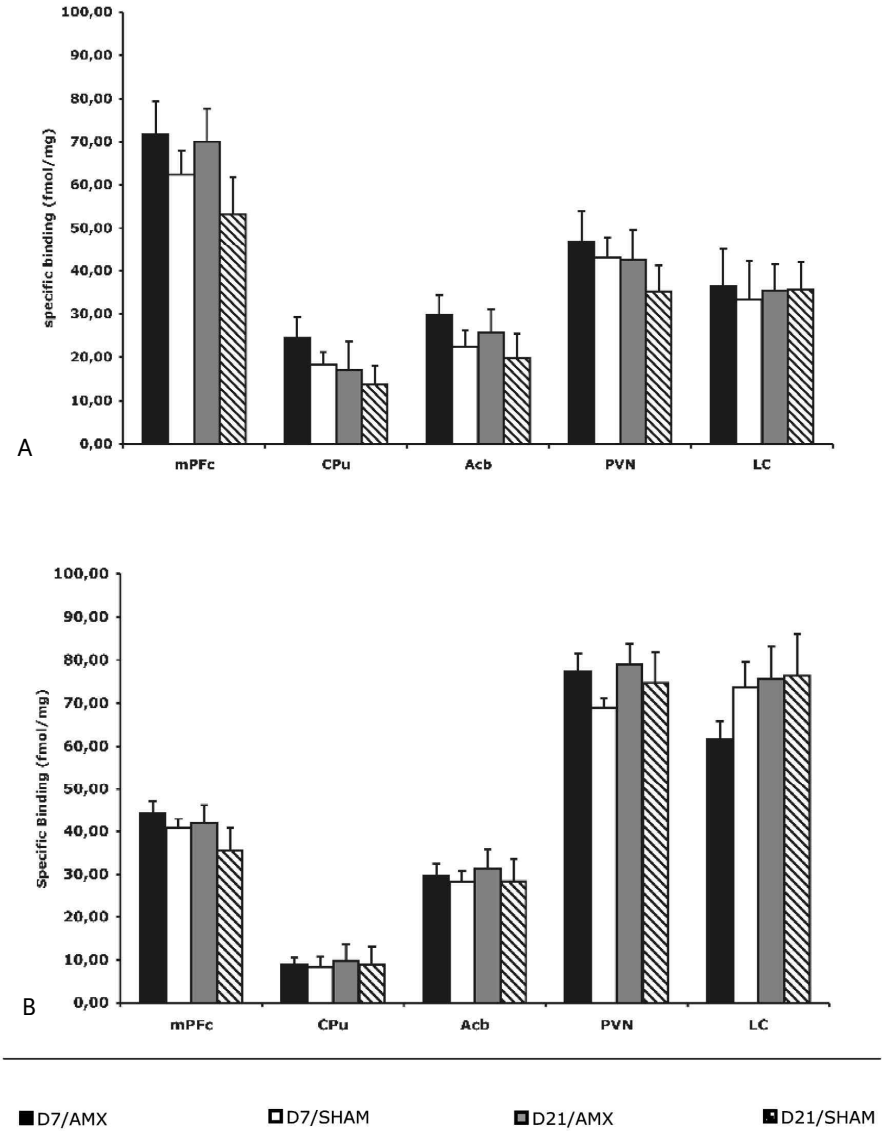


Fig. 3. Specific receptor binding (fmol/mg) of adrenoceptors in adult animals lesioned in the basolateral nucleus of the amygdala (AMX) at postnatal day 7 (D7) or postnatal day 21 (D21) as compared to sham operated animals (SHAM). A: α_1 [^{3}H]Prazosin binding, B: α_2 [^{3}H]UK14.304 binding. Depicted is the receptor binding in the regions of interest: medial prefrontal cortex (mPFC), caudate putamen (CPu), accumbens nucleus (Acb), paraventricular nucleus of the hypothalamus (PVN), and locus coeruleus (LC).

$p = 0.474$), indicating that there were no differences between the experimental groups in α_2-adrenoceptor density in these brain regions (fig. 3B).

α_1- and α_2-adrenoceptor balance

Post-hoc analysis of α_1- versus α_2-radioligand binding revealed differences in the mPFC ($F_{(1,47)} = 20.907$, $p < 0.001$), CPu ($F_{(1,47)} = 6.941$, $p = 0.011$), PVN ($F_{(1,47)} = 61.141$, $p < 0.001$), and LC ($F_{(1,47)} = 50.802$, $p < 0.001$). The density of the α_1-adrenoceptor was higher than the density of the α_2-adrenoceptor in the mPFC and the CPu, and the density of the α_2-adrenoceptor was higher than the density of the α_1-adrenoceptor in the PVN and LC. There was no difference between α_1- and α_2-binding in the Acb ($F_{(1,47)} = 3.584$, $p = 0.065$).

Discussion

In the present study, no differences in specific α_1- and α_2-adrenoceptor binding were observed between the experimental groups (D7 AMX, D7 SHAM, D21 AMX and D21 SHAM), suggesting that the adrenoceptor density was not different across the groups. Since saturating ligand concentrations were selected, the specific receptor binding measured is a fairly accurate indication of the B_{max} or receptor density. The lack of a difference in α_1- and α_2-adrenoceptor density was found despite previous results of our group, showing a lower NA neurotransmission in the mPFC, Acb and CPu of D7 AMX as compared to D7 SHAM rats (Bouwmeester, 2002). Prolonged exposure to altered levels of endogenous or to exogenous adrenoceptor ligands has been shown to alter adrenoceptor expression (Grimm et al., 1992; Flugge et al., 2003). We therefore expected receptor densities in mPFC, Acb and CPu to be changed. Given the relatively small within group variance in our data, it is unlikely that receptor density changes of any significance in the D7 AMX model would have escaped detection due to lack of power.

Little is known about changes in transmitter systems and receptors during life after neonatal disturbances in these systems. For example, neonatal (D5) 6-hydroxydopamine lesioning in rats resulted in motor hyperactivity at D25, but not at D37 or 60, a reduction in DA transporter binding at D25, D37 and D60, an increase and decrease in D4 receptor levels in the caudate-putamen and nucleus accumbens, respectively at D25, but not at D37 or D60, and minor changes in D1- and D2-receptor binding (Zhang et al., 2001). Thus, depletion of dopamine early in life, leads to adaptive changes in the DA receptor systems, which resulted in marked differences depending on the developmental stage of the animal. Thus, the discrepancy between the NA transmission and the NA receptor density may be due to adaptations that occurred in the time between the lesion (D7) and the assessment (adult).

Further, it should be kept in mind that there exist a variety of subtypes of the α_1- and

α_2-adrenoceptors (i.e. $\alpha_{1A\text{-}D}$ and $\alpha_{2A\text{-}C}$ (Bylund et al., 1994)), that may be involved in different processes (Han et al., 1987a, b; Han and Minneman, 1991; Scheinin et al., 1994). In the mPFC, for instance, the α_{2A}-adrenoceptor subtype, predominantly but not exclusively located on the presynaps, is most abundant (Aoki et al., 1994; Scheinin et al., 1994). Yet, the cortically less abundant α_{2C}-adrenoceptor, which is reported to be present postsynaptically (Aoki et al., 1994), is preferentially targeted by atypical neuroleptic drugs (Kalkman and Loetscher, 2003). The α_{2C}-adrenoceptor is able to significantly influence behavior (Bjorklund et al., 1999). In the basal ganglia (CPu and Acb), the α_{2C}-adrenoceptor is the predominant receptor subtype, while the α_{2B}-adrenoceptor is almost exclusively present in the thalamus (Scheinin et al., 1994). It can thus not be excluded that changes had occurred in D7 AMX rats, but only in (a) subtype(s) of adrenoceptors.

The presently found α_1- and α_2-adrenoceptor balance in the PVN ($\alpha_1 < \alpha_2$) is in line with a previous report (Plotsky et al., 1989). The α_1- and α_2-receptor binding in the LC ($\alpha_1 < \alpha_2$) apparently conflicts with previously published results (Chamba et al., 1991), but it should be noted that Chamba and co-workers used ligand concentrations that did not approach saturation of the binding sites, and their data therefore cannot be accepted as indicative of receptor density. Our data on the α_1- and α_2-receptor balance in the mPFC ($\alpha_1 > \alpha_2$), CPu ($\alpha_1 > \alpha_2$) and Acb ($\alpha_1 = \alpha_2$) are novel. They may be of functional importance, because the α_1- and α_2-receptors have been shown to mediate opposite effects in mPFC (Birnbaum et al., 1999; Li et al., 1999), Acb (Nurse et al., 1984; Tuinstra and Cools, 2000), and PVN (Brooks et al., 1986).

Our data revealed that the α_1- and α_2-adrenoceptor density is not changed in the mPFC, Acb, CPu, PVN, and LC in the D7 AMX rats, even though previous results have indicated altered NA signaling in brain regions of such animals. However, more information is needed, e.g. on receptor density changes over time and differences between the various subtypes, before definite conclusions can be drawn about the relation between the two observations and their significance for the disturbed behaviors found in this neurodevelopmental model.

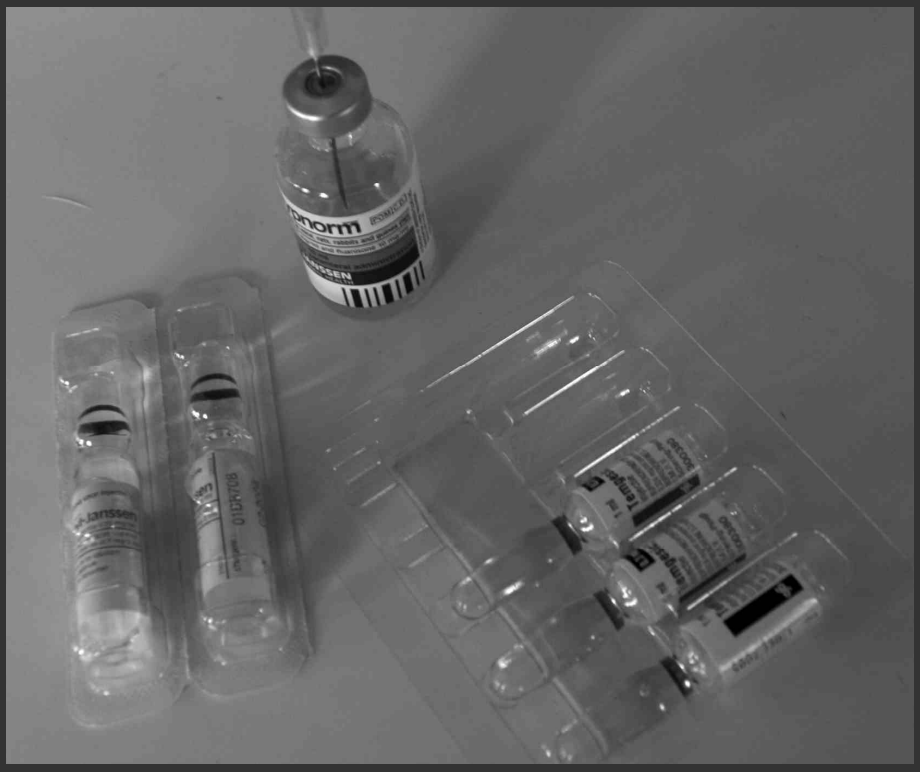
Acknowledgments

We would like to thank Heidi Lesscher for her expert guidance and advice with regard to the quantitative receptor autoradiography, Inge Wolterink-Donselaar and Annemarie van der Linden for their expert surgical assistance, and Leo van Halewijn for his experimental assistance.

chapter 5

Effect of chronic neuroleptic treatment on stress responsiveness in adult rats with neonatal amygdala lesions

Jeroen Terpstra, Christine C. Gispen-De Wied, Babs Fabriek, Mark H. Broekhoven, Henk Spierenburg, Jan M. van Ree, Victor M. Wiegant



Abstract

Adult rats with amygdala lesions made on postnatal day 7 after birth (D7 AMX), are considered a representative model for neurodevelopmental psychopathological disorders such as schizophrenia. Beyond other similarities with schizophrenia, these rats display a diminished hypothalamic-pituitary-adrenal axis (HPA) response to stress. An impaired stress response has been hypothesized to underlie increased stress sensitivity. Neuroleptics are to some extent protective to the effects of stress in schizophrenic patients. In this, atypical neuroleptics are in advantage over typical ones. We investigated the stress response in D7 AMX rats before and after chronic treatment with haloperidol or clozapine, at the level of behavior and endocrinology.

Adult rats (D7 AMX, D7 SHAM) were treated with daily s.c. injections of haloperidol, clozapine or saline for 14 consecutive days. A small open field (SOF) test was used to follow behavior during the treatment period. Blood samples were taken for the assessment of baseline HPA axis activity, and during footshock-stress (FS) on day 14.

D7 AMX rats displayed a blunted HPA response to footshock-stress compared to D7 SHAM rats, which agrees with previous results. Neuroleptic treatments did not change the level of the stress response in D7 AMX rats, nor did they hamper a normal stress response in SHAM lesioned rats. Yet, in rats chronically treated with clozapine the peak of the ACTH response occurred earlier after the footshock stress than in haloperidol and saline treated rats, irrespective of lesion status.

At the behavioral level, D7 AMX rats appeared to be more active in the SOF, and less responsive to the footshock stress than D7 SHAM rats. These effects were not affected by neuroleptic treatment.

It can be concluded that clozapine alters the physiology of the HPA axis response, and that this may have some bearing upon the way atypical neuroleptics, such as clozapine, exert their stress protective effect.

Introduction

Adult rats with bilateral amygdala lesions made on day 7 after birth (D7 AMX rats) have been proposed as a model for neurodevelopmental psychopathological disorders such as schizophrenia (Daenen et al., 2001; Wolterink et al., 2001; Daenen et al., 2002*a*). D7 AMX rats show a number of deficits that are also seen in schizophrenia, for instance impaired pre-pulse inhibition, impaired social behavior, stereotyped hyperactivity, and increased sensitivity to phencyclidine and apomorphine (Daenen, 1999; Daenen et al., 2001; Daenen et al., 2002*a*; Daenen et al., 2003*a*). Since these deficits are not present in adult rats with amygdala lesions made on postnatal day 21 (D21 AMX), they are not dependent on the amygdala lesions per se, but likely result from a disruption of amygdala-dependent neurodevelopmental processes that occur beyond postnatal day 7 but are completed at postnatal day 21. Indeed, recent studies on the development of neural projections from the amygdala to other brain regions have demonstrated that early lesions of the amygdala may affect the development of prefrontal cortical areas in the rat (Bouwmeester et al., 2002).

Recently we have shown that adult D7 AMX rats display a blunted hypothalamus-pituitary-adrenal (HPA) axis response to footshock stress. Also in this respect, D7 AMX rats appeared to differ from adult D21 AMX rats, that showed an HPA axis response similar to SHAM operated controls (Terpstra et al., 2003). Interestingly, this feature adds to the relevance of the D7 AMX rat as a neurodevelopmental model of schizophrenia, since it has been reported that schizophrenic patients and children at high risk for schizophrenia display a blunted HPA response to stress (Albus et al., 1982; Breier et al., 1988; Kudoh et al., 1997; Jansen et al., 1998; Jansen et al., 1999; Jansen et al., 2000*a*; Jansen et al., 2000*b*).

Schizophrenic patients have difficulties with the interpretation of environmental stimuli, and this cognitive impairment might relate to the fact that they are probably not well adapted and may not respond properly to stressors (Gispen-de Wied, 2000). Neuroleptics are in some way protective against stress, since they are able to reduce the risk of decompensation as a result of stress. Treatment with atypical neuroleptics, like clozapine, is more effective in this respect than treatment with typical neuroleptics, like haloperidol (Kane et al., 1988*a*; Kane et al., 1988*b*). Notably, clozapine has more cognition enhancing properties than haloperidol (Buchanan et al., 1994*a*; Gallhofer et al., 1996; Fujii et al., 1997; Murphy et al., 1997; Schall et al., 1998; Grabe et al., 1999; Manschreck et al., 1999; Galletly et al., 2000), and may therefore be more effective than haloperidol in improving the interaction of patients with their environment, and thus their adaptation.

In the present study, the effect of chronic neuroleptic treatment on footshock stress was investigated in D7 AMX animals. The reasoning was twofold. When neuroleptic

treatment would restore the impaired stress response, this might explain their purported stress reducing properties in patients. Conversely when neuroleptics would hamper a proper stress response in rats, the results would lend support to the often-proposed explanation that neuroleptic treatment causes impaired stress responsivity in schizophrenic patients. Several reports in the literature indicate that neuroleptic treatment (acute, chronic) can interfere with basal HPA-axis activity in experimental animals (Keim and Sigg, 1977; Meador-Woodruff et al., 1990). Few studies have dealt with the effects of chronic neuroleptic treatment on the responsivity of the axis. It was shown that chronic clozapine treatment attenuated the HPA axis response to serotonergic agents (Sebens et al., 2000), and haloperidol treatment reduced the response to restraint stress (Keim and Sigg, 1977). For their different mode of action, and the assumed preference of the atypical neuroleptics, chronic treatment with these two neuroleptics was used to investigate their effect on footshock stress in D7 AMX rats.

Experimental procedures

All procedures were performed in accordance with the guidelines established by the Committee for the Ethical Treatment of Animals Utrecht (DEC Utrecht), The Netherlands, and the Society for Neuroscience Policy on the Use of Animals in Research.

Animals and housing

Pregnant female Wistar rats [U:WU] were obtained at day 18 of gestation and were housed individually in Macrolon® Type III cages with sawdust bedding. After delivery, male and female pups were separated; male pups were randomized among nests. All nests contained at least two female pups and up to seven male pups (all nests were made to consist of nine pups). Male pups were lesioned on postnatal day 7 and were weaned at 21 days. After weaning, all male rats were housed socially (two to three rats per cage) in Macrolon® Type III cages, with sawdust bedding, until they reached adulthood (approximately 13 weeks). Thereafter a chronic jugular vein cannula was surgically implanted and the rats were individually housed in transparent Perspex™ cages, measuring 25 × 25 × 34 cm (l × w × h) with sawdust bedding, and were allowed to recover for two weeks. During this recovery period, the cannulas were flushed regularly and the rats were habituated to the blood sampling procedure. The rats were weighed daily after surgery until they reached their pre-surgical weight and were then weighed weekly. They were given standard chow (Hope Farms, standard laboratory animal food) and water ad libitum. A 12-hour light/dark cycle was used, with lights on from 07.00 to 19.00. Ambient temperature was held constant at 21 ± 1 ˚C with humidity at 50 ± 10%.

Surgical procedures and histology

The lesioning procedure was performed according to the technique described by Wolterink (Wolterink et al., 2001). 60 Male Wistar rats were anesthetized with fentanyl (4 µg/rat, s.c.). The heads of the D7 rat pups were placed in a specially constructed head mould to enable stable fixation of the head. The coordinates for positioning of the needles for the amygdala lesions were 3.8 mm lateral to the midline, 1.0 mm posterior to bregma, and 6.0 mm below the surface of the skull, at an angle of 4˚. The tip of the needles was aimed at the basolateral nucleus of the amygdala (BLA). The lesions were made by bilateral infusion of ibotenic acid (3 µg/0.3 µl phosphate-buffered saline) over two minutes, using an infusion pump. After infusion, the needles were left in place for four minutes. SHAM-lesioned rats received the corresponding amount of vehicle. Three months after the AMX procedure all animals were given a jugular vein cannula to facilitate repeated blood sampling.

Within two weeks after the end of the experiments all rats were killed with an overdose of intravenous pentobarbital. After decapitation, the brains were collected in formaldehyde-containing vials, sectioned (50 µm) and stained with hematoxylin and eosin. Histological evaluation showed that AMX lesions in six rats (approximately 21% of the lesions) were either too large or outside the BLA (Paxinos and Watson, 1997), and data from these animals were discarded.

Experimental design

After the two-week recovery period following the jugular vein cannulation, the animals were divided among six groups (AMX/haloperidol; SHAM/haloperidol; AMX/clozapine; SHAM/clozapine; AMX/saline; SHAM/saline). Animals were given equal volumes of either 0.5 mg/kg haloperidol (standard 5mg/ml injection fluid containing lactic acid as a solvent, Janssen-Cilag BV, Tilburg, The Netherlands), 10 mg/kg clozapine (standard 5mg/2ml injection fluid containing laurinic acid as a solvent, Novartis Pharma Sweiz AG, Bern, Switzerland), or saline s.c. once daily for 14 days. All tests were performed between 10.00 and 14.00 to avoid effects of circadian rhythmicity.

Novelty testing

Prior to the start of drug treatment all animals were tested in a Small Open Field (SOF; 30 cm in diameter) for three minutes to assess baseline behavior. One day after start of the treatment, and one hour after the last dose, the animals were again tested in a SOF to assess the acute effects of medication. Two weeks after the start of the treatment, and one hour after the last dose, the animals were again tested in a SOF to assess the chronic effect of treatment. All behavior was videotaped for later analysis.

Baseline ACTH

Blood samples (250 µl) for the assessment of basal plasma ACTH concentrations were taken on: T = -1, T = 1, T = 7 and T = 14 (T in days; start of treatment on day 0). Time points for sampling were chosen in such a manner that acute and chronic effects of treatment could be discriminated. All samples were taken before treatment and testing procedures and in the home cage.

Footshock testing

Two weeks after the start of the medication-period, and twenty hours after the last dose, all animals were submitted to a 10-minute footshock (FS) procedure. The procedure was as follows: all animals were attached, in their home cage, to the sampling cannula 90 minutes before stress exposure (t = -90 minutes). A basal blood sample (250 µl) was taken at t = -10 minutes, and the animals were transferred from their home cage to the novel footshock-test cage (measuring 31 × 32 × 45; l × w × h) at t = 0 minutes. The test started at t = 0 minutes, and consisted of a 10-minute period during which a total of ten individual footshocks with a current of 0.25 mA and lasting 1 second were given (at 1:00, 1:40, 2:40, 4:00, 4:40, 5:40, 7:00, 7:40, 8:40, and 9:30 minutes). Blood samples (250 µl) were taken at t = 5, 10, 20 and 30 minutes. At the end of the 10-minute test, a blood sample was collected (t = 10 min), and the animals were put back into their home cage.

Analysis of ACTH concentration

Samples were collected in heparin/EDTA-containing vials and kept on ice, centrifuged within four hours of sampling, and plasma was stored at -80˚C. ACTH concentration was assessed with an in-house ^{125}I RIA using the S-5 230676 antibody as described previously (Van Oers and Tilders, 1991).

Behavioral analysis

Behavior was analyzed with Observer® software (Noldus Information Technology, Wageningen, The Netherlands). The SOF ethogram consisted of four behavioral parameters: ambulation (hind-paws are rotated 180˚), locomotion, immobility (includes freezing), and attention (sitting while head and whiskers are moving). The FS ethogram consisted of three behavioral parameters (both frequency and duration): locomotion, immobility (includes freezing), and attention (sitting while head and whiskers are moving).

Statistical analysis

ACTH data were analyzed using repeated measures analysis of variance (ANOVA, SPSS 10.0.8 for Macintosh®). The analysis of the basal sample data included the with-

in subjects factor 'time' (the endocrine response over time; four levels: T = -1, 1, 7 and 14 days), 'treatment' (three levels: Haloperidol, Clozapine and Saline), and 'lesion' (two levels: AMX and SHAM). The analysis of the footshock data included the within subjects factor 'time' (the endocrine response over time; five levels: t = -10, 5, 10, 20 and 30 minutes), 'treatment' (three levels: Haloperidol, Clozapine and Saline), and 'lesion' (two levels: AMX and SHAM). When relevant, post-hoc analyses were performed using an ANOVA repeated measures. Significance was reached when $p \leq 0.05$.
Behavioral SOF data were analyzed using analysis of variance (ANOVA, SPSS 10.0.8 for Macintosh®). The analysis included the within subjects factor 'time' (the behavioral response over time; three levels: T = -1, 1 and 14 days), 'treatment' (three levels: Haloperidol, Clozapine and Saline), and 'lesion' (two levels: AMX and SHAM). Significance was reached when $p \leq 0.05$. Two post-hoc analyses were performed using ANOVA repeated measures. The first analysis was performed to look at 'acute' effects, and included the within subjects factor 'time' (the behavioral response over time; two levels: T = -1 and 1 days), 'treatment' (three levels: Haloperidol, Clozapine and Saline), and 'lesion' (two levels: AMX and SHAM). The second analysis was performed to look at 'chronic' effects and included the within subjects factor 'time' (the behavioral response over time; two levels: T = -1 and 14 days), 'treatment' (three levels: Haloperidol, Clozapine and Saline), and 'lesion' (two levels: AMX and SHAM). Cutoff for statistical significance was set at $p \leq 0.025$ (Bonferroni correction). Behavioral FS data were analyzed using a multivariate analysis of variance (MANOVA, SPSS 10.0.8 for Macintosh®). Significance was reached when $p \leq 0.05$. All results are given as means ± SEM.

Results

Histological evaluation showed that the size and location of the AMX lesions (fig. 1) were similar to those previously described (Wolterink et al., 2001).

Responses to footshock stress

ACTH response: Two weeks after the start of the treatment (90 minutes after the last saline or drug injection), rats were subjected to the 10 minutes footshock stress (FS), and ACTH was determined in blood samples taken 10 minutes before, and 5, 10, 20 and 30 minutes after the start of the FS procedure. The results are shown in figure 2. Statistical analysis of the data (table 1) revealed no 'lesion × treatment' interaction effects. In accord with our previous results (Terpstra et al., 2003), we found a 'lesion × time' interaction effect, due to an attenuated ACTH response in D7 AMX as compared to SHAM rats. In addition, there was a 'treatment × time' interaction, and post hoc analysis revealed a difference between clozapine and saline treated rats ($F_{(4,92)} = 3.422$,

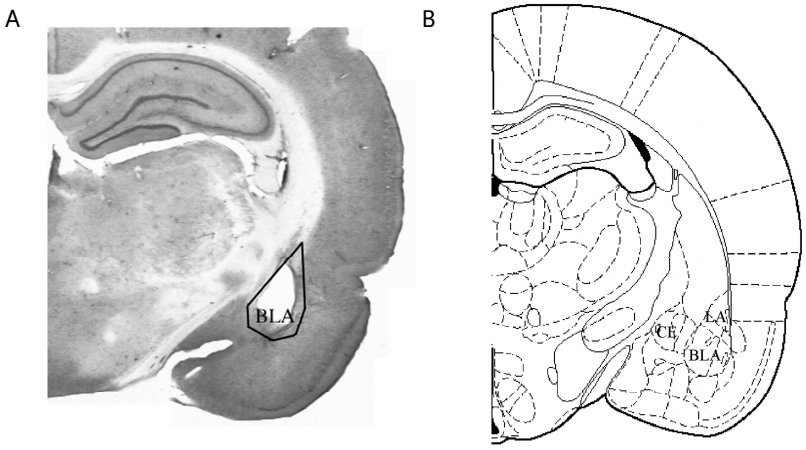


Fig. 1. Micrograph of a representative amygdala lesion. Highlighted, after histological evaluation, is the location of the basolateral amygdala. A: D7 AMX, B: anatomical drawing of the important amygdala nuclei, BLA: basolateral nucleus of the amygdala, LA: lateral nuclei of the amygdala, CE: central nuclei of the amygdala (figure derived from Paxinos and Watson, 1997).

footshock		
within subjects effects	ACTH	
	$F_{(4,132)}$	p
time	29.166	**0.000**
lesion × time	3.536	**0.009**
treatment × time	2.990	**0.004**
lesion × treatment × time	0.673	0.715
between subjects effects		
	$F_{(1,33)}$	p
lesion	16.018	**0.000**
treatment	0.113	0.893
lesion × treatment	0.108	0.898

Table 1: Statistical analysis of the ACTH response (pg/ml) to footshock-stress (ANOVA repeated measures). Number of rats per group: AMX/Saline = 5, AMX/Haloperidol = 5, AMX/Clozapine = 7, SHAM/Saline = 7, SHAM/Haloperidol = 7, SHAM/Clozapine = 8. Time = endocrine response over time, Lesion = AMX vs. Sham, and Treatment = Haloperidol vs. Clozapine vs. Saline.

$p = 0.012$), and between clozapine and haloperidol treated rats ($F_{(4,100)} = 4.983$, $p = 0.001$), but not between haloperidol and saline treated rats ($F_{(4,88)} = 0.679$, n.s.). These data indicate that the ACTH response to FS peaked at an earlier time point in rats treated with clozapine than in haloperidol or saline treated rats (fig. 2).

Behavioral response: The behavioral results are shown in table 2. Statistical analysis of the behavioral response during the FS procedure (table 3) revealed no 'lesion × treatment' interaction or main 'treatment' effects. Main effects of 'lesion' were due to more locomotion (frequency, duration) and attention (frequency, duration), and less immobility (frequency, duration) in D7 AMX than SHAM rats. Thus, in line with our previous observations, D7 AMX rats showed less immobility to FS than SHAM rats.

Basal plasma ACTH

Basal plasma ACTH concentrations were determined in blood samples taken 1 day before, and 1, 7 and 14 days after the start of drug treatment. The results are shown in figure 2. Within subjects analysis and between subjects analysis revealed no differences. This indicates that there is no change in basal ACTH levels over time, no difference between D7 AMX and D7 SHAM rats in basal ACTH levels, and no effect of treatment on basal ACTH levels within this experimental context.

Table 2: Means ± SEM of behavioral elements during Footshock. FR = frequency (per 10 minutes), DR = duration (seconds), S = saline, H = haloperidol, and C = clozapine.

		footshock		
		S	H	C
locomotion FR	AMX	39.4 ± 4.9	35.7 ± 7.1	31.0 ± 7.4
	SHAM	21.2 ± 1.5	20.3 ± 1.6	16.1 ± 1.9
locomotion DR	AMX	87.6 ± 14.2	95.5 ± 23.8	71.0 ± 17.1
	SHAM	43.7 ± 4.2	45.1 ± 2.3	40.1 ± 5.0
immobility FR	AMX	8.6 ± 2.8	11.0 ± 2.2	7.4 ± 1.6
	SHAM	12.8 ± 1.5	14.1 ± 1.2	12.3 ± 1.0
immobility DR	AMX	65.0 ± 32.1	131.5 ± 38.6	137.3 ± 76.2
	SHAM	173.0 ± 21.6	240.3 ± 6.7	234.6 ± 29.6
attention FR	AMX	56.9 ± 3.7	47.5 ± 4.8	40.4 ± 7.8
	SHAM	33.5 ± 2.3	32.1 ± 2.6	32.6 ± 3.9
attention DR	AMX	401.7 ± 24.1	330.3 ± 30.5	346.3 ± 59.6
	SHAM	220.0 ± 26.7	214.1 ± 28.3	218.1 ± 21.9

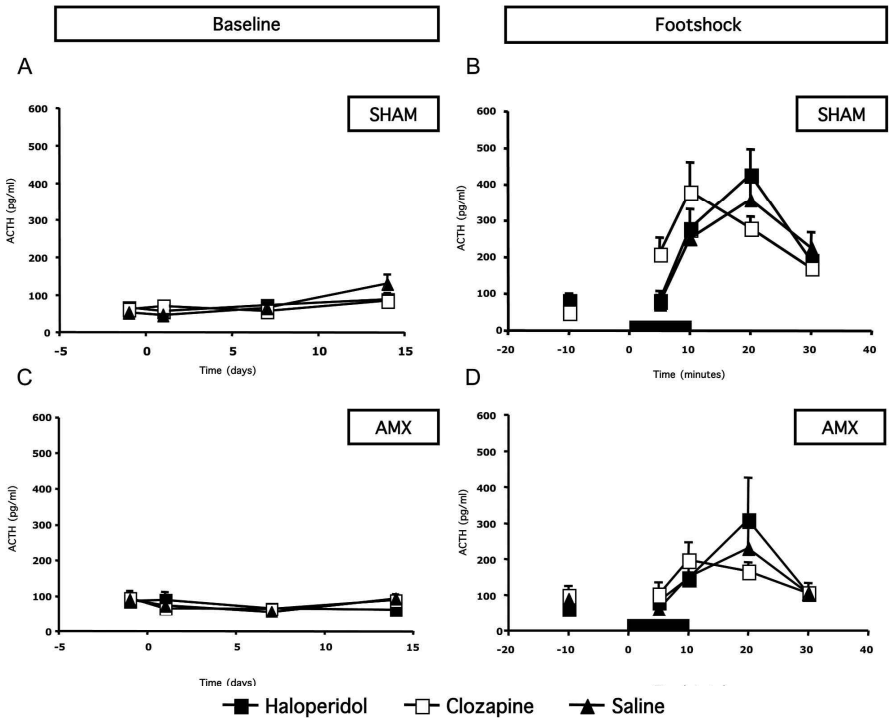


Fig. 2. A: Baseline plasma ACTH concentration (pg/ml) in SHAM rats over the 14 day treatment period, B: Plasma ACTH response (pg/ml) in SHAM rats to a 10-minute footshock session (black bar), C: Baseline plasma ACTH concentration (pg/ml) in AMX rats over the 14 day treatment period, D: Plasma ACTH response (pg/ml) in AMX rats to a 10-minute footshock session (black bar).

	footshock lesion	
	$F_{(1,35)}$	$p \leq$
locomotion FR	17.9	**0.000**
locomotion DR	15.5	**0.000**
immobility FR	7.7	**0.009**
immobility DR	9.8	**0.003**
attention FR	16.7	**0.000**
attention DR	25.3	**0.000**

Table 3: Statistical analysis of the behavioral response to a footshock stimulus (MANOVA, between subject effects). AMX/Saline = 7, AMX/Haloperidol = 6, AMX/Clozapine = 7, SHAM/Saline = 6, SHAM/Haloperidol = 7, SHAM/Clozapine = 8. Lesion = AMX vs. Sham, FR = frequency (per 10 minutes), DR = duration (seconds).

Table 4: Means ± SEM of behavioral elements in a Small Open Field (SOF) test during treatment.

		day	S	H	C
ambulation FR	AMX	-1	6.5 ± 1.6	5.1 ± 1.1	7.4 ± 2.0
		1	2.3 ± 0.7	0.6 ± 0.4	3.1 ± 0.9
		14	2.6 ± 0.6	1.7 ± 0.5	2.4 ± 1.3
	SHAM	-1	4.4 ± 0.8	4.7 ± 1.0	4.2 ± 0.5
		1	2.0 ± 0.4	0.1 ± 0.1	1.8 ± 0.7
		14	1.6 ± 0.4	0.4 ± 0.3	0.9 ± 0.3
locomotion FR	AMX	-1	14.3 ± 1.1	11.1 ± 2.0	13 ± 1.3
		1	11.9 ± 1.4	3.4 ± 1.1	10.7 ± 2.0
		14	12.9 ± 2.1	8.6 ± 1.6	10.1 ± 3.0
	SHAM	-1	11.3 ± 1.2	9.7 ± 0.9	10.9 ± 0.9
		1	9.6 ± 1.0	3.7 ± 0.7	8.8 ± 1.7
		14	8.3 ± 1.1	7.6 ± 1.0	6.1 ± 1.1
locomotion DR	AMX	-1	44.8 ± 6.8	42.5 ± 8.3	49.3 ± 8.9
		1	34.3 ± 4.4	10.4 ± 4.5	39.0 ± 7.9
		14	29.1 ± 5.4	25.4 ± 5.5	25.9 ± 7.5
	SHAM	-1	39.1 ± 4.9	28.6 ± 3.9	33.8 ± 3.8
		1	24.1 ± 2.5	10.1 ± 2.4	22.8 ± 4.7
		14	21.1 ± 3.4	17.9 ± 2.6	15.4 ± 2.7
immobility FR	AMX	-1	3.2 ± 1.1	5.3 ± 1.0	3.4 ± 1.0
		1	2.7 ± 1.1	4.0 ± 0.7	4.6 ± 0.8
		14	2.7 ± 0.6	5.0 ± 1.1	4.1 ± 1.5
	SHAM	-1	2.1 ± 0.8	1.0 ± 0.4	1.3 ± 0.2
		1	2.1 ± 0.6	5.9 ± 0.8	4.0 ± 0.9
		14	3.0 ± 1.2	3.3 ± 0.9	4.3 ± 1.3
immobility DR	AMX	-1	12.7 ± 4.5	41.1 ± 15.2	12 ± 4.7
		1	22.7 ± 12.5	133.4 ± 15.4	43.3 ± 18.8
		14	22.1 ± 11.2	50.9 ± 13.8	44.3 ± 18.8
	SHAM	-1	11.7 ± 6.1	3.0 ± 1.5	3.7 ± 1.4
		1	16.6 ± 10.8	82.7 ± 9.2	32.6 ± 8.6
		14	18.7 ± 9.8	11.0 ± 3.7	28.8 ± 9.7
attention FR	AMX	-1	13.5 ± 1.2	14.8 ± 1.2	15.3 ± 1.7
		1	15.4 ± 0.9	5.0 ± 1.0	13.1 ± 1.7
		14	15.4 ± 2.2	14.9 ± 2.5	16.0 ± 2.4
	SHAM	-1	14.1 ± 0.6	12.9 ± 1.0	12.1 ± 1.4
		1	12.6 ± 0.9	10.1 ± 1.2	13.4 ± 1.4
		14	12.7 ± 2.0	14.4 ± 0.9	12.4 ± 0.6
attention DR	AMX	-1	67.3 ± 6.8	66.8 ± 6.3	70.3 ± 11.9
		1	73.6 ± 9.8	36.1 ± 12.0	65.7 ± 8.2
		14	77.3 ± 8.2	87.1 ± 9.1	71.1 ± 8.7
	SHAM	-1	50.3 ± 5.7	66.0 ± 5.8	56.5 ± 7.8
		1	60.1 ± 10.1	70.3 ± 8.4	72.1 ± 7.1
		14	75.1 ± 11.5	74.3 ± 6.3	75.5 ± 7.3

	baseline (day-1) lesion	
	$F_{(1,44)}$	p ≤
ambulation FR	4.071	**0.050**
locomotion FR	4.416	**0.041**
locomotion DR	6.339	**0.016**
immobility FR	13.678	**0.001**
immobility DR	6.413	**0.015**
attention FR	2.118	0.153
attention DR	2.192	0.146

Table 5: Behavioral effects of baseline (day -1) small open field (SOF) behavior (ANOVA repeated measures, within subject effects). Number of rats per group: AMX/Saline = 7, AMX/Haloperidol = 8, AMX/Clozapine = 7, SHAM/Saline = 7, SHAM/Haloperidol = 7, SHAM/Clozapine = 10. FR = frequency (per three minutes), DR = duration (seconds), Lesion = AMX vs SHAM.

acute (day -1 vs day 1)

	time		time × lesion		time × treatment		time × lesion × treatment	
	$F_{(1,39)}$	p ≤	$F_{(1,39)}$	p ≤	$F_{(2,39)}$	p ≤	$F_{(2,39)}$	p ≤
ambulation FR	57.307	**0.000**	1.681	0.202	0.728	0.489	0.325	0.724
locomotion FR	31.085	**0.000**	0.312	0.580	4.271	**0.021**	0.061	0.941
locomotion DR	25.949	**0.000**	0.160	0.691	1.405	0.257	0.304	0.740
immobility FR	6.047	**0.018**	9.538	**0.004**	1.777	0.183	4.643	**0.016**
immobility DR	52.081	**0.000**	0.232	0.635	15.003	**0.000**	0.019	0.981
attention FR	6.443	**0.015**	1.505	0.227	6.105	**0.005**	3.626	0.036
attention DR	0.128	0.723	4.298	0.045	2.504	0.095	1.282	0.289

chronic (day -1 vs. day 14)

	time		time × lesion		time × treatment		time × lesion × treatment	
	$F_{(1,37)}$	p ≤	$F_{(1,37)}$	p ≤	$F_{(2,37)}$	p ≤	$F_{(2,37)}$	p ≤
ambulation FR	53.393	**0.000**	0.610	0.440	0.120	0.888	0.599	0.554
locomotion FR	17.164	**0.000**	0.687	0.413	0.692	0.507	0.059	0.943
locomotion DR	65.274	**0.000**	0.521	0.475	1.537	0.228	0.132	0.876
immobility FR	4.536	0.040	5.464	**0.025**	0.993	0.380	0.452	0.640
immobility DR	10.250	**0.003**	0.077	0.783	2.461	0.099	0.132	0.876
attention FR	0.685	0.413	0.673	0.417	0.158	0.854	0.525	0.596
attention DR	10.686	**0.002**	0.122	0.729	0.591	0.559	1.105	0.342

Behavioral responses in the Small Open Field

Effects of lesion: To assess the effects of the lesion on novelty-induced behavior, all animals were subjected to a Small Open Field test (SOF) one day before the start of the treatment. The results are presented in table 4. Univariate analysis (table 5) revealed lesion effects on ambulation (frequency), locomotion (frequency, duration), and immobility (frequency, duration). This is due to the fact that, in accord with previous results (Daenen et al., 2001; Terpstra et al., 2003), D7 AMX rats showed more ambulations, locomotion (frequency, duration) and immobility (frequency, duration) in the novel environment.

Effects of neuroleptics: On days 1 and 14 of the treatment, the rats were again tested in the SOF to assess the acute and chronic effects of neuroleptic treatment respectively. The results are presented in table 4. Combined statistical analysis of the results of the three SOF tests revealed effects of 'time' on all behavioral elements except attention duration ($p \leq 0.035 - p \leq 0.001$), a 'time × lesion' interaction effect on immobility frequency ($p \leq 0.009$), and 'time × treatment' interaction effects on locomotion frequency, immobility duration and attention frequency ($p \leq 0.025 - p \leq 0.001$). Post-hoc statistical tests were performed to further analyze the acute and chronic effects of the treatments.

Acute effects: Post hoc analysis of the behavioral response on day 1 of treatment versus that on the day before treatment (table 6) revealed a 'time × lesion × treatment' interaction effect on immobility frequency, due to the fact that neuroleptic treatment lead to more frequent bouts of immobility in SHAM rats but not in D7 AMX rats. There also were 'time x treatment' interaction effects on locomotion frequency, immobility duration and attention frequency, due to the fact that treatment with haloperidol resulted in behavioral inactivation in both SHAM and D7 AMX rats. Finally, there were main effects of 'time' on ambulation frequency and locomotion duration, due to a decrease in these active behaviors across treatment groups, possibly reflecting habituation to the SOF as a result of repeated testing.

Table 6, *page 76 below*: Behavioral effects of Acute and Chronic neuroleptic treatment (ANOVA repeated measures, within subject effects). Cutoff for significance was set at 0.025 (Bonferroni correction). Number of rats per group in the Acute analysis: AMX/Saline = 7, AMX/Haloperidol = 7, AMX/Clozapine = 7, SHAM/Saline = 7, SHAM/Haloperidol = 7, SHAM/Clozapine = 10. Number of rats per group in the Chronic analysis: AMX/Saline = 7, AMX/Haloperidol = 7, AMX/Clozapine = 7, SHAM/Saline = 7, SHAM/Haloperidol = 7, SHAM/Clozapine = 8. FR = frequency (per three minutes), DR = duration (seconds), Time = endocrine response over time, Lesion = AMX vs. Sham, and Treatment = Haloperidol vs. Clozapine vs. Saline. Significant effects are indicated in bold.

Chronic effects: Post hoc comparison of the behavioral response in the SOF test on day 14 with that on the day before treatment (table 6) revealed a 'time × lesion' interaction effect on immobility frequency, due to the fact that SHAM rats showed an increase in this behavioral element compared to AMX rats. In addition, there were main effects of 'time' on ambulation and locomotion frequency, and locomotion, immobility and attention duration, indicating a decrease in active, exploratory behaviors across all experimental groups, pointing to habituation to the test.

Discussion

In accord with previous results (Terpstra et al., 2003) we found that D7 AMX rats, as compared to D7 SHAM rats, displayed a blunted ACTH response to footshock-stress. This difference between D7 AMX and D7 SHAM rats remained present after chronic treatment with haloperidol or clozapine. Thus, chronic neuroleptic treatment apparently did not restore stress responsivity in D7 AMX rats, but did also not interfere with normal stress responsivity in D7 SHAM rats. This notion is supported by our finding that there were no appreciable differences between D7 AMX and SHAM rats in the chronic effects of neuroleptics on the behavioral response in the SOF, a test employing novelty as a mild stressor.

Yet, we found that clozapine treatment affected the dynamics of the ACTH response to footshock stress irrespective of lesioned state of the rats. In particular we found that peak ACTH levels occurred earlier during the response of rats chronically treated with clozapine than of those treated with haloperidol or saline. Although this is rather speculative at present, this may point to adaptational effects induced by clozapine in brain mechanisms involved in the regulation of HPA-axis activity during stress, and should be investigated much more thoroughly.

In accord with our previous findings (Terpstra et al., 2003), D7 AMX rats displayed less immobility when submitted to the footshock stimulus than D7 SHAM rats D7 AMX rats share this deficit with D21 AMX rats, indicating that it is likely caused by the absence of a functional amygdala per se, and not developmental in nature. However, D7 AMX rats also showed more ambulations in the SOF test, in line with previous findings (Daenen et al., 2002*a*; Terpstra et al., 2003), and this effect was not found in D21 AMX rats. It therefore is likely caused by disturbed development and function of targets of the amygdala. Impaired behavioral and endocrine stress responses are thus consistently found in D7 AMX rats. To attribute these findings to the array of abnormalities in the amygdala lesion animal model that mimic aspects of schizophrenia is therefore justified.

Clozapine did not specifically affect the behavioral parameters tested in the SOF. Moreover, neither haloperidol, nor clozapine affected behavior during the footshock

stress. Thus, chronic neuroleptic treatment does not seem to alter stress related behavior in the paradigms used.

Protection against stressful events has been employed as therapy in schizophrenia. Although no studies have specifically focused on the stress-protective effects of neuroleptics, those effects are assumed to exist (Johnson, 1977; Strachan, 1986; Leff et al., 1994; Liberman, 1994). The effect of clozapine that we found in our present study (shifting the ACTH response forward) may have some bearing upon the idea that interference with stress-responsivity could be one of the mechanisms whereby atypical neuroleptics exert stress protective properties. It suggests that the animals could have been more alert in responding to the aversive stimulus. However, there was no relation between the endocrine and behavioral findings to support this idea. Future studies relating physiology and behavior both in the animal and human experiment should therefore be performed to further explore this issue.

Acknowledgments

We thank Inge Wolterink-Donselaar and Annemarie van der Linden for their expert surgical assistance.

chapter 6

Summary and concluding remarks

The aim of the research described in this thesis was to investigate the stress response in adult rats with amygdala lesions made on postnatal day 7 (D7 AMX), a putative animal model for schizophrenia, and generate new hypotheses and insights that may benefit future (pre)clinical research.

Chapter 1 provides an introductory review of literature relating to the subject of this thesis. The project was initiated based on two lines of recent evidence. Firstly, evidence indicates that schizophrenic patients have an adaptational deficit. Not only do life events appear to increase the risk of psychotic relapse and the number of daily life stressors relate to the number of schizophrenic symptoms (Norman and Malla, 1994), but schizophrenic patients also have a diminished HPA response to a variety of stressful stimuli (Kudoh et al., 1997; Jansen et al., 2000*a*). In addition, cognitive-emotional deficits in schizophrenia, that involve the prefrontal cortex and the limbic system, run in families (Johnson et al., 2003). This indicates that heritable factors are involved that likely affect brain development and functions.
Secondly, evidence indicates that rats bearing lesions of the amygdala made on postnatal day 7 (D7 AMX) model aspects of neurodevelopmental psychopathologies, such as schizophrenia (Wolterink et al., 2001). Thus, adult D7 AMX rats display impaired prepulse inhibition, impaired behavioral responses to stress, impaired social behavior, and increased sensitivity to phencyclidine. These symptoms become manifest after puberty, as is the case in schizophrenic patients, and are not observed in rats in which the amygdalae are lesioned on postnatal day 21 (D21 AMX), suggesting altered development of brain structures that are innervated by the amygdala under normal conditions. Indeed, during normal development, major reorganization of the innervation of the prefrontal cortex by the amygdala was found to occur after postnatal day 7 (Bouwmeester et al., 2002).

In **chapter 2** experiments are reported that were conducted to investigate the effects of neonatal amygdala lesions on stress responsivity later in life, and assess the neurodevelopmental nature of these effects. To that end, we compared the endocrine, sympathetic-adrenomedullary and behavioral responses to footshock stress and novelty stress in groups of D7 AMX and D7 SHAM rats with those of D21 AMX and D21 SHAM rats.
In both stress paradigms, the HPA axis response of D7 AMX was found markedly reduced, while D21 AMX rats only showed moderately blunted ACTH and corticosterone (CORT) responses to novelty stress. In addition, D7 AMX rats showed an even less active behavioral response to novelty than D21 AMX. The plasma noradrenalin

response was similarly reduced in both lesioned groups, while the adrenaline response was not affected.
Thus, both D7 AMX and D21 AMX rats showed attenuated behavioral, endocrine and sympathetic stress responses. This hyporesponsivity of AMX rats accords with the general notion that the amygdala plays a critical in cognitive-emotional processes and adaptational responses to environmental stimuli inducing fear and anxiety. However, the effects of the amygdala lesions, in particular the effects on HPA axis responses, were much more pronounced in D7 than D21 AMX rats. This indicates that, in addition to the damage to the amygdala itself, developmental deficits in target structures of the amygdala likely contribute to the stress hyporesponsiveness of D7 AMX rats. These results add to the validity of the D7 AMX rat as an animal model for neurodevelopmental psychopathologies, such as schizophrenia.

In **chapter 3**, we investigated the mechanism underlying the blunted HPA axis response to stress of D7 AMX versus D7 SHAM rats. To that end, we assessed the sensitivity of the pituitary-adrenal system to corticotrophin-releasing hormone (CRH) in those rats by monitoring the CORT response following i.v. administration of synthetic ovine CRH (oCRH). In addition, we assessed the sensitivity of the HPA axis to glucocorticoid feedback by testing the inhibitory effect of pretreatment with a low dose of CORT on the novelty induced CORT response. Finally, we determined the basal plasma CORT concentration throughout a complete circadian cycle.
D7 AMX rats displayed a reduced CORT response to oCRH, whereas the inhibitory effect of CORT pretreatment on the novelty-induced HPA response was similar in D7 AMX and D7 SHAM rats. These data suggest normal glucocorticoid feedback, and indicate that impaired CRH signaling, most likely at the level of the pituitary, underlies the deficit of D7 AMX rats in HPA responding to stress. In addition, D7 AMX rats displayed elevated basal plasma CORT levels, particularly during the nocturnal phase of the circadian cycle, when rats are awake and active. In absence of changes in glucocorticoid feedback, this might suggest increased arousal during wakefulness. These data add to evidence that amygdala lesions early in life lead to deficits in stress responsive systems in the rat.

Recently, the concentrations of noradrenalin (NA) and its metabolite 3-methoxy-4-hydroxyphenylglycol were found decreased in the medial prefrontal cortex (mPFC), nucleus accumbens (Acb), and caudate putamen (CPu) of D7 AMX rats, indicating reduced NA neurotransmission in these brain structures (Bouwmeester, 2002). In view of these findings and the known role of NA in the regulation of the HPA axis, we

further investigated the disturbances in brain NA systems focusing on NA receptor density (**chapter 4**). To that end, the density of α_1- and α_2-adrenoreceptors was determined by in vitro autoradiography of brain slices incubated with saturating concentrations of the α_1- and α_2-specific radioligands [^{3}H]Prazosin and [^{3}H]UK14.304. The study focused on brain regions known to be involved in the stress response, namely the mPFC, Acb, CPu, paraventricular nucleus of the hypothalamus (PVN), and locus coeruleus (LC).
The density of α_1- and α_2-adrenoreceptors varied considerably between the brain regions studied, as expected based on the literature. We found no differences in binding between D7 AMX and D7 SHAM rats. This indicates that the density of NA receptors and NA transmission were apparently not altered at the same time in D7 AMX rats. Before definite conclusions can be drawn, however, further research is needed addressing the dynamics over time of the various adrenoceptor subtypes.

Antipsychotics are to some extent protective to the effects of stress in schizophrenic patients, and atypical neuroleptics are advantageous over typical ones in this respect (Kane et al., 1988*a*). Since D7 AMX rats share, amongst a number of other deficits, stress hyporesponsiveness with schizophrenic patients, we investigated the effect of chronic treatment with a typical (haloperidol) or an atypical neuroleptic (clozapine) on the stress response in D7 AMX compared with D7 SHAM rats (**chapter 5**).
Rats were treated daily with haloperidol, clozapine or saline. Before and during the treatment, basal plasma ACTH levels were determined, and behavior was monitored using a 'small open field' test. Footshock stress induced endocrine and behavioral responses were determined after two weeks of treatment.
Neuroleptic treatment did not restore HPA hyporesponsiveness in D7 AMX rats, nor did it affect the behavioral deficits in the small open field and footshock tests. Irrespective of the lesion status of the rats, however, chronic treatment with clozapine altered the dynamics of the HPA response, yielding ACTH responses peaking at an earlier point in time after the stressor. From these results it is concluded that chronic treatment with clozapine alters processes, possibly in the brain, that are involved in generating the HPA axis response to stress. This may have some bearing upon the way atypical neuroleptics exert their stress protective effect in schizophrenic patients.

The results presented in this thesis highlight the importance of the amygdala for adaptational responses to environmental stimuli. They indicate that dysfunction of the amygdala in early life, for instance as a consequence of trauma, can result in disturbed development and function of the HPA axis, one of the organism's major adaptational

systems. In view of the similarities in symptoms between the D7 AMX rat model and schizophrenia, the results call for further research on the role of the amygdala complex and the HPA axis in this disease.

chapter 7

References

Albert DJ, Walsh ML (1984)
Neural systems and the inhibitory modulation of agonistic behavior: a comparison of mammalian species.
Neuroscience and Biobehavioral Reviews 8:5-24.

Albus M, Ackenheil M, Engel RR, Müller F (1982)
Situational reactivity of autonomic functions in schizophrenic patients.
Psychiatry Research 6:361-370.

Allen RM, Young SJ (1978)
Phencyclidine-induced psychosis.
American Journal of Psychiatry 135:1081-1084.

Altar CA, Wasley AM, Neale RF, Stone GA (1986)
Typical and atypical antipsychotic occupancy of D2 and S2 receptors: an autoradiographic analysis in rat brain.
Brain Research Bulletin 16:517-525.

Amaral DG (1986)
Amygdalohippocampal and amygdalocortical projections in the primate brain.
Advances in Experimental Medicine and Biology 203:3-17.

Amaral DG, Bauman MD, Capitanio JP, Lavenex P, Mason WA, Mauldin-Jourdain ML, Mendoza SP (2003)
The amygdala: is it an essential component of the neural network for social cognition?
Neuropsychologia 41:517-522.

Amaral DG, Cowan WM (1980)
Subcortical afferents to the hippocampal formation in the monkey.
Journal of Comparative Neurology 189:573-591.

Amaral DG, Insausti R (1992)
Retrograde transport of D-[^{3}H]-aspartate injected into the monkey amygdaloid complex.
Experimental Brain Research 88:375-388.

Amaral DG, Price JL (1984)
Amygdalo-cortical projections in the monkey (Macaca fascicularis).
Journal of Comparative Neurology 230:465-496.

Amaral DG, Veazey RB, Cowan WM (1982)
Some observations on hypothalamo-amygdaloid connections in the monkey.
Brain Research 252:13-27.

Anderson JE, Wible CG, McCarley RW, Jakab M, Kasai K, Shenton ME (2002)
An MRI study of temporal lobe abnormalities and negative symptoms in chronic schizophrenia.
Schizophrenia Research 58:123-134.

Andreasen NC, Rezai K, Alliger R, Swayze VW^{2nd}, Flaum M, Kirchner P, Cohen G, O'Leary DS (1992)
Hypofrontality in neuroleptic-naive patients and in patients with chronic schizophrenia. Assessment with xenon 133 single-photon emission computed tomography and the Tower of London.
Archives of General Psychiatry 49:943-958.

Antoni FA (1986)
Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of 41-residue corticotropin-releasing factor.
Endocrine Reviews 7:351-378.

Aoki C, Go CG, Venkatesan C, Kurose H (1994)
Perikaryal and synaptic localization of alpha 2A-adrenergic receptor-like immunoreactivity.
Brain Research 650:181-204.

APA (1994)
Diagnostic and Statistical Manual of Mental Disorders, 4th edition: DSM-IV Edition.
Washington DC: American Psychiatric

Association.

Aston-Jones G (1985)
The Locus Coeruleus: Behavioral functions of locus coeruleus derived from cellular attributes.
Physiological Psychology 13:118-126.

Aston-Jones G, Akaoka H, Charléty P, Chouvet G (1991)
Serotonin selectively attenuates glutamate-evoked activation of noradrenergic locus coeruleus neurons.
Journal of Neuroscience 11:760-769.

Aston-Jones G, Ennis M, Pieribone VA, Nickell WT, Shipley MT (1986)
The brain nucleus locus coeruleus: restricted afferent control of a broad efferent network.
Science 234:734-737.

Beatty WW, Jocic Z, Monson N, Staton RD (1993)
Memory and frontal lobe dysfunction in schizophrenia and schizoaffective disorder.
Journal of Nervous and Mental Disease 181:448-453.

Beaulieu S, Di Paolo T, Barden N (1986)
Control of ACTH secretion by the central nucleus of the amygdala: implication of the serotoninergic system and its relevance to the glucocorticoid delayed negative feedback mechanism.
Neuroendocrinology 44:247-254.

Beaulieu S, Di Paolo T, Cote J, Barden N (1987)
Participation of the central amygdaloid nucleus in the response of adrenocorticotropin secretion to immobilization stress: opposing roles of the noradrenergic and dopaminergic systems.
Neuroendocrinology 45:37-46.

Bellack AS, Mueser KT, Wade J, Sayers S, Morrison RL (1992)
The ability of schizophrenics to perceive and cope with negative affect.
British Journal of Psychiatry 160:473-480.

Berman KF, Illowsky BP, Weinberger DR (1988)
Physiological dysfunction of dorsolateral prefrontal cortex in schizophrenia. IV. Further evidence for regional and behavioral specificity [see comments].
Archives of General Psychiatry 45:616-622.

Birbaumer N, Grodd W, Diedrich O, Klose U, Erb M, Lotze M, Schneider F, Weiss U, Flor H (1998)
fMRI reveals amygdala activation to human faces in social phobics.
Neuroreport 9:1223-1226.

Birley JL, Brown GW (1970)
Crises and life changes preceding the onset or relapse of acute schizophrenia: clinical aspects.
British Journal of Psychiatry 116:327-333.

Birnbaum S, Gobeske KT, Auerbach J, Taylor JR, Arnsten AF (1999)
A role for norepinephrine in stress-induced cognitive deficits: alpha-1-adrenoceptor mediation in the prefrontal cortex.
Biological Psychiatry 46:1266-1274.

Bjorklund M, Sirvio J, Sallinen J, Scheinin M, Kobilka BK, Riekkinen P, Jr. (1999)
Alpha2C-adrenoceptor overexpression disrupts execution of spatial and non-spatial search patterns.
Neuroscience 88:1187-1198.

Bogerts B, Meertz E, Schonfeldt-Bausch R (1985)
Basal ganglia and limbic system pathology in schizophrenia. A morphometric study of brain volume and shrinkage.
Archives of General Psychiatry 42:784-791.

Bouwmeester H (2002)

Neurodevelopmental aspects of neonatal amygdala lesions in the rat.
Thesis, Utrecht University. ISBN 90 393 4048 3

Bouwmeester H, Wolterink G, Van Ree JM (2002)
Neonatal development of projections from the basolateral amygdala to prefrontal, striatal, and thalamic structures in the rat.
Journal of Comparative Neurology 442:239-249.

Brake WG, Flores G, Francis D, Meaney MJ, Srivastava LK, Gratton A (2000)
Enhanced nucleus accumbens dopamine and plasma corticosterone stress responses in adult rats with neonatal excitotoxic lesions to the medial prefrontal cortex.
Neuroscience 96:687-695.

Brann DW, Mahesh VB (1994)
Excitatory amino acids: function and significance in reproduction and neuroendocrine regulation.
Fronteers in Neuroendocrinology 15:3-49.

Breier A, Wolkowitz OM, Doran AR, Bellar S, Pickar D (1988)
Neurobiological effects of lumbar puncture stress in psychiatric patients and healthy volunteers.
Psychiatry Research 25:187-194.

Brooks DP, Share L, Crofton JT (1986)
Central adrenergic control of vasopressin release.
Neuroendocrinology 42:416-420.

Brown GW, Birley JL (1968)
Crises and life changes and the onset of schizophrenia.
Journal of Health and Social Behavior 9:203-214.

Buchanan RW, Holstein C, Breier A (1994*a*)
The comparative efficacy and long-term effect of clozapine treatment on neuropsychological test performance.
Biological Psychiatry 36:717-725.

Buchanan SL, Thompson RH, Maxwell BL, Powell DA (1994*b*)
Efferent connections of the medial prefrontal cortex in the rabbit.
Experimental Brain Research 100:469-483.

Burchfield SR (1979)
The stress response: a new perspective.
Psychosomatic Medicine 41:661-672.

Buwalda B, de Boer SF, Schmidt ED, Felszeghy K, Nyakas C, Sgoifo A, Van der Vegt BJ, Tilders FJ, Bohus B, Koolhaas JM (1999)
Long-lasting deficient dexamethasone suppression of hypothalamic-pituitary-adrenocortical activation following peripheral CRF challenge in socially defeated rats.
Journal of Neuroendocrinology 11:513-520.

Bylund DB, Eikenberg DC, Hieble JP, Langer SZ, Lefkowitz RJ, Minneman KP, Molinoff PB, Ruffolo RR, Jr., Trendelenburg U (1994)
International Union of Pharmacology nomenclature of adrenoceptors.
Pharmacological Review 46:121-136.

Cabib S, Giardino L, Calzá L, Zanni M, Mele A, Puglisi-Allegra S (1998)
Stress promotes major changes in dopamine receptor densities within the mesoaccumbens and nigrostriatal systems.
Neuroscience 84:193-200.

Cannon WB (1929)
Organization for physiological homeostasis.
Physiological Review 399–431.

Cannon WB (1935)
Stresses and strains of homeostasis.

American Journal of Medicine: 1–14.

Carpenter WT, Jr., Buchanan RW (1994)
Schizophrenia.
New England Journal of Medicine 330:681-690.

Carrasco GA, Van de Kar LD (2003)
Neuroendocrine pharmacology of stress.
European Journal of Pharmacology 463:235-272.

Carter CJ, Pycock CJ (1980)
Behavioural and biochemical effects of dopamine and noradrenaline depletion within the medial prefrontal cortex of the rat.
Brain Research 192:163-176.

Castellon SA, Asarnow RF, Goldstein MJ, Marder SR (1994)
Persisting negative symptoms and information-processing deficits in schizophrenia: implications for subtyping.
Psychiatry Research 54:59-69.

Chamba G, Weissmann D, Rousset C, Renaud B, Pujol JF (1991)
Distribution of alpha-1 and alpha-2 binding sites in the rat locus coeruleus.
Brain Research Bulletin 26:185-193.

Chambers RA, Moore J, McEvoy JP, Levin ED (1996)
Cognitive effects of neonatal hippocampal lesions in a rat model of schizophrenia.
Neuropsychopharmacology 15:587-594.

Chance SA, Esiri MM, Crow TJ (2002)
Amygdala volume in schizophrenia: post-mortem study and review of magnetic resonance imaging findings.
British Journal of Psychiatry 180:331-338.

Civelli O, Bunzow JR, Grandy DK (1993)
Molecular diversity of the dopamine receptors.
Annual Review of Pharmacology and Toxicology 33:281-307.

Clifford JJ, Usiello A, Vallone D, Kinsella A, Borrelli E, Waddington JL (2000)
Topographical evaluation of behavioural phenotype in a line of mice with targeted gene deletion of the D2 dopamine receptor.
Neuropharmacology 39:382-390.

Cole BJ, Robbins TW (1992)
Forebrain norepinephrine: role in controlled information processing in the rat.
Neuropsychopharmacology 7:129-142.

Collingridge G (1987)
Synaptic plasticity. The role of NMDA receptors in learning and memory.
Nature 330:604-605.

Collingridge GL, Lester RA (1989)
Excitatory amino acid receptors in the vertebrate central nervous system.
Pharmacological Reviews 41:143-210.

Collins P, Roberts AC, Dias R, Everitt BJ, Robbins TW (1998)
Perseveration and strategy in a novel spatial self-ordered sequencing task for nonhuman primates. Effects of excitotoxic lesions and dopamine depletions of the prefrontal cortex.
Journal of Cognitive Neuroscience 10:332-354.

Cramer P, Bowen J, O'Neill M (1992)
Schizophrenics and social judgement. Why do schizophrenics get it wrong?
British Journal of Psychiatry 160:481-487.

Creese I, Burt DR, Snyder SH (1976)
Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs.
Science 192:481-483.

Csernansky JG, Kerr S, Pruthi R, Prosser ES (1988)
Mesolimbic dopamine receptor increases two weeks following hippocampal kindling.

Brain Research 449:357-360.

Cunningham ET, Jr., Sawchenko PE (1988)
Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus.
Journal of Comparative Neurology 274:60-76.

Daenen EW, Wolterink G, Gerrits MA, Van Ree JM (2002*a*)
Amygdala or ventral hippocampal lesions at two early stages of life differentially affect open field behaviour later in life; an animal model of neurodevelopmental psychopathological disorders.
Behavioural Brain Research 131:67-78.

Daenen EW, Wolterink G, Gerrits MA, Van Ree JM (2002*b*)
The effects of neonatal lesions in the amygdala or ventral hippocampus on social behaviour later in life.
Behavioural Brain Research 136:571-582.

Daenen EW, Van der Heyden JA, Kruse CG, Wolterink G, Van Ree JM (2001)
Adaptation and habituation to an open field and responses to various stressful events in animals with neonatal lesions in the amygdala or ventral hippocampus.
Brain Research 918:153-165.

Daenen EW, Wolterink G, Van Der Heyden JA, Kruse CG, Van Ree JM (2003*a*)
Neonatal lesions in the amygdala or ventral hippocampus disrupt prepulse inhibition of the acoustic startle response; implications for an animal model of neurodevelopmental disorders like schizophrenia.
European Neuropsychopharmacology 13:187-197.

Daenen EWPM (1999)
Neonatal lesions of the rat brain; a model of neurodevelopmental psychopathological disorders.
Thesis. Utrecht University. ISBN 90 393 2258 9

Daenen EWPM, Wolterink G, Van Ree JM (2003*b*)
Hyperresponsiveness to phencyclidine in animals lesioned in the amygdala on day 7 of life: Implications for an animal model of schizophrenia.
Neuropsychopharmacology 13:273-279.

Dahlstrom A, Fuxe K (1964)
Localization of monoamines in the lower brain stem.
Experientia 20:398-399.

Daniel DG, Berman KF, Weinberger DR (1989)
The effect of apomorphine on regional cerebral blood flow in schizophrenia.
Journal of Neuropsychiatry and Clinical Neuroscience 1:377-384.

Daniel DG, Weinberger DR, Jones DW, Zigun JR, Coppola R, Handel S, Bigelow LB, Goldberg TE, Berman KF, Kleinman JE (1991)
The effect of amphetamine on regional cerebral blood flow during cognitive activation in schizophrenia.
Journal of Neuroscience 11:1907-1917.

Davis JO, Phelps JA, Bracha HS (1995)
Prenatal development of monozygotic twins and concordance for schizophrenia.
Schizophrenia Bulletin 21:357-366.

Davis M (1992)
The role of the amygdala in fear and anxiety.
Annual Review of Neuroscience 15:353-375.

Daw NW, Stein PS, Fox K (1993)
The role of NMDA receptors in information processing.
Annual Review of Neuroscience 16:207-222.

Day R, Nielsen JA, Korten A, Ernberg G,

Dube KC, Gebhart J, Jablensky A, Leon C, Marsella A, Olatawura M, al e (1987)
Stressful life events preceding the acute onset of schizophrenia: a cross-national study from the World Health Organization.
Culture, Medicine and Psychiatry 11:123-205.

Day TA (1989)
Control of neurosecretory vasopressin cells by noradrenergic projections of the caudal ventrolateral medulla.
Progress in Brain Research 81:303-317.

Day TA, Ferguson AV, Renaud LP (1985)
Noradrenergic afferents facilitate the activity of tuberoinfundibular neurons of the hypothalamic paraventricular nucleus.
Neuroendocrinology 41:17-22.

Dayas CV, Buller KM, Day TA (1999)
Neuroendocrine responses to an emotional stressor: evidence for involvement of the medial but not the central amygdala.
European Journal of Neuroscience 11:2312-2322.

De Kloet ER, Van Acker SA, Sibug RM, Oitzl MS, Meijer OC, Rahmouni K, De Jong W (2000)
Brain mineralocorticoid receptors and centrally regulated functions.
Kidney International 57:1329-1336.

DeLisi LE, Dauphinais ID, Gershon ES (1988)
Perinatal complications and reduced size of brain limbic structures in familial schizophrenia.
Schizophrenia Bulletin 14:185-191.

Dess NK, Linwick D, Patterson J, Overmier JB, Levine S (1983)
Immediate and proactive effects of controllability and predictability on plasma cortisol responses to shocks in dogs.
Behavioral Neuroscience 97:1005-1016.

Deutch AY (1993)
Prefrontal cortical dopamine systems and the elaboration of functional corticostriatal circuits: implications for schizophrenia and Parkinson's disease.
Journal of Neural Transmission General Section 91:197-221.

Deutch AY, Clark WA, Roth RH (1990)
Prefrontal cortical dopamine depletion enhances the responsiveness of mesolimbic dopamine neurons to stress.
Brain Research 521:311-315.

Diaz-Cabiale Z, Petersson M, Narvaez JA, Uvnas-Moberg K, Fuxe K (2000)
Systemic oxytocin treatment modulates alpha 2-adrenoceptors in telencephalic and diencephalic regions of the rat.
Brain Research 887:421-425.

Doering S, Müller E, Köpcke W, Pietzcker A, Gaebel W, Linden M, Müller P, Müller-Spahn F, Tegeler J, Schüssler G (1998)
Predictors of relapse and rehospitalization in schizophrenia and schizoaffective disorder.
Schizophrenia Bulletin 24:87-98.

Dolan RJ, Fletcher P, Frith CD, Friston KJ, Frackowiak RS, Grasby PM (1995)
Dopaminergic modulation of impaired cognitive activation in the anterior cingulate cortex in schizophrenia.
Nature 378:180-182.

Domeney A, Feldon J (1998)
The disruption of prepulse inhibition by social isolation in the Wistar rat: how robust is the effect?
Pharmacology Biochemistry and Behavior 59:883-890.

Dori I, Petrou M, Parnavelas JG (1989)
Excitatory transmitter amino acid-containing

neurons in the rat visual cortex: a light and electron microscopic immunocytochemical study.
Journal of Comparative Neurology 290:169-184.

Ellenbroek BA, Geyer MA, Cools AR (1995)
The behavior of APO-SUS rats in animal models with construct validity for schizophrenia.
Journal of Neuroscience 15:7604-7611.

Ellenbroek BA, Van den Kroonenberg PT, Cools AR (1998)
The effects of an early stressful life event on sensorimotor gating in adult rats.
Schizophrenia Research 30:251-260.

Engberg G (1992)
Citalopram and 8-OH-DPAT attenuate nicotine-induced excitation of central noradrenaline neurons.
Journal of Neural Transmission General Section 89:149-154.

Ennis M, Aston-Jones G (1988)
Activation of locus coeruleus from nucleus paragigantocellularis: a new excitatory amino acid pathway in brain.
Journal of Neuroscience 8:3644-3657.

Enrico P, Bouma M, de Vries JB, Westerink BH (1998)
The role of afferents to the ventral tegmental area in the handling stress-induced increase in the release of dopamine in the medial prefrontal cortex: a dual-probe microdialysis study in the rat brain.
Brain Research 779:205-213.

Epstein J, Stern E, Silbersweig D (1999)
Mesolimbic activity associated with psychosis in schizophrenia. Symptom-specific PET studies.
Annals of the New York Academy of Sciences 877:562-574.

Familari M, Smith AI, Smith R, Funder JW (1989)
Arginine vasopressin is a much more potent stimulus to ACTH release from ovine anterior pituitary cells than ovine corticotropin-releasing factor. 1. In vitro studies.
Neuroendocrinology 50:152-157.

Feenstra MG, Botterblom MH, van Uum JF (1998)
Local activation of metabotropic glutamate receptors inhibits the handling-induced increased release of dopamine in the nucleus accumbens but not that of dopamine or noradrenaline in the prefrontal cortex: comparison with inhibition of ionotropic receptors.
Journal of Neurochemistry 70:1104-1113.

Fiore M, Talamini L, Angelucci F, Koch T, Aloe L, Korf J (1999)
Prenatal methylazoxymethanol acetate alters behavior and brain NGF levels in young rats: a possible correlation with the development of schizophrenia-like deficits.
Neuropharmacology 38:857-869.

Flores G, Wood GK, Liang JJ, Quirion R, Srivastava LK (1996)
Enhanced amphetamine sensitivity and increased expression of dopamine D2 receptors in postpubertal rats after neonatal excitotoxic lesions of the medial prefrontal cortex.
Journal of Neuroscience 16:7366-7375.

Flugge G, van Kampen M, Meyer H, Fuchs E (2003)
Alpha2A and alpha2C-adrenoceptor regulation in the brain: alpha2A changes

persist after chronic stress.
European Journal of Neuroscience 17:917-928.

Foote SL, Bloom FE, Aston-Jones G (1983)
Nucleus locus ceruleus: new evidence of anatomical and physiological specificity.
Physiological Reviews 63:844-914.

Fudge JL, Powers JM, Haber SN, Caine ED (1998)
Considering the role of the amygdala in psychotic illness: a clinicopathological correlation.
Journal of Neuropsychiatry and Clinical Neurosciences 10:383-394.

Fujii DE, Ahmed I, Jokumsen M, Compton JM (1997)
The effects of clozapine on cognitive functioning in treatment-resistant schizophrenic patients.
Journal of Neuropsychiatry and Clinical Neurosciences 9:240-245.

Gaillet S, Lachuer J, Malaval F, Assenmacher I, Szafarczyk A (1991)
The involvement of noradrenergic ascending pathways in the stress-induced activation of ACTH and corticosterone secretions is dependent on the nature of stressors.
Experimental Brain Research 87:173-180.

Gaillet S, Alonso G, Le Borgne R, Barbanel G, Malaval F, Assenmacher I, Szafarczyk A (1993)
Effects of discrete lesions in the ventral noradrenergic ascending bundle on the corticotropic stress response depend on the site of the lesion and on the plasma levels of adrenal steroids.
Neuroendocrinology 58:408-419.

Gainetdinov RR, Mohn AR, Caron MG (2001)
Genetic animal models: focus on schizophrenia.
Trends in Neurosciences 24:527-533.

Galletly CA, Clark CR, McFarlane AC, Weber DL (2000)
The effect of clozapine on the speed and accuracy of information processing in schizophrenia.
Progress in Neuropsychopharmacology and Biological Psychiatry 24:1329-1338.

Gallhofer B, Bauer U, Lis S, Krieger S, Gruppe H (1996)
Cognitive dysfunction in schizophrenia: comparison of treatment with atypical antipsychotic agents and conventional neuroleptic drugs.
European Neuropsychopharmacology 6 Supplement 2:S13-20.

Gambill JD, Kornetsky C (1976)
Effects of chronic d-amphetamine on social behavior of the rat: implications for an animal model of paranoid schizophrenia.
Psychopharmacology 50:215-223.

Gannon L, Pardie L (1989)
The importance of chronicity and controllability of stress in the context of stress-illness relationships.
Journal of Behavioral Medicine 12:357-372.

Geyer MA, Wilkinson LS, Humby T, Robbins TW (1993)
Isolation rearing of rats produces a deficit in prepulse inhibition of acoustic startle similar to that in schizophrenia.
Biological Psychiatry 34:361-372.

Giardino L, Zanni M, Pozza M, Bettelli C, Covelli V (1998)
Dopamine receptors in the striatum of rats exposed to repeated restraint stress and

alprazolam treatment.
European Journal of Pharmacology 344:143-147.

Gingrich JA, Caron MG (1993)
Recent advances in the molecular biology of dopamine receptors.
Annual Review of Neuroscience 16:299-321.

Gispen-de Wied CC (2000)
Stress in schizophrenia: an integrative view.
European Journal of Pharmacology 405:375-384.

Glavin GB, Hall AM (1994)
Clozapine, a dopamine DA4 receptor antagonist, reduces gastric acid secretion and stress-induced gastric mucosal injury.
Life sciences 54:PL261-264.

Gogos JA, Morgan M, Luine V, Santha M, Ogawa S, Pfaff D, Karayiorgou M (1998)
Catechol-O-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior.
Proceedings of the National Academy of Sciences of the United States of America 95:9991-9996.

Goto Y, O'Donnell P (2002)
Delayed mesolimbic system alteration in a developmental animal model of schizophrenia.
Journal of Neuroscience 22:9070-9077.

Grabe HJ, Wolf T, Gratz S, Laux G (1999)
The influence of clozapine and typical neuroleptics on information processing of the central nervous system under clinical conditions in schizophrenic disorders: implications for fitness to drive.
Neuropsychobiology 40:196-201.

Graeff FG, Guimarães FS, De Andrade TG, Deakin JF (1996)
Role of 5-HT in stress, anxiety, and depression.
Pharmacology, Biochemistry and Behavior 54:129-141.

Greengrass P, Bremner R (1979)
Binding characteristics of ^{3}H-prazosin to rat brain alpha-adrenergic receptors.
European Journal of Pharmacology 55:323-326.

Grimm LJ, Blendy JA, Kellar KJ, Perry DC (1992)
Chronic reserpine administration selectively up-regulates beta 1- and alpha 1b-adrenergic receptors in rat brain: an autoradiographic study.
Neuroscience 47:77-86.

Groenewegen HJ, Vermeulen-Van der Zee E, Te Kortschot A, Witter MP (1987)
Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of Phaseolus vulgaris leucoagglutinin.
Neuroscience 23:103-120.

Gur RE, Cowell P, Turetsky BI, Gallacher F, Cannon T, Bilker W, Gur RC (1998)
A follow-up magnetic resonance imaging study of schizophrenia. Relationship of neuroanatomical changes to clinical and neurobehavioral measures.
Archives of General Psychiatry 55:145-152.

Gur RE, Turetsky BI, Cowell PE, Finkelman C, Maany V, Grossman RI, Arnold SE, Bilker WB, Gur RC (2000)
Temporolimbic volume reductions in schizophrenia.
Archives of General Psychiatry 57:769-775.

Hafner H, Van der Heiden W, Behrens S, Gattaz WF, Hambrecht M, Loffler W, Maurer K, Munk-Jorgensen P, Nowotny B, Riecher-Rossler A, Stein A (1998)
Causes and consequences of the gender difference in age at onset of schizophrenia.
Schizophrenia Bulletin 24:99-113.

Han C, Abel PW, Minneman KP (1987*a*)
Heterogeneity of alpha 1-adrenergic receptors revealed by chlorethylclonidine.
Molecular Pharmacology 32:505-510.

Han C, Abel PW, Minneman KP (1987*b*)
Alpha 1-adrenoceptor subtypes linked to different mechanisms for increasing intracellular Ca2+ in smooth muscle.
Nature 329:333-335.

Han CD, Minneman KP (1991)
Interaction of subtype-selective antagonists with alpha 1-adrenergic receptor binding sites in rat tissues.
Molecular Pharmacology 40:531-538.

Hanlon FM, Sutherland RJ (2000)
Changes in adult brain and behavior caused by neonatal limbic damage: implications for the etiology of schizophrenia.
Behavioural Brain Research 107:71-83.

Haroutunian V, Knott P, Davis KL (1988)
Effects of mesocortical dopaminergic lesions upon subcortical dopaminergic function.
Psychopharmacology Bulletin 24:341-344.

Herman JP, Guillonneau D, Dantzer R, Scatton B, Semerdjian-Rouquier L, Le Moal M (1982)
Differential effects of inescapable footshocks and of stimuli previously paired with inescapable footshocks on dopamine turnover in cortical and limbic areas of the rat.
Life Sciences 30:2207-2214.

Huether G (1996)
The central adaptation syndrome: psychosocial stress as a trigger for adaptive modifications of brain structure and brain function.
Progress in Neurobiology; Archives of General Psychiatry 48:569-612.

Hulshoff Pol HE, Schnack HG, Mandl RC, Van Haren NE, Koning H, Collins DL, Evans AC, Kahn RS (2001)
Focal gray matter density changes in schizophrenia.
Archives of General Psychiatry 58:1118-1125.

Jacobs BL, Abercrombie ED, Fornal CA, Levine ES, Morilak DA, Stafford IL (1991)
Single-unit and physiological analyses of brain norepinephrine function in behaving animals.
Progress in Brain Research 88:159-165.

Jakob H, Beckmann H (1986)
Prenatal developmental disturbances in the limbic allocortex in schizophrenics.
Journal of Neural Transmission 65:303-326.

Jansen LM, Gispen-de Wied CC, Kahn RS (2000*a*)
Selective impairments in the stress response in schizophrenic patients.
Psychopharmacology 149:319-325.

Jansen LM, Gispen-de Wied CC, Gademan PJ, De Jonge RC, van der Linden JA, Kahn RS (1998)
Blunted cortisol response to a psychosocial stressor in schizophrenia.
Schizophrenia Research 33:87-94.

Jansen LM, Gispen-de Wied CC, Jansen MA, Van der Gaag RJ, Matthys W, van Engeland H (1999)
Pituitary-adrenal reactivity in a child psychiatric population: salivary cortisol response to stressors.
European Neuropsychopharmacology 9:67-75.

Jansen LM, Gispen-de Wied CC, Van der Gaag RJ, ten Hove F, Willemsen-Swinkels SW, Harteveld E, Van Engeland H (2000*b*)

Unresponsiveness to psychosocial stress in a subgroup of autistic-like children, multiple complex developmental disorder. *Psychoneuroendocrinology 25:753-764.*

Javitt DC, Zukin SR (1991)
Recent advances in the phencyclidine model of schizophrenia.
American Journal of Psychiatry 148:1301-1308.

Jentsch JD, Elsworth JD, Redmond DEJ, Roth RH (1997)
Phencyclidine increases forebrain monoamine metabolism in rats and monkeys: modulation by the isomers of HA966.
Journal of Neuroscience 17:1769-1775.

Joel D, Weiner I, Feldon J (1997)
Electrolytic lesions of the medial prefrontal cortex in rats disrupt performance on an analog of the Wisconsin Card Sorting Test, but do not disrupt latent inhibition: implications for animal models of schizophrenia.
Behavioural Brain Research 85:187-201.

Johnson DA (1977)
Treatment of chronic schizophrenia.
Drugs 14:291-299.

Johnson JK, Tuulio-Henriksson A, Pirkola T, Huttunen MO, Lonnqvist J, Kaprio J, Cannon TD (2003)
Do schizotypal symptoms mediate the relationship between genetic risk for schizophrenia and impaired neuropsychological performance in co-twins of schizophrenic patients?
Biological Psychiatry 54:1200-1204.

Jones LS, Gauger LL, Davis JN (1985)
Anatomy of brain alpha 1-adrenergic receptors: in vitro autoradiography with [^{125}I]-heat.
Journal of Comparative Neurology 231:190-208.

Joyce JN (2001)
Dopamine D3 receptor as a therapeutic target for antipsychotic and antiparkinsonian drugs.
Journal of Pharmacology and Experimental Therapeutics 90:231-259.

Kaczmarek L, Kossut M, Skangiel-Kramska J (1997)
Glutamate receptors in cortical plasticity: molecular and cellular biology.
Physiological Reviews 77:217-255.

Kahn RS, Davidson M, Siever LJ, Sevy S, Davis KL (1994)
Clozapine treatment and its effect on neuroendocrine responses induced by the serotonin agonist, m-chlorophenylpiperazine.
Biological Psychiatry 35:909-912.

Kahn RS, Siever L, Davidson M, Greenwald C, Moore C (1993)
Haloperidol and clozapine treatment and their effect on M-chlorophenylpiperazine-mediated responses in schizophrenia: implications for the mechanism of action of clozapine.
Psychopharmacology 112:S90-94.

Kalkman HO, Loetscher E (2003)
alpha(2C)-Adrenoceptor blockade by clozapine and other antipsychotic drugs.
European Journal of Pharmacology 462:33-40.

Kamphuis PJ, Bakker JM, Broekhoven MH, Kunne C, Croiset G, Lentjes EG, Tilders FJ, Van Bel F, Wiegant VM (2002)
Enhanced glucocorticoid feedback inhibition of hypothalamo-pituitary-adrenal responses to stress in adult rats neonatally treated with dexamethasone.
Neuroendocrinology 76:158-169.

Kane J, Honigfeld G, Singer J, Meltzer H

(1988*a*)
Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine.
Archives of General Psychiatry 45:789-796.

Kane JM, Honigfeld G, Singer J, Meltzer H (1988*b*)
Clozapine in treatment-resistant schizophrenics.
Psychopharmacology Bulletin 24:62-67.

Kaneko M, Yokoyama F, Hoshino Y, Takahagi K, Murata S, Watanabe M, Kumashiro H (1992)
Hypothalamic-pituitary-adrenal axis function in chronic schizophrenia: association with clinical features.
Neuropsychobiology 25:1-7.

Kapit RM (1977)
Schizophrenia and tardive dyskinesia: is schizophrenia also a "denervation hypersensitivity"?
Medical Hypotheses 3:207-210.

Kathol RG, Gehris TL, Carroll BT, Samuelson SD, Pitts AF, Meller WH, Carter JL (1992)
Blunted ACTH response to hypoglycemic stress in depressed patients but not in patients with schizophrenia.
Journal of Psychiatric Research 26:103-116.

Keim KL, Sigg EB (1977)
Plasma corticosterone and brain catecholamines in stress: effect of psychotropic drugs.
Pharmacology, Biochemistry and Behavior 6:79-85.

Kesner RP, Berman RF, Tardif R (1992)
Place and taste aversion learning: role of basal forebrain, parietal cortex, and amygdala.
Brain Research Bulletin 29:345-353.

Kitchen I, Slowe SJ, Matthes HW, Kieffer B (1997)
Quantitative autoradiographic mapping of mu-, delta- and kappa-opioid receptors in knockout mice lacking the mu-opioid receptor gene.
Brain Research 778:73-88.

Klepstad P, Maurset A, Moberg ER, Oye I (1990)
Evidence of a role for NMDA receptors in pain perception.
European Journal of Pharmacology 187:513-518.

Knable MB, Weinberger DR (1995)
Are mental diseases brain diseases? The contribution of neuropathology to understanding of schizophrenic psychoses.
European Archives of Psychiatry and Clinical Neuroscience 245:224-230.

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MBJ, Charney DS (1994)
Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses.
Archives of General Psychiatry 51:199-214.

Kudoh A, Ishihara H, Matsuki A (1999)
Pituitary-adrenal and parasympathetic function in chronic schizophrenic patients with postoperative ileus or hypotension.
Neuropsychobiology 39:125-130.

Kudoh A, Kudo T, Ishihara H, Matsuki A (1997)
Depressed pituitary-adrenal response to surgical stress in chronic schizophrenic patients.
Neuropsychobiology 36:112-116.

Kulagina NV, Zigmond MJ, Michael AC (2001)
Glutamate regulates the spontaneous and evoked release of dopamine in the rat

striatum.
Neuroscience 102:121-128.

Kushner MG, Riggs DS, Foa EB, Miller SM (1992)
Percieved controllability and the development of posttraumatic stress disorder (PTSD) in crime victims.
Behaviour Research and Therapy 31:105-110.

Lacroix L, Spinelli S, White W, Feldon J (2000)
The effects of ibotenic acid lesions of the medial and lateral prefrontal cortex on latent inhibition, prepulse inhibition and amphetamine-induced hyperlocomotion.
Neuroscience 97:459-468.

Lawrie SM, Whalley H, Kestelman JN, Abukmeil SS, Byrne M, Hodges A, Rimmington JE, Best JJ, Owens DG, Johnstone EC (1999)
Magnetic resonance imaging of brain in people at high risk of developing schizophrenia.
Lancet 353:30-33.

Leccese AP, Lyness WH (1987)
Lesions of dopamine neurons in the medial prefrontal cortex: effects on self-administration of amphetamine and dopamine synthesis in the brain of the rat.
Neuropharmacology 26:1303-1308.

Lee JH, Woo JI, Meltzer HY (2001)
Effects of clozapine on sleep measures and sleep-associated changes in growth hormone and cortisol in patients with schizophrenia.
Psychiatry Research 103:157-166.

Leff J, Thornicroft G, Coxhead N, Crawford C (1994)
The TAPS Project. 22: A five-year follow-up of long-stay psychiatric patients discharged to the community.
British Journal of Psychiatry Supplement: 13-17.

Lehmann J, Pryce CR, Feldon J (2000)
Lack of effect of an early stressful life event on sensorimotor gating in adult rats.
Schizophrenia Research 41:365-371.

Lesscher HM, Bailey A, Burbach JP, Van Ree JM, Kitchen I, Gerrits MA (2003)
Receptor-selective changes in mu-, delta- and kappa-opioid receptors after chronic naltrexone treatment in mice.
European Journal of Neuroscience 17:1006-1012.

Li BM, Mao ZM, Wang M, Mei ZT (1999)
Alpha-2 adrenergic modulation of prefrontal cortical neuronal activity related to spatial working memory in monkeys.
Neuropsychopharmacology 21:601-610.

Liberman RP (1994)
Psychosocial treatments for schizophrenia.
Psychiatry 57:104-114.

Linn GS, O'Keeffe RT, Schroeder CE, Lifshitz K, Javitt DC (1999)
Behavioral effects of chronic phencyclidine in monkeys.
Neuroreport 10:2789-2793.

Lipska BK, Weinberger DR (1993)
Delayed effects of neonatal hippocampal damage on haloperidol-induced catalepsy and apomorphine-induced stereotypic behaviors in the rat.
Brain Research and Developmental Brain Research 75:213-222.

Lipska BK, Weinberger DR (1995)
Genetic variation in vulnerability to the behavioral effects of neonatal hippocampal damage in rats.
Proceedings of the National Academy of Sciences of the United States of America 92:8906-8910.

Lipska BK, Weinberger DR (2000)
To model a psychiatric disorder in animals: schizophrenia as a reality test.
Neuropsychopharmacology 23:223-239.

Lipska BK, Weinberger DR (2002)
A neurodevelopmental model of schizophrenia: neonatal disconnection of the hippocampus.
Neurotoxicity Research 4:469-475.

Lipska BK, Jaskiw GE, Weinberger DR (1993)
Postpubertal emergence of hyper-responsiveness to stress and to amphetamine after neonatal excitotoxic hippocampal damage: a potential animal model of schizophrenia.
Neuropsychopharmacology 9:67-75.

Lipska BK, al Amin HA, Weinberger DR (1998)
Excitotoxic lesions of the rat medial prefrontal cortex. Effects on abnormal behaviors associated with neonatal hippocampal damage.
Neuropsychopharmacology 19:451-464.

Lipska BK, Chrapusta SJ, Egan MF, Weinberger DR (1995)
Neonatal excitotoxic ventral hippocampal damage alters dopamine response to mild repeated stress and to chronic haloperidol.
Synapse 20:125-130.

Lipska BK, Khaing ZZ, Weickert CS, Weinberger DR (2001)
BDNF mRNA expression in rat hippocampus and prefrontal cortex: effects of neonatal ventral hippocampal damage and antipsychotic drugs.
European Journal of Neuroscience 14:135-144.

Lipska BK, Halim ND, Segal PN, Weinberger DR (2002*a*)
Effects of reversible inactivation of the neonatal ventral hippocampus on behavior in the adult rat.
Journal of Neuroscience 22:2835-2842.

Lipska BK, Jaskiw GE, Chrapusta S, Karoum F, Weinberger DR (1992)
Ibotenic acid lesion of the ventral hippocampus differentially affects dopamine and its metabolites in the nucleus accumbens and prefrontal cortex in the rat.
Brain Research 585:1-6.

Lipska BK, Aultman JM, Verma A, Weinberger DR, Moghaddam B (2002*b*)
Neonatal damage of the ventral hippocampus impairs working memory in the rat.
Neuropsychopharmacology 27:47-54.

Lukoff D, Snyder K, Ventura J, Nuechterlein KH (1984)
Life events, familial stress, and coping in the developmental course of schizophrenia.
Schizophrenia Bulletin 10:258-292.

Malaspina D, Goetz RR, Yale S, Berman A, Friedman JH, Tremeau F, Printz D, Amador X, Johnson J, Brown A, Gorman JM (2000)
Relation of familial schizophrenia to negative symptoms but not to the deficit syndrome.
American Journal of Psychiatry 157:994-1003.

Manschreck TC, Redmond DA, Candela SF, Maher BA (1999)
Effects of clozapine on psychiatric symptoms, cognition, and functional outcome in schizophrenia.
Journal of Neuropsychiatry and Clinical Neuroscience 11:481-489.

Marcilhac A, Siaud P (1996)
Regulation of the adrenocorticotrophin response to stress by the central nucleus of the amygdala in rats depends upon the nature

of the stressor.
Experimental Physiology 81:1035-1038.

Martins JM, Kastin AJ, Banks WA (1996)
Unidirectional specific and modulated brain to blood transport of corticotropin-releasing hormone.
Neuroendocrinology 63:338-348.

Mason JW (1971)
A re-evaluation of the concept of non-specificity in stress theory.
Journal of Psychiatric Research 8:323-333.

McEwen BS (1998)
Protective and damaging effects of stress mediators.
New England Journal of Medicine 338:171-179.

McGuffin P, Asherson P, Owen M, Farmer A (1994)
The strength of the genetic effect. Is there room for an environmental influence in the aetiology of schizophrenia?
British Journal of Psychiatry 164:593-599.

McKenna PJ, Tamlyn D, Lund CE, Mortimer AM, Hammond S, Baddeley AD (1990)
Amnesic syndrome in schizophrenia.
Psychological Medicine 20:967-972.

Meador-Woodruff JH, Pellerito B, Bronstein D, Lin HL, Ling N, Akil H (1990)
Differential effects of haloperidol on the rat pituitary: decreased biosynthesis, processing and release of anterior lobe pro-opiomelanocortin.
Neuroendocrinology 51:294-303.

Meltzer HY, Stahl SM (1976)
The dopamine hypothesis of schizophrenia: a review.
Schizophrenia Bull 2:19-76.

Miyamoto Y, Yamada K, Noda Y, Mori H, Mishina M, Nabeshima T (2001)
Hyperfunction of dopaminergic and serotonergic neuronal systems in mice lacking the NMDA receptor epsilon1 subunit.
Journal of Neuroscience 21:750-757.

Monteleone P, Maj M, Fusco M, Kemali D, Reiter RJ (1992)
Depressed nocturnal plasma melatonin levels in drug-free paranoid schizophrenics.
Schizophrenia Research 7:77-84.

Morien A, Cassone VM, Wellman PJ (1999)
Diurnal changes in paraventricular hypothalamic alpha1 and alpha2-adrenoceptors and food intake in rats.
Pharmacological Biochemical Behaviour 63:33-38.

Morris RG, Anderson E, Lynch GS, Baudry M (1986)
Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5.
Nature 319:774-776.

Murphy BL, Roth RH, Arnsten AF (1997)
Clozapine reverses the spatial working memory deficits induced by FG7142 in monkeys.
Neuropsychopharmacology 16:433-437.

Nicholas AP, Pieribone V, Hokfelt T (1993)
Distributions of mRNAs for alpha-2 adrenergic receptor subtypes in rat brain: an in situ hybridization study.
Journal of Comparative Neurology 328:575-594.

Nicolson R, Lenane M, Hamburger SD, Fernandez T, Bedwell J, Rapoport JL (2000)
Lessons from childhood-onset schizophrenia.
Brain Research Reviews 31:147-156.

Nielsen EB, Lyon M, Ellison G (1983)
Apparent hallucinations in monkeys during

around-the-clock amphetamine for seven to fourteen days. Possible relevance to amphetamine psychosis.
Journal of Nervous and Mental Disease 171:222-233.

Nijsen M, Croiset G, Diamant M, Stam R, Delsing D, de Wied D, Wiegant V (1998)
Conditioned fear-induced tachycardia in the rat: vagal involvement.
European Journal of Pharmacology 350:211-222.

Nijsen MJ, Croiset G, Stam R, Bruijnzeel A, Diamant M, de Wied D, Wiegant VM (2000)
The role of the CRH type 1 receptor in autonomic responses to corticotropin-releasing hormone in the rat.
Neuropsychopharmacology 22:388-399.

Noda Y, Yamada K, Furukawa H, Nabeshima T (1995)
Enhancement of immobility in a forced swimming test by subacute or repeated treatment with phencyclidine: a new model of schizophrenia.
British Journal of Pharmacology 116:2531-2537.

Norman RM, Malla AK (1993)
Stressful life events and schizophrenia. I: A review of the research.
British Journal of Psychiatry 162:161-166.

Norman RM, Malla AK (1994)
A prospective study of daily stressors and symptomatology in schizophrenic patients.
Social Psychiatry and Psychiatric Epidemiology 29:244-249.

Nuechterlein KH, Dawson ME, Gitlin M, Ventura J, Goldstein MJ, Snyder KS, Yee CM, Mintz J (1992)
Developmental Processes in Schizophrenic Disorders: longitudinal studies of vulnerability and stress.
Schizophrenia Bulletin 18:387-425.

Nuechterlein KH, Dawson ME, Ventura J, Gitlin M, Subotnik KL, Snyder KS, Mintz J, Bartzokis G (1994)
The vulnerability/stress model of schizophrenic relapse: a longitudinal study.
Acta Psychiatrica Scandinavica Supplementum 382:58-64.

Nurse B, Russell VA, Taljaard JJ (1984)
Alpha 2 and beta-adrenoceptor agonists modulate [^{3}H]dopamine release from rat nucleus accumbens slices: implications for research into depression.
Neurochemical Research 9:1231-1238.

O'Callaghan E, Gibson T, Colohan HA, Buckley P, Walshe DG, Larkin C, Waddington JL (1992)
Risk of schizophrenia in adults born after obstetric complications and their association with early onset of illness: a controlled study.
British Medical Journal 305:1256-1259.

O'Donnell P, Lewis BL, Weinberger DR, Lipska BK (2002)
Neonatal hippocampal damage alters electrophysiological properties of prefrontal cortical neurons in adult rats.
Cerebral Cortex 12:975-982.

Oranje B, Gispen-de Wied CC, Verbaten MN, Kahn RS (2002)
Modulating sensory gating in healthy volunteers: the effects of ketamine and haloperidol.
Biological Psychiatry 52:887-895.

Paxinos G, Watson C (1986)
The Rat Brain in Stereotaxic Coordinates, 2nd Edition.
San Diego, CA: Academic Press.
ISBN 0 12 547621 3

Paxinos G, Watson C (1997)
The Rat Brain in Stereotaxic Coordinates, compact 3rd Edition.
San Diego, CA: Academic Press.
ISBN 0 12 547623 x

Paxinos G, Watson C (1998)
The Rat Brain in Stereotaxic Coordinates, 4th Edition.
San Diego, CA: Academic Press.
ISBN 0 12 547619 1

Pieribone VA, Nicholas AP, Dagerlind A, Hokfelt T (1994)
Distribution of alpha 1 adrenoceptors in rat brain revealed by in situ hybridization experiments utilizing subtype-specific probes.
Journal of Neuroscience 14:4252-4268.

Plotsky PM (1987)
Facilitation of immunoreactive corticotropin-releasing factor secretion into the hypophysial-portal circulation after activation of catecholaminergic pathways or central norepinephrine injection.
Endocrinology 121:924-930.

Plotsky PM, Cunningham ET, Jr., Widmaier EP (1989)
Catecholaminergic modulation of corticotropin-releasing factor and adrenocorticotropin secretion.
Endocrinology Review 10:437-458.

Posse S, Fitzgerald D, Gao K, Habel U, Rosenberg D, Moore GJ, Schneider F (2003)
Real-time fMRI of temporolimbic regions detects amygdala activation during single-trial self-induced sadness.
Neuroimage 18:760-768.

Prewitt CM, Herman JP (1994)
Lesion of the central nucleus of the amygdala decreases basal CRH mRNA expression and stress-induced ACTH release.
Annals of the New York Academy of Sciences 746:438-440.

Puri S, Ray A, Chakravarti AK, Sen P (1994)
Role of dopaminergic mechanisms in the regulation of stress responses in experimental animals.
Pharmacology, Biochemistry and Behavior 48:53-56.

Rabkin JG (1980)
Stressful life events and schizophrenia: a review of the research literature.
Psychological Bulletin 87:408-425.

Rao ML, Strebel B, Halaris A, Gross G, Braunig P, Huber G, Marler M (1995)
Circadian rhythm of vital signs, norepinephrine, epinephrine, thyroid hormones, and cortisol in schizophrenia.
Psychiatry Research 57:21-39.

Reul JM, de Kloet ER (1985)
Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation.
Endocrinology 117:2505-2511.

Rivier C, Rivier J, Mormede P, Vale W (1984)
Studies of the nature of the interaction between vasopressin and corticotropin-releasing factor on adrenocorticotropin release in the rat.
Endocrinology 115:882-886.

Robbins TW (1990)
The case of frontostriatal dysfunction in schizophrenia.
Schizophrenia Bulletin 16:391-402.

Romero LM, Sapolsky RM (1996)
Patterns of ACTH secretagog secretion in response to psychological stimuli.
Journal of Neuroendocrinology 8:243-258.

Roozendaal B, Koolhaas JM, Bohus B (1991*a*)
Central amygdala lesions affect behavioral and autonomic balance during stress in rats.
Physiology and Behaviour 50:777-781.

Roozendaal B, Koolhaas JM, Bohus B (1991*b*)
Attenuated cardiovascular, neuroendocrine, and behavioral responses after a single footshock in central amygdaloid lesioned male rats.
Physiology and Behavior 50:771-775.

Rossi A, Stratta P, Mancini F, Gallucci M, Mattei P, Core L, Di Michele V, Casacchia M (1994)
Magnetic resonance imaging findings of amygdala-anterior hippocampus shrinkage in male patients with schizophrenia.
Psychiatry Research 52:43-53.

Sah P, Faber ES, Lopez De Armentia M, Power J (2003)
The amygdaloid complex: anatomy and physiology.
Physiology Review 83:803-834.

Sams-Dodd F (1997)
Effect of novel antipsychotic drugs on phencyclidine-induced stereotyped behaviour and social isolation in the rat social interaction test.
Behavioural Pharmacology 8:196-215.

Sams-Dodd F, Lipska BK, Weinberger DR (1997)
Neonatal lesions of the rat ventral hippocampus result in hyperlocomotion and deficits in social behaviour in adulthood.
Psychopharmacology (Berlin) 132:303-310.

Sander K, Brechmann A, Scheich H (2003)
Audition of laughing and crying leads to right amygdala activation in a low-noise fMRI setting.
Brain Research Protocols 11:81-91.

Sapolsky RM, Romero LM, Munck AU (2000)
How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions.
Endocrine Reviews 21:55-89.

Sarter M, Markowitsch HJ (1985)
Involvement of the amygdala in learning and memory: a critical review, with emphasis on anatomical relations.
Behavioural Neuroscience 99:342-380.

Sasaki K, Suzuki K, Ueno M, Takako K, Yoshizaki F (1998)
Increase in monoamine levels caused by emotional stress in mice brain regions is attenuated by Saiko-ka-ryukotsu-borei-to.
Methods and Findings in Experimental and Clinical Pharmacology 20:27-30.

Sawaguchi T, Matsumura M, Kubota K (1990)
Effects of dopamine antagonists on neuronal activity related to a delayed response task in monkey prefrontal cortex.
Journal of Neurophysiology 63:1401-1412.

Saykin AJ, Gur RC, Gur RE, Mozley PD, Mozley LH, Resnick SM, Kester DB, Stafiniak P (1991)
Neuropsychological function in schizophrenia. Selective impairment in memory and learning.
Archives of General Psychiatry 48:618-624.

Scaccianoce S, Muscolo LA, Cigliana G, Navarra D, Nicolai R, Angelucci L (1991)
Evidence for a specific role of vasopressin in sustaining pituitary-adrenocortical stress response in the rat.
Endocrinology 128:3138-3143.

Schall U, Catts SV, Chaturvedi S, Liebert B,

Redenbach J, Karayanidis F, Ward PB (1998)
The effect of clozapine therapy on frontal lobe dysfunction in schizophrenia: neuropsychology and event-related potential measures.
International Journal of Neuropsychopharmacology 1:19-29.

Schauz C, Koch M (1999)
Lesions of the nucleus basalis magnocellularis do not impair prepulse inhibition and latent inhibition of fear-potentiated startle in the rat.
Brain Research 815:98-105.

Scheinin M, Lomasney JW, Hayden-Hixson DM, Schambra UB, Caron MG, Lefkowitz RJ, Fremeau RT, Jr. (1994)
Distribution of alpha 2-adrenergic receptor subtype gene expression in rat brain.
Molecular Brain Research21:133-149.

Schulz SC, Koller MM, Kishore PR, Hamer RM, Gehl JJ, Friedel RO (1983)
Ventricular enlargement in teenage patients with schizophrenia spectrum disorder.
American Journal of Psychiatry 140:1592-1595.

Schwartz JC, Diaz J, Pilon C, Sokoloff P (2000)
Possible implications of the dopamine D(3) receptor in schizophrenia and in antipsychotic drug actions.
Brain Research Reviews 31:277-287.

Sebens JB, Kuipers SD, Koch T, Ter Horst GJ, Korf J (2000)
Limited participation of 5-HT(1A) and 5-HT(2A/2C) receptors in the clozapine-induced Fos-protein expression in rat forebrain regions.
European Journal of Pharmacology 408:11-17.

Selye H (1936)
A Syndrome produced by Diverse Nocuous Agents.
Nature 138:32.

Sesack SR, Pickel VM (1990)
In the rat medial nucleus accumbens, hippocampal and catecholaminergic terminals converge on spiny neurons and are in apposition to each other.
Brain Research 527:266-279.

Sham PC, O'Callaghan E, Takei N, Murray GK, Hare EH, Murray RM (1992)
Schizophrenia following pre-natal exposure to influenza epidemics between 1939 and 1960.
British Journal of Psychiatry 160:461-466.

Shenton ME, Dickey CC, Frumin M, McCarley RW (2001)
A review of MRI findings in schizophrenia.
Schizophrenia Research 49:1-52.

Shih JC, Chen K (1999)
MAO-A and -B gene knock-out mice exhibit distinctly different behavior.
Neurobiology (Bp) 7:235-246.

Strachan AM (1986)
Family intervention for the rehabilitation of schizophrenia: toward protection and coping.
Schizophrenia Bulletin 12:678-698.

Susser ES, Lin SP (1992)
Schizophrenia after prenatal exposure to the Dutch Hunger Winter of 1944-1945.
Archives of General Psychiatry 49:983-988.

Suzuki T, Ishigooka J, Watanabe S, Miyaoka H (2002)
Enhancement of delayed release of dopamine in the amygdala induced by conditioned fear stress in methamphetamine-sensitized rats.
European Journal of Pharmacology 435:59-65.

Swerdlow NR, Braff DL, Geyer MA, Koob GF (1986)
Central dopamine hyperactivity in rats

mimics abnormal acoustic startle response in schizophrenics.
Biological Psychiatry 21:23-33.

Swerdlow NR, Taaid N, Oostwegel JL, Randolph E, Geyer MA (1998)
Towards a cross-species pharmacology of sensorimotor gating: effects of amantadine, bromocriptine, pergolide and ropinirole on prepulse inhibition of acoustic startle in rats.
Behavioural Pharmacology 9:389-396.

Talamini LM, Koch T, Ter Horst GJ, Korf J (1998)
Methylazoxymethanol acetate-induced abnormalities in the entorhinal cortex of the rat; parallels with morphological findings in schizophrenia.
Brain Research 789:293-306.

Talamini LM, Koch T, Luiten PG, Koolhaas JM, Korf J (1999)
Interruptions of early cortical development affect limbic association areas and social behaviour in rats; possible relevance for neurodevelopmental disorders.
Brain Research 847:105-120.

Terpstra J, Gispen-de Wied CC, Broekhoven MH, Frankhuijzen AC, Kahn RS, Van Ree JM, Wiegant VM (2003)
Attenuated stress responsiveness in an animal model for neurodevelopmental psychopathological disorders.
European Neuropsychopharmacology 13:249-256.

Thierry AM, Tassin JP, Blanc G, Glowinski J (1976)
Selective activation of mesocortical DA system by stress.
Nature 263:242-244.

Tuinstra T, Cools AR (2000)
High and low responders to novelty: effects of adrenergic agents on the regulation of accumbal dopamine under challenged and non-challenged conditions.
Neuroscience 99:55-64.

Udelsman R, Harwood JP, Millan MA, Chrousos GP, Goldstein DS, Zimlichman R, Catt KJ, Aguilera G (1986)
Functional corticotropin releasing factor receptors in the primate peripheral sympathetic nervous system.
Nature 319:147-150.

Van Bockstaele EJ, Bajic D, Proudfit H, Valentino RJ (2001)
Topographic architecture of stress-related pathways targeting the noradrenergic locus coeruleus.
Physiology and Behaviour 73:273-283.

Van Cauter E, Linkowski P, Kerkhofs M, Hubain P, L'Hermite-Balériaux M, Leclercq R, Brasseur M, Copinschi G, Mendlewicz J (1991)
Circadian and sleep-related endocrine rhythms in schizophrenia.
Archives of General Psychiatry 48:348-356.

Van den Bosch RJ, van Asma MJ, Rombouts R, Louwerens JW (1992)
Coping style and cognitive dysfunction in schizophrenic patients.
British Journal of Psychiatry Supplement: 123-128.

Van Oers JW, Tilders FJ (1991)
Antibodies in passive immunization studies: characteristics and consequences.
Endocrinology 128:496-503.

Van Venrooij J, Terpstra J, Heijnen C, Kahn R, Gispen-de Wied C
Impairments in the stress response system of

medication-naive, first episode, schizophrenic patients.
(in preparation)

Varty GB, Higgins GA (1995)
Examination of drug-induced and isolation-induced disruptions of prepulse inhibition as models to screen antipsychotic drugs.
Psychopharmacology (Berlin) 122:15-26.

Walker E, Bollini AM (2002)
Pubertal neurodevelopment and the emergence of psychotic symptoms.
Schizophrenia Research 54:17-23.

Wallace DM, Magnuson DJ, Gray TS (1992)
Organization of amygdaloid projections to brainstem dopaminergic, noradrenergic, and adrenergic cell groups in the rat.
Brain Research Bulletin 28:447-454.

Washburn M, Moises HC (1989)
Electrophysiological correlates of presynaptic alpha 2-receptor-mediated inhibition of norepinephrine release at locus coeruleus synapses in dentate gyrus.
Journal of Neuroscience 9:2131-2140.

Weinberger DR (1987)
Implications of normal brain development for the pathogenesis of schizophrenia.
Archives of General Psychiatry 44:660-669.

Weinberger DR, Berman KF (1996)
Prefrontal function in schizophrenia: confounds and controversies.
Philosophical Transactions of the Royal Society of London Series B: Biological Sciences 351:1495-1503.

Weinberger DR, Berman KF, Illowsky BP (1988)
Physiological dysfunction of dorsolateral prefrontal cortex in schizophrenia. III. A new cohort and evidence for a monoaminergic mechanism [see comments].
Archives of General Psychiatry 45:609-615.

Weiner I, Lubow RE, Feldon J (1984)
Abolition of the expression but not the acquisition of latent inhibition by chronic amphetamine in rats.
Psychopharmacology 83:194-199.

Willenberg HS, Bornstein SR, Hiroi N, Path G, Goretzki PE, Scherbaum WA, Chrousos GP (2000)
Effects of a novel corticotropin-releasing-hormone receptor type I antagonist on human adrenal function.
Molecular Psychiatry 5:137-141.

Wolterink G, Daenen EWPM, Dubbeldam S, Gerrits MAFM, Van Rijn R, Kruse CG, Van der Heyden JA, Van Ree JM (1998)
Long-term behavioural disorders after early amygdala damage in rats as a model for neurodevelopmental psychopathological disorders.
European Neuropsychopharmacology 8:S93-S94.

Wolterink G, Daenen LEWPM, Dubbeldam S, Gerrits MAFM, Van Rijn R, Kruse CG, Van der Heyden JAM, Van Ree JM (2001)
Early amygdala damage in the rat as a model for neurodevelopmental psychopathological disorders.
European Neuropsychopharmacology 11:51-59.

Wood GK, Lipska BK, Weinberger DR (1997)
Behavioral changes in rats with early ventral hippocampal damage vary with age at damage.
Developmental Brain Research 101:17-25.

Woods BT (1998)
Is schizophrenia a progressive neuro-developmental disorder? Toward a unitary pathogenetic mechanism.

American Journal of Psychiatry 155:1661-1670.

Wright IC, Rabe-Hesketh S, Woodruff PW, David AS, Murray RM, Bullmore ET (2000) Meta-analysis of regional brain volumes in schizophrenia.
American Journal of Psychiatry 157:16-25.

Yang CR, Mogenson GJ (1985) An electrophysiological study of the neural projections from the hippocampus to the ventral pallidum and the subpallidal areas by way of the nucleus accumbens.
Neuroscience 15:1015-1024.

Yank GR, Bentley KJ, Hargrove DS (1993) The vulnerability-stress model of schizophrenia: advances in psychosocial treatment.
American Journal of Orthopsychiatry 63:55-69.

Young WS, 3rd, Kuhar MJ (1980) Noradrenergic alpha 1 and alpha 2 receptors: light microscopic autoradiographic localization.
Proceedings of the National Academy of Sciences of the United States of Amrica 77:1696-1700.

Zhang K, Tarazi FI, Baldessarini RJ (2001) Role of dopamine D(4) receptors in motor hyperactivity induced by neonatal 6-hydroxy-dopamine lesions in rats.
Neuropsychopharmacology 25:624-632.

chapter 8

Nederlandse samenvatting

Schizofrenie is een ernstig psychiatrisch syndroom dat zich bij ongeveer één procent van de bevolking voordoet. Het syndroom kenmerkt zich door zijn chronisch verloop, waarbij met name de psychotische episodes (met wanen en/of hallucinaties) en de duidelijke afname in het sociaal-maatschappelijk functioneren op de voorgrond staan.

De amygdala (ofwel amandelkern) is een een amandelvormige structuur in de mediale temporaalkwab van de hersenen van alle vertebraten (gewervelde dieren), die verondersteld wordt betrokken te zijn bij regulatie van emoties (met name negatieve emoties, zoals angst). Feitelijk is de amygdala geen 'echte' kern maar een verzameling van verschillende, doch onderling nauw verbonden, kerngebieden en corticale gebieden (ook wel het amygdala-complex genoemd). Omdat de amygdala wederkerige verbindingen met vele hersengebieden heeft, is gepostuleerd dat een intacte amygdala belangrijk is voor de normale ontwikkeling van de gebieden waarnaar deze projecteert.

Ratten waarvan de amygdalakernen (waaronder in ieder geval de basolaterale nucleus van de amygdala, die wederkerige verbindingen heeft met de mediale prefrontale cortex) zeven dagen na de geboorte zijn geledeerd (D7 AMX), vertonen op latere leeftijd een aantal stoornissen die ook waargenomen worden bij patiënten met schizofrenie. Het gaat hierbij om stoornissen in pre-puls inhibitie, stress-gerelateerd gedrag en sociaal gedrag, en een verhoogde gevoeligheid voor phencyclidine. Deze stoornissen ontwikkelen zich pas na de pubertijd, hetgeen ook het geval is bij patiënten met schizofrenie, en worden niet waargenomen bij ratten waarvan de amygdala niet op dag 7, maar pas op dag 21 na de geboorte is geledeerd (D21 AMX). Dit suggereert dat een verandering in de ontwikkeling van de hersenen, met name van structuren die door de amygdala worden geïnnerveerd, een rol belangrijke speelt bij het ontstaan van deze stoornissen. De D7 AMX rat vormt aldus een interessant diermodel voor aspecten van psychiatrische hersenontwikkelingsstoornissen, zoals schizofrenie.

In **hoofdstuk 1** wordt onder andere een overzicht gegeven van de literatuur aangaande het onderwerp van dit proefschrift, te weten 'stress-responsiviteit bij neonataal amygdala geledeerde ratten, en de relevantie hiervan voor schizofrenie'. Met name worden hierin de twee belangrijkste waarnemingen uit de literatuur die de basis vormden voor het in dit proefschrift beschreven onderzoek naar voren gebracht. De eerste van die waarnemingen is dat patiënten met schizofrenie zich slecht kunnen aanpassen aan veranderende omstandigheden. Belangrijke, stressvolle gebeurtenissen ('life events') vergroten bij dergelijke patienten de kans op psychotische decompensatie, en de hoeveelheid dagelijkse stress ('daily life stress') is gecorreleerd met het aantal schizofrene symptomen. Ook zijn er bij patiënten met schizofrenie cognitieve en emotionele

stoornissen gevonden die gerelateerd zijn aan (dys)functies van de prefrontale cortex en het limbisch systeem (waaronder de amygdala). Tenslotte hebben patiënten met schizofrenie een verminderde respons van de hypothalamus-hypofyse-bijnieras (HHB), een voor stress-adaptatie zeer belangrijk hormonaal systeem. De tweede waarneming is, dat D7 AMX ratten een aantal stoornissen vertonen die ook zijn waargenomen bij patiënten met schizofrenie. Recent onderzoek bij dergelijke dieren suggereert dat hieraan mogelijk een uitrijpingsstoornis van de prefrontale cortex ten grondslag ligt die zou kunnen ontstaan als gevolg van de door de lesie verstoorde innervatie van de prefrontale cortex vanuit de amygdala.

In **hoofdstuk 2** worden de resultaten van een experiment gepresenteerd waarin de stress-responsiviteit van volwassen ratten met neonatale amygdala lesies werd onderzocht. De vraag die in dit experiment gesteld werd was, of de stress-responsiviteit bij ratten met neonatale amygdala lesies net als bij patiënten met schizofrenie is verminderd, en in hoeverre een verandering in de ontwikkeling van de hersenen daarbij een rol speelt. Om dit te onderzoeken werden de HHB-respons (plasma adreno-corticotrophic-hormone [ACTH] en corticosteron [CORT]), de sympatico-adrenomedullaire-respons (noradrenaline [NA] en adrenaline [A]) en de gedragsrespons op een 'footshock' en een 'novelty' stress in kaart gebracht bij groepen geledeerde en schijngeopereerde (SHAM) dieren, te weten D7 AMX, D7 SHAM, D21 AMX en D21 SHAM. De resultaten van deze studie lieten zien dat de HHB respons bij D7 AMX ratten in beide stressparadigmata verlaagd was, terwijl de HHB respons bij D21 AMX alleen in de 'novelty' stress test, en dan nog slechts in geringe mate, verlaagd was. Bovendien toonden de D7 AMX ratten minder actief gedrag in de 'novelty' stress test dan de D21 AMX ratten. De sympatico-adrenomedullaire respons was in beide amygdala geledeerde groepen gelijkelijk verminderd.
D7 en D21 AMX ratten lieten dus verminderde gedrags-, endocriene- en sympathische responsen zien op stress. Dat is in overeenstemming met de essentiële rol die de amygdala volgens de literatuur speelt in cognitief-emotionele processen en adaptieve responsen op stressvolle stimuli. Echter, het effect van de amygdala lesies op de HHB respons was veel sterker bij D7 AMX ratten dan bij D21 AMX ratten. Dit wijst erop dat, naast een effect van de amygdala lesie zelf, ook stoornissen in de ontwikkeling van hersenstructuren die door de amygdala worden geïnnerveerd naar alle waarschijnlijkheid bijdragen aan de verminderde HHB respons van D7 AMX ratten.
Onze huidige bevinding dat D7 AMX ratten een verminderde HHB respons op stress laten zien voegt een element toe aan de stoornissen die zij delen met schizofrene patiënten, en versterkt daardoor de validiteit van de D7 AMX rat als diermodel voor psychiatrische hersenontwikkelinggsstoornissen, zoals schizofrenie.

In **hoofdstuk 3** werd onderzocht welke mechanismen mogelijk ten grondslag zouden kunnen liggen aan de verstoorde HHB respons van D7 AMX ratten, zoals die in hoofdstuk 2 werd beschreven. De vraag die in dit experiment gesteld werd, was of neonatale amygdala lesies leiden tot een verstoring in de fysiologie van de HHB. Derhalve werd enerzijds de gevoeligheid van de HHB voor corticotrophin-releasing-hormone (CRH), en anderzijds de gevoeligheid van de as voor de negatieve feedback door CORT getoetst. Allereerst werd daartoe ovine CRH (oCRH) intraveneus ingespoten bij twee groepen ratten (D7 AMX en D7 SHAM), en de daaropvolgende plasma CORT respons gemeten. Vervolgens werden de twee groepen dieren voorbehandeld met een lage dosis CORT waarna ze werden blootgesteld aan 'novelty' stress. De daaropvolgende plasma CORT respons werd gemeten. Tenslotte werden van beide groepen gedurende een volledige 24-uurs cyclus de basale plasma CORT spiegels gemeten.
De resultaten lieten zien dat de door oCRH geïnduceerde plasma CORT response bij D7 AMX ratten verminderd was ten opzichte van die bij D7 SHAM dieren, terwijl het effect van CORT voorbehandeling op de 'novelty' geïnduceerde plasma CORT respons bij beide groepen niet verschilde. Dit wijst erop dat de in hoofdstuk 1 gevonden verstoring van HHB respons op stress waarschijnlijk (mede) het gevolg is van een verminderde gevoeligheid van de hypofyse voor CRH, en niet van een versterkte glucocorticoïd-feedback. Uit de 24-uurs basale CORT registratie kwam naar voren dat D7 AMX ratten gedurende de donker-fase van de cyclus (de actieve fase van de rat) een verhoogde basale plasma CORT spiegel hebben. Gezien het feit dat deze verhoging gepaard gaat met een ongestoorde glucocorticoïd-feedback, zou dit kunnen wijzen op een verhoogdestaat van arousal tijdens waken. De bevindingen uit dit experiment geven verder inzicht in het niveau waarop neonatale amygdala lesies leiden tot verstoring van de stress responsiviteit.

In een recente studie werd aangetoond dat de concentraties van de neurotransmitter noradrenaline (NA) en de belangrijke NA metaboliet 3-methoxy-4-hydroxyphenylglycol verlaagd zijn in de mediale prefrontale cortex (mPFC), nucleus accumbens (Acb) en caudate putamen (CPu) van D7 AMX ratten. Dit wijst op een verminderde NA neurotransmissie in deze hersenstructuren. Omdat NA een belangrijke rol speelt bij de regulatie van de HHB werd een experiment gedaan (**hoofdstuk 4**) om de NA receptor dichtheden te analyseren in hersengebieden die betrokken zijn bij de stress-respons. Daarvoor werd in vitro autoradiografie van α_1- en α_2-adrenoceptoren toegepast op hersencoupes, waarbij gekeken werd naar de mPFC, Acb, CPu, paraventriculaire kern van de hypothalamus (PVN) en de locus coeruleus (LC). Voor de analyse van de α_1-adrenoceptor werd gebruik gemaakt van het α_1-selectieve radioligand [^{3}H]Prazosin. Voor de analyse van de α_2-adrenoceptor werd gebruik gemaakt van het α_2-selectieve

radioligand [^{3}H]UK14.304. Hoewel de α_1- en α_2-adrenoceptor dichtheden, in overeenstemming met de literatuur, verschilden per hersengebied, werd er geen verschil in radioligand-binding tussen D7 AMX en D7 SHAM ratten gevonden. Dit wijst erop dat de NA receptor dichtheid en de NA neurotransmissie klaarblijkelijk niet gelijktijdig verstoord zijn bij D7 AMX ratten. Voordat er echter definitieve conclusies getrokken kunnen worden uit deze bevindingen, dient de dynamiek van de verschillende subtypen van de α_1- en α_2-adrenoceptoren over de tijd bij D7 AMX ratten onderzocht te worden.

De effecten van stress op patiënten met schizofrenie kunnen voor een deel bestreden worden door behandeling met antipsychotica. Atypische antipsychotica, zoals clozapine, lijken daarbij beter te werken dan typische (ofwel klassieke) antipsychotica, zoals haloperidol. Daar D7 AMX ratten, naast de eerder in de literatuur beschreven stoornissen, ook een verminderde stress-respons gemeen hebben met patiënten die lijden aan schizofrenie, werd het effect van langdurige behandeling met een typisch en een atypisch antipsychoticum op de stress-responsiviteit van D7 AMX ratten onderzocht (**hoofdstuk 5**). Daartoe werden D7 AMX en D7 SHAM ratten twee weken lang dagelijks behandeld met haloperidol, clozapine of een fysiologische zoutoplossing. Vóór en tijdens aanvang van de behandeling werden basaalwaarden van ACTH en de gedragsrespons in een 'klein open veld' test gemeten. Aan het einde van de behandelperiode van twee weken werden de door footshock-stress geïnduceerde ACTH- en gedragsresponsen bepaald.
De resultaten uit deze studie lieten zien dat behandeling met antipsychotica de verminderde endocriene stressrespons van D7 AMX ratten niet herstelt. Ook werd de gedragsrespons van D7 AMX ratten op stress niet door de behandeling beïnvloed. Wel veranderde langdurige behandeling met clozapine de dynamiek van de HHB respons bij zowel D7 AMX als D7 SHAM ratten (dus onafhankelijk van lesie). Bij clozapine behandelde ratten trad de piek van de ACTH respons eerder op. Hieruit kan geconcludeerd worden dat behandeling met clozapine, mogelijk op het niveau van het centrale zenuwstelsel, fysiologische processen verandert die betrokken zijn bij de aansturing van de HHB tijdens stress. Deze bevinding zou mogelijk relevant kunnen zijn voor de manier waarop atypische antipsychotica hun stress-beschermende werking bij patiënten teweeg brengen.

Conclusie

De in dit proefschift gepresenteerde resultaten benadrukken eens te meer dat de amygdala een belangrijke rol speelt bij adaptatie van het organisme aan zijn omgeving. Ze tonen bovendien aan, dat wanneer de amygdala vroeg in het leven wordt bescha-

digd, dit leidt tot een ontwikkelingsstoornis die ook de HHB, één van de belangrijkste adaptieve systemen van een gewerveld organisme, betreft. Gezien de overeenkomsten tussen het D7 AMX model en schizofrenie, onderstrepen de in dit proefschrift beschreven resultaten het belang van verder onderzoek naar de rol van de amygdala en de HHB bij schizofrenie.

Dankwoord

Bij de totstandkoming van ieder proefschrift zijn veel mensen betrokken. Van het meedenken over experimentele vragen, het kritisch bijschaven van protocollen, het assisteren bij de uitvoering van de experimenten en het discussiëren over de resultaten tot en met de onmisbare correcties bij het schrijven. Aan iedere facet van de ruwe steen wordt door vele handen gewerkt, totdat er een zorgvuldig gepolijste edelsteen ligt, waar de promovendus dan mee mag pronken. Al deze mensen ben ik derhalve dank verschuldigd en hoewel ik niemand te kort wil doen, wil ik een aantal mensen er specifiek uitlichten.

Als eerste wil ik mijn begeleiders bedanken voor hun kundige begeleiding, oog voor detail en onmisbare hulp bij het wetenschappelijk verantwoord op papier zetten van mijn gedachten. Zij hebben het mogelijk gemaakt dat ik de afgelopen jaren op het terrein van de wetenschap heb kunnen groeien. Christine, als co-promotor was je vanaf het begin nauw betrokken bij het ontstaan van dit project. Als 'brug-AIO' tussen kliniek en pre-kliniek viel ik wel eens tussen wal en schip, maar jij wist mij dan altijd weer te motiveren. En hoewel je in de laatste fase een andere betrekking in 'het Haagse' aannam, bleef je zeer betrokken. Ook al werd het onderzoek steeds pre-klinischer, je bleef je inzetten om met mij de 'brug naar de kliniek' te blijven behouden. Jouw enthousiasme was enorm aanstekelijk en ik heb een groot respect voor het geduld dat je deze afgelopen jaren met mijn eigenwijsheid hebt gehad. Jouw laatste AIO gaat nu daadwerkelijk promoveren, en ik denk dat je trots kunt zijn op het werk dat jij verricht hebt de afgelopen jaren op het gebied van stress en schizofrenie, waar dit proefschift slechts een klein onderdeel van is. Victor, als eerste promotor was ook jij vanaf het begin betrokken bij het ontstaan van het project. Jouw oog voor detail en wetenschappelijke scherpzinnigheid is volgens mij onovertroffen. Dit was onmisbaar tijdens de wetenschappelijke maandagochtend-besprekingen, waar protocollen en data nauwkeurig tegen het licht werden gehouden, en essentiëel tijdens het laatste deel van mijn promotietraject waarin ik de data aan het papier moest toevertrouwen. Dankzij jou heeft deze eenvoudige dokter een meer wetenschapelijke houding kunnen ontwikkelen. Mijn schrijfstijl was niet optimaal, maar is mede dankzij jouw kritische correcties aanzienlijk verbeterd. Jan, hoewel jij pas in een later stadium als promotor in beeld kwam, kon ik vanaf het begin bij jou terecht voor advies. Ik heb jouw encyclopedische kennis en laagdrempelig advies enorm gewaardeerd. René, jij hebt bij aanvang van het project een deel van de wetenschappelijke begeleiding voor jouw rekening genomen, ik heb de samenwerking altijd als prettig ervaren en ik hoop deze, nu jij mijn opleider bent, de komende jaren voort te zetten.

Mijn studenten, Aysun, Babs, Maarten en Souad, jullie hebben een belangrijke bijdrage geleverd aan de experimenten, waarvan de data in dit proefschrift zijn opgenomen. Hoewel niet alles altijd volgens plan verliep, zoals dat nu eenmaal gaat in de wetenschap, hebben jullie alle lof verdiend.

Verder wil ik natuurlijk mijn collega AIO's en OIO's bedanken voor de gezellige samenwerking, de mogelijkheid tot het delen van het AIO-leed en niet te vergeten de legendarische AIO-uitjes in Tivoli de afgelopen vier jaren. Mijn oud-kamergenoten Johanneke, Femke en Roelof, wat hebben we gelachen. Ik geloof dat onze kamer een tijd lang bekend heeft gestaan als 'de disco', vanwege de gezamelijk aangeschafte gettoblaster, die het AIO leed aanzienlijk verzachtte. Johanneke, buuv, jou en Robert (succes in New York jongen, ga ervoor!) zien wij gelukkig nog geregeld omdat jullie achter ons wonen. Femke en Roelof, jullie zie ik helaas minder vaak, en ik zal julie missen. (Oud-)stressgroepers van het RMI, Patrick (wanneer kom je nou 'ns trainen), Teus-Jan, Rianne, Mark, Ank, Henk, Leon, Bo en Hugo. Nieuwe kamergenoten, Gert-Jan (koop nou 'ns een echte computer) en John (ik hoop dat je een geweldige wereldreis gehad hebt en ik wil er alles van horen). Verder Heidi, Leontien, Hans, Inge, Annemarie, Suzanne van de groep van Van Ree, reuze bedankt voor de fijne samenwerking. (Oud-) AIO's van de 4e, Jildau en Joost, van de verhuisde groep van Mathijs, ik vind het fijn dat wij elkaar nog geregeld zien (dat moeten we zeker zo houden), Marjan wij zien elkaar gelukkig nu dagelijks (als jullie huis af is dan mogen we het vast bekijken), Ineke, Caroline, Daniel, Patrick, Lars, Simone en Cornelle bedankt. Marijke, Ria, Jetty, Sjaak, Martin, Marco, Leo, Wout, Bert en alle andere RMI'ers. Verder natuurlijk de stressgroepers van psychiatrie Lucres, Sjoerd, Janine, Annemieke en Bob. Oud kantoortuin I-ers Wouter, Melanie, Astrid, Nienke, Indrag, Mechteld, Iris, Martijn en alle anderen die ik vergeten ben bij naam te noemen.

Mijn ouders, jullie hebben mij altijd gestimuleerd en het mogelijk gemaakt al die dingen te doen waarvan ik droomde. Betere ouders zijn er gewoon niet, echt niet! Mijn paranimfen Guido en Remco, ik ben blij dat ik jullie vriendschap heb en dat jullie mij terzijde willen staan. Remco, naast je paranimfschap heb je ook nog de layout van het proefschrift verzorgd, en mij aldus bijgestaan in de stress rondom de laatste fase van de afronding. Wij hebben een lange geschiedenis met elkaar en onze vriendschap is voor mijn gevoel uniek, dank je dat je er ook nu weer voor mij bent.

Daniëlle, mijn liefste, zoals beloofd een aparte alinea alleen voor jouw, dat heb je wel verdiend. Jij hebt me als geen ander gesteund, en waar nodig een schop onder de kont gegeven, en daar ben ik je eeuwig dankbaar voor, je bent de liefste.

Curriculum vitae

curriculum vitae

Jeroen Terpstra werd op 19 januari 1974 te Heemskerk geboren. In 1992 haalde hij het gymnasiumdiploma aan het Gymnasium Felisenum, te Velsen-Zuid. Hierna volgde hij de propedeuse en het eerste doctoraal jaar Bewegingswetenschappen aan de Vrije Universiteit Amsterdam. In 1993 begon hij met de studie Geneeskunde aan de Universiteit van Amsterdam (UvA). In 1994 haalde hij zijn propedeuse-examen en liep hij een wetenschappelijke stage bij het SLICC/Haemorheologie laboratorium. Daar onderzocht hij onder leiding van dr. M. Hardeman en emeritus prof. dr. J. Vreeken 'de rheologie van het bloed in klinisch ischaemische extremiteiten'. Ook volgde hij een bijvak Neurale Netwerken aan de Faculteit der Wiskunde, Informatica, Natuurkunde en Sterrenkunde van de UvA, en legde het tentamen met goed gevolg af. Hij liep een extra wetenschappelijke stage aan het Nederlands Instituut voor Hersenonderzoek, onder leiding van dr. R.W.H. Verwer en A.A. Sluiter, met als onderwerp 'Cytochroom-oxidase immunocytochemie in paraffine coupes en in-situ hybridisatie in cryostaat coupes van de humane hersenen in relatie tot de ziekte van Alzheimer'. In 1997 haalde hij zijn doctoraal-examen Geneeskunde en begon hij aan zijn co-schappen. In 1998 begon hij in de functie van onderzoeksassistent aan de voorbereiding van zijn promotieonderzoek bij de afdeling Psychiatrie van het Rudolf Magnus Instituut voor Neurowetenschappen (RMI) onder leiding van C.C. Gispen-De Wied. In 1999 haalde hij het arts-examen, waarna een aanstelling volgde als assistent in opleiding bij de afdeling Farmacologie & Anatomie en de afdeling Psychiatrie van het RMI. De resultaten van het promotieonderzoek dat hij daar uitvoerde onder begeleiding van C.C. Gispen-De Wied, prof. dr. V.M. Wiegant, prof. dr. J.M. van Ree en prof. dr. R.S. Kahn, staan beschreven in dit proefschrift. Sinds september 2003 is Jeroen Terpstra in opleiding tot psychiater aan het Universitair Medisch Centrum Utrecht.