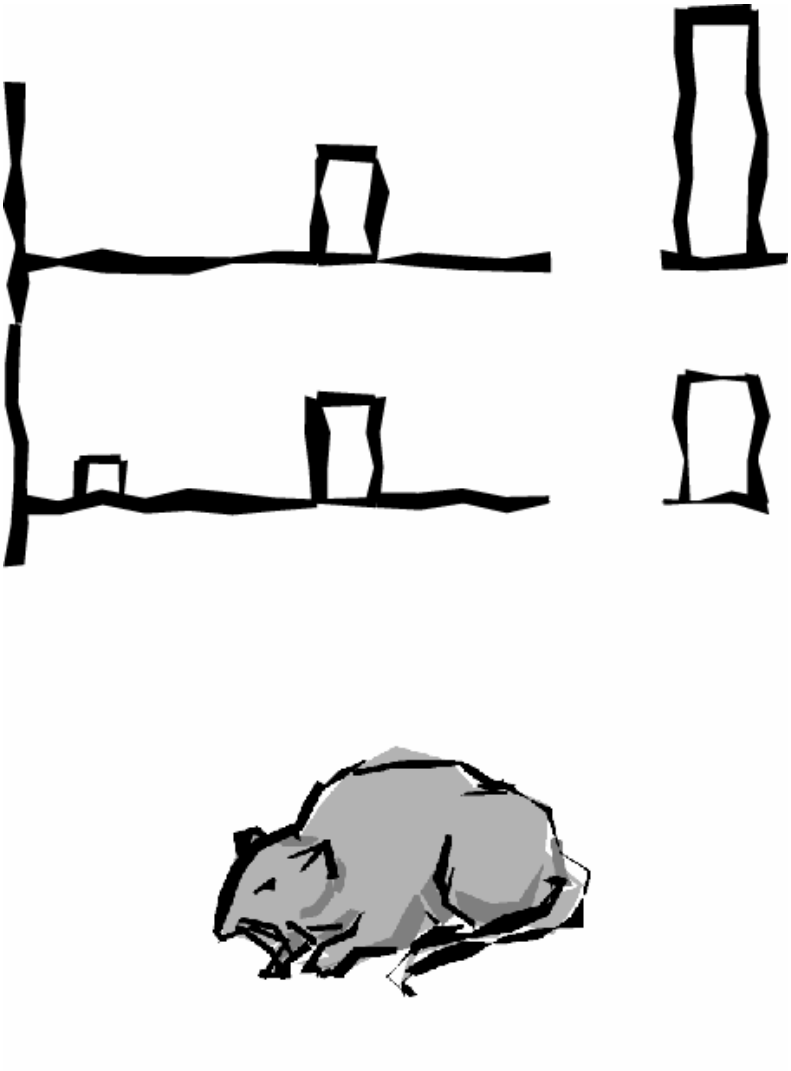
THE ROLE OF STRESS AND NORADRENALINE IN PREPULSE INHIBITION,

A RODENT STUDY

IMPLICATIONS FOR SCHIZOPHRENIA



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A RODENT STUDY

Implications for Schizophrenia

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Medische Wetenschappen

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Chapter 1

GENERAL INTRODUCTION

Schizophrenia

Schizophrenia is a severe mental disorder that is marked by distortions of reality, disturbances in perception, intellectual functioning, motivation and motor behaviour. Statistical analyses of schizophrenic patients and their symptoms led to the identification of three groups of symptoms (Liddle, 1987; Arndt, Alliger et al., 1991; Bilder, Mukherjee et al., 1985; Kulhara, Kota et al., 1986; Malla, Norman et al., 1993).

The first group of these symptoms is called reality distortion. It consists of delusions and hallucinations. Although all kind of hallucinations can occur in schizophrenic patients auditory hallucinations are most common. Persecutory delusions or delusions of reference are also quite common. However, bizarre and not understandable delusions are most characteristic for schizophrenia since the other delusions are also found in other psychotic diseases. The second group of symptoms is disorganization, characterised by incoherent or irrelevant speech, inappropriate affect and behaviour. The third group finally is the psychomotor poverty syndrome. These symptoms are associated with slowing down of (mental) activity and consist of symptoms like blunted affect, poverty of speech, decreased spontaneous movement and planning difficulties. Recent psychopathological research has provided strong evidence that these three different groups of symptoms are associated with different brain activity patterns (Liddle, 2000). Reality distortion is accompanied by an increased blood flow in the medial temporal cortex and a decreased blood flow in the posterior cingulated cortex. Disorganization is associated with an increased blood flow in the right anterior cingulated cortex and a decreased blood flow in the right ventral frontal cortex. The psychomotor poverty syndrome, finally, is accompanied by increased blood the caudate nucleus and decreased blood flow in the left frontal and parietal cortex.

As an alternative, the symptoms of schizophrenia can also be classified as positive and negative symptoms. Negative symptoms are symptoms that reflect a decrease or absence of a process that is present in normal individuals, like poverty of speech, flat affect and decreased amount of voluntary activity. These symptoms perfectly correspond to the symptoms of psychomotor poverty. Positive symptoms are those that reflect the presence of a process that is not present in normal individuals, like delusions, hallucinations, formal thought disorder, bizarre behaviour and inappropriate affect.

The aetiology of schizophrenia is still unclear however; the involvement of factors like genetics and stress has been mentioned in this respect. These factors will be discussed in detail in the following sections.

Genetics in Schizophrenia

The study from Gottesman and Shields (Gottesman and Shields, 1982) has shown an increased risk of developing schizophrenia in affected families. The risk of developing this illness in the general population is about 1% whereas the risk of siblings and offspring of schizophrenics is in the range of 10% and it is even increased up to 46% in children with two schizophrenic parents. However one should be aware that relatives of schizophrenics not only share parts of their genes but also their social environment. Against the background of the influence of psychosocial factors and stress on schizophrenia (see below) this should not be overlooked. This problem was attacked by comparing the vulnerability risk of monozygotic and dizygotic twins. Usually both groups of twins share equal part of their social environment but monozygotic twins share 100% of their genes whereas dizygotic twins only share 50% of their genes. Thus if schizophrenia is genetically based, the risk must be higher in monozygotic twins then in dizygotic twins. In fact monozygotic twins have a risk of about 57% to become schizophrenic compared to dizygotic twins which have a risk of about 12 % (Faraone and Tsuang, 1985). However the statement that monozygotic twins and dizygotic twin share equal part of their environment has been criticised because monozygotic twins are often dressed alike or share more similar interests then dizygotic twins. Basically it has been argued that the "microenvironment" between monozygotic and dizygotic twins differs and this may account for the increased concordance rate in monozygotic twins. Gottesman and colleague (Gottesman and Shields, 1982) have investigated the concordance rate in monozygotic twins that were reared separately and found a concordance rate of 58% which is similar to that of monozygotic twins reared together. Based on these results, the authors have concluded that the shared microenvironment contributes little to the development of schizophrenia. Further evidence about the influence of genes on schizophrenia comes from adoption studies. The study by Heston (Heston, 1966) for example has compared a group of adopted children whose biological mothers were schizophrenic with a control group of adopted children whose biological mothers were not schizophrenic. None of the children in the control group became schizophrenic whereas 11% of the children in the group with schizophrenic biological mother developed this disease.

Thus the evidence that the genetic background contributes to this illness is quite clear. However if schizophrenia were only genetically based one would expect to find concordance rates around

100% in monozygotic twins which is not the case. Thus other non-genetic factors must also contribute to the development of this disease. In these respect environmental factors, and in particular stress have been proposed.

Stress in Schizophrenia

Stress appears to play a dual role in schizophrenia. First, exposure to stress early in life has been proposed to contribute to the development of the disease itself probably by disturbing the normal brain development. The second effect of stress becomes apparent when the disease is manifest. In schizophrenic patients stress has been found to increase the risk of relapse or, in other words, to trigger the occurrence of a psychotic episode.

Perinatal stressful experiences like early parental loss (Agid, Shapira et al., 1999) or growing up in an urban region compared to a rural region increase the risk to develop schizophrenia (David, Lewis et al., 1992; Takei, O´Callaghan et al., 1992). Also obstetric complications during delivery (Owen, Lewis et al., 1988) have been found to increase the risk for developing this disease. In addition, other early experiences in life, like prenatal exposure to influenza (Mednick, Machon et al., 1988; O´Callaghan, Sham et al., 1991; Takei, Mortensen et al., 1996) or maternal malnutrition (Susser and Lin, 1992) have been found to be involved in the development of schizophrenia.

These findings have led to the so-called neurodevelopmental hypothesis of schizophrenia, which states that early life events affect the normal development resulting in abnormal brain and body morphology and ultimately in schizophrenia. In fact several abnormalities in the brain of schizophrenic patients have been found, like enlarged ventricles, reduced volume of the basal ganglia, temporal lobe and the limbic region, many of which appear to be present at the onset of the disease and do not change much during the course of the illness (Raz and Raz, 1990). Studies about the cytoarchitecture of the brain are of special interest since it is largely determined during foetal brain development. In 1986 Jacob and Beckman (Jakob and Beckmann, 1986) reported abnormalities in the superficial layers of the rostral entorhinal cortex in schizophrenic patients. They found that certain neurons (the so called pre-alpha neurons) are ordered atypically instead of being ordered in clusters. Furthermore they found a reduction in neuron number and a poorer development. Since the laminar pattern in the entorhinal cortex is set in the second trimester of gestation, the authors have concluded that an abnormal neuronal migration in schizophrenics occurs during this prenatal period. This finding was recently confirmed by Arnold et al (Arnold, Han et al., 2000). Furthermore even though the position of the pre-alpha cells in the layer II of the

entorhinal cortex is not equally distributed (in the anterior compartment the position is deeper relative to the pinal surface), the pre-alpha cells are nevertheless located deeper and at different places in the entorhinal cortex in schizophrenics compared to controls (Falkai, Schneider-Axmann et al., 2000). Thus there are clear indications that schizophrenic patients display impairments in the normal brain development.

As soon as schizophrenia has become manifest, usually after puberty, abrupt changes in life or stressful experiences have been found to increase the risk of relapse (Brown and Birley, 1968; Bebbington, Wilkins et al., 1993; Jones, Bebbington et al., 1993). In addition, the expression of criticism and hostility towards a schizophrenic patient by relatives has been found to increase the risk of relapsing. If the family behaves supportive and positive, the risk of a breakdown is much lower (Brown, Birley et al., 1972; Vaughn and Leff, 1976). These findings have led to the concept that "Expressed Emotion" (EE) increases the risk of relapse. Based on a standard interview with relatives of the patient, dealing with the illness, the families can be divided in high EE or low EE families. Based on this concept several studies have confirmed the finding of Brown and colleagues for an increased risk of relapse in a high EE-family (for review see : Koenigsberg and Handley, 1986; Butzlaff and Hooley, 1998). Based on these studies one can conclude that stress is worsening the symptoms of schizophrenia.

The effects of stress on the body are among others regulated by the so-called hypothalamo-pituitary-adrenal (HPA) axis. Basically, stress induces the release of corticotropine releasing hormone (CRH) from the paraventricular nuclei of the hypothalamus (PVN) which induces the release of the adrenocorticotropine hormone (ACTH) and arginine vasopressine in the pituitary. Finally ACTH induces the release of cortisol (and corticosteroids in animals) from the adrenal cortex.

It should be noted that alterations in this system have been found in schizophrenic patients for example hypercortisolemia (Gil-Ad, Dickerman et al., 1986; Whalley, Christie et al., 1989; Breier and Buchanan, 1992). In addition, negative symptoms have been found to correlate with the plasma levels of ACTH and cortisol (Shirayama, Hashimoto et al., 2002). Furthermore a stress induced activation of the HPA-axis induces higher levels of ACTH and cortisol in the plasma of schizophrenic patients than in control subjects (Elman, Adler et al., 1998). Finally, after suppression of the HPA-axis with dexamethasone, CRH induces the release of higher levels of cortisol in

schizophrenic patients compared to controls (Lammers, Garcia-Borreguero et al., 1995). Thus the system itself that is affected by stress is also affected in schizophrenia.

Stress also activates the dopaminergic system. For example, tail shocks increase dopamine release in the nucleus accumbens and the frontal cortex in rats (Abercrombie, Keefe et al., 1989; Klitenick, Taber et al., 1996). Likewise, even very mild stressors, such as novelty or handling can induce the release of dopamine from the nucleus accumbens and the prefrontal cortex (Feenstra, Botterblom et al., 1995; Saigusa, Tuinstra et al., 1999). Moreover, repeated stress has been found to alter brain dopamine receptors (Puglisi-Allegra, Kempf et al., 1991).

It is well known that amphetamine and other dopamine releasing drugs can induce a state resembling paranoid schizophrenia in normal individuals (Connell, 1958; Griffiths, Cavanaugh et al., 1972; Angrist, Sathanathan et al., 1974). Furthermore, increased levels of dopamine (Bird, Spokes et al., 1977) and increased numbers of D_2 receptors have been found in the nucleus accumbens, basal ganglia and substantia nigra in post-mortem brains of schizophrenics (Owen, Cross et al., 1978; Mackay, Iversen et al., 1982; Seeman, Ulpian et al., 1984). In addition, it has been recently shown that amphetamine induces a much stronger dopamine release in schizophrenic patients than in controls (Abi-Dargham, Gil et al., 1998) indicating not only differences in dopamine receptor levels but also functional differences in the dopamine release of the patients. In other words there is clear evidence that the dopaminergic system in schizophrenic patients is impaired. Thus the dopaminergic system that is affected by stress is also itself affected in schizophrenia.

The third important system involved in the regulation of the stress response is the noradrenergic system (Chrousos, 1988). Stress activates the sympathetic nervous system, which releases noradrenaline from the adrenal medulla into the blood stream. In addition, stress activates central noradrenergic cells, such as the locus coeruleus and the nucleus of the tractus solitarius (Cullinan, Herman et al., 1995; Dent, Smith et al., 2001; Saavedra and Torda, 1980; Teppema, Veening et al., 1997). This is also reflected in an increased release of noradrenaline in the nucleus accumbens, prefrontal cortex and hippocampus (Feenstra, Botterblom, and Mastenbroek, 2000; Ihalainen, Riekkinen et al., 1999).

There is clear evidence that also the noradrenergic system is impaired in schizophrenic patients. For example, abnormal levels of noradrenaline and its metabolites have been found in post-mortem brains (Farley, Price et al., 1978; Bird, Spokes et al., 1979; Crow, Baker et al., 1979). In addition

elevated levels of noradrenaline and it metabolites have been found in cerebrospinal fluid (csf) of schizophrenics (van Kammen, Peters et al., 1989; Gomes, Shanley et al., 1980; Lake, Sternberg et al., 1980; Kemali, Del Vecchio et al., 1982; Hornykiewicz, 1986; van Kammen and Antelman, 1984). Even more important the symptoms of paranoid schizophrenia correlate with cerebrospinal fluid noradrenaline levels (van Kammen and Gelernter, 1987). Likewise, Dajas and colleagues have found a correlation between plasma noradrenaline, positive symptoms, overall psychopathology and paranoid symptoms (Dajas, Barbeito et al., 1983). Thus the noradrenergic system that is affected by stress is also itself affected in schizophrenia.

Interestingly, noradrenaline is known to control both the HPA-axis as well as the dopaminergic system. For example noradrenaline has been found to alter the dopamine activity at the level of the substantia nigra (Donaldson, Dolphin et al., 1976). Furthermore, there are several indications about an interaction between noradrenaline and dopamine in the prefrontal cortex (for review see: Tassin, 1992). For example, stimulation of prefrontal alpha-adrenoceptors has been found to inhibit the dopamine activity at the level of the D_1 receptors in the prefrontal cortex (Tassin, Studler et al., 1986). Furthermore, recent microdialysis study by Kawahara and colleagues have shown that local activation of the locus coeruleus increases both noradrenaline and dopamine in the prefrontal cortex (Kawahara, Kawahara et al., 2001). However noradrenaline also controls dopamine in other brain nuclei. For example local activation of accumbal $beta_2$ adrenoceptors in rats has been found to increase dopamine overflow in nucleus accumbens slices (Nurse, Russell et al., 1985). A recent microdialysis study showed that not only $beta_2$-adrenoceptor agonists but also $alpha_1$-adrenoceptor agents influence mesolimbic dopamine release (Tuinstra and Cools, 2000).

Noradrenaline also interferes with the HPA-axis. Several studies have shown that noradrenaline stimulate the HPA-axis through activation of CRH-release (for review see: Plotsky, Cunningham et al., 1989). Thus, intracerebroventricular injections of noradrenaline elicits a dose dependent release of CRH an effect that is blocked by an $alpha_1$-antagonist (Plotsky, 1987). CRH is synthesised mainly in the parvocellular division of the PVN (Liposits, Phelix et al., 1987), a structure that is innervated by noradrenergic neurons arising in the locus coeruleus (Sawchenko and Swanson, 1982).

The fact that the dopaminergic system and the HPA-axis are impaired in schizophrenics together with the fact that noradrenaline appears to control both systems suggests a central role of noradrenaline in schizophrenia. This is in line with the elevated levels of noradrenaline found in these patients. Furthermore the functional connection between the locus coeruleus and the PVN

on the one hand, and between the locus coeruleus and the dopaminergic cells on the other hand further suggest a central role of noradrenaline in the symptoms of schizophrenia. Therefore, in the present thesis, we focus on the role of stress and noradrenaline in schizophrenia. In order to investigate this, we used the so called apomorphine-susceptible (APO-SUS) and apomorphine unsusceptible (APO-UNSUS) rats because these rats have several features that make them a perfect model for these investigations as discussed below.

Apomorphine-Susceptible and Apomorphine-unsusceptible rats

APO-SUS and APO-UNSUS rats were originally developed in our laboratory on the basis of their response to apomorphine (Cools, Brachten et al., 1990). Whereas APO-SUS rats show an intense gnawing response to apomorphine, APO-UNSUS rats show only a very weak gnawing response to this drug. From approximately 1985 these rats have been pharmacogenetically bred using a procedure to minimize inbreeding. In 1995 a replication breeding line was started with an identical procedure (Ellenbroek, Sluyter et al., 2000).

Like in schizophrenics, the APO-SUS rats display impairments in their stress response, in their noradrenergic and dopaminergic system as well as in their HPA-axis. For example APO-SUS rats show a much stronger locomotor response to novelty stress than APO-UNSUS rats (Cools, Brachten, Heeren, Willemen, and Ellenbroek, 1990).

With respect to the noradrenergic system, pharmaco-behavioural studies have indicated that APO-UNSUS rats have a high functional noradrenergic activity in the ventral striatum at rest, whereas the APO-SUS have a low functional noradrenergic activity in the ventral striatum at rest. Furthermore there is evidence that stress increases the functional noradrenergic activity in APO-SUS rats but decreases the functional noradrenergic activity in APO-UNSUS rats (Cools, Brachten, Heeren, Willemen, and Ellenbroek, 1990). In addition, APO-SUS rats show a stronger locomotor response than APO-UNSUS rats after application of the $alpha_1$ adrenoceptor agonist phenylephrine into the nucleus accumbens (Ellenbroek and Cools, 1993).

Given the differential sensitivity of APO-SUS and APO-UNSUS rats to the dopaminergic agonist apomorphine, it is not surprising that these animals also show other differences in their dopaminergic system. Thus an increase in mRNA for tyrosine hydroxylase in APO-SUS rats has been reported (Rots, Cools et al., 1996). Furthermore binding studies have indicated that APO-SUS rats have increased number of D_2 receptors in the projection area of the substantia nigra (Rots, Cools, Berod, Voorn, Rostene, and deKloet, 1996).

As far as the HPA-axis is concerned, plasma levels of ACTH and CRH-mRNA in the paraventricular nucleus are elevated in APO-SUS rats under baseline conditions. Furthermore mild stress induces a stronger and longer lasting increase of ACTH in APO-SUS rats compared to APO-UNSUS rats (Rots, Cools et al., 1995).

Given the alterations in stress sensitivity and in the noradrenergic, dopaminergic and HPA-axis systems, it is not surprising that the APO-SUS rats show a number of abnormalities that resemble schizophrenia (for a recent review see: Ellenbroek and Cools, 2002). Apart from the above mentioned ones, APO-SUS rats display abnormalities in the immune system (i.e. a T_{H2} dominance) that has also been observed in schizophrenic patients. Recently we also could show that APO-SUS rats are more resistant to cancer metastasis, similar to schizophrenic patients (Teunis, Kavelaars et al., 2002). Finally APO-SUS rats show an abnormal prepulse inhibition and latent inhibition (Ellenbroek, Geyer et al., 1995). Especially the reduced prepulse inhibition is of interest, as it has been repeatedly proposed to represent an interesting animal model for specific aspects of schizophrenia (Ellenbroek and Cools, 2000; Ellenbroek and Cools, 2002). For this reason, we decided to use this paradigm in our experiment.

The prepulse inhibition

A contraction of skeleton and facial muscles as a response to a sudden stimulus is known as the startle reflex. Whereas in humans the eyeblink component of the startle is measured, the whole body flinch is measured in rats or mice. In either case similar stimuli are used. The primary startle circuit consists of the auditory nerve, the cochlear root neurons, the nucleus reticularis pontis caudalis (PnC) and the spinal motor neurons (Koch, 1999). The startle response displays several forms of plasticity like habituation, fear potentation and prepulse inhibition. Prepulse inhibition is a reduction of the startle response to a stimulus (the so-called pulse) induced by a relative weak preceding stimulus (the so-called prepulse). It should be noted that the prepulse itself is too weak to induce a startle response in the subject. In order to inhibit the startle response the prepulse must precede the pulse by 30-500 ms in both humans and rats. The prepulse inhibition is not a form of learning or conditioning, since it occurs on the first exposure to the combination of pulse and prepulse and, furthermore, it shows no sign of habituation or extinction after multiple trials.

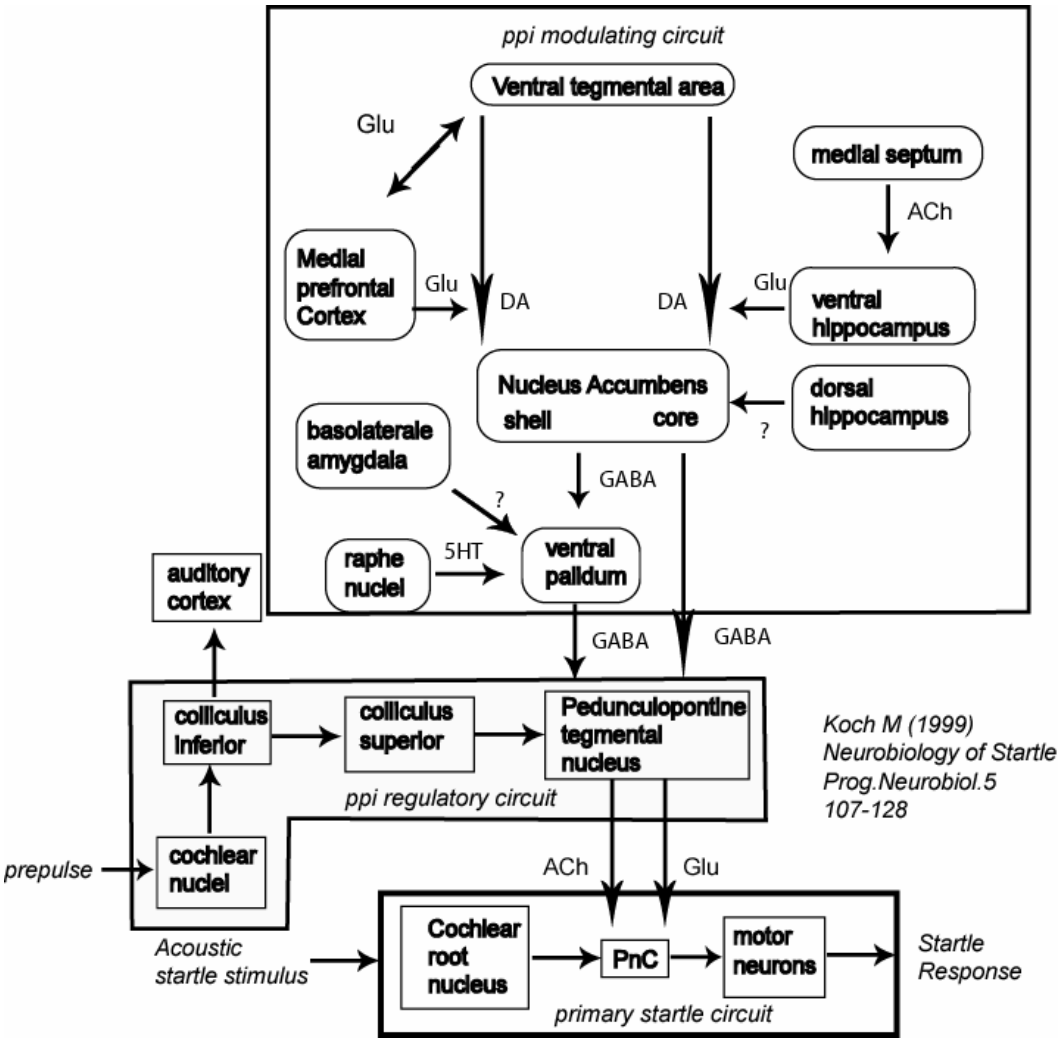

Fig 1 The prepulse inhibition circuit

The neuronal elements that contribute to prepulse inhibition can be divided in those that modulate prepulse inhibition versus those that regulate prepulse inhibition. The elements that regulate prepulse inhibition are activated by the prepulse itself and transmit the neuronal and behavioural consequences of the prepulse; namely a reduction in startle response. Basically this circuit consists out of the auditory nerve, the cochlear nuclei, the colliculus inferior, the colliculus superior and the pedunculoponine tegmental nucleus. This circuit impinges on the pontis caudalis (PnC) as part of the primary startle circuit. Modulation of the prepulse inhibition is carried out by neuronal connections between limbic cortex, the nucleus accumbens, and the ventral palidum (Swerdlow, Caine et al., 1992; Koch and Schnitzler, 1997; Swerdlow and Geyer, 1998; Swerdlow and Geyer, 1999). At the level of the PnC this modulating circuit interacts with the primary startle circuit (Fendt, Li et al., 2001). This circuit modulates the prepulse inhibition by altering the impact of the prepulse on the startle response (see: Fig 1).

Reductions in prepulse inhibition have been found in schizophrenic patients (Braff, Stone et al., 1978; Braff, Grillon et al., 1992; Braff, Geyer et al., 2001) and even drug naïve schizophrenics (Weike, Bauer et al., 2000; Mackeprang, Kristiansen et al., 2002). These deficits in prepulse inhibition correlate with various symptoms in patients (Braff, Swerdlow et al., 1999). However it should be noted that disturbances in prepulse inhibition are not exclusive for schizophrenia since these disturbances are also present in other neuropsychiatric diseases like obsessive compulsive disorder (Braff, Geyer, and Swerdlow, 2001) Huntington's disease (Swerdlow, Paulsen et al., 1995), attention deficit disorder (Ornitz, Hanna et al., 1992) Tourette's syndrome (Castellanos, Fine et al., 1996) and posttraumatic stress disorder (Grillon, Morgan et al., 1996).

Given the fact that dopamine plays a dominant role in schizophrenia it is important to realise that the prepulse inhibition is closely related to the dopamine function. Thus Hutchinson and Swift have demonstrated that d-amphetamine reduces prepulse inhibition in normal human subjects (Hutchison and Swift, 1999). In addition the D_2 agonist bromocriptine reduces the prepulse inhibition significantly in healthy subjects an effect that is antagonised by the D_2 antagonist haloperidol (Abduljawad, Langley et al., 1999). It is less clear whether antipsychotics can also reverse the deficit observed in schizophrenic patients. In the only longitudinal study reported so far, no effects of risperidone or zuclopenthixol on the prepulse inhibition deficit have been observed in drug naïve patients, in spite of a clear therapeutic effect of both drugs (Mackeprang, Kristiansen, and Glenthoj, 2002). Based on this study it appears that the disturbance in prepulse inhibition in schizophrenic patients is a phenomenon that is independent from the treatment with antipsychotic drugs, although the effects of other antipsychotic drugs have not been studied in any detail yet.

The role of dopamine in prepulse inhibition has been investigated in detail in rodents (for extensive review see: Geyer, Krebs-Thomson et al., 2001). Thus Mansbach and colleagues (Mansbach, Geyer et al., 1988) have shown that the dopamine agonist apomorphine disrupts prepulse inhibition in naïve rats and that this effect is blocked by haloperidol. On the other hand, the selective D_1 agonist SKF38393 does not disturb prepulse inhibition (Peng, Mansbach et al., 1990; Wan, Caine et al., 1996; Zhang, Bast et al., 2000). However, one study has reported a reduction in prepulse inhibition after application of the D_1-agonist SKF82958 (Wan, Caine, and Swerdlow, 1996). This might have been due to a relative weak selectivity to D_1-receptors of this drug over D_2-receptors. So far there is little evidence that the D_3 or D_4 receptors are involved in modulating prepulse inhibition since in knockout-mice the disruptive effect of amphetamine on prepulse inhibition is attenuated only in

mice lacking the D_2 receptors but not in D_3 or D_4 knockouts (Ralph, Varty et al., 1999). Furthermore, there are clear indications that especially the D_2 receptors in the nucleus accumbens are involved in the dopamine effect on prepulse inhibition (Swerdlow, Braff et al., 1990). Thus at present these data suggest that the disruptive effects of dopamine agonists on prepulse inhibition are based on their activity at the level of the D_2 receptors most likely located in the nucleus accumbens.

Despite the fact that noradrenaline plays a role in schizophrenia and has a modulatory influence on dopamine, only a few studies have investigated the role of noradrenaline in prepulse inhibition. Basically it has been found that the $alpha_2$ agonist clonidine and $alpha_2$ antagonist RX821002 have no effect on prepulse inhibition (Kehne, Padich et al., 1996; Bakshi and Geyer, 1997). However the $alpha_1$ agonist cirazoline reduces the prepulse inhibition (Carasso, Bakshi et al., 1998; Varty, Bakshi et al., 1999) and at least one study has shown that the $alpha_1$-antagonist prazosin increases the prepulse inhibition in rats (Depoortere, Perrault et al., 1997).

At present the noradrenergic agents have been given systemically, thus it is not known which brain structures are involved in the modulatory effect of noradrenaline on prepulse inhibition. However the fact that noradrenaline can increase the dopamine release in the nucleus accumbens (Tuinstra and Cools, 2000) together with the fact that an increase in dopamine activity in this nucleus can reduce prepulse inhibition (Swerdlow, Geyer et al., 2001) suggests that this nucleus which contains both beta and alpha-adrenoceptors (Nurse, Russell, and Taljaard, 1985) plays a central role. The noradrenergic input to the nucleus accumbens is provided by the locus coeruleus (Speciale, Crowley et al., 1978) and the medullary group (Delfs, Zhu et al., 1998; Lindvall and Bjorklund, 1983; Rainbow, Parsons et al., 1984; Sawaya, Dolphin et al., 1977). Based on these notions it seems likely that noradrenaline modulates prepulse inhibition by controlling the dopamine release in the nucleus accumbens. Since the noradrenergic input of the nucleus accumbens is provided by the locus coeruleus and the medullary group, it is likely that also these nuclei play a role in modulating prepulse inhibition.

Aim and outline of the thesis

Thus an impaired stress response and altered noradrenaline levels appear to be crucial in schizophrenia. However, the exact role of noradrenaline, and the brain mechanisms involved in this are far from clear. The aim of this study is therefore to investigate the role of stress and noradrenaline in relation to schizophrenic symptoms. Because of their known similarities with

schizophrenic patients we used the APO-SUS rats, and since disturbances in prepulse inhibition have been found in schizophrenics as well as in APO-SUS rats we used this paradigm as the read-out parameter.

Since stress worsens the symptoms of schizophrenia, we first investigated whether stress also disrupts prepulse inhibition by subjecting rats to a short term social stress, namely one day of isolation. These experiments are described in **chapter 2.** It was found that only stressed APO-SUS rats show a reduction in prepulse inhibition, whereas stressed APO-UNSUS rats even show an increase in prepulse inhibition. We know that stress increases the functional noradrenergic activity in APO-SUS rats but decreases it in APO-UNSUS rats. This links stress with the noradrenergic activity of these rats and suggest that an increased noradrenergic activity reduces prepulse inhibition. This is in line with the elevated levels of noradrenaline and the reductions of prepulse inhibition that has been found in schizophrenic patients. In order to investigate whether an increase in noradrenergic activity indeed reduces prepulse inhibition, we tested the effect of the $alpha_1$-adrenoceptor agonist cirazoline and the $beta_2$-agonist clenbuterol in non-stressed APO-SUS and APO-UNSUS rats **(chapter 3)**.We were able to demonstrate that cirazoline reduces prepulse inhibition in non-stressed APO-SUS rats, giving clear evidence that the noradrenergic activity modulates the prepulse inhibition in these rats. However it is not known which brain nuclei are involved in this process. As mentioned in the Introduction the noradrenergic terminals of the locus coeruleus innervates the PVN known to release CRH. Furthermore, this nucleus projects to the nucleus accumbens, a structure that is well known to modulate prepulse inhibition. This strongly suggests that the locus coeruleus plays a role in modulating prepulse inhibition. In order to investigate the role of this nucleus in prepulse inhibition we destroyed the terminals of the locus coeruleus with the help of the neurotoxin DSP4 **(chapter 4)**. These experiments were carried out in stressed and non-stressed APO-SUS and APO-UNSUS rats. It was found that the prepulse inhibition is reduced in stressed APO-SUS and increased in stressed APO-UNSUS rats. No effect was found in non-stressed rats. These data confirm that the locus coeruleus is indeed involved in modulating prepulse inhibition. As mentioned above the locus coeruleus projects to the nucleus accumbens and several studies have shown that the dopamine activity within this nucleus modulates prepulse inhibition. Interestingly noradrenaline is known to control the dopamine release in this nucleus. These findings suggest that the noradrenergic activity in the nucleus accumbens modulates prepulse inhibition. Therefore we investigated the effect of accumbal injected noradrenergic drugs on prepulse inhibition in **chapter 5**. We tested the $alpha_1$-adrenoceptor agonist phenylephrine, the $alpha_1$-adrenoceptor antagonist phentolamine and the $beta_2$-adrenoceptor

agonist isoproterenol and found that the noradrenergic activity in the nucleus accumbens modulates prepulse inhibition. Notably both types of accumbal adrenoceptors are involved in modulating prepulse inhibition. In **chapter 6** gives a summary of the main results. Finally in **chapter 7** we will discuss the consequences of the present findings for the neuronal regulation of the prepulse inhibition and the impact of theses findings for the treatment of schizophrenia.

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C h a p t e r 2

REMOVAL OF SHORT-TERM ISOLATION STRESS DIFFERENTIALLY INFLUENCES PREPULSE INHIBITION IN APO-SUS AND APO-UNSUS RATS

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Abstract

Epidemiological studies have reported that the risk of developing schizophrenia increases with the number of genes one shares with patients suffering from schizophrenia (Gottesman, 1991). In addition, stressful life events are known to increase the risk of developing schizophrenia (Ellenbroek, van-den Kroonenberg et al., 1998) resulting in the stress hypothesis of schizophrenia. Remarkably, stress increases the release of dopamine and noradrenaline in the nucleus accumbens, (Puglisi, Imperato et al., 1991), which links the stress hypothesis with the known dopamine hypothesis of schizophrenia. Additionally an increased dopamine transmission in the nucleus accumbens (nacc) is known to disturb prepulse inhibition (ppi) (Wan, Geyer et al., 1994), a phenomenon observed in, among others, schizophrenics (Braff and Geyer, 1990).

Some years ago we have genetically selected two rat-lines which are marked by a high (APO-SUS) and by a low (APO-UNSUS) apomorphine susceptibility. Similar to schizophrenics the APO-SUS rat-line shows a reduced prepulse inhibition (Ellenbroek, Geyer et al., 1995). However, these data were obtained after a period of mild stress, namely a 24h period of social isolation. Mild stress changes the line specific differences of APO-SUS and APO-UNSUS rats. The stress pushes the APO-SUS rat in the direction of an APO-UNSUS and vice versa, especially as far as it concerns the dopamine and noradrenaline activity in the nucleus accumbens. (Cools, van den Bos et al., 1991;Cools, Rots et al., 1994;Cools, Brachten et al., 1990;Roozendaal and Cools, 1994). Therefore, in the present paper we investigated the prepulse inhibition response in non-stressed, i.e. non-isolated APO-SUS and APO-UNSUS rats. In agreement with this hypothesis, we found that

removal of the stress led to an increase of prepulse inhibition in the APO-SUS, but a decrease in the APO-UNSUS.

These data clearly shows that the prepulse inhibition is stress-dependent in APO-SUS and APO-UNSUS rats. It is suggested that the differential stress-induced change in the dopaminergic and the noradrenergic system influences the reaction of APO-SUS and APO-UNSUS rats on prepulse inhibition.

Introduction

Epidemiological studies have shown that the risk of developing schizophrenia is in part determined by the number of genes shared with someone suffering from this psychiatric illness (Gottesman, 1991;Prescott and Gottesman, 1993). However, although genes play a crucial role in developing schizophrenia further epidemiological studies have shown that environmental factors are also of crucial importance. Most notably stressful life events occurring early in life or at adult age have been reported to increase the risk of schizophrenia (Ellenbroek, van-den Kroonenberg, and Cools, 1998). Schizophrenic patients show elevated noradrenaline and cortisol levels in plasma and cerebrospinal fluid (Meltzer, 1987), which might reflect an increased response to stress. Moreover, the cortisol response to a stressor is greatly increased in schizophrenic patients (Lammers, Garcia-Borreguero et al., 1995). This data are in concordance with the stress-hypothesis of schizophrenia (Walker and Diforio, 1997). Stress can enhance the release of dopamine especially in the nucleus accumbens (Puglisi, Imperato, Angelucci, and Cabib, 1991), which might link the stress hypothesis to the well known dopamine hypothesis of schizophrenia. Interestingly, an increase of dopamine transmission in the nucleus accumbens disrupts prepulse inhibition (Wan, Geyer, and Swerdlow, 1994), a phenomenon also observed in, among others, schizophrenic patients (Braff and Geyer, 1990)

These facts clearly show that a genetic predisposition and stress contribute to schizophrenia. Several years ago, we have developed two rat lines based on the differential susceptibility to the dopaminergic agonist apomorphine: The APO-SUS rats show a strong stereotyped gnawing response to apomorphine, whereas the APO-UNSUS rats show a very weak gnawing response to this dopaminergic agonist. We have been able to show that both genetic and early environmental factors underlie this line-specific difference in apomorphine-susceptibility (Ellenbroek, Sluyter et al., 2000). Similar to schizophrenic patients, APO-SUS rats show a heightened response to stress (Rots, Cools et al., 1996) as well as a reduced prepulse inhibition (Ellenbroek, Geyer, and Cools, 1995). However, this reduced prepulse inhibition has been observed in rats that were socially isolated, i.e. after a period of mild stress. As discussed elsewhere, mildly stressed APO-SUS rats are among

others characterised by a relatively high mesolimbic noradrenergic and dopamine D_2 activity, whereas mildly stressed APO-UNSUS rats are, among others, characterized by a relatively low mesolimbic noradrenergic and dopaminergic D_2 activity (Cools, van den Bos, Ploeger, and Ellenbroek, 1991;Cools, Rots, and de Kloet, 1994;Cools, Brachten, Heeren, Willemen, and Ellenbroek, 1990; Roozendaal and Cools, 1994). Given the fact that both noradrenaline and dopamine agonists reduce prepulse inhibition (Carasso, Bakshi et al., 1998), these data may explain the line-specific difference in prepulse inhibition.

Interestingly, when APO-SUS and APO-UNSUS rats are not stressed, the APO-SUS rats show a relative low noradrenergic activity but the non-stressed APO-UNSUS rats show a relative high noradrenergic activity. This change of noradrenergic activity has an influence on the dopaminergic activity, since noradrenaline facilitates dopamine release in the nucleus accumbens (Cools, van den Bos, Ploeger, and Ellenbroek, 1991;Cools, Rots, and de Kloet, 1994;Cools, Brachten, Heeren, Willemen, and Ellenbroek, 1990;Roozendaal and Cools, 1994). If the line-specific differences in dopamine and/or noradrenaline are causally related to the differences in prepulse inhibition, one would expect that removal of stress decreases prepulse inhibition in APO-UNSUS rats (in parallel to the increase in noradrenaline and dopamine D_2 activity), and increases it in APO-SUS (in parallel to the decrease in noradrenaline and dopamine D_2 activity). The present study was designed to investigate the influence of removal of mild stress on prepulse inhibition performance in APO-SUS and APO-UNSUS rats.

Material and Methods

Animals

All APO-SUS and APO-UNSUS rats were bred in the Central Animal Laboratory of the University of Nijmegen. They weighed between 200 and 250 g at the time of experiment and had water and food freely available except during the testing in the prepulse inhibition-boxes. They were housed with a maximum of three animals per cage (42 x 26 x 15 cm). The rooms were temperature-controlled with a standard 12 L: 12 D cycle: lights on from 07:00 to 19:00 h.

Pharmacogenetic selection

Several years ago, Cools et al. started a breeding program to pharmacogenetically select APO-SUS and APO-UNSUS rats (Cools, Brachten, Heeren, Willemen, and Ellenbroek, 1990). The present experiments were performed with male APO-SUS and APO-UNSUS rats belonging to the 28th generation. APO-SUS rats are defined as animals born from an APO-SUS mother and father. Likewise, APO-UNSUS rats are defined as animals born from an APO-UNSUS father and mother.

Treatment

We used two groups of animals. The control group was treated according to Ellenbroek et al. (Ellenbroek, Geyer, and Cools, 1995). They were individually housed one day before testing. The animals of the experimental group were housed in groups (2-3 animals per cage, 42 x 26 x 15 cm). Then, we had four groups of animals: Socially housed APO-SUS (N = 6), isolated APO-SUS (N=7), socially housed APO-UNSUS (N = 8) and isolated APO-UNSUS (N = 6).

Prepulse inhibition of the acoustic startle response

The prepulse inhibition experiments were performed in four acoustic startle chambers of San Diego Instruments. One cage consists of a plexiglas tube (8.2 cm in diameter, 25 cm in length) resting on a plastic frame. A piezoelectric accelerometer mounted under the tube detected and transduced the motion of the tube. Stimulus delivery was done using the SR-LAB software, via a speaker mounted 10 cm above the cylinder. The computer software also digitized, rectified, and recorded the response of the accelerometer; with 100 ms readings collected beginning at stimulus onset. Startle amplitude was defined as the average of 100 readings. The whole system was mounted within a sound-attenuating chamber. Throughout the startle session, a background noise of 70dB was maintained. The experiment started with a 5 min habituation session with background noise in the startle system. After this habituation period, ten blocks of five trials were delivered to measure prepulse inhibition. Each of these blocks consisted of one startle trial (120 dB, 20 ms broad band burst), one no-stimulus condition, and three different prepulse-startle pairing administered pseudorandomly. In these pairings, the prepulse was 3, 5, or 10 dB above background. These prepulse were always 20msec broadband burst and always followed by the 120 dB startle pulse 100 ms later. The interval between two trials was between 10 and 20 sec.

The startle amplitude was calculated as the mean of 10 delivered startle trials.

The degree of prepulse inhibition (in percentage) was calculated according to the formula

$$100 - \frac{\text{mean of all prepulses trials}}{\text{startle amplitude on startle trial}} \text{ x } 100$$

Statistics

To evaluate the data of the startle response and prepulse inhibition a two-way ANOVA with the factors line and treatment was used. The Fisher's LSD post-hoc test was performed when the two way ANOVA was significant with either line or treatment as factor

Results

Effect on the startle response (Fig 2)

Removal of social isolation did not affect the startle response in APO-SUS or APO-UNSUS rats. Statistical analyses revealed no significant treatment, line or interaction effect.

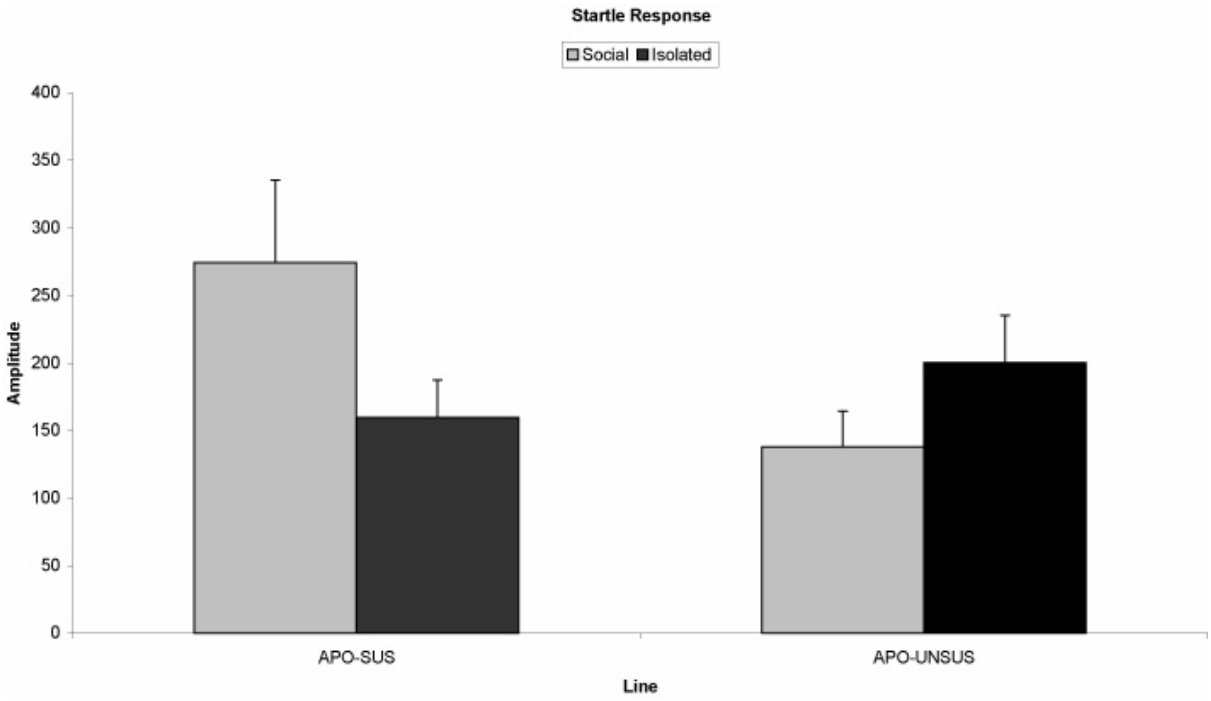

Fig 2: Effect of stress-removal on the Startle Response: Removal of stress had no effect on the startle response in APO-SUS and APO-UNSUS rats. The values are means + SEM

Effect on the prepulse inhibition in APO-SUS and APO-UNSUS Rats (Fig 3)

As expected the removal of isolation stress had a differential effect in APO-SUS and APO-UNSUS rats (treatment x line :($F_{1,23}$ = 11.93; p = < 0.001); though the treatment had no overall effect ($F_{1,23}$ = 0.33; p = > 0.05). Removal of the stress increased the prepulse inhibition in APO-SUS rats (Fisher's LSD post-hoc p = 0.05) and decreased it in the APO-UNSUS rats (Fisher's LSD post-hoc p =0.008).

The socially housed groups differed too, APO-SUS rats showed a greater prepulse inhibition than APO-UNSUS rats ($F_{1,12}$ = 13,0 ; p = 0,004) whereas the isolated groups did not differ ($F_{1,11}$ = 1,22 ; p = > 0.05). However, analysis of the effect of various intensities revealed that the isolated APO-SUS rats had less prepulse inhibition than the isolated APO-UNSUS rats at 75 dB prepulse intensity. ($F_{1,11}$ = 5.290; p = 0.042) (data not shown).

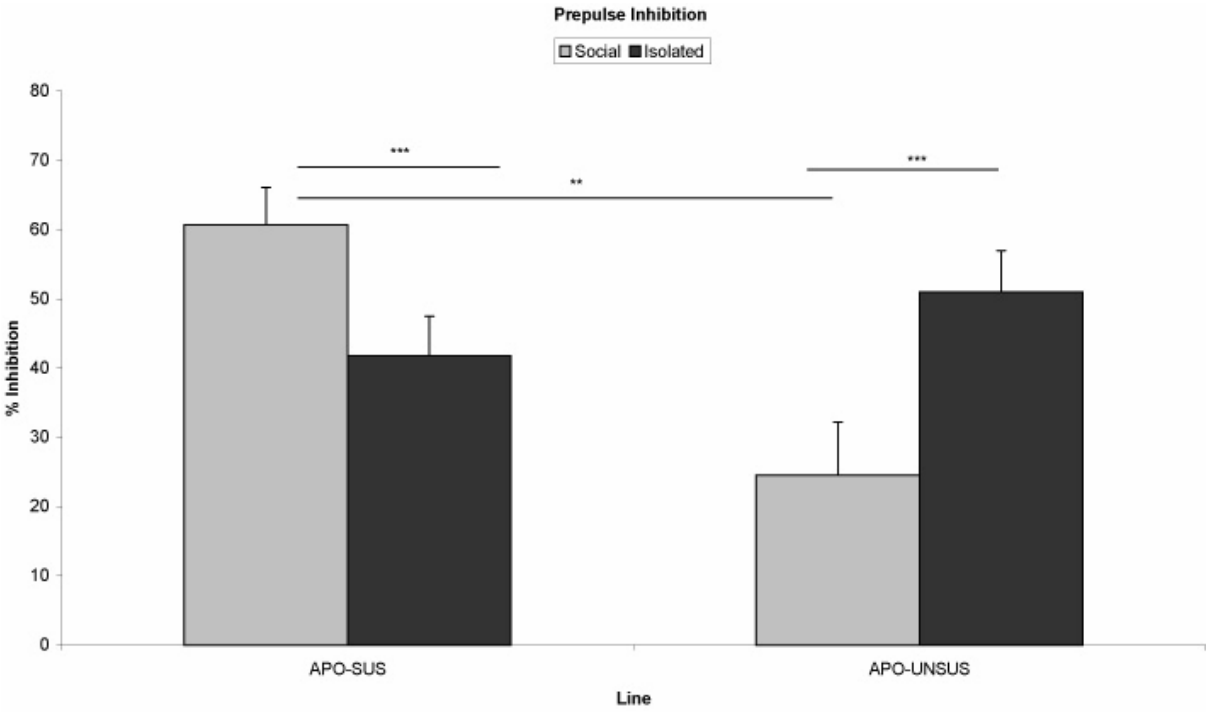

Fig 3: Effect of stress removal on the prepulse inhibition in APO-SUS and APO-UNSUS: Removal of stress increased the prepulse inhibition in the APO-SUS rats (* $p < 0.05$) and decreased it in the APO-UNSUS rats (***$p < 0.01$). Consequently the social housed APO-SUS and APO-UNSUS rats differ in prepulse inhibition (*** $p < 0.01$)

Discussion

This study shows that removal of isolation stress differentially affected the prepulse inhibition in APO-SUS and APO-UNSUS rats: In line with our earlier hypothesis, it increased the prepulse inhibition in APO-SUS rats and decreased it in APO-UNSUS rats. Data from earlier studies showed that mildly stressed APO-SUS rats had a reduced prepulse inhibition at certain prepulse inhibition intensities (Ellenbroek, Geyer, and Cools, 1995). This deficit in APO-SUS rats was found at 72 and 74 dB prepulse intensity. Since somewhat different prepulse intensities were used in the present study, the data are not fully comparable. Nonetheless, as in the study in 1995, the stressed APO-SUS rats show a significantly reduced prepulse inhibition at the lower prepulse intensities compared to APO-UNSUS rats. Due to a relatively large variance, this reduction was not statistically significant at 73 dB, but it was at 75 dB.

More importantly, however, the findings of this study complement the earlier data by showing that stress is a significant factor in prepulse inhibition performance, at least in APO-SUS and APO-UNSUS rats. So far, very few people have investigated the relationship between stress and prepulse inhibition. We have previously found that maternally deprived normal Wistar rats, which are characterized by an increased sensitivity to stress, show a reduction in prepulse inhibition (Ellenbroek, van-den Kroonenberg, and Cools, 1998). Likewise, CRH-over expressing mice show

reduction in prepulse inhibition (Dirks, Groenink et al., 2002). Previous papers have also shown that social isolation from rearing on leads to reductions in prepulse inhibition in several rat strains (Geyer, Wilkinson et al., 1993;Varty and Geyer, 1998), including Wistar rats (Ellenbroek and Cools, 2000). However, these data cannot be compared to the present set of experiments, since isolation rearing involves a very long period of isolation (up to 8 weeks) and the timing of the isolation seems to be crucial, since similar long periods of isolation in adulthood do not affect prepulse inhibition (Wilkinson, Killcross et al., 1994).

As described in the introduction, exposing APO-SUS and APO-UNSUS rats to a mild stressor has, among others, a differential effect on the noradrenergic activity at the level of mesolimbic $alpha_1$ adrenoceptors: it enhances the relatively low noradrenergic activity in APO-SUS rats, whereas it decreases the relatively high noradrenergic activity in APO-UNSUS rats. Because stimulation of $alpha_1$ adrenoceptor activity is known to reduce prepulse inhibition (Carasso, Bakshi, and Geyer, 1998) it is possible that the stress-induced change of noradrenergic activity plays an important role in regulating prepulse inhibition in APO-SUS and APO-UNSUS rats. Based on this change of noradrenergic activity it was predicted that the removal of isolation-induced stress results in an enhancement of prepulse inhibition in APO-SUS rats and in a reduction of prepulse inhibition in APO-UNSUS rats. Although this is precisely what was found, it is evident that future studies are required to prove that it is indeed the mesolimbic $alpha_1$ adrenoceptor activity that mediates the effects of systemically administered $alpha_1$ adrenoceptor agonists and antagonists on prepulse inhibition. The precise mechanism how noradrenaline disturbs the prepulse inhibition is not clear yet; however, mesolimbic noradrenaline is known, among others to facilitate the dopamine release in the nucleus accumbens (Cools, 1988). Several studies have shown that an increased dopamine activity in the nucleus accumbens also disturbs prepulse inhibition (Swerdlow, Braff et al., 1990). Given the role of mesolimbic dopamine in prepulse inhibition, these findings suggest that noradrenaline might influence the prepulse inhibition by changing the dopamine release in the nucleus accumbens. Independent of the precise mechanism of action that underlies the line-specific effects of isolation-induced stress on prepulse inhibition, the present study clearly shows that exposure (or removal) to stress has a differential effect on prepulse inhibition in APO-SUS and APO-UNSUS rats.

Overall impact

First, the present study shows that prepulse inhibition is not fully invariant in rats that have, among others, a genetic difference in the mesolimbic noradrenergic system; it is clearly under control of internal and/or environmental conditions. For, unchallenged APO-SUS rats are marked by an increased prepulse inhibition in comparison with challenged APO-SUS rats, whereas unchallenged

APO-UNSUS rats are marked by a decreased prepulse inhibition in comparison with challenged APO-UNSUS rats. As mentioned in the introduction, stress is a central factor in schizophrenia. Studies have found an elevated level of cortisol (Muck-Seler, Pivac et al., 1999) and noradrenaline (Meltzer, 1987) in blood and plasma of patients. Furthermore, elevated plasma noradrenaline correlates with positive symptoms, overall psychopathology and paranoid symptoms (Dajas, Barbeito et al., 1983;Hultman, Wieselgren et al., 1997;Leff, 1981). Moreover, schizophrenic patients have an enhanced cortisol response to stress, and stressful life events might precipitate a relapse (Walker, Diforio et al., 1999). Given this differential sensitivity of schizophrenic patients and controls to stress, the present data may have consequences for the assessment of prepulse inhibition in human studies. Moreover, stress may also be a confounding factor in prepulse inhibition studies in animals, especially when different strains are being investigated.

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C h a p t e r 3

THE EFFECT OF ADRENOCEPTOR AGONISTS ON PREPULSE INHIBITION IN APOMORPHINE SUSCEPTIBLE (APO-SUS) AND APOMORPHINE UNSUSCEPTIBLE (APO-UNSUS) RATS

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Abstract

Many years ago we started to breed two lines of Wistar rats on the basis of their sensitivity to the dopaminergic agonist apomorphine, namely the APO-SUS and APO-UNSUS rats. Several experiments in our laboratory have shown that APO-SUS rats share features such as a reduced prepulse inhibition (ppi) and an impaired stress response with schizophrenic patients. Recent experiments in our laboratory have shown that mild stressors reduce prepulse inhibition in APO-SUS rats and increase prepulse inhibition in APO-UNSUS rats. Since mild stressors are known to increase the normally low functional noradrenergic activity in APO-SUS rats, but to decrease the normally high functional noradrenergic activity in APO-UNSUS rats, these data suggest that increasing the functional noradrenaline activity reduces prepulse inhibition. Aim of the present study was to investigate whether noradrenergic agonists would indeed reduce prepulse inhibition in APO-SUS and APO-UNSUS rats. Moreover, since it is known that the sensitivity of postsynaptic adrenoceptor agonist is inversely related to the functional noradrenergic activity, we expected a stronger effect in non-stressed APO-SUS rats, compared to non-stressed APO-UNSUS rats. We found that the $alpha_1$ agonist cirazoline dose-dependently decreased prepulse inhibition in non-stressed APO-SUS rats, but not in non-stressed APO-UNSUS rats, an effect which could be reversed by the $alpha_1$-antagonist prazosin. The $beta_2$ adrenoceptors agonist clenbuterol on the other hand did not affect prepulse inhibition in either APO-SUS or APO-UNSUS rats, when tested under non-stressed conditions. Neither adrenergic agonist affected baseline startle amplitude in these rats. These data suggest that $alpha_1$ but not $beta_2$-adrenoceptors modulates prepulse inhibition in APO-SUS and APO-UNSUS rats.

Introduction

Several years ago we started to genetically breed two lines of rats, marked by a high (APO-SUS) and low (APO-UNSUS) response to apomorphine, respectively. As described elsewhere in detail these animals are further characterized by several behavioural and neurochemical differences (Cools, Brachten et al., 1990; Cools, Rots et al., 1993; Cools and Gingras, 1998). The differences in dopaminergic sensitivity make these animals an interesting model for investigating illnesses that are related to this neurotransmitter, such as schizophrenia and addiction. Indeed, we have previously shown that APO-SUS rats share a number of features with schizophrenic patients, including a reduced prepulse inhibition (Ellenbroek, Geyer et al., 1995) an increased stress response (Rots, Cools et al., 1995; Kemali, Maj et al., 1985) an increase in mRNA for tyrosine hydroxylase (Rots, Cools et al., 1996) and a dominance of Th2 helper cells (Breivik, Sluyter et al., 2000). Furthermore the APO-SUS rats display a reduced tumour growth which is in line with the finding that schizophrenic patients are relative protected from cancer (Teunis, Kavelaars et al., 2002). The fact that the differences between APO-SUS and APO-UNSUS rats are not only due to genetic differences but are also regulated by (early) environmental factors (Ellenbroek, Sluyter et al., 2000) emphasizes the validity of APO-SUS rats as a model for schizophrenia, since it is well known that both genetic (Freedman, Adler et al., 1999; Joober, Benkelfat et al., 1999) and early environmental factors (Agid, Shapira et al., 1999) contribute to the aetiology of schizophrenia

We have shown recently that the prepulse inhibition response of APO-SUS and APO-UNSUS rats is not invariant, but sensitive to mild stressors (Sontag, Cools et al., 2003a). In fact a short term social isolation reduces prepulse inhibition in the APO-SUS rats, but enhances it in APO-UNSUS rats. Mild stressors, such as social isolation, are also known to enhance the functional mesolimbic noradrenergic activity in APO-SUS rats and to reduce it in APO-UNSUS rats (Cools, Rots et al., 1994; Cools and Gingras, 1998) Thus in a non-stressed situation, APO-SUS rats show a relatively low functional noradrenergic activity, and APO-UNSUS rats a relatively high functional noradrenergic activity in the forebrain (Cools, Brachten, Heeren, Willemen, and Ellenbroek, 1990; Cools and Gingras, 1998). Combining these data results in the notion that rats with a high functional noradrenergic activity, i.e. stressed APO-SUS and non-stressed APO-UNSUS rats, have a reduced prepulse inhibition, whereas rats with a low functional noradrenergic activity, i.e. non-stressed APO-SUS and stressed APO-UNSUS rats, have an increased prepulse inhibition. Thus, the differential response of stressed and non-stressed APO-SUS and APO-UNSUS rats in the prepulse inhibition might be related to the mesolimbic noradrenergic tone of these animals.

It is well know that the noradrenergic tone determines the sensitivity of postsynaptic alpha-and beta-adrenoceptors (Starke, 1981; Reisine, Chesselet et al., 1982). An increased noradrenaline tone

leads to a reduced sensitivity of postsynaptic receptors for adrenergic agonists, whereas a reduced noradrenaline tone leads to an increased sensitive of these receptors for adrenergic agonists. Aim of the present paper was to investigate to what extent noradrenergic agonists reduce prepulse inhibition in non-stressed APO-SUS and APO-UNSUS rats, and whether the effect depends on the noradrenergic tone. Since we used non-stressed rats, we expected that naïve APO-SUS rats, having a low functional noradrenergic activity at rest, to be more sensitive to adrenergic agonist than naïve APO-UNSUS rats, which have a high functional noradrenergic activity at rest.

Materials and Methods

Animals

All APO-SUS and APO-UNSUS rats were bred in the Central Animal Laboratory of the University of Nijmegen. They weighed between 200 and 250 g at the time of the experiments and had water and food freely available, except during the testing in the prepulse inhibition-boxes. They were housed with a maximum of three animals per cage (42 x 26 x 15 cm). The rooms were temperature-controlled with a standard 12 L: 12 D cycle: lights on from 07:00 to 19:00 h. All experiments were performed according to institutional, national and international guidelines for animals care and welfare. The rats were only used once in these experiments.

Pharmacogenetic selection

Several years ago, we started a breeding program to pharmacogenetically select APO-SUS and APO-UNSUS rats (Cools, Brachten, Heeren, Willemen, and Ellenbroek, 1990). The present experiments were performed with male APO-SUS and APO-UNSUS rats belonging to the 17th generation of the replication line that was started in 1995 (Ellenbroek, Sluyter, and Cools, 2000). APO-SUS rats are defined as animals born from an APO-SUS male and female. Likewise, APO-UNSUS rats are defined as animals born from an APO-UNSUS male and female. The number of animals per group tested is shown in table 1

Prepulse inhibition of the acoustic startle response

The prepulse inhibition experiments were performed in four acoustic startle chambers of San Diego Instruments. (San Diego, USA). Each cage consisted of a plexiglas tube (8.2 cm in diameter, 25 cm in length) resting on a plastic frame. A piezoelectric accelerometer mounted under the tube detected and transduced the motion of the tube. Stimulus delivery was done using the SR-LAB software, via a speaker mounted 10 cm above the cylinder. The computer software also digitized, rectified and recorded the response of the accelerometer; with 100 ms readings, collected at the beginning of stimulus onset. Startle amplitude was defined as the average of these 100 readings.

The whole system was mounted within a sound-attenuating chamber. Throughout the startle session, a background noise of 70dB was maintained.

The experiment started with a 5 min habituation session with background noise (70 dB) in the startle system. After this habituation period, ten blocks of five trials were delivered to measure prepulse inhibition. Each of these blocks consisted of one startle trial (120 dB, 20 ms broad band burst), one no-stimulus condition and three different prepulse-startle pairing administered pseudorandomly. In these pairings, the prepulse was 3, 5 or 10 dB above background. These prepulses were always 20 ms broadband burst and always followed by the 120 dB startle pulse 100 ms later. The interval between two trials was between 10 and 20 s.

The startle amplitude was calculated as the mean of 10 delivered startle trials.

The degree of prepulse inhibition (in percentage) was calculated according to the formula:

$$100 - \frac{\text{mean of all prepulses trials}}{\text{startle amplitude on startle trial}} \text{ x } 100$$

Treatment

All rats were housed in groups of 2 or 3 rats until immediately before testing in the prepulse inhibition-boxes. In contrast to previous experiments these rats were not isolated one day prior to the experiment since we have demonstrated that this form of mild stress reduces prepulse inhibition in APO-SUS rats but increase it in APO-UNSUS rats (Sontag, Cools, and Ellenbroek, 2003a).

Drugs	APO-SUS	APO-UNSUS
saline	9	9
cirazoline (0.1mg/kg)	8	8
cirazoline (0.5mg/kg)	7	7
cirazoline (1.0mg/kg)	6	8
clenbuterol (0.001mg/kg)	8	8
clenbuterol (0.004mg/kg)	8	7
clenbuterol (0.016mg/kg)	7	6
clenbuterol (0.064mg/kg)	8	8
prazosin (2.5mg/kg) and cirazoline (1.0mg/kg)	8	-

Table 1: number of animals per experiment

Before testing they received an i.p. injection and were transferred to the prepulse inhibition boxes and testing started. The control animals were injected with saline (1ml/kg). We used two drugs in

different concentrations. The alpha$_1$-adrenoceptor agonist cirazoline (Research Biochemical Incorporated, Natick MA USA) was given in the dosage 0.1, 0.5 and 1.0 mg/kg. These doses were chosen on the basis of the outcome of the study by Carasso et al. (Carasso, Bakshi et al., 1998) who showed that these doses were behaviourally active. The beta$_2$-adrenoceptor agonist clenbuterol (Sigma, Schnelldorf Germany) was given in the dosage 0.001, 0.004, 0.016 and 0.064 mg/kg, namely doses that were found to be behaviourally active in rats (Dooley, Mogilnicka et al., 1983). For antagonizing the alpha$_1$-adrenoceptor agonist cirazoline, the alpha$_1$-antagonist prazosin (Sigma, Schnelldorf Germany) in the dosage of 2.5 mg/kg was used. This dose was chosen based on the study from Bakshi et al (Bakshi and Geyer, 1997) who showed that this dose is behavioural active. The antagonist was given twenty minutes prior to the agonist. All drugs were dissolved in saline and the injected volume was 1ml/kg.

Statistics

The data were analyzed separately for each line, because APO-SUS and APO-UNSUS rats are characterized by a diametrically opposite adrenergic activity in the brain during rest as well as during challenging conditions (Cools, Brachten, Heeren, Willemen, and Ellenbroek, 1990;Cools and Gingras, 1998). The data of the startle response and prepulse inhibition were analyzed with a univariate Analysis of Variance (ANOVA) with the factor treatment. The post-hoc tests were done with Fisher LSD test. A p-value of < 0.05 was considered to be significant.

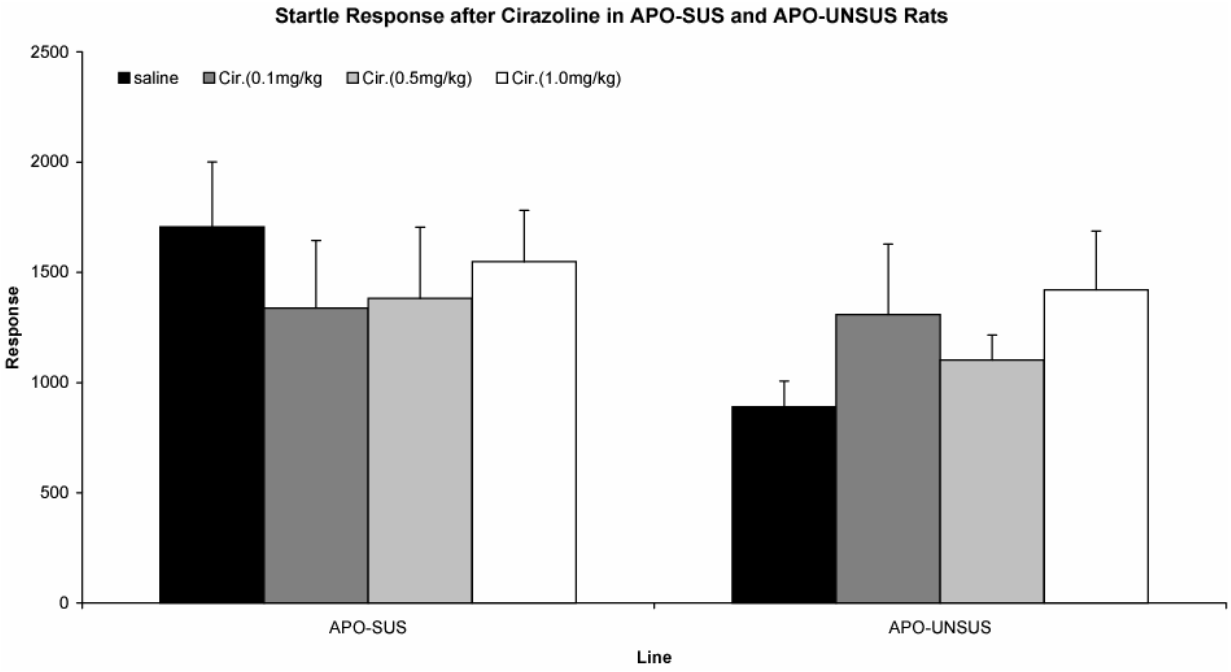

Fig 4: Effect of cirazoline on the Startle response: Cirazoline had no effect on startle response in APO-SUS and APO-UNSUS rats. The values are means ± S.E.M

Results

Startle Response

Cirazoline (Fig 4)

Stimulation of the $alpha_1$ adrenoceptors with cirazoline did not significantly alter the baseline startle response, neither in APO-SUS rats (Treat: $F_{(3, 26)} = 0.348$; $p = 0.791$) nor in APO-UNSUS rats (Treat: $F_{(3, 28)} = 1.160$; $p = 0.343$).

Clenbuterol (Fig 5)

Stimulation of the $beta_2$ adrenoceptors with clenbuterol likewise did not alter the baseline startle amplitude in APO-SUS (Treat: $F_{(4, 35)} = 0.97$; $p = 0.983$) or APO-UNSUS rats (Treat: $F_{(4, 33)} = 2.297$; $p = 0.080$), though there was a tendency for an increase at 0.004 mg/kg dose in the latter rats.

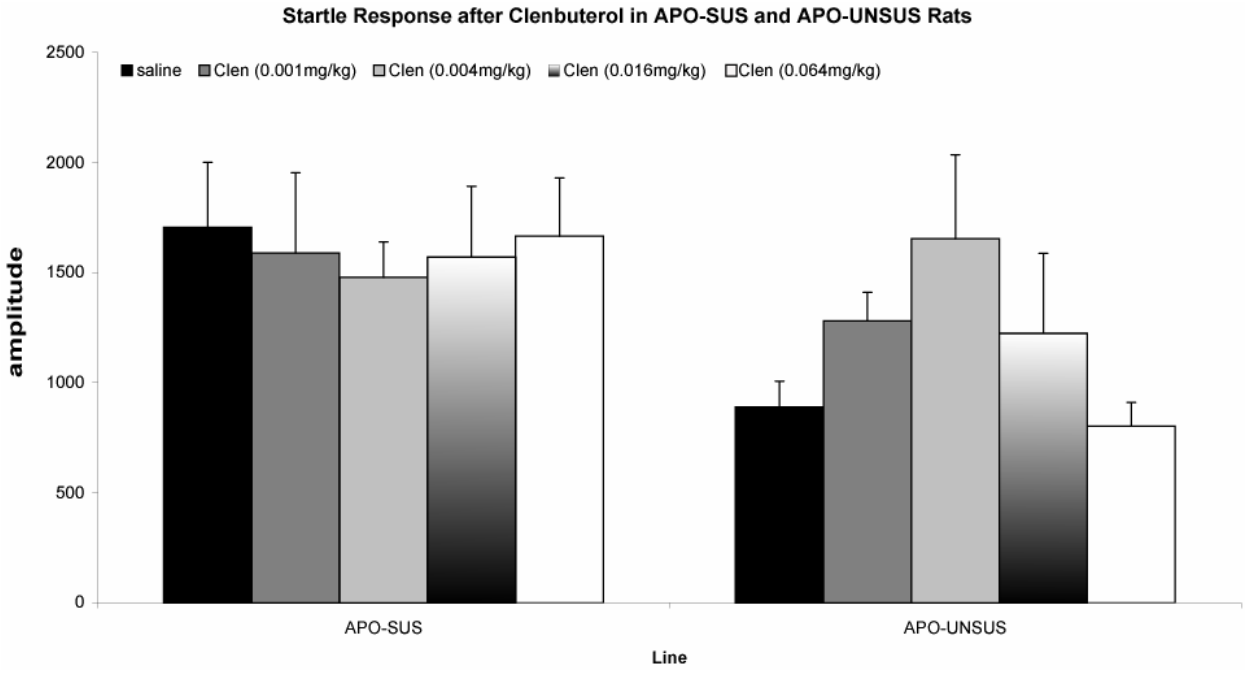

Fig 5: Effect of clenbuterol on startle response: clenbuterol had no effect on startle response in APO-SUS and APO-UNSUS rats

Cirazoline and Prazosin (Fig 6)

The basal startle response after the combined treatment of prazosin and cirazoline was not different from either saline or cirazoline treatment alone ($F_{(2,20)} = 0.450$; $p > 0.05$).

Prepulse Inhibition

Cirazoline (Fig 7)

The effect of the $alpha_1$-adrenoceptor agonist cirazoline on prepulse inhibition differed between APO-SUS and APO-UNSUS rats. Cirazoline dose-dependently reduced prepulse inhibition in

APO-SUS rats (Treat: $F_{(3, 26)} = 6.261$; $p = 0.002$). Post-hoc analyses showed that the two highest doses (0.5 and 1.0 mg/kg) significantly differed from the saline control group. On the other hand, cirazoline did not significantly alter prepulse inhibition in APO-UNSUS rats (Treat: $F_{(3, 28)} = 2.266$; $p = 0.103$), though inspection of Fig 7 shows that there was a clear tendency for a reduction at the highest dose (1.0 mg/kg).

Clenbuterol (Fig 8)

The $beta_2$ adrenoceptor agonist clenbuterol had no effect on prepulse inhibition in APO-SUS (Treat: $F_{(4, 35)} = 1.593$; $p = 0.198$) and APO-UNSUS rats (Treat: $F_{(4, 33)} = 0.466$; $p = 0.760$).

Cirazoline and Prazosin (Fig 9)

The $alpha_1$-antagonist prazosin antagonized the disruptive effect of cirazoline on prepulse inhibition in APO-SUS rats ($F_{(2,20)} = 18.090$; $p < 0.05$).

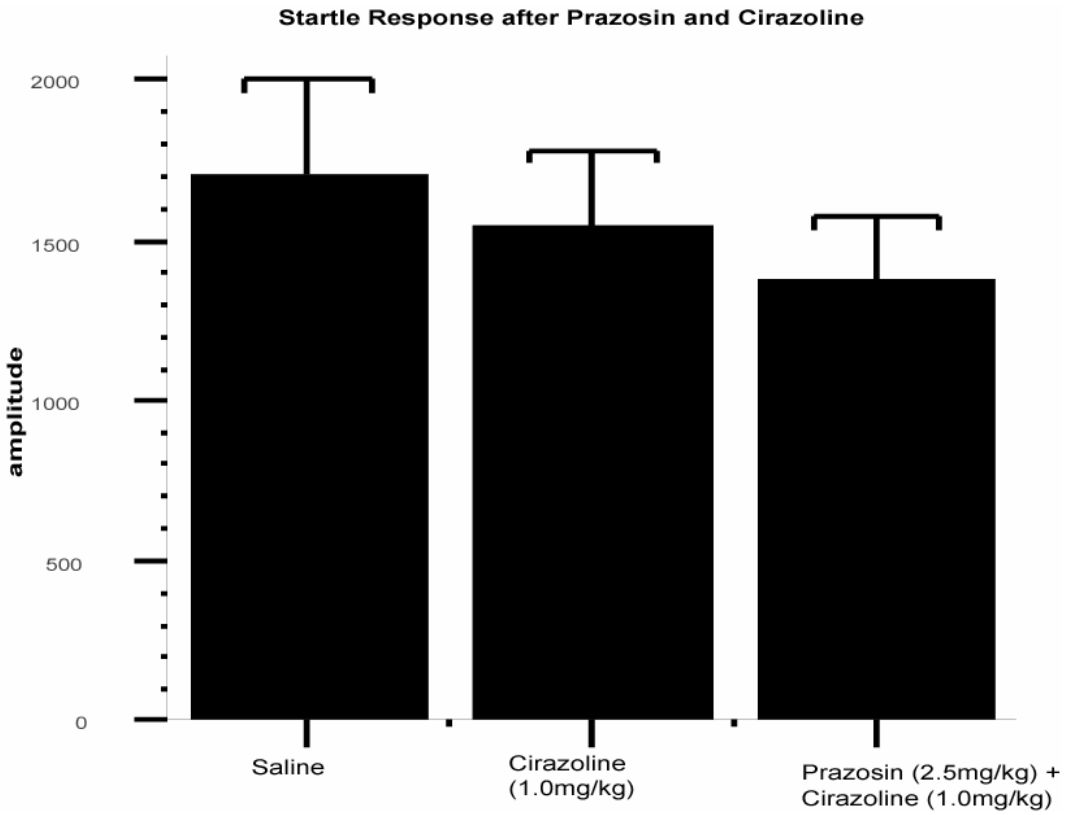

Fig 6: Effect of cirazoline on startle response after pre-treatment with prazosin

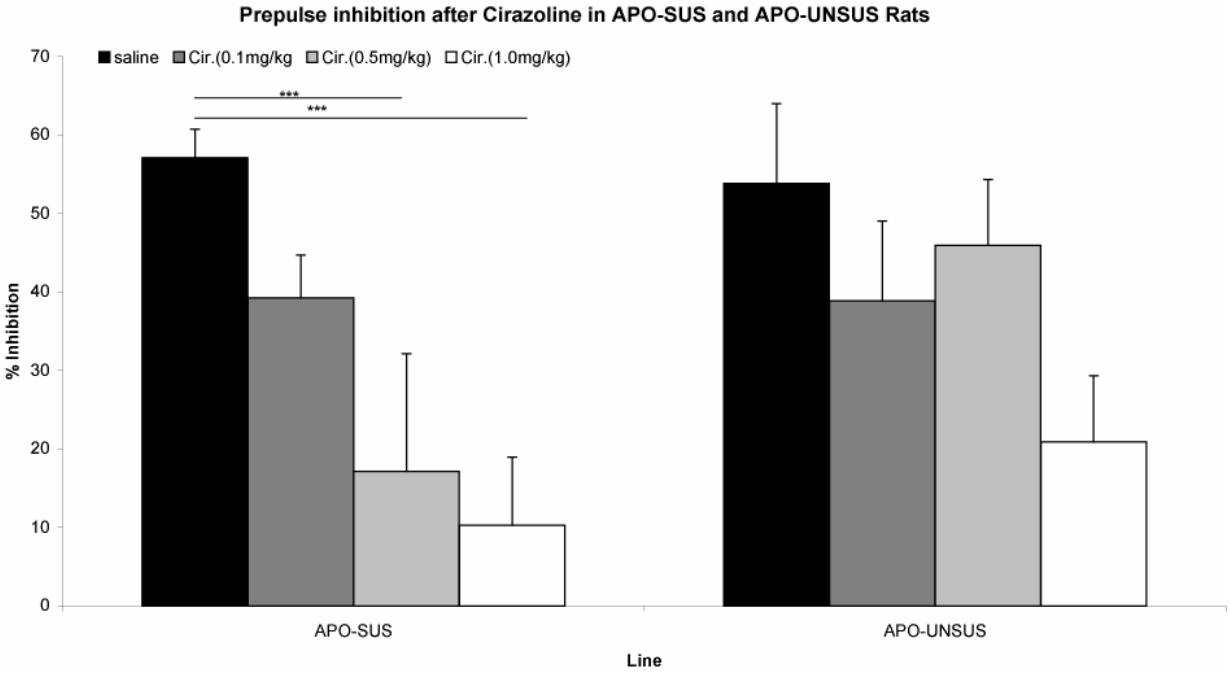

Fig 7: Effect of cirazoline on the prepulse inhibition: Cirazoline did not change the prepulse inhibition in the APO-UNSUS rats, but reduced it dose-dependently in APO-SUS rats (0.5mg/kg, ***p = 0.01; 1.0 mg/kg, ***p < 0.01

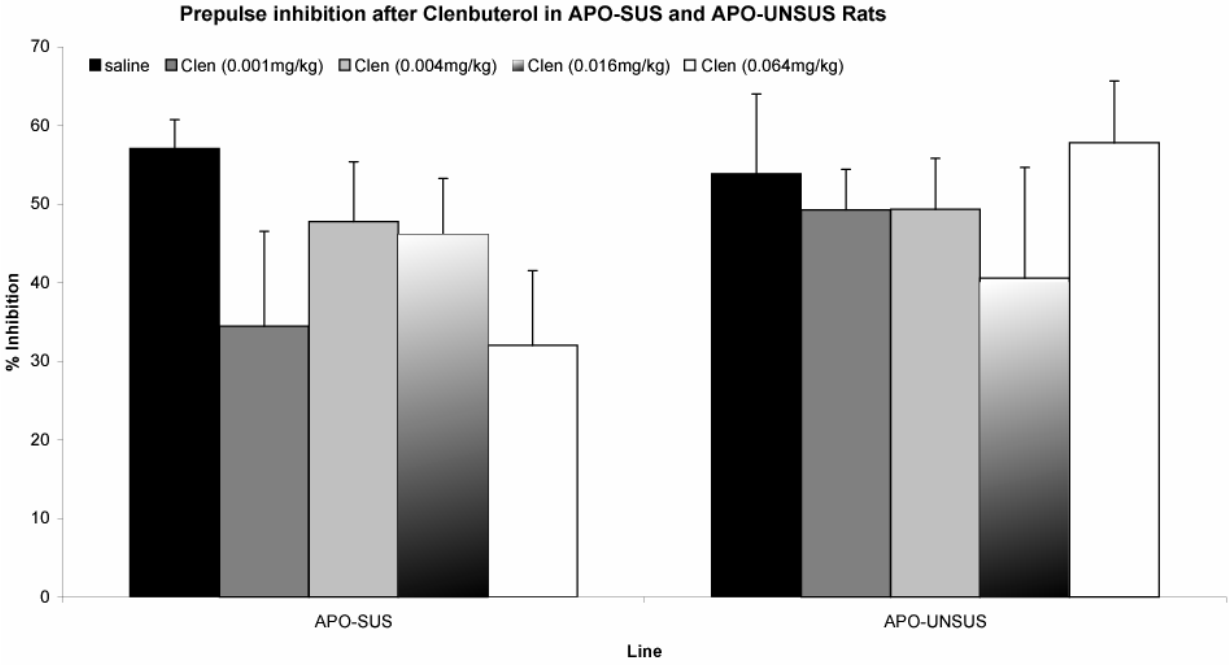

Fig 8: Effect of clenbuterol on prepulse inhibition: Clenbuterol did not change the prepulse inhibition in APO-SUS and APO-UNSUS rats.

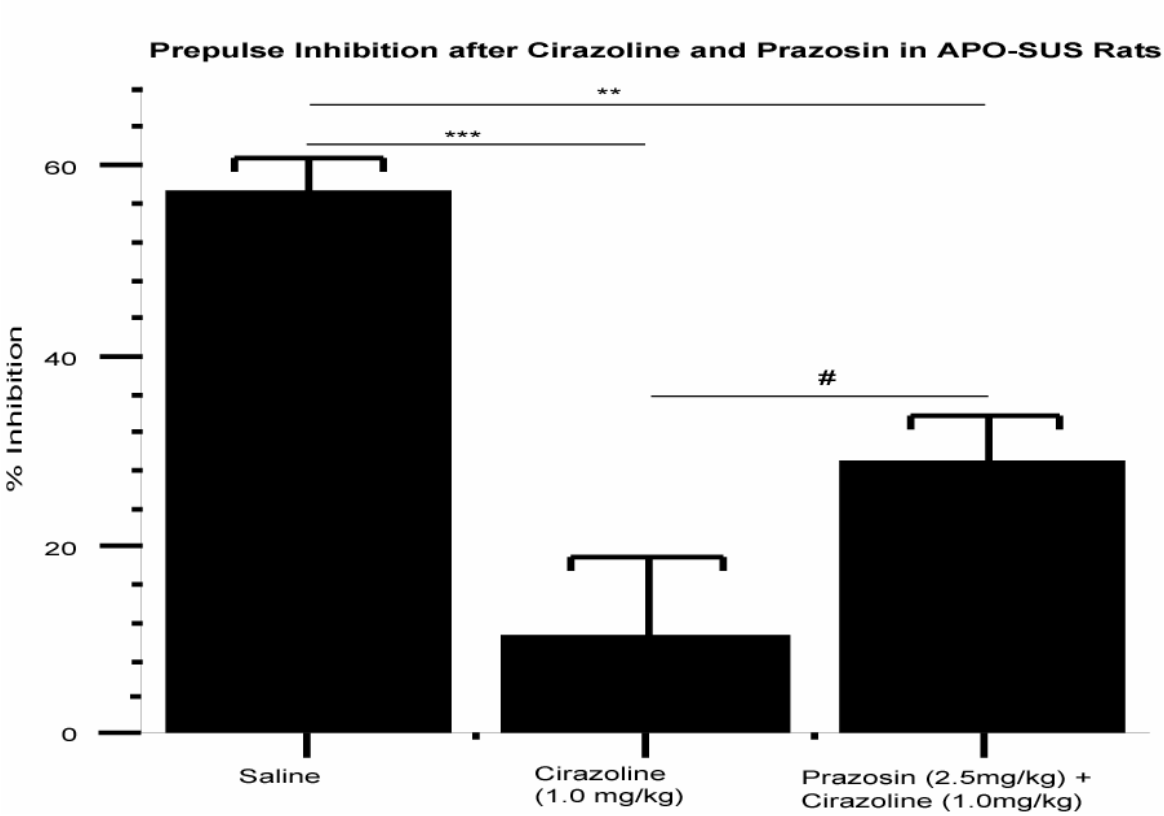

Fig 9: The effect of cirazoline and prazosin on prepulse inhibition: Prazosin antagonised the effect of cirazoline on prepulse inhibition in APO-SUS rats (***$p < 0.001$; **$p < 0.01$; * $p < 0.05$; # $p < 0.05$

Discussion

The data of the present study clearly show that $alpha_1$ adrenoceptor stimulation dose-dependently decreased prepulse inhibition in APO-SUS but not in APO-UNSUS rats, an effect that could be antagonized with the $alpha_1$-antagonist prazosin. On the other hand, stimulation of the $beta_2$-adrenoceptor did not affect prepulse inhibition in APO-SUS or APO-UNSUS rats. Neither adrenoceptor agonist affected baseline startle amplitude.

The finding that the $beta_2$ adrenoceptor agonist did not affect the baseline startle amplitude is in agreement with the study of Astrachan & Davis who also showed that beta-adrenoceptor agonists are ineffective in this respect (Astrachan and Davis, 1981). Our finding that the $alpha_1$-adrenoceptor agonist was ineffective in changing the baseline startle amplitude is somewhat surprising since the aforementioned authors found an increase in baseline startle amplitude after intrathecal application of phenylephrine. Even though we used the same dose range as Carasso et al (Carasso, Bakshi, and Geyer, 1998) who also found an increase in baseline startle amplitude, we did not find this effect in our APO-SUS and APO-UNSUS rats. It is at present difficult to explain these discrepancies, though it should be noted that Carasso et al (1998) and Astrachan and Davis (1981) used Sprague Dawley rats, whereas we used APO-SUS and APO-UNSUS rats and distinct

strains are known to respond differently to drugs as far as it concerns prepulse inhibition (Kinney, Wilkinson et al., 1999).

The main goal of this study was to investigate the effects of adrenergic agonists on prepulse inhibition. The data shows that the alpha$_1$-adrenoceptor agonist cirazoline dose-dependently reduced prepulse inhibition in non-stressed APO-SUS rats. In non-stressed APO-UNSUS rats a tendency for a reduced prepulse inhibition was seen at the highest dose (1.0 mg/kg); however, the overall effect was not significant. The dose of 0.5 mg/kg was not effective in APO-UNSUS, whereas it was highly effective in APO-SUS rats. Thus non-stressed APO-SUS rats were more sensitive for the alpha$_1$-adrenoceptor agonist cirazoline than non-stressed APO-UNSUS rats. This is in line with our hypothesis, mentioned in the introduction, that the effects of alpha agonists depend on the functional activity of the noradrenergic system, which differs between non-stressed APO-SUS and non-stressed APO-UNSUS rats. The relatively high functional noradrenergic activity in the non-stressed APO-UNSUS is accompanied by a relatively low adrenoceptor sensitivity for cirazoline, whereas the relatively low functional noradrenergic activity in the non-stressed APO-SUS is accompanied by relatively high adrenoceptor sensitivity for cirazoline.

In contrast to the alpha$_1$ agonist, the beta$_2$ agonist clenbuterol had no effect on prepulse inhibition. It is unlikely that this lack of effect was due to the dose range chosen. Dooley and colleagues have shown that these doses are behaviourally active (Dooley, Mogilnicka, Delini, Waechter, Truog, and Wood, 1983). Moreover, we have also found that theses doses are behaviourally active in our APO-SUS and APO-UNSUS rats (Sontag, Cools et al., 2003b submitted). Thus it seems more likely that beta$_2$ adrenoceptors do not contribute to the expression of prepulse inhibition.

The neuronal mechanism by which alpha$_1$ adrenoceptor agonists modulates prepulse inhibition is not clear yet. The noted differences in noradrenergic activity between APO-SUS and APO UNSUS rats are, among others, present in the nucleus accumbens (Nacc) (Cools, Brachten, Heeren, Willemen, and Ellenbroek, 1990; Tuinstra and Cools, 2000).The nucleus accumbens is known to play a crucial role in prepulse inhibition, since increasing the dopamine (D_2) activity within this nucleus disrupts prepulse inhibition (Swerdlow, Mansbach et al., 1990;Wan and Swerdlow, 1993;Wan, Geyer et al., 1994).

As described elsewhere in detail stimulation of accumbal alpha$_1$-adrenoceptors results in the inhibition of the dopamine activity at the level of a subtype of dopamine receptors that differ from the classic dopamine D_1 and D_2 receptors (Cools, van den Bos, Ploeger, and Ellenbroek, 1991). Because such a low dopamine activity at these receptors is always associated with a high dopamine

activity at the level of the accumbal, dopamine D_2 receptors it might be this functional relation that gives rise to the cirazoline induced decrease in prepulse inhibition (Cools, van den Bos, Ploeger, and Ellenbroek, 1991).

In summary, we found that non-stressed APO-SUS rats show a strong reduction in prepulse inhibition, after intraperitoneal application of the $alpha_1$-adrenoceptor agonist cirazoline, while non-stressed APO-UNSUS rats were much less sensitive to this drug. This is in agreement with the notion that non-stressed APO-SUS rats are characterized by a relatively low functional noradrenergic activity, and subsequently a relatively high sensitivity of the postsynaptic $alpha_1$ adrenoceptors for the agonist. Non-stressed APO-UNSUS rats, on the other hand, are characterized by a relatively high functional noradrenergic activity, and subsequently a relatively low sensitivity of the postsynaptic $alpha_1$ adrenoceptors for the agonist. On the other hand stimulation of the $beta_2$ adrenoceptors, by application of clenbuterol, had no effect on prepulse inhibition in either APO-SUS or APO-UNSUS rats. It is concluded that $alpha_1$ but not $beta_2$ adrenoceptors are involved in modulating prepulse inhibition.

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C h a p t e r 4

PREPULSE INHIBITION IN APOMORPHINE SUSCEPTIBLE (APO-SUS) AND APOMORPHINE UNSUSCEPTIBLE (APO-UNSUS) RATS: EFFECTS OF DEPLETION OF NORADRENALINE

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Abstract

As described elsewhere in detail the so called apomorphine susceptible (APO-SUS) and apomorphine unsusceptible (APO-UNSUS) rats are characterised by several behavioural and neurochemical differences. Furthermore APO-SUS rats share some symptoms with schizophrenic patients like a reduced prepulse inhibition (ppi). However recent studies showed that mild stress reduces prepulse inhibition in APO-SUS rats and increases it in APO-UNSUS rats. Parallel mild stress is known to increase the functional noradrenergic activity in APO-SUS and to decrease it in APO-UNSUS rats. Noteworthy, alpha$_1$-adrenergic agonists were found to disrupt prepulse inhibition whereas alpha$_1$-antagonists were found to increase prepulse inhibition. This strongly suggests that a high noradrenergic activity reduces prepulse inhibition whereas a low noradrenergic activity increases prepulse inhibition. In the present study we depleted central noradrenaline with the help of the neurotoxin DSP4 which destroy locus coeruleus (LC) terminals to investigate whether a decrease in noradrenergic activity leads to an increase in prepulse inhibition in APO-SUS and APO-UNSUS rats. The effects of DSP4 on prepulse inhibition were tested under non-stressed conditions and after exposing the rats to mild stress. We also investigated the effects of DSP4 on the adrenoceptor sensitivity with the help of counting the number of head-dips. DSP4 did not alter the prepulse inhibition in non-stressed rats. However DSP4 increased prepulse inhibition in stressed APO-UNSUS, but reduced it in stressed APO-SUS rats. Furthermore, the pre-treatment with DSP4 strengthened the effect of the alpha$_1$ agonist cirazoline in reducing the number of head-dips in APO-SUS rats. Such an effect was not seen in APO-UNSUS rats. Likewise, the pre-treatment with DSP4 strengthened the effect of the alpha$_2$ antagonist clonidine in reducing the number of head-dips in APO-UNSUS rats; again, such an effect was not seen in APO-SUS rats. These data indicate the development of supersensitive alpha$_1$-adrenoceptors in the APO-SUS rats

and the development of supersensitive alpha$_2$ adrenoceptors in APO-UNSUS rats after DSP4 treatment. In addition these data show that the alteration of prepulse inhibition in DSP4-treated APO-SUS and APO-UNSUS rats is dependent on mild stress. In conclusion these data give strong evidence that the locus coeruleus is somehow involved in modulating prepulse inhibition.

Introduction

The prepulse inhibition of the acoustic startle is a decrease in startle response that occurs when the startle stimulus is preceded by a weaker stimulus. Disturbances in this phenomenon have been found in patients with neuropsychiatric diseases like Huntington's disease (Swerdlow, Paulsen et al., 1995) and schizophrenia (Braff and Geyer, 1990). Several years ago we started breeding the so called apomorphine-susceptible (APO-SUS) and apomorphine-unsusceptible (APO-UNSUS) rats. Notably, the APO-SUS rats share several abnormalities with schizophrenic patients like an enhanced stress response (Rots, Cools et al., 1995), and a reduced prepulse inhibition and latent inhibition (Ellenbroek, Geyer et al., 1995). Recent data from our laboratory have shown that the degree of prepulse inhibition also depends on the level of stress. Thus a short term social isolation reduces prepulse inhibition in APO-SUS and increases it in APO-UNSUS rats (Sontag, Cools et al., 2003a). In the past we have found that these rats differ both in their functional noradrenergic activity as well as in the reactivity of this system to mild stress. The non-stressed APO-SUS rats are marked by a relatively low functional noradrenergic activity whereas the non-stressed APO-UNSUS rats are marked by a relatively high functional noradrenergic activity. Exposure to a mild stressor increases the functional noradrenergic activity in the APO-SUS rats, but decreases it in the APO-UNSUS rats (Cools, Brachten et al., 1990; Cools and Gingras, 1998). Taken these data together it appears that rats with a high functional noradrenergic activity (viz. stressed APO-SUS and non-stressed APO-UNSUS rats) show a reduced prepulse inhibition, whereas rats with a low functional noradrenergic activity (viz. non-stressed APO-SUS and stressed APO-UNSUS rats) show an enhanced prepulse inhibition. In agreement with this, we have recently found that the alpha$_1$-adrenoceptor agonist cirazoline reduces prepulse inhibition in non-stressed APO-SUS, rats but not in non-stressed APO-UNSUS rats (Sontag, Cools et al., 2003b submitted).

In the present paper we investigated whether a decrease in noradrenergic activity would lead to an increase in prepulse inhibition in APO-SUS and APO-UNSUS rats. For that purpose, we depleted the central noradrenergic system using the neurotoxin DSP4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine). This neurotoxin is known to selectively destroy the noradrenergic terminals of the locus coeruleus (LC) fibres without affecting the serotonergic or dopaminergic fibres (Jonsson, Hallman et al., 1981). The effect on the peripheral noradrenergic system is reversible and returns to normal after approximately 7 to 10 days (Jonsson, Hallman, Ponzio, and Ross, 1981).

We used four experimental groups to investigate the effect of DSP4 on prepulse inhibition. The effects under baseline conditions were investigated in non-stressed APO-SUS and APO-UNSUS rats. To investigate the effect of DSP4 under mildly stressful conditions we isolated the APO-SUS and APO-UNSUS rats one day prior to the prepulse inhibition experiment (Sontag, Cools, and Ellenbroek, 2003a). The prepulse inhibition experiments were performed 14 days after the administration of DSP4.

Since DSP4 reduces the noradrenergic activity, we expected to see an increase in prepulse inhibition in the rats with a high functional noradrenergic activity (i.e. stressed APO-SUS and non-stressed APO-UNSUS rats). Less effect on prepulse inhibition was expected in the rats which already have a low functional noradrenergic activity (i.e. non-stressed APO-SUS and stressed APO-UNSUS rats). Surprisingly, we found a decrease in prepulse inhibition in stressed APO-SUS rats after DSP4 treatment. Since the most likely explanation for this phenomenon is the development of supersensitive receptors, we decided to investigate whether DSP4 indeed induces a change in the sensitivity of the noradrenergic receptors using the adrenoceptor agonist-induced reduction in head dips (Dooley, Mogilnicka et al., 1983).

Material and Methods

Animals

APO-SUS	Naïve (Sontag, Cools, and Ellenbroek, 2003a)	saline	DSP4
Non-isolated	6	6	6
Isolated	7	7	6
APO-UNSUS	Naïve (Sontag, Cools, and Ellenbroek, 2003a)	saline	DSP4
Non-isolated	8	8	7
Isolated	6	10	6

Table 2: Amount of rats used

All APO-SUS and APO-UNSUS rats were obtained from the Central Animal Laboratory of the University of Nijmegen. They weighed between 200 and 250 g at the time of experiment and had water and food freely available except during the testing. They were grouped housed (2-3 animals per cage) in temperature-controlled rooms with a standard 12 L: 12 D cycle: lights on from 0700 to 1900 hr. All experiments were performed according to institutional, national and international guidelines for animal care and welfare. The number of animals used in each group is indicated in table 2

Pharmacogenetic selection

Several years ago we started a breeding program to pharmacogenetically select APO-SUS and APO-UNSUS rats (Cools, Brachten, Heeren, Willemen, and Ellenbroek, 1990). The present experiments were performed with naive male APO-SUS and APO-UNSUS rats belonging to the 29th generation. APO-SUS rats are defined as animals born to an APO-SUS male and female; likewise APO-UNSUS rats are defined as animals born to an APO-UNSUS male and female.

Treatment

DSP4 Injection

Non-isolated Group

The rats received a single dose on DSP4 (Sigma-Aldrich, Steinheim Germany; 25 mg/ kg; 1ml / kg) injected i.p. The drug was dissolved in saline; consequently, the control animals received the same volume of saline. The dose was chosen on the basis of the study of Lapiz et al (Lapiz, Mateo et al., 2000) who showed a significant reduction in noradrenaline content after DSP4 treatment. The animals stayed grouped housed in their home cages for two weeks, allowing the peripheral noradrenergic system to recover (Jonsson, Hallman, Ponzio, and Ross, 1981).

Isolated Group

The rats were treated similar to the rats of the "non-isolated group", except that they were isolated in new cages one day prior to the prepulse inhibition experiment.

Prepulse Inhibition of the acoustic startle response

Two weeks after the administration of DSP4, the rats were tested for their performance in prepulse inhibition. The prepulse inhibition experiments were performed in acoustic startle chambers of San Diego Instruments (San Diego USA). Basically, the cage consists of a plexiglas tube (8.2 cm in diameter, 25 cm in length) resting on a plastic frame. A piezoelectric accelerometer mounted under the tube detected and transduced the motion of the tube. Stimulus delivery was done using the SR-LAB software, via a speaker mounted 10 cm above the cylinder. The computer software also digitized, rectified and recorded the response of the accelerometer, with hundred 1 ms readings collected beginning at stimulus onset. Startle amplitude was defined as the average of 100 readings. The whole system was mounted within a sound attenuating chamber. Throughout the startle session a background noise of 70dB was maintained.

The experiment started with a 5 min habituation period in the startle system with background noise. After this habituation period ten blocks of five trials were delivered to measure prepulse inhibition. Each of these blocks consisted of one startle trial (20 ms 120 dB broadband burst), one

no-stimulus condition and three different prepulse-startle pairing administered pseudorandomly. In these pairings the prepulse was 3, 5 or 10 dB above background. The prepulses were 20 ms broadband burst, followed by a startle pulse 100 ms later. The interval between two trials was between 10 and 20 s.

The startle amplitude was calculate as the mean of the 10 delivered startle trials

The degree of prepulse inhibition (in percentage) was calculated according to the formula

$$100 - \frac{\text{mean of all prepulses trials}}{\text{startle amplitude on startle trial}} \text{ x } 100$$

Head-dips

This test was based on the study of Dooley and colleagues. (Dooley, Mogilnicka, Delini, Waechter, Truog, and Wood, 1983). After injection of the drug, the rats were placed in a square box where the numbers of head-dips were detected automatically for 12 minutes. This box consists of a black plastic box of 70 x 70 cm. The bottom plate of this box is equipped with 32 holes. In the centre of the plate is a central platform (25 cm x 25 cm). The activity of the rats is measured with the help of infrared beams located at the side walls; 10 beams above the plate for measuring locomotor activity and another 12 beams underneath the plate for measuring the head-dips. This test was performed one week after the prepulse inhibition experiment. First we tried to replicate the experiment performed by Dooley and colleagues (Dooley, Mogilnicka, Delini, Waechter, Truog, and Wood, 1983) with the $alpha_2$ adrenoceptor agonist clonidine (Sigma, Steinheim Germany) in the dosage 0.065 and 0.13 mg/kg. Since it is known from previous studies (Carasso, Bakshi et al., 1998; Sontag, Cools, and Ellenbroek, 2003b submitted) that especially the $alpha_1$ adrenoceptors play a role in mediating prepulse inhibition, we also used the $alpha_1$-adrenergic agonist cirazoline (Research Biochemical Incorporated, Natick MA USA) in the dosage 0.1 and 0.5 mg/kg to evaluate the DSP4 induced changes in $alpha_1$-adrenoceptor sensitivity. The doses were chosen on the basis of the outcome of our previous study (Sontag, Cools, and Ellenbroek, 2003b submitted) where we showed that these doses were behaviourally active.

All drugs were dissolved in saline and given i.p. and the injected volume was 1ml/kg. The control groups were injected with the same volume of saline.

Statistics

The prepulse inhibition and startle response data were analyzed with a one-way analysis of variances (ANOVA) with the factor pre-treatment. For analysis the animals were separate in

isolated and non-isolated and each line was analysed separately. This was done because of the known difference in stress sensitivity and the known difference in the noradrenergic activity of these rats. A p-value of < 0.05 was considered to be significant.

The data of the head-dip experiment were analysed with a two-way analysis of variances. (ANOVA) with the factors pre-treatment (saline vs. DSP-4) and drug (saline vs. clonidine or cirazoline). The data were analysed separately for line and alpha-adrenergic drugs. The LSD-Fisher Test was used as post-hoc test. A p-value of < 0.05 was considered to be significant.

Results

Non-Isolated Group

Startle Response (Fig 10)

DSP4 did not significantly affect the startle amplitude in either APO-UNSUS rats ($F_{1,13} = 3.74$; $p > 0.05$) or APO-SUS rats ($F_{1,10} = 0.23$; $p > 0.05$), though a notable (non-significant) reduction was found in the non-isolated DSP4-treated APO-UNSUS rats.

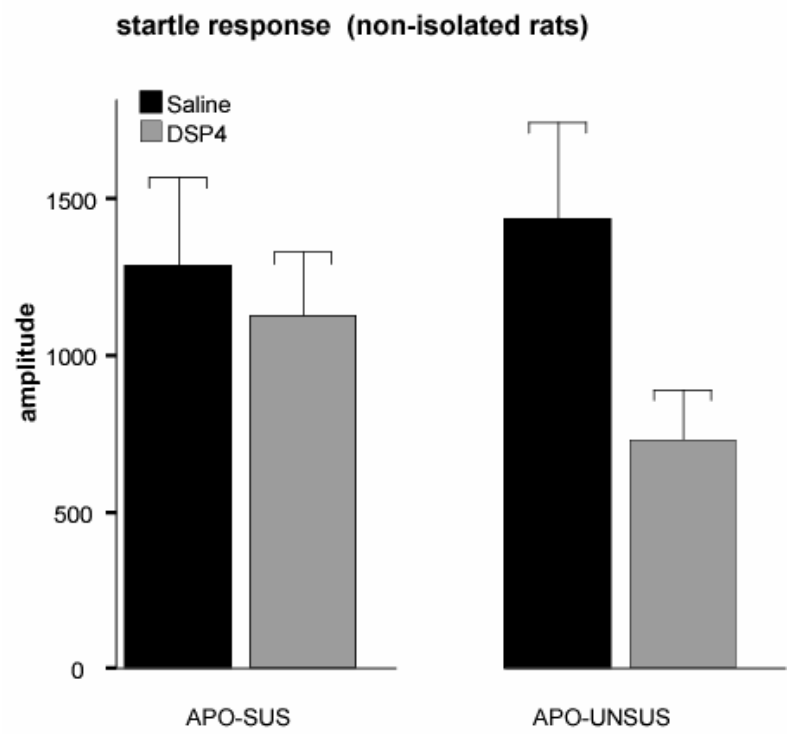

Fig 10: The Treatment with DSP4 had no effect on the startle response in non-isolated rats.

Prepulse inhibition (Fig 11)

No significant effect of DSP4 treatment on prepulse inhibition in non-isolated APO-SUS ($F_{1,10} = 0.25$; $p > 0.05$) and non-isolated APO-UNSUS rats was found ($F_{1,13} = 0.083$; $p > 0.05$).

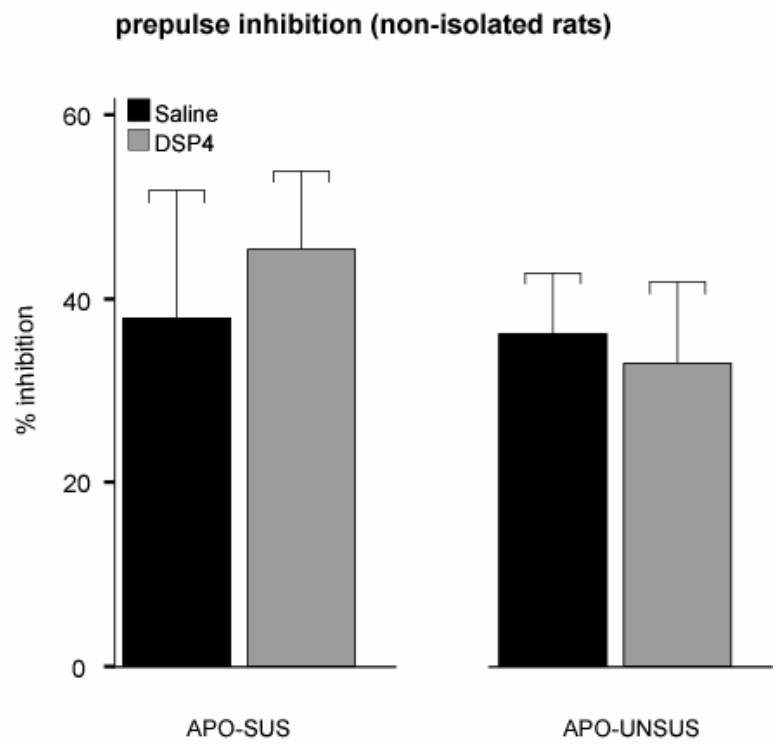

Fig 11: The treatment with DSP4 had no effect on the prepulse inhibition in non-isolated rats

Isolated Group

Startle Response (Fig 12)

DSP4 significantly reduced the startle response in isolated APO-SUS rats ($F_{1,11} = 6.59$; $p < 0.05$), but not in isolated APO-UNSUS rats ($F_{1,14} = 0.08$; $p > 0.05$).

Prepulse Inhibition (Fig 13)

DSP4 increased prepulse inhibition in the isolated APO-UNSUS rats ($F_{1,14} = 6.92$; $p < 0.05$) and reduced prepulse inhibition in the DSP4-pretreated isolated APO-SUS rats, though this just missed significance ($F_{1,11} = 4.35$; $p = 0.06$).

Head-Dips

Clonidine (Fig 14)

Clonidine significantly reduced the number of head-dips in both APO-SUS ($F_{5,26} = 10.29$; $p < 0.05$) and APO-UNSUS rats ($F_{5,39} = 9.67$; $p < 0.05$). At the lowest dose (0.065 mg/kg), clonidine significantly reduced the number of head-dips only in the DSP4 pre-treated ($p < 0.01$), but not in the saline pre-treated APO-UNSUS rats ($p > 0.05$). This dose did not significantly alter the number of head-dips in either DSP4 or saline pre-treated APO-SUS rats. The highest dose of clonidine reduced the number of head-dips in all animals.

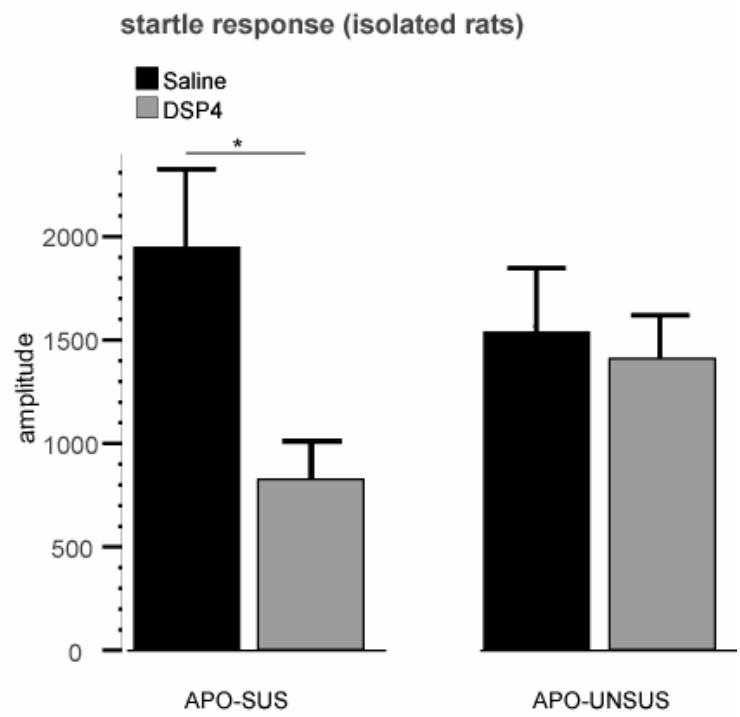

Fig 12: The treatment with DSP4 reduced the startle response in isolated APO-SUS rats; * $p < 0.05$

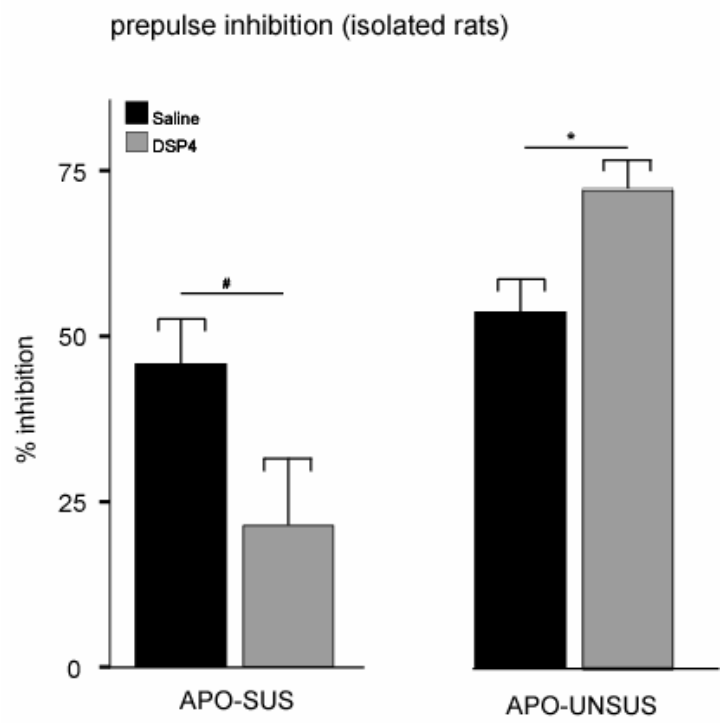

Fig 13: DSP4 increased the prepulse inhibition in isolated APO-UNSUS rats and showed a strong trend for a reduced prepulse inhibition in APO-SUS rats; * $p < 0.05$, # $p = 0.06$

Cirazoline (Fig 15)

The number of head-dips decreased in both APO-SUS ($F_{5,31} = 6.96$; $p < 0.01$) and APO-UNSUS rats ($F_{5,38} = 6.53$; $p < 0.01$) after cirazoline treatment. The lowest dose (0.1mg/kg) reduced the

number of head-dips in DSP4 pre-treated APO-SUS rats only ($p < 0.05$) but not in saline pre-treated APO-SUS rats ($p > 0.05$). The highest dose (0.5mg/kg) reduced the number of head-dips in both the saline ($p < 0.01$) and DSP4 pre-treated ($p < 0.01$) APO-SUS rats to a similar amount. In the APO-UNSUS rats, both the lowest and the highest dose reduced the number of head-dips to an equal amount in both saline and DSP-4 treated rats.

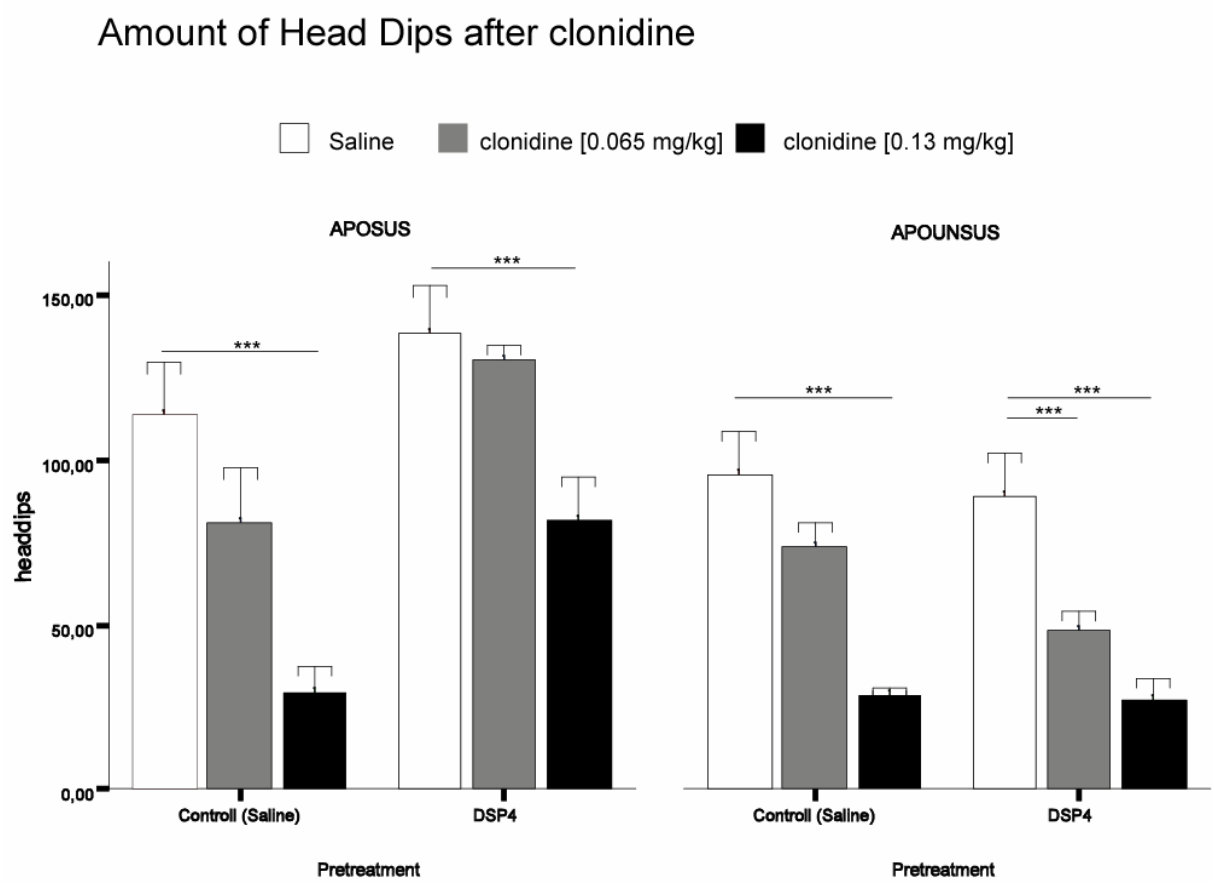

Fig 14: clonidine reduced the head dips in both lines of rats. The lowest dose clonidine reduced the head dips only in DSP4 pre-treated APO-SUS rats. The same dose had no effect in either saline or DSP4 pre-treated APO-SUS rats ; *** $p < 0.001$

Discussion

DSP4 did not affect prepulse inhibition (Fig 11) or the startle response (Fig 10) in non-isolated APO-SUS or APO-UNSUS rats. However, when the rats were isolated for one day DSP4 reduced the startle response in APO-SUS rats (Fig 13). The same treatment increased prepulse inhibition in APO-UNSUS rats and decreased it in APO-SUS rats. In addition, the lower dose of the $alpha_2$-agonist clonidine reduced the number of head-dips only in the DSP4 pre-treated APO-UNSUS rats, indicating supersensitive $alpha_2$-adrenoceptors in these animals. In APO-SUS rats, on the other hand, the lowest dose of cirazoline reduced the head-dips only in the DSP4 pre-treated rats indicating the development of supersensitive $alpha_1$-adrenoceptors in these rats.

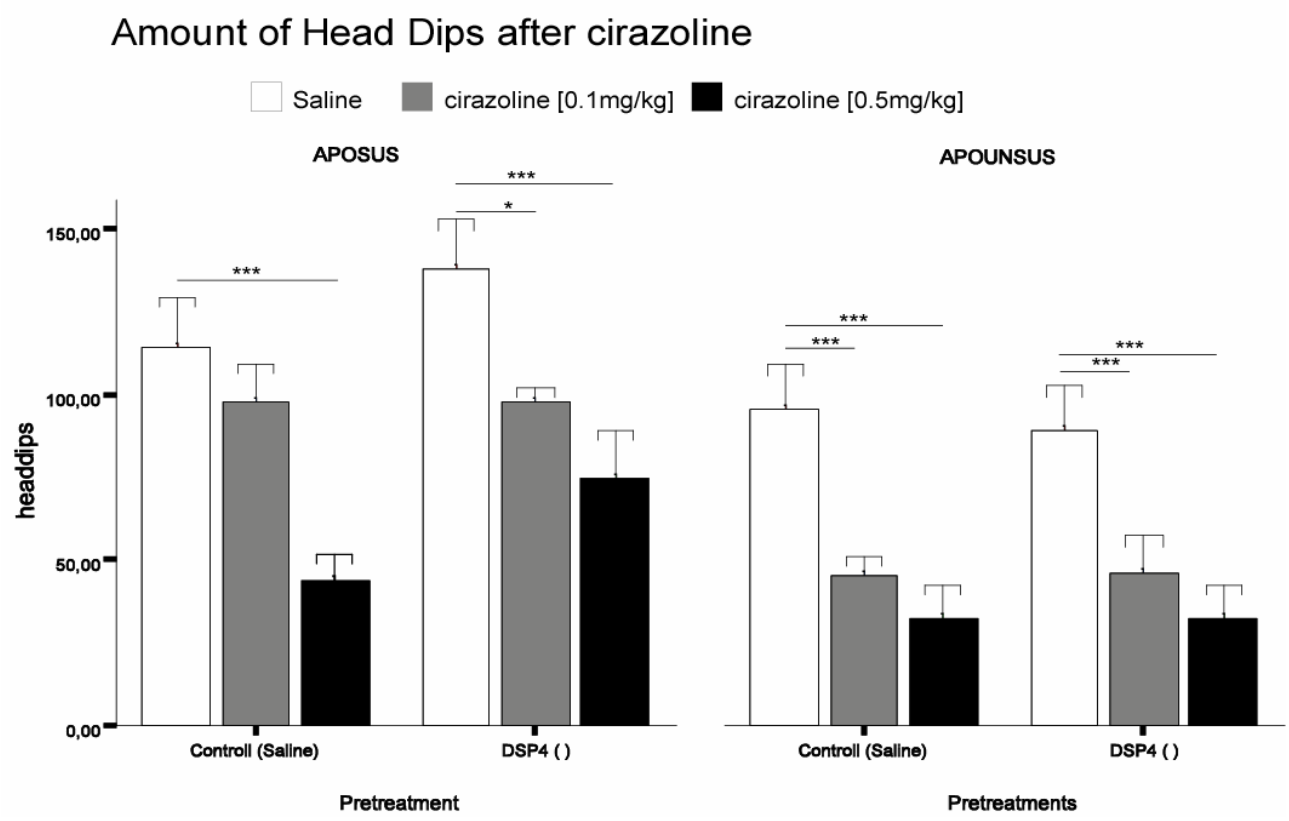

Fig 15: The treatment with cirazoline decrease the head dips in both lines of rats. The lowest dose reduced the head dips in DSP4 pre-treated APO-SUS rats but not in saline pre-treated APO-SUS rats. In APO-UNSUS rats both doses reduced the head dips to a similar amount independent from the pre-treatment; *** $p < 0.001$, * $p < 0.05$

Prepulse Inhibition

The data are not in complete agreement with our original hypothesis, namely that a reduction in noradrenaline levels would lead to an increase in prepulse inhibition, especially in animals with a high functional noradrenergic activity (i.e. non-isolated APO-UNSUS rats and isolated APO-SUS rats). In fact we found no effect of DSP4 on prepulse inhibition in non-isolated animals at all. When our data are compared to those of our previous study (Sontag, Cools, and Ellenbroek, 2003a), it is clear that the prepulse inhibition in the non-isolated APO-SUS rats in the present experiment is relatively low (see

Table 3). Likewise, the prepulse inhibition of the non-isolated APO-UNSUS animals is relatively high. The only difference between the two experiments is that the non-isolated animals in the present study received a saline injection two weeks prior to the prepulse inhibition experiment. Saline injections are known to induce a mild stress in animals (Radu, Brodin et al., 2001; Kiyatkin and Wise, 2001; Baumann, Elmer et al., 2000). Moreover, there is evidence that these saline injections can induce long-lasting alterations. Indeed, Antelman and colleagues have shown that such a saline injection increased the sensitivity to haloperidol two weeks after a single saline

injection (Antelman, Kocan et al., 1986). Since mild stress is known to reduce prepulse inhibition in the APO-SUS and increase it the APO-UNSUS rat, (Sontag, Cools, and Ellenbroek, 2003a) we can only conclude that the saline injection indeed induced a mild stress in these animals, which lasted for at least 2 weeks.

APO-SUS	**Naïve (Sontag, Cools, and Ellenbroek, 2003a)**	**saline**	**DSP4**
Non-isolated	61%	38%	47%
Isolated	42%	46%	21%
APO-UNSUS	**Naïve (Sontag, Cools, and Ellenbroek, 2003a)**	**saline**	**DSP4**
Non-isolated	25%	36%	33%
Isolated	51%	54%	72%

Table 3: Effects on the prepulse inhibition

One of the most intriguing findings of the present study is that depletion of noradrenaline with the neurotoxin DSP4 did not affect the prepulse inhibition in rats that were non-isolated, but altered it in animals that were isolated for 24 hr prior to the test (see Table 3). As discussed (Sontag, Cools, and Ellenbroek, 2003a), short term isolation also acts as a mild stressor. Since both the non-isolated and the isolated animals received an injection two weeks prior to the experiment, this strongly suggests that the stress of the injection differs from the stress of the isolation, since DSP-4 affected the prepulse inhibition only in the isolated animals.

It has been shown that noradrenaline, especially at level of the $alpha_1$-adrenoceptors regulate prepulse inhibition: an increase in noradrenergic activity decreases prepulse inhibition (Sontag, Cools, and Ellenbroek, 2003b submitted; Carasso, Bakshi, and Geyer, 1998) whereas a decrease in noradrenergic activity increases prepulse inhibition (Depoortere, Perrault et al., 1997). The treatment with DSP4 reduces the noradrenergic activity by destroying the terminals of the locus coeruleus; therefore we expected to find an increased prepulse inhibition after this treatment. The increased prepulse inhibition found in the isolated APO-UNSUS rats fits perfectly with this expectation, indicating that the noradrenergic activity of the locus coeruleus is involved in modulating the prepulse inhibition. However, DSP4 was found to decrease the prepulse inhibition

in isolated APO-SUS rats, namely a phenomenon that occurs after an increase rather than a decrease in the noradrenergic activity.

Dooley and colleagues have reported a significant reduction in the number of head-dips in DSP4 treated rats after the injection of the alpha$_2$ antagonist clonidine (Dooley, Mogilnicka, Delini, Waechter, Truog, and Wood, 1983). Moreover, they showed that DSP4 treatment can indeed induce supersensitivity, at least with respect to the alpha$_2$ adrenoceptors (Dooley, Bittiger et al., 1983). In agreement with Dooley and colleagues we found that the highest dose of clonidine reduced the number of head-dips in both APO-SUS and APO-UNSUS rats, independent of the pre-treatment. However the low dose reduced the number of head-dips only in the DSP4 pre-treated but not the saline pre-treated APO-UNSUS rats, indicating increased alpha$_2$-adrenoceptor sensitivity. From previous studies it is known that the alpha$_1$-adrenoceptor (Sontag, Cools, and Ellenbroek, 2003b submitted), rather than the alpha$_2$-adrenoceptor is involved in modulating prepulse inhibition (Abduljawad, Langley et al., 1997; Kehne, Padich et al., 1996). Thus we used the alpha$_1$-adrenoceptor agonist cirazoline to investigate the changes in adrenoceptor sensitivity. The lowest dose of cirazoline reduced the head-dips in the DSP4 pre-treated but not in the saline pre-treated APO-SUS rats, indicating supersensitive alpha$_1$-adrenoceptors in these rats. In APO-UNSUS rats cirazoline reduced the head-dips to an equal amount in both saline and DSP4-pretreated rats indicating the same receptor sensitivity in both treatment groups.

APO-SUS	**Naïve (Sontag, Cools, and Ellenbroek, 2003a)**	**saline**	**DSP4**
Non-isolated	1079.7	1229.5	1154.3
Isolated	705.7	1947.4	825.0
APO-UNSUS	**Naïve (Sontag, Cools, and Ellenbroek, 2003a)**	**saline**	**DSP4**
Non-isolated	639	1434.9	730.6
Isolated	776.0	1527.3	1402.8

Table 4: Effects on the Startle response

This increased sensitivity on the basis of the alpha$_1$-adrenoceptors might explain the unexpected finding of a reduced prepulse inhibition in isolated DSP4 pre-treated APO-SUS rats. Stress is known to increase the functional noradrenergic activity in these rats. The treatment with DSP4 destroys locus coeruleus terminals selectively, therefore other noradrenergic sources like the

tegmental cell system or the medullary group are still active. In addition it can not be fully excluded that some locus coeruleus neurons have survived the treatment. Thus it may well be that the reduced prepulse inhibition found in these rats was caused by an increase in functional noradrenergic activity in combination with supersensitive $alpha_1$-adrenoceptors.

Startle Response

The noradrenergic activity can also influence the startle response. The treatment with DSP4 reduced the startle response in isolated APO-SUS rats. This finding is in line with the study from Adams and colleagues (Adams and Geyer, 1981); they found that lesioning of the locus coeruleus reduced the startle response. Both sets of data suggest that the reduction of the noradrenergic activity from the locus coeruleus reduce the startle response. However we discussed above that the reduction in prepulse inhibition in these rats can best be explained by an increased noradrenergic activity at the level of the $alpha_1$-adrenoceptors. Thus these results suggest that the startle response and the prepulse inhibition are modulated by different adrenoceptors. In agreement with this, it was found that the $alpha_2$-adrenoceptor agonist clonidine (Davis, Cedarbaum et al., 1977), but not the $alpha_1$ antagonist prazosin (Depoortere, Perrault, and Sanger, 1997) reduced the basal startle amplitude.

It should be noted that the startle response in the present experiments was quite high when compared to that found in our previous study (see Table 2). However, the rats were treated slightly different in these two studies. In the previous study the rats that were used for measuring startle response weighted between 200 and 250 g. In the present study the rats weighted between 200 and 250 g when they were injected with DSP4 or saline respectively. Since the peripheral noradrenergic system has to recover from this treatment we waited two weeks before measuring the startle response (see Material and Methods). Therefore the rats in the present experiment were heavier than the ones used in our previous study. Because the startle amplitude positively correlates with weight (Schaeffer, 1987; Hall, 1984; Blaszczyk and Tajchert, 1996), we speculate that differences in weight underlie the noted differences in startle amplitudes.

In conclusion

The present data show that lesioning of the noradrenergic neurotransmission using the neurotoxin DSP4 differentially alters the prepulse inhibition in APO-SUS and APO-UNSUS rats. Thus it reduced the prepulse inhibition in isolated APO-SUS but increased it in isolated APO-UNSUS rats. Moreover, we could show that the behavioural alterations after DSP4 pre-treatment critically depend upon the level of stress of these rats. Thus DSP4 did not affect prepulse inhibition in non-isolated (non-stressed) rats but altered it in isolated (stressed) APO-SUS and APO-UNSUS rats.

Finally we could show that DSP4 treatment induced supersensitivity of the alpha$_1$-adrenoceptors in APO-SUS rats and of alpha$_2$-adrenoceptors in APO-UNSUS rats. In summary these data show that the noradrenergic terminals of the locus coeruleus (which are selectively destroyed by DSP4) modulate the prepulse inhibition in APO-SUS and APO-UNSUS rats, but that the effect depends on the level of stress of these animals.

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C h a p t e r 5

NORADRENERGIC ACTIVITY WITHIN THE NUCLEUS ACCUMBENS MODULATES PREPULSE INHIBITION.

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Abstract

Recent experiments in our laboratory suggested that the noradrenergic activity modulates the performance in prepulse inhibition in rats. However the mechanism by which noradrenaline reduces prepulse inhibition is unclear. Noradrenaline is known to control dopamine release in the nucleus accumbens and several studies have shown that the increase in accumbal dopamine reduce prepulse inhibition. Therefore we hypothesised that increased noradrenergic activity in the nucleus accumbens contributes to reductions in prepulse inhibition. In addition according to the gaiting theory by Cools and colleagues noradrenaline controls the information input from the basolateral amygdala and hippocampus to the nucleus accumbens, structures that have been identified to modulate prepulse inhibition. We implanted cannulas in the nucleus accumbens of rats in order to test the effects of the $beta_2$- adrenoceptor agonist isoproterenol, the $alpha_1$ adrenoceptor agonist phenylephrine and the $alpha_1$ adrenoceptor antagonist phentolamine, drugs that are known to control accumbal dopamine release and control the information input to the nucleus accumbens. We found that none of the drugs given separately was able to alter the prepulse inhibition. However when given isoproterenol and phentolamine together the prepulse inhibition was significantly reduced. Although the mechanism is not fully understood these data show that the noradrenergic activity in the nucleus accumbens modulates prepulse inhibition.

Introduction

In patients suffering from neuropsychiatric diseases like Huntington's disease (Swerdlow, Paulsen et al., 1995) and schizophrenia (Braff and Geyer, 1990) a reduced prepulse inhibition was found. This phenomenon refers to a decrease in startle response that occurs when the startle stimulus is preceded by a weaker stimulus. Recent experiments in our laboratory showed that environmental

factors like mild stress modulate prepulse inhibition in apomorphine susceptible (APO-SUS) and apomorphine unsusceptible (APO-UNSUS) rats. Thus a single short-term social isolation was found to reduce prepulse inhibition in APO-SUS rats but to increase it in APO-UNSUS rats (Sontag, Cools et al., 2003a). Mild stress is known to increase the functional noradrenergic activity in APO-SUS rats but decrease it in APO-UNSUS rats (Cools, Brachten et al., 1990; Cools and Gingras, 1998). In addition the $alpha_1$-agonist cirazoline was found to reduce prepulse inhibition in non-stressed APO-SUS rats, but not in non-stressed APO-UNSUS rats (Sontag, Cools et al., 2003b submitted) a finding that is in line with the study by Carasso and colleagues (Carasso, Bakshi et al., 1998). Furthermore it was found that the destruction of locus coeruleus (LC) terminals with the selective neurotoxin DSP4 increased prepulse inhibition in stressed APO-UNSUS (Sontag, Cools et al., 2003c submitted). These data suggest that a high noradrenergic activity is associated with a reduction in prepulse inhibition and that a low noradrenergic activity is associated with an increase in noradrenergic activity. However the mechanism by which noradrenaline disrupts prepulse inhibition is still unclear.

The locus coeruleus sends noradrenergic fibers to, among others, the nucleus accumbens (Cedarbaum and Aghajanian, 1978), a structure often implicated in the modulation of prepulse inhibition for (review see Swerdlow, Geyer et al., 2001). Thus, an increase in dopamine activity within this nucleus was found to disrupt prepulse inhibition (Swerdlow, Geyer, and Braff, 2001; Swerdlow, Caine et al., 1992; Wan and Swerdlow, 1993; Reijmers, Vanderheyden et al., 1995; Kretschmer and Koch, 1998). Interestingly, noradrenaline is known to control the dopamine release in the nucleus accumbens. This function is associated with regulating the information input of the basolateral amygdala (BLA) and the hippocampus into the nucleus accumbens. The gating theory by Cools and colleagues (Cools, van den Bos et al., 1991) states that activation of the $beta_2$-adrenoceptors blocks the information input of the hippocampus closing thereby the hippocampal gate. This is always associated with an increase in dopamine activity at the level of the D_2 receptors. On the other hand inhibition of the $alpha_1$-adrenoceptors blocks the information input from the basolateral amygdala (BLA) closing thereby the amygdala gate. This is always associated with an increase in dopaminergic activity at the level of a subtype of dopamine receptor that differs from the classical D_1 and D_2 receptors (Cools, van den Bos, Ploeger, and Ellenbroek, 1991). Recently, using the microdialysis technique, Tuinstra and colleagues showed that stimulation of $beta_2$ and inhibition of $alpha_1$ adrenoceptors increase the release of accumbal dopamine (Tuinstra and Cools, 2000). Remarkably, also the stimulation of accumbal $alpha_1$-adrenoceptors was found to increase accumbal dopamine. It was concluded that the agonist acts indirectly at the presynaptic site thereby inhibiting the release of noradrenaline from its terminal and subsequently disinhibits the release of

dopamine. The antagonist has a direct effect by inhibiting the postsynaptic receptors on the corresponding dopamine terminals thereby facilitating the release of dopamine.

Given the fact that noradrenaline regulates the accumbens dopamine release, and that accumbal dopamine plays a crucial role in prepulse inhibition (Swerdlow, Geyer, and Braff, 2001), we hypothesised that the accumbal noradrenaline transmission is involved in modulating prepulse inhibition. In order to investigate the effect of noradrenaline in the nucleus accumbens we locally applied $alpha_1$ or $beta_2$ adrenoceptor agents and studied their effect on prepulse inhibition. Because activation of the $beta_2$-adrenoceptors enhances the release of mesolimbic dopamine at the level of the D_2 receptors we expected to find a decrease in prepulse inhibition after intra-accumbal application of the $beta_2$ antagonist isoproterenol. Other studies (Sontag, Cools, and Ellenbroek, 2003b submitted; Carasso, Bakshi, and Geyer, 1998; Varty, Bakshi et al., 1999) indicated that especially the $alpha_1$-adrenoceptors mediate prepulse inhibition. Therefore we also tested the $alpha_1$-agonist phenylephrine and the $alpha_1$-antagonist phentolamine since both drugs increase accumbal dopamine. According to the gaiting theory there are two distinct dopamine systems in the nucleus accumbens. By the combined administration of isoproterenol and phentolamine we tried to activate these two systems.

Materials and Methods

Animals

All male Wistar rats were obtained from the Central Animal Laboratory of the University of Nijmegen. They weighed between 200 and 250 g at the time of experiment and had water and food freely available except during the experiment. They were grouped housed (2-3 animals per cage) in temperature-controlled rooms with a standard 12 L: 12 D cycle: lights on from 0700 to 1900 hr. All experiments were performed according to institutional, national and international guidelines for animals care and welfare.

Stereotactic Operation

The rats were anaesthetized with sodium pentobarbital (60mg/kg, i.p., Narcovet®) and placed in a stereotaxic apparatus. A guide cannula (5.5 mm in length, 0.65 mm outer diameter, 0.3 mm inner diameter), was aimed at the right and left nucleus accumbens according to previously described procedures (Saigusa, Tuinstra et al., 1999). The following coordinates were used: anterior, 10.6 mm; lateral, 1.5 mm and vertical, 3.5 mm according to the atlas of Paxinos and Watson (Paxinos and Watson, 1986). The cannula was angled 10° laterally. The cannulas were fixed on to the skull with the use of stainless steel screws and dental cement. The guide cannula contained an inner cannula to prevent infections as well as occlusions. The rats were placed in single cages and were allowed to

recover from surgery for the next two weeks. To reduce the stress of injection, the animals were habituated to the injection procedure. Three days before the start of the experiment the animals were handled, the next day the screws of the cannulas were opened and one day prior to the prepulse inhibition test the injection itself was simulated by the use of an empty Hamilton syringe.

Treatment and drugs

Immediately before placing the rats in the prepulse inhibition chamber the rats received a bilateral injection in the nucleus accumbens. The injection depth was 12 mm. We used the $beta_2$-adrenoceptor agonist isoproterenol (Sigma, Schnelldorf Germany) in the doses of 0.5μg and 1μg; the $alpha_1$ adrenoceptor agonist phenylephrine (Sigma Schnelldorf Germany) was given in the doses of 3μg, 5μg and 10μg. Furthermore we used the $alpha_1$-antagonist phentolamine (Sigma Schnelldorf Germany) in the dose 2.5 μg. The doses were chosen on the basis of the studies from Tuinstra and colleagues (Tuinstra and Cools, 2000). Based on the outcome of the experiments we further used a combination of 1μg isoproterenol and 2.5μg phentolamine. All drugs were dissolved in saline and applied in a volume of 0.5μl per side. The control rats were injected with saline and all rats were used only once.

Prepulse Inhibition of the acoustic startle response

Two weeks after the operation the rats were placed in the experimental room and had 30 minutes to habituate. The prepulse inhibition experiments were performed in an acoustic startle chamber of San Diego Instruments. Basically, the cage consists of a plexiglas tube (8.2 cm in diameter, 25 cm in length) resting on a plastic frame. A piezoelectric accelerometer mounted under the tube detected and transduced the motion of the tube. Stimulus delivery was done using the SR-LAB software, via a speaker mounted 10 cm above the cylinder. The computer software also digitized, rectified and recorded the response of the accelerometer, with 100 1 ms readings collected beginning at stimulus onset. Startle amplitude was defined as the average of 100 readings. The whole system was mounted within a sound attenuating chamber. Throughout the startle session a background noise of 70dB was maintained.

The experiment started with a 5 min habituation session in the startle system with background noise. After this habituation period ten blocks of five trials were delivered to measure prepulse inhibition. Each of these blocks consisted of one startle trial (120 dB), one no-stimulus condition and three different prepulse-startle pairing administered pseudorandomly. In these pairings the prepulse was 3, 5 or 10 dB above background. These prepulse were always 20 ms broad band burst and always followed by startle pulse 100 ms later. The interval between two trials was between 10 and 20 sec.

The startle amplitude was calculate as the mean of 10 delivered startle trials

The degree of prepulse inhibition (in percentage) was calculated according to the formula

$$100 - \frac{\text{mean of all prepulses trials}}{\text{startle amplitude on startle trial}} \times 100$$

Histology

After testing the rats in the prepulse inhibition they were deeply anaesthetized with pentobarbital (60mg/kg, i.p., Narcovet®) and perfused intracardially with 4% formaldehyde solution. The brains were removed and postfixed in the same fixative for at least 24h. Vibratome sections (100µm) were cut out to determine the location of the injection.

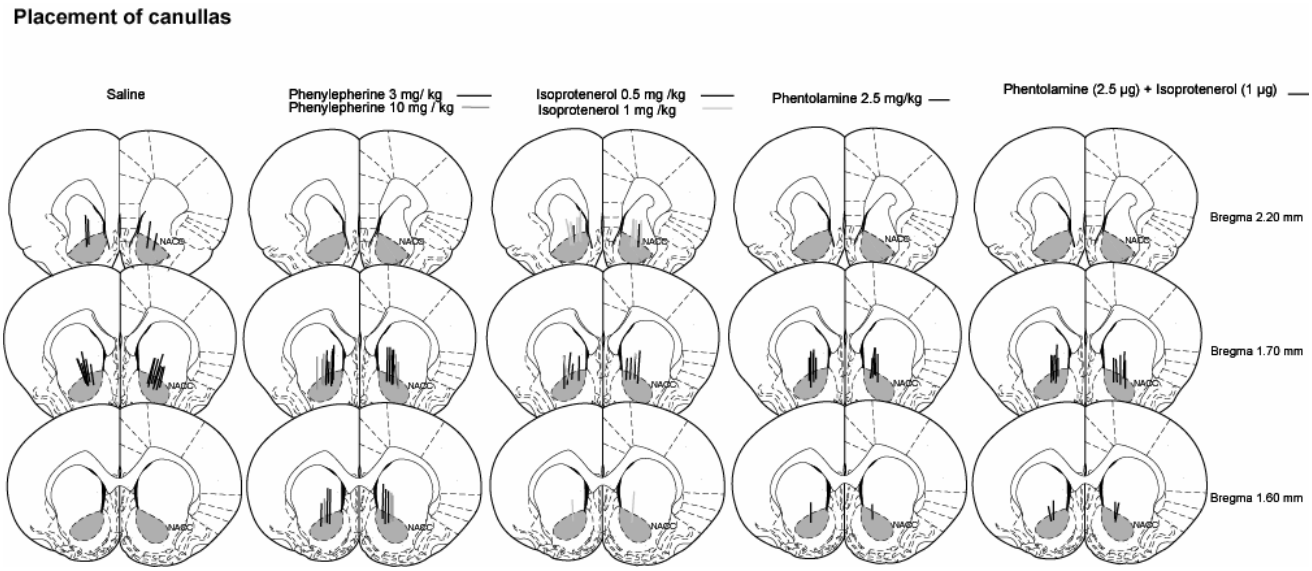

Fig 16: Placement of cannulas

Statistics

The prepulse inhibition and startle response data were analysed with a one-way analysis of variances (ANOVA) with the factor dose. Post-hoc analyses were done with the LSD-Fisher Test. A p-value of < 0.05 was considered to be significant. Each drug was analysed separately.

Results:

Histology

Placement of cannulas (Fig 16)

Only rats with a correct placement of the cannula were included in the analysis. In total 57 rats were operated, 9 rats have been taken out because the placement was incorrect.

Fig 16 shows the placement of the cannulas of the rats that were analysed.

Startle:

Isoproterenol (Fig 17)

The beta$_2$-adrenoceptor agonist isoproterenol induced an increase in the startle response ($F_{2,18}$ = 18.57 ; $p < 0.05$) with the highest dose (1µg) being significantly larger than that seen in the saline control ($p < 0.001$).

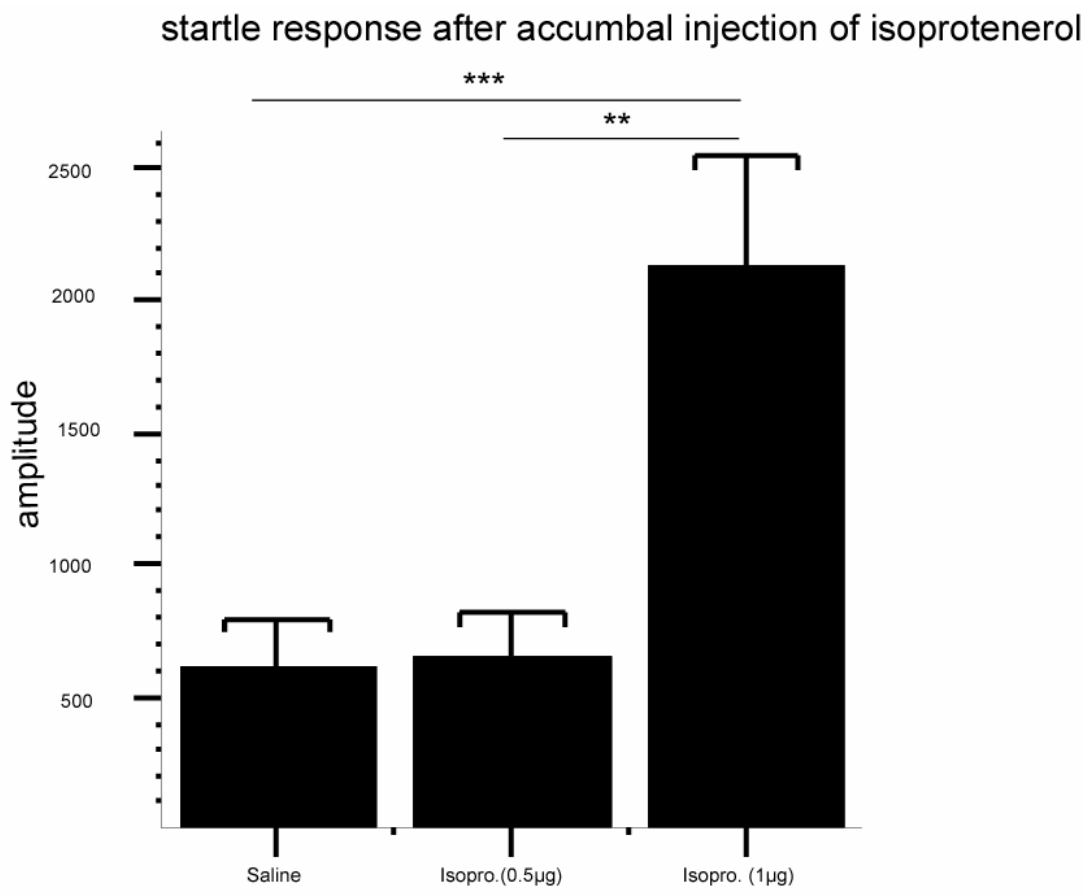

Fig 17: startle response after accumbal injection of isoproterenol. Only the highest dose of isoproterenol increased the startle significantly; ** $p < 0.01$, *** $p < 0.001$

Phenylephrine and Phentolamine (Fig 18)

Neither the alpha$_1$-adrenoceptor agonist phenylephrine nor the alpha$_1$-antagonist phentolamine significantly altered the startle response ($F_{3,25}$ = 2.75 ; $p > 0.05$).

Combination of alpha-antagonist and beta agonist (Fig 19)

The accumbal injection of isoproterenol and phentolamine did not alter the startle response compared to saline injection ($F_{3,24}$ = 7.98; $p < 0.001$; posthoc $p > 0.05$). However compared to

isoproterenol, the combined injection of isoproterenol and phentolamine significantly reduced the startle ($p < 0.01$)

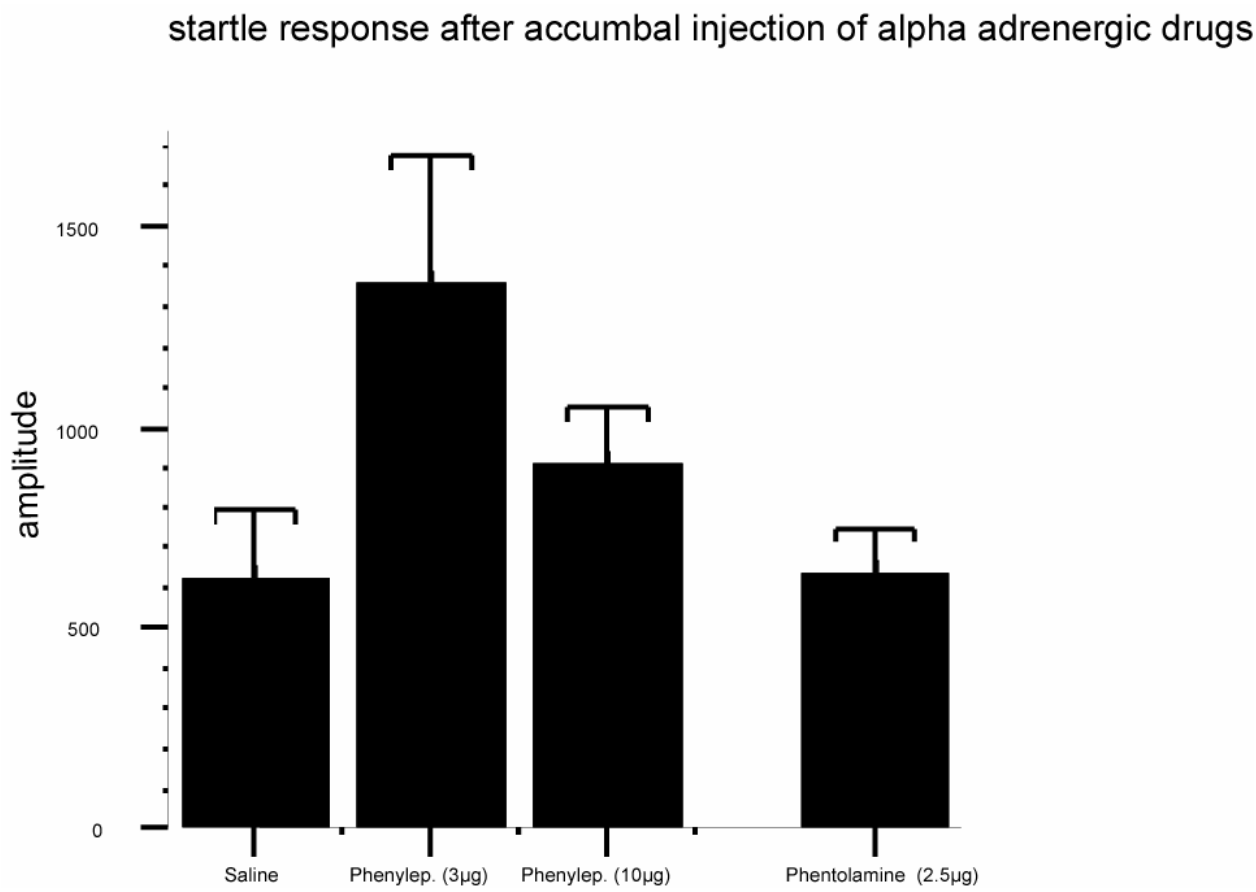

Fig 18: Startle response after accumbal injection of alpha-adrenergic drugs

Prepulse inhibition

Isoproterenol (Fig 20)

The local application of the $beta_2$ agonist isoproterenol into the nucleus accumbens had no effect on the prepulse inhibition ($F_{2,18} = 1.83$; $p > 0.05$)

Phenylephrine and Phentolamine (Fig 21)

After local application of the $alpha_1$ agonist phenylephrine or the $alpha_1$ antagonist phentolamine into the nucleus accumbens the prepulse inhibition was not significantly altered ($F_{3,25} = 1.31$; $p > 0.05$)

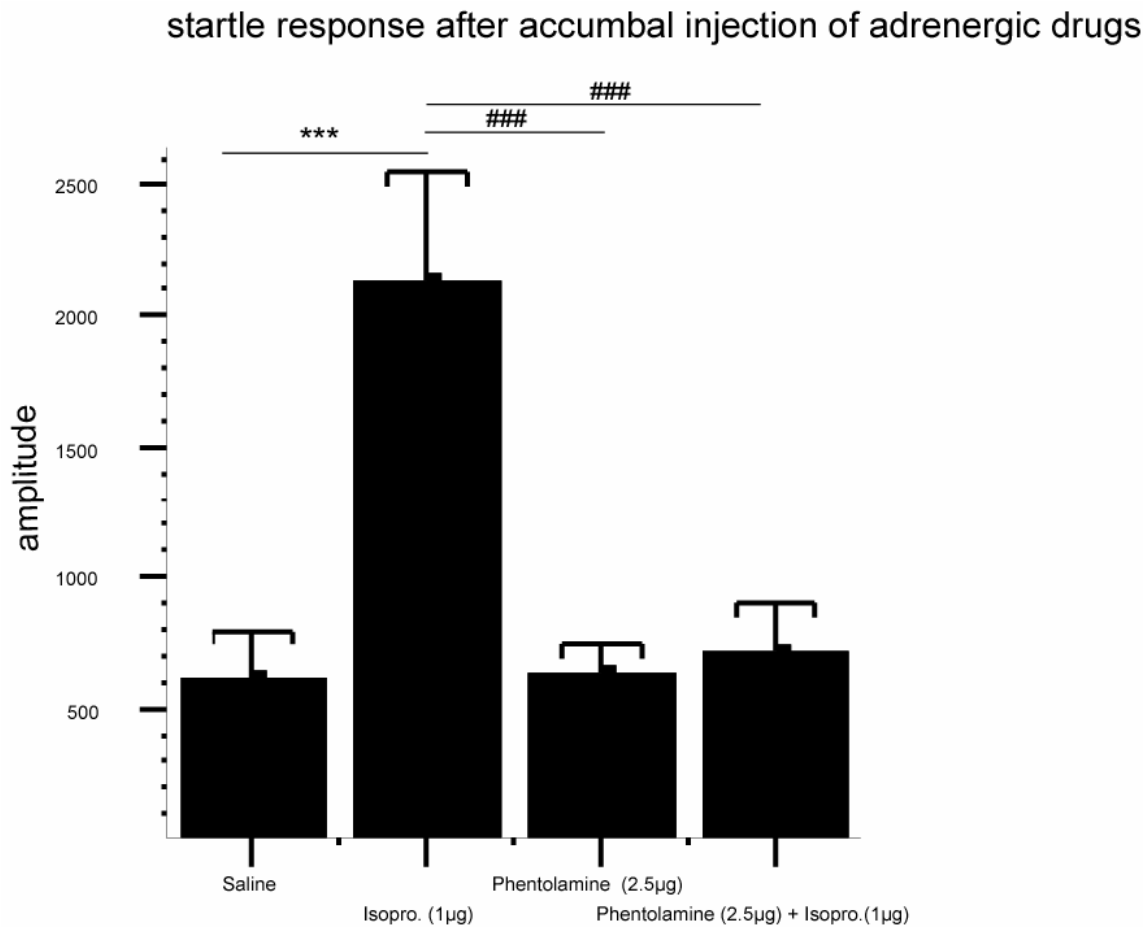

Fig 19: startle response after accumbal injection of adrenergic drugs: The increase in startle after isoproterenol was blocked by phentolamine. *** $p < 0.001$; ## $p < 0.01$, ### $p < 0.001$ against isoproterenol 1µg

Combination of alpha-antagonist and beta agonist (Fig 22)

In contrast to the single injection of either phentolamine or isoproterenol, the combined administration of phentolamine and isoproterenol induced a significant reduction in prepulse inhibition ($F_{3,24} = 3.30$; $p < 0.05$).

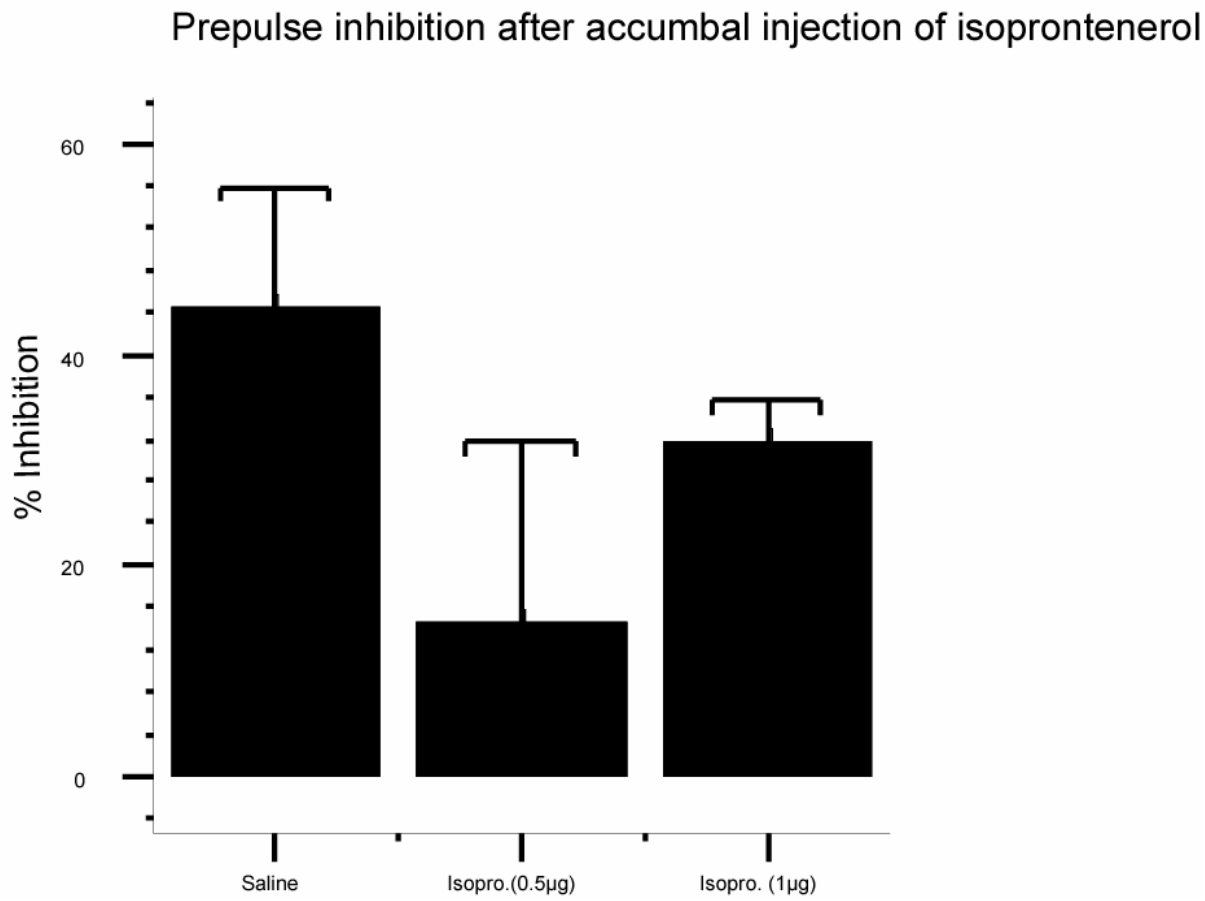

Fig 20: PPI after accumbal injection of isoproterenol

Discussion

The present data show that stimulation of accumbal adrenoceptors differentially modulates startle response and prepulse inhibition. The $beta_2$-agonist isoproterenol increased the startle response without affecting prepulse inhibition. Both the $alpha_1$ adrenoceptor agonist phenylephrine and the $alpha_1$ adrenoceptor antagonist phentolamine did not alter the startle response or prepulse inhibition. Noteworthy, a joint application of isoproterenol and phentolamine reduced the startle response compared to isoproterenol alone. Furthermore this combination decreased prepulse inhibition compared to saline treated rats.

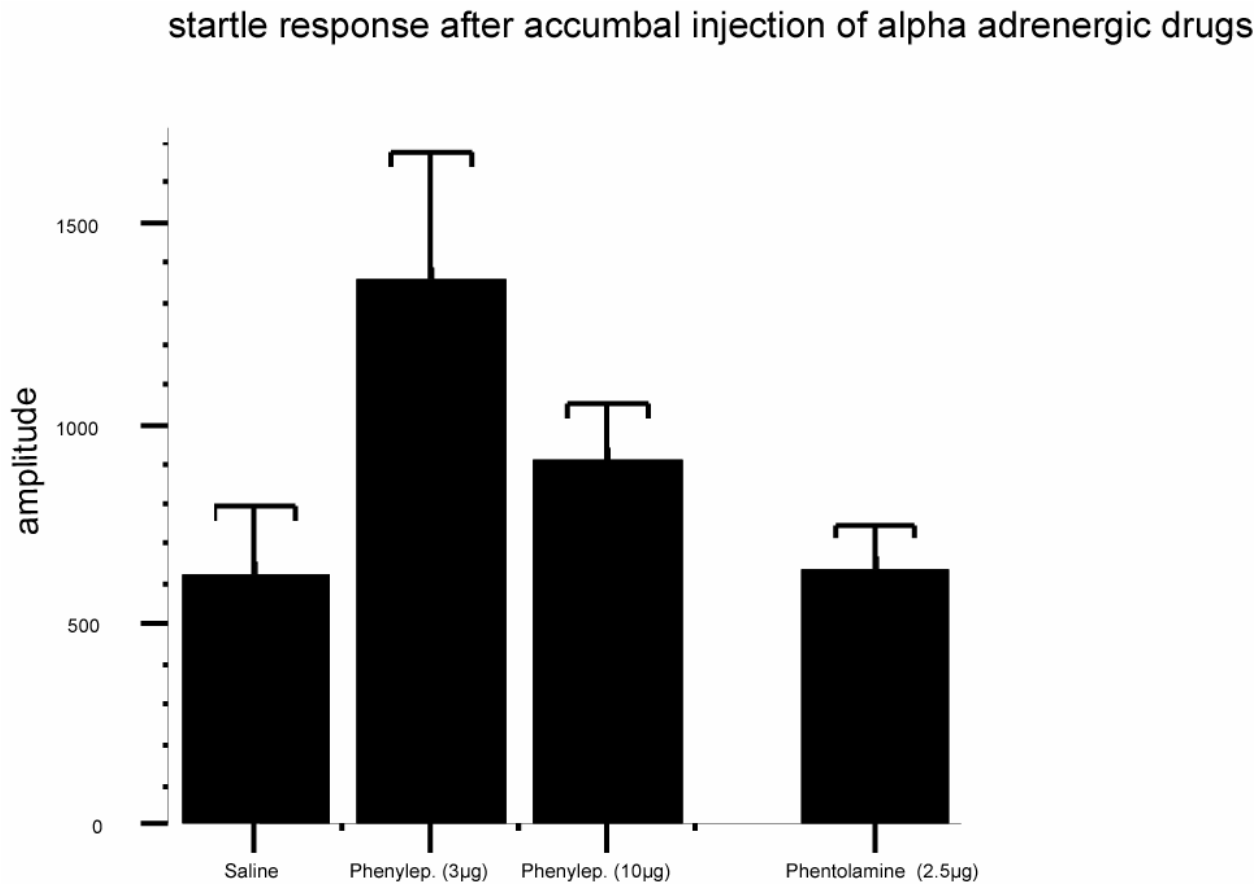

Fig 21: PPI after accumbal injection of alpha adrenergic drugs

Startle response

Intraperitoneal (Carasso, Bakshi, and Geyer, 1998) or intrathecal (Astrachan and Davis, 1981) application of $alpha_1$-agonist were found to increase the startle response whereas a $beta_2$-agonist had no effect. This is in line with a previous study from our laboratory (Sontag, Cools, and Ellenbroek, 2003b submitted), where we reported no effect of the $beta_2$- agonist clenbuterol on the startle after intraperitoneal injection. In the present study we found the opposite after accumbal application: phenylephrine had no effect on startle, whereas isoproterenol in the highest dose significantly increased the startle response. Thus the present study suggests that accumbal $alpha_1$ adrenoceptors are unlikely to contribute to the increased startle response seen after systemic application. It is more difficult to explain why local accumbal, but not systemic application of a beta agonist increased the startle response. One possibility might be that the effects of stimulation of accumbal beta receptors are counteracted by beta receptors in another brain structure. Alternatively, different beta receptors might be involved. Thus, clenbuterol (which is ineffective after systemic application) is a $beta_2$ agonist, whereas isoproterenol (which is effective after local application) is a mixed $beta_1$/$beta_2$ agonist.

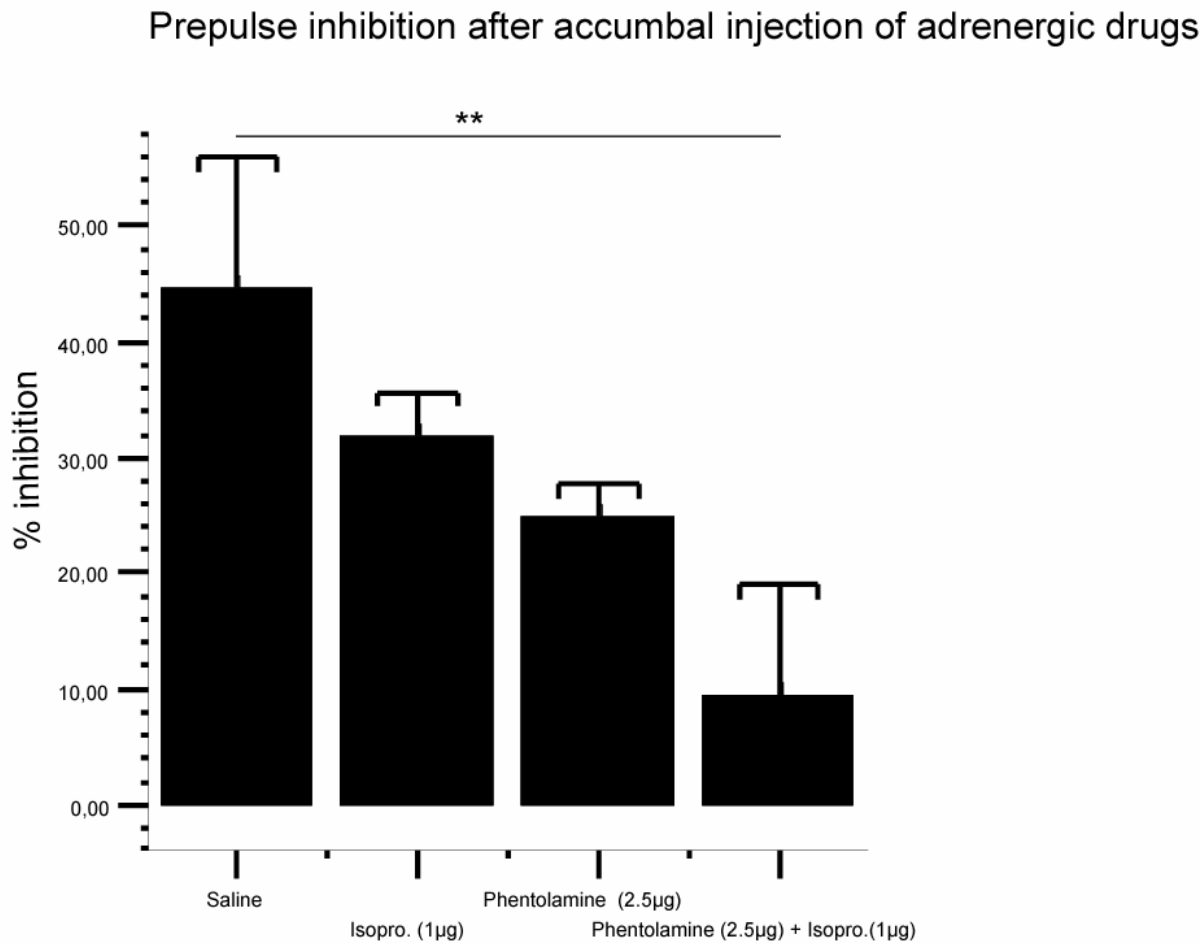

Fig 22: PPI after accumbal injection of adrenergic drugs. The combined injection of phentolamine and isoproterenol reduced the ppi. ** $p < 0.01$

Prepulse inhibition

The main interest of this study was to investigate the effects of intra-accumbal applied noradrenergic drugs on prepulse inhibition in rats. As discussed in the introduction, both $alpha_1$ agonists and antagonists and $beta_2$ agonists can increase the release of dopamine when applied to the nucleus accumbens (Tuinstra and Cools, 2000). However, even though several papers have shown that increasing the dopamine transmission in the nucleus accumbens reduces prepulse inhibition (Swerdlow, Mansbach et al., 1990), we did not find any reduction after application of a adrenoceptor agonist or antagonist. Interestingly, when the $beta_2$ agonist isoproterenol was combined with the $alpha_1$ antagonist phentolamine, prepulse inhibition was significantly reduced. It has been suggested that, in order to disrupt prepulse inhibition, the increase in dopamine release in the accumbens must be very high (Zhang, Pouzet et al., 2000), which would be in line with the current data. However, the data actually suggest that it is not just the amount of dopamine that is relevant, but that it might be the combined activation of two independent dopaminergic systems that is essential for disruption of prepulse inhibition. Thus according to the gating theory proposed

by Cools and colleagues (Cools, van den Bos, Ploeger, and Ellenbroek, 1991) the accumbal dopamine release is controlled by two distinct systems. The stimulation of $beta_2$-adrenoceptors increases the dopaminergic activity at a system that is associated with the D_2-receptors, and inhibition of the $alpha_1$ adrenoceptors increases the dopaminergic activity at a system which is associated with a dopamine receptor that differs from the classical D_1 and D_2 receptor. Furthermore, the noradrenergic activity in the nucleus accumbens is known to regulate the information input of the hippocampus (via the $beta_2$ and D_2 receptor system) and the basolateral amygdala (via the $alpha_1$ and non D_2 receptor system). According to the gating theory an increase in noradrenergic activity results in a closed hippocampal gate because of the activation of the $beta_2$ adrenoceptors and a open amygdala gate because of the activation of the $alpha_1$-adrenoceptors. The opposite is found when the noradrenergic activity is decreased: the hippocampus gate is open because of the inhibition of the $beta_2$-adrenoceptors and the amygdala gate is closed because of the inhibition of the alpha1-adrenoceptors. (for further details see (Cools, van den Bos, Ploeger, and Ellenbroek, 1991)).This implies that under normal circumstances either the hippocampal gate is closed and the amygdala gate is open or the hippocampal gate is open and the amygdala gate is closed.

Based on this model, the $beta_2$-adrenoceptor agonist isoproterenol blocked the information input from the hippocampus but allows the information input from the basolateral amygdala. Likewise the $alpha_1$-adrenoceptor antagonist phentolamine is expected to block the information input from the basolateral amygdala, but allows information input from the hippocampus. Neither of these treatments affected prepulse inhibition. However the combined infusion of both drugs, thus blocking the information input from both structures significantly reduces prepulse inhibition. A similar finding was reported by Roosendaal and colleagues (Roozendaal and Cools, 1994). They showed that the neophobic response of rats was only altered when the information input of both structures was blocked. These data suggest that an imbalance of information input from the basolateral amygdala and the hippocampus are involved in prepulse inhibition. Interestingly, both the basolateral amygdala and the hippocampus are known to modulate prepulse inhibition. For example lesions of the amygdala (Decker, Curzon et al., 1995; Wan and Swerdlow, 1997) and the ventral subilicum, as part of the hippocampus (Swerdlow, Taaid et al., 2000) were found to reduce prepulse inhibition. Furthermore infusion of the glutamate agonist NMDA in the ventral hippocampus reduced prepulse inhibition, an effect that was blocked by the NMDA-Antagonist AP5 (Wan, Caine et al., 1996; Wan and Swerdlow, 1996). On the other hand Bakshi and colleagues reported that infusion of the NMDA-antagonist dizocilpine in the dorsal hippocampus reduced prepulse inhibition (Bakshi and Geyer, 1998). Furthermore, dizocilpine or picrotoxin infusion in

the basolateral amygdala disrupted prepulse inhibition an effect that was blocked by the dopamine antagonist haloperidol (Fendt, Schwienbacher et al., 2000). In contrast to our findings the aforementioned authors found alterations in prepulse inhibition when either the basolateral amygdala or the hippocampus was manipulated. However this does not exclude that these manipulations induced an imbalance of information input from the basolateral amygdala and the hippocampus to the nucleus accumbens, reducing prepulse inhibition.

In summary these data suggest that the noradrenergic activity in the nucleus accumbens modulates prepulse inhibition, most likely by increasing the dopaminergic activity. Furthermore since noradrenaline regulates the information input from the basolateral amygdala and the hippocampus to the nucleus accumbens, the presented data suggest that an imbalance in this information flow might contribute to disturbances in prepulse inhibition.

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Chapter 6

SUMMARY OF THE RESULTS

The aim of this study was to investigate the role of stress and noradrenaline in schizophrenia and to further understand the neuronal mechanism involved. Among other symptoms, reductions in prepulse inhibition have been found in these patients. This paradigm is a very value tool for instigating the neurobiology of schizophrenia since this behavioural response can be measured both in humans and in animals. This opens the possibility to use animals in schizophrenia research. In our experiments we used the so-called APO-SUS and APO-UNSUS rats because APO-SUS rats share a large number of similarities with schizophrenic patients like impairments in their response to stress, as well as alterations in their noradrenergic and dopaminergic system, and, even more importunately, a reduction in prepulse inhibition.

It is known that stress can worsen the symptoms of schizophrenia, for example abrupt changes in life increase the risk of relapse in schizophrenic patients (Jones, Babington et al., 1993). We therefore expected to find that stress would worsen the prepulse inhibition in APO-SUS rats (**chapter 2**). We used one day of social isolation as a stressor. In line with our expectation stressed APO-SUS rats displayed a decrease in prepulse inhibition and furthermore APO-UNSUS rats displayed an increase in prepulse inhibition. Further evidence for the effect of stress on prepulse inhibition in APO-SUS and APO-UNSUS rats was obtained in **chapter 4**. We found evidence that a single saline injection, known to act as a stressor, given two weeks before testing reduces the prepulse inhibition in APO-SUS rats and increases it in APO-UNSUS rats.

Thus stress can elicit deficits in prepulse inhibition in APO-SUS rats, an effect that is similar to the finding that stress can elicit psychotic episodes in schizophrenic patients. Therefore these data further validate the APO-SUS rats as a good model for schizophrenia.

One characteristic of APO-SUS and APO-UNSUS rats is that stress increases the functional noradrenergic activity in APO-SUS rats but decreases the functional noradrenergic activity in APO-UNSUS rats (Cools, Brachten et al., 1990; Roozendaal and Cools, 1994). The fact that stress decreased prepulse inhibition in APO-SUS rats and increased prepulse inhibition in APO-UNSUS rats indicates that an increase in noradrenergic activity reduces prepulse inhibition. This is in line with the elevated levels of noradrenaline that are found in schizophrenic patients (van Kammen, Peters et al., 1989). Furthermore as mentioned in the Introduction these levels correlate with schizophrenic symptoms (van Kammen and Gelernter, 1987; Dajas, Barbeito et al., 1983). These

data strongly suggest that there exists a functional relation between stress, increased noradrenaline levels and the worsening of schizophrenic symptoms. The situation in the stressed APO-SUS rats is similar since they have a high functional noradrenergic activity, induced by stress, and a reduced prepulse inhibition. The nature of the involved adrenergic receptors is not studied in detail. Therefore we investigated the effects of the $beta_2$-adrenoceptor agonist clenbuterol and the $alpha_1$-adrenoceptor agonist cirazoline on prepulse inhibition in non-stressed APO-SUS and APO-UNSUS rats (**chapter 3**). According to our expectation we found that the $alpha_1$-adrenoceptor agonist cirazoline reduced prepulse inhibition in non-stressed APO-SUS rats, an effect that could be antagonised with the $alpha_1$-adrenoceptor antagonist prazosin. Only a weak, non-significant effect was seen in non-stressed APO-UNSUS rats. In addition the $beta_2$-adrenoceptor agonist clenbuterol had no effect on the prepulse inhibition. These findings show that the $alpha_1$-adrenoceptors are involved in modulating the prepulse inhibition a finding that is in line with previous studies (Carasso, Bakshi et al., 1998). Even more interesting, we show that only the APO-SUS rats display reductions in prepulse inhibition after the treatment with prazosin, indicating that the APO-SUS rats are more sensitive to the $alpha_1$ agonist than the APO-UNSUS rats. This is again in line with the idea that the APO-SUS rats are more sensitive to stress and thus are a good animal model for schizophrenia. To our knowledge no one has so far investigated the sensitivity of schizophrenic patients to noradrenergic agonists.

Taken these two sets of data together (chapter 2 and 3), it seems highly likely that the disruptive effect of a mild stressor on prepulse inhibition in a valid animal model of schizophrenia is due to an increased activity of noradrenaline.

At present it is not known which neuronal structures are involved in the modulation of prepulse inhibition by stress or noradrenaline. As mentioned in the Introduction, noradrenergic fibres from the locus coeruleus innervate the part of the PVN that contains terminals of CRH-releasing neurons that, in turn modulate the stress response of the HPA-axis. Thus the noradrenergic terminals from the locus coeruleus regulate the stress response. In addition the locus coeruleus innervates the nucleus accumbens, namely a structure known to play a role in prepulse inhibition. We therefore hypothesized that the locus coeruleus is also involved in modulating prepulse inhibition.

In order to investigate this we blocked the noradrenergic input by selectively destroying the terminals of the locus coeruleus with the help of the neurotoxin DSP4. Given the inverse relationship between noradrenaline and prepulse inhibition, we expected an improvement in prepulse inhibition since the destruction of the locus coeruleus terminals reduces the noradrenergic

activity. The effects of DSP4 on the prepulse inhibition in stressed and non-stressed APO-SUS and APO-UNSUS rats are described in **chapter 4.** We found that DSP4 had no effect on prepulse inhibition in non-stressed (non-isolated) APO-SUS rats or APO-UNSUS rats. However in line with the expectations, stressed DSP4-treated APO-UNSUS rats displayed an improved prepulse inhibition. Interestingly the stressed DSP4-treated APO-SUS rats displayed a reduced prepulse inhibition suggestive for an increased noradrenergic activity or an increased adrenoceptor sensitivity. We could indeed show that the treatment with DSP4 induced supersensitive $alpha_1$-adrenoceptors in APO-SUS rats, but not in APO-UNSUS rats. Although the treatment with DSP4 reduces the noradrenergic activity, it should be noted that this treatment selectively destroys the locus coeruleus terminals. Therefore, other noradrenergic sources in the brain like the medullary group are still active. As mentioned above, stress increases the functional noradrenergic activity in APO-SUS rats. Thus, since the DSP4 treated APO-SUS rats have developed supersensitive $alpha_1$-adrenoceptors a small increase in noradrenergic activity can already reduce prepulse inhibition. It is, at present unclear why only APO-SUS rats developed $alpha_1$ adrenoceptor supersensitivity. One possibility might be that DSP4 induced a larger reduction in noradrenaline levels in APO-SUS than in APO-UNSUS rats. DSP4 is taken up by the cell via the noradrenaline reuptake mechanism (Jonsson, Hallman et al., 1981). Since the adrenoceptor sensitivity is known to adapt to the noradrenergic activity, one can imagine that the same holds true for the sensitivity of the reuptake mechanism. Thus, rats with a high noradrenergic activity would have a high reuptake, whereas rats with a low noradrenergic activity would have a low reuptake. This implies that DSP4 is more toxic to rats that have high noradrenergic activity because more of this substance is taken up by the cell. As mentioned above, the injection stressed the rats and accordingly enhances the noradrenergic activity in APO-SUS rats, but decreases it in APO-UNSUS rats. Therefore, more DSP4 is taken up by APO-SUS rats resulting in more destroyed terminals and a stronger reduction in noradrenergic activity. To compensate this reduction the postsynaptic $alpha_1$-adrenoceptors develop supersensitivity. Since the injection reduces the noradrenergic activity in APO-UNSUS, less DSP4 is taken up and fewer terminals are destroyed. Therefore the noradrenergic activity is less reduced in APO-UNSUS rats and accordingly not compensated by the development of supersensitive $alpha_1$-adrenoceptors receptors. However, until noradrenaline levels have been measured in APO-SUS rats and APO-UNSUS rats, this explanation remains speculative.

The data show that the noradrenergic terminals of the locus coeruleus are an important modulator of the prepulse inhibition. The role of the medullary group in this respect remains to be investigated

Among others the locus coeruleus sends fibers to the nucleus accumbens (Speciale, Crowley et al., 1978), a structure that is known to modulate prepulse inhibition. For example increasing the dopaminergic activity in this nucleus has been found to reduce prepulse inhibition (Swerdlow, Geyer et al., 2001). This is in line with the fact that amphetamine and other dopamine releasing drugs can induce a state resembling paranoid schizophrenia in normal individuals (Connell, 1958) and reduce prepulse inhibition in both humans (Hutchison and Swift, 1999) and rats (Mansbach, Geyer et al., 1988). Within the nucleus accumbens noradrenaline is known to control dopamine release (Tuinstra and Cools, 2000). This leads to the hypothesis that noradrenaline modulates the prepulse inhibition by altering the dopamine activity in the nucleus accumbens. In order to test this hypothesis we applied different noradrenergic agents in the nucleus accumbens and tested the effects on prepulse inhibition in rats.

This experiment was based on the microdialysis study by Tuinstra and colleagues (Tuinstra and Cools, 2000). The authors showed that both the $alpha_1$-adrenoceptor agonist phenylephrine and the $beta_2$-agonist isoproterenol increase the accumbal dopamine release. Notably also the $alpha_1$-adrenoceptor antagonist phentolamine was found to increase accumbal dopamine. The authors concluded that the agonist acts at the presynaptic site thereby inhibiting the release of noradrenaline from its terminals and subsequently disinhibits the release of dopamine. The antagonist on the other hand acts directly by inhibiting the postsynaptic receptors on the corresponding dopamine terminals facilitating the release of dopamine. Based on these facts we expected to find a reduction in prepulse inhibition in rats after the application of phentolamine, isoproterenol and phenylephrine **(chapter 5)**.

Intra-accumbal injection of neither the $alpha_1$-adrenoceptor agonist phenylephrine nor the $beta_2$-adrenoceptor agonist isoproterenol altered prepulse inhibition in rats. Also the $alpha_1$-adrenoceptor antagonist phentolamine was ineffective in this respect. Interestingly, when given together, isoproterenol and phentolamine significantly reduced prepulse inhibition. This suggests a cumulative effect of these two drugs on dopamine. As shown by Zhang and colleagues, the increase in accumbal dopamine must be up to 100% to disrupt prepulse inhibition (Zhang, Pouzet et al., 2000). More importantly, it should be noted that there are two distinct noradrenergic-dopaminergic systems in the nucleus accumbens. According to the gating theory by Cools and colleagues (Cools, van den Bos et al., 1991) $beta_2$-adrenoceptor agonists increase the dopaminergic activity at the level of the D_2-receptors, whereas $alpha_1$-adrenoceptor antagonists increase the dopaminergic activity at the level of a subtype of dopamine receptors that differs from the classical D_1 and D_2 type of receptors. Since neither isoproterenol nor phentolamine alone altered prepulse inhibition, these data strongly suggests that activation of both types of dopamine receptors is

required for a reduction in prepulse inhibition. Furthermore, according to the gating theory the increase in accumbal dopamine is associated with regulation of the information input to the nucleus accumbens. Increasing dopaminergic activity at the level of the D_2 receptors, by activating the accumbal $beta_2$-adrenoceptors, blocks the information input from the hippocampus. On the other hand increasing the dopaminergic activity at the level of the non-classical D_1/D_2 subtype of dopamine receptors, by inhibiting the accumbal $alpha_1$-adrenoceptors, blocks the information input of the amygdala. Since it was found that prepulse inhibition was only reduced when isoproterenol and phentolamine were given together, these data strongly suggests that reductions in prepulse inhibition can only be observed when the information flow from both nuclei is blocked. This is in line with previous data on neophobia, in which a disturbance was only observed when both the hippocampal and the amygdale gate were closed (Roozendaal and Cools, 1994). This finding suggests that information input from both the amygdala and the hippocampus is necessary for a correct prepulse inhibition. It was already found that hippocampus and amygdala modulate prepulse inhibition. For example infusion of the NMDA antagonist dizocilipine in the basolateral amygdala reduces prepulse inhibition (Fendt, Schwienbacher et al., 2000). In addition infusion of the of the glutamate agonist NMDA in the ventral hippocampus reduces prepulse inhibition (Wan, Caine et al., 1996). Lesions of the entorhinal cortex (which constitutes the principal output connection of the hippocampus to the nucleus accumbens) reduces prepulse inhibition (Goto, Ueki et al., 2002). Interestingly, this effect is reversed by systemic application of the D_2 dopamine antagonist haloperidol. Although it is not clear whether this reversal occurs at the level of the nucleus accumbens, it is in line with the gating theory, which states that the hippocampal gate is controlled by D_2 receptors in the nucleus accumbens.

Anyhow, these data show that accumbal noradrenaline modulates prepulse inhibition. The fact that noradrenaline increases accumbal dopamine together with the fact that an increased dopamine activity in the nucleus accumbens reduces prepulse inhibition suggests that noradrenaline reduced prepulse inhibition by increasing accumbal dopamine. Furthermore these data indicate that the information input from both the amygdala and the hippocampus is required for a correct prepulse inhibition.

In summary the data presented in this thesis demonstrate that stress modulates prepulse inhibition. Furthermore we were able to show that this effect is based on an increase in noradrenergic activity, most likely by activating the locus coeruleus. Finally we found evidence that noradrenaline modulates prepulse inhibition in the nucleus accumbens, presumably by modulating accumbal dopamine and thus regulating the information input from the amygdala and the hippocampus to the nucleus accumbens.

In the following chapter we discuss the consequences of these findings for the modulation of the prepulse inhibition and the treatment for schizophrenia.

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Chapter 7

CONCLUDING REMARKS

Neuronal regulation of prepulse inhibition: Koch's model revised.

As discussed in the Introduction, there is extensive knowledge about the neuronal structures that are mediating and regulating prepulse inhibition (see Fig 1, Chapter 1). Nevertheless, this study has provided the original evidence that a hitherto unstudied brain structure in this respect has to be added, namely the locus coeruleus. In this thesis hard evidence is presented that the locus coeruleus as well as its axons that terminate in the nucleus accumbens are crucial for the presence of a normal prepulse inhibition. Apart from this, the present study shows that beta-adrenoceptors in the nucleus accumbens that are known to belong to the noradrenergic fibres that arise in the locus coeruleus (see: Cools, van den Bos, Ploeger, and Ellenbroek, 1991) are important, though stimulation or inhibition of these receptors alone is insufficient for altering the prepulse inhibition. In addition, this study shows that alpha-adrenoceptors in the nucleus accumbens are also important for the regulation of prepulse inhibition, though, like the beta-adrenoceptors in this nucleus, just stimulation or inhibition of these receptors is insufficient for altering prepulse inhibition. Previously, it has been postulated that the alpha-adrenoceptors in the nucleus accumbens belong to noradrenergic fibres that arise in the medullary cell group, the so-called A2-A5 group (see: Cools, van den Bos, Ploeger, and Ellenbroek, 1991). In view of this, we suggest that also this medullary group is a neuronal structure that plays a role in prepulse inhibition. In other words, both the locus coeruleus and the medullary cell groups should be added to the brain nuclei that regulate or mediate prepulse inhibition. Analysis of Figure 1 in chapter 1 reveals that noradrenaline is not at all included as a neurotransmitter that plays a crucial role in prepulse inhibition. As extensively discussed above, this thesis provides hard evidence that central noradrenaline is crucial for the regulation and mediation of prepulse inhibition. Interestingly, the accumbal adrenoceptors are known to control accumbal dopamine, a neurotransmitter that, in turn, is known to control the glutaminergic input from the basolateral amygdala and the ventral hippocampus (for details see: Cools, van den Bos, Ploeger, and Ellenbroek, 1991). Just briefly, activating the accumbal $beta_2$ adrenoceptors increases the activity at the level of the accumbal D_2 receptors thereby blocking the glutaminergic input from the ventral hippocampus to the nucleus accumbens. On the other hand, inhibition of the accumbal $alpha_1$-adrenoceptors increases the activity at the level of a non D_2-subtype of dopaminergic receptor (Cools, van den Bos, Ploeger, and Ellenbroek, 1991) thereby blocking the glutaminergic input from the basolateral amygdala. The data of this thesis clearly shows that the prepulse

inhibition is only reduced if an inhibition of accumbal alpha$_1$-adrenoceptors is combined with a stimulation of beta$_2$-adrenoceptors. According to the earlier described and above-mentioned gating theory, this implies that a combined inhibition of the amygdala and the hippocampal input of the nucleus accumbens results in an impaired prepulse inhibition. Indeed, it is already known that manipulations with the amygdala or the hippocampus alter prepulse inhibition. The notion that there exists both an accumbal dopamine D_2-dependent and an accumbal dopamine D_2-independent mechanism that is involved in prepulse inhibition, is fully in line with the findings of Wan and colleagues (Wan and Swerdlow, 1996): they have found that the dopamine D_2 antagonist haloperidol antagonizes the AMPA-induced impairment of prepulse inhibition, when this drug is administered into the core of the nucleus accumbens, whereas it does not antagonize the AMPA-induced impairment of the prepulse inhibition, when AMPA is infused into the shell of the nucleus accumbens. These data open the perspective that both the beta$_2$-adrenoceptors and the dopamine D_2 receptors that regulate prepulse inhibition in the nucleus accumbens, are primarily localized in the core, and that both the alpha$_1$-adrenoceptors and the dopamine non-D_2 receptors that regulate prepulse inhibition in the nucleus accumbens, are primarily localized in the shell of the nucleus accumbens. Because no additional evidence in this respect is available, future studies are required to (in) validate these hypotheses. As a final remark in this context, there is evidence that the nucleus accumbens contains at least two distinct output pathways of the nucleus accumbens by which prepulse inhibition is transmitted (Kretschmer and Koch, 1998). This knowledge was already included in Koch's diagram depicting the brain nuclei involved in prepulse inhibition, namely one accumbal output pathway that terminates in the ventral pallidum and one accumbal output pathway that terminates in the pedunculopontine tegmental nucleus. Because it has been reported that the impairment of prepulse inhibition following accumbal administration of the dopamine D_2/ D_1 agonist apomorphine is fully inhibited by a lesion in the ventral pallidum, it becomes attractive to postulate that dopamine D_2 controlled hippocampal input of the nucleus accumbens impinges upon neurons that innervate the ventral pallidum. On the other hand, the prepulse inhibition impairment induced by accumbal administration of a glutaminergic antagonist of NMDA-receptors is not inhibited by a lesion of the ventral pallidum. In view of this, one might speculate that the basolateral amygdala input of the nucleus accumbens impinges upon neurons that bypasses the ventral pallidum and, possibly, terminate on the pedunculopontine tegmental nucleus. In view of all these data, we decided to revise the original diagram of Koch (Koch, 1999). The results are depicted in Fig 23. The putative input-output connections between the ventral subiculum of the hippocampus, core and ventral pallidum on one hand and those between the basolateral part of the amygdala, the shell and the pedunculopontine nucleus on the other hand are incorporated in Fig 24. It is evident that this model gives rise to a number of hypotheses that can be tested.

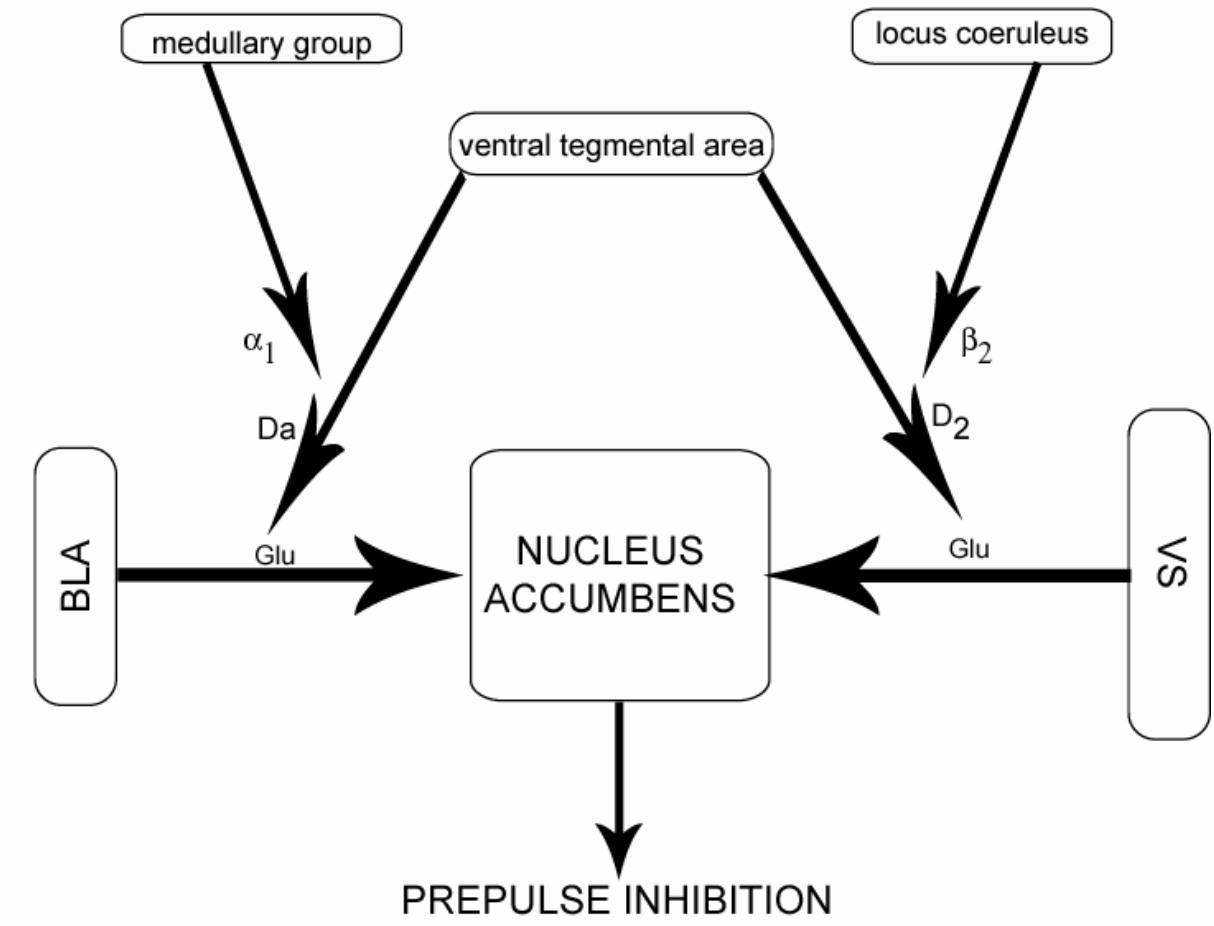

Fig 23: noradrenaline modulates prepulse inhibition. BLA: basolateral amygdala; VS: ventral subilicum ; Da: Dopamine; Glu: glutamate

First, our model states that both the hippocampal and the amygdala input of the nucleus accumbens have to be inhibited in order to impair prepulse inhibition. Therefore it becomes worthwhile to study whether or not simultaneous stimulation of the involved glutamate receptors in both the ventral subiculum of the hippocampus and the basolateral part of the amygdala is required to disturb prepulse inhibition. Second, our model states that stimulation of both types of dopamine receptors in the nucleus accumbens is required for producing an impaired prepulse inhibition. Accordingly, the question arises to what extent simultaneous stimulation of these receptors is effective or not in this respect. Furthermore, since these dopamine receptors are associated with different noradrenergic receptors it is important to validate to what extent these effects are dependent on these different noradrenergic receptors. Third, our model states that both the nucleus accumbens output towards the ventral pallidum and the nucleus accumbens output to the pedeunculopontine tegmental nucleus have to be blocked in order to disrupt prepulse inhibition. Accordingly, the question arises to what extent a simultaneous blockade of the GABA-ergic receptors in the ventral pallidum and peduculopontine tegmental nucleus is required in order to worsen prepulse inhibition. It is clear that our model provides a firm foundation for extending

our present-day knowledge about the involvement of distinct brain structures in the mediation and regulation of prepulse inhibition. Future studies are necessary to collect hard data in this respect.

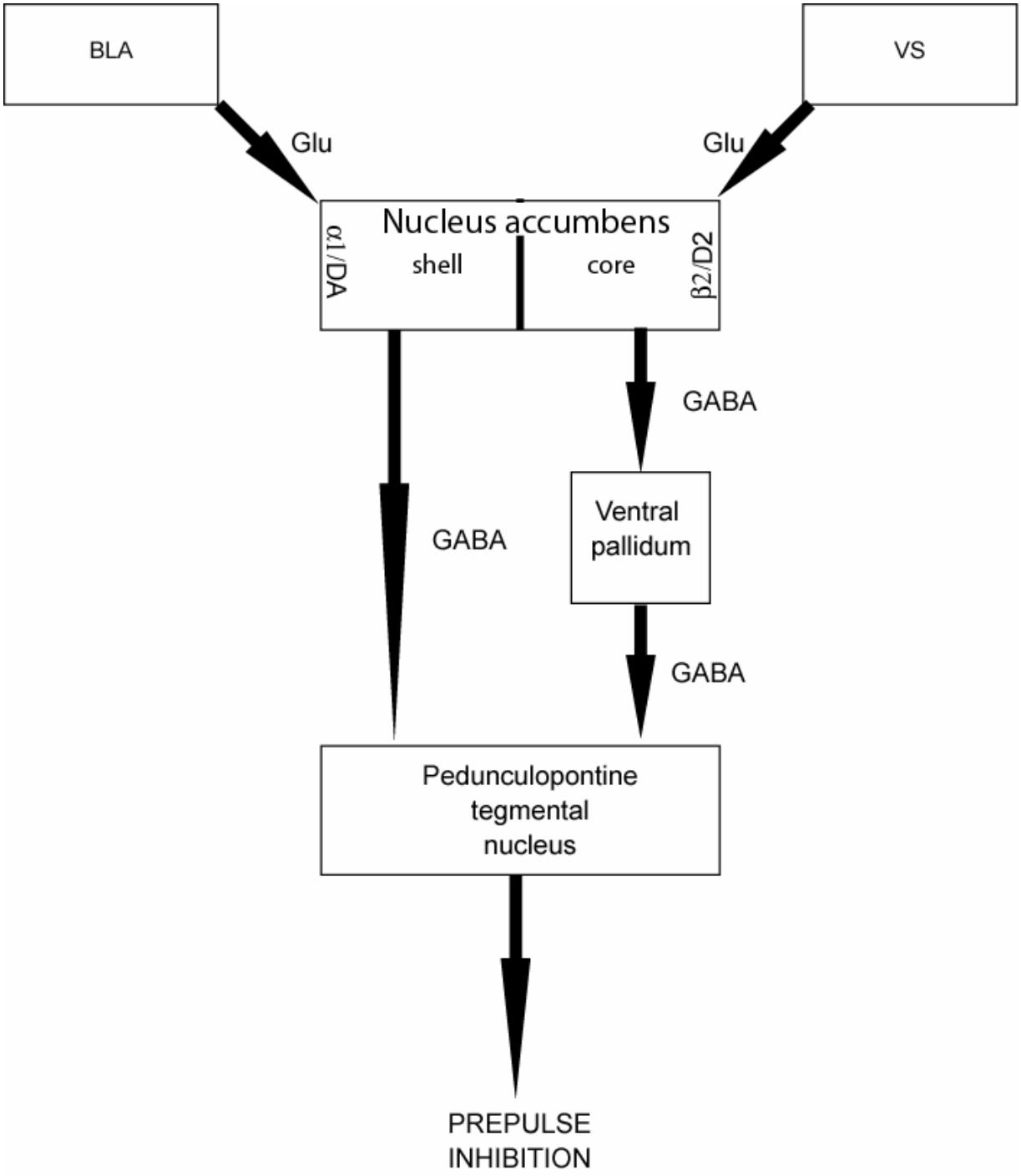

Fig 24: Putative neuronal connection in the nucleus accumbens, modulating prepulse inhibition:BLA: basolateral amygdala; VLS: ventral subilicum ; Da: Dopamine; Glu: glutamate

Implications for schizophrenia

As discussed in the Introduction, stress is known to increase the risk of relapse in schizophrenic patients: it can actually elicit psychotic episodes in schizophrenic patients. One of the symptoms

seen in schizophrenic patients is a reduced prepulse inhibition (Braff, Stone et al., 1978). As elaborated in the Introduction, this can be mimicked in animals by administering amphetamine (Mansbach, Geyer et al., 1988), a treatment known to elicit reduced prepulse inhibition in normal humans (Hutchison and Swift, 1999). Previously, it has been shown that the APO-SUS rat is a valid animal of certain aspects of schizophrenia. Among others these rats show an impaired prepulse inhibition (Ellenbroek, Geyer et al., 1995). The present study presents hard evidence that it is stress that triggers this phenomenon. The data presented in this thesis may provide an adequate explanation for the clinical finding that stress influences the display of symptoms in patients with schizophrenia (Koenigsberg and Handley, 1986). It is known that stress among others increases the central noradrenergic activity (Cullinan, Herman et al., 1995) and we were able to demonstrate that an increased noradrenergic activity gives rise to the display of an impaired prepulse impulse inhibition.

In other words, stress can enhance the central noradrenergic activity that, in turn, triggers a schizophrenic symptom like a reduction of the prepulse inhibition in patients. This may explain why families marked by a so-called high EE, but not low EE, can have such a negative effect upon the relapse of schizophrenic patients in such families (Koenigsberg and Handley, 1986). The present study also shows that the ability of stress to elicit an impaired prepulse inhibition is limited to our schizophrenia-prone rats: stress reduced prepulse inhibition only in APO-SUS rats, but not in APO-UNSUS rats, the controls of APO-SUS rats. This suggests that especially individuals with a predisposition for schizophrenia are sensitive to the effect of stress upon prepulse inhibition. As discussed in the Introduction, increased levels of noradrenaline have been found in the brain of schizophrenic patients (van Kammen, Peters et al., 1989). Because the present study has provided hard evidence that an abnormally high activity of central noradrenaline results in a reduced prepulse inhibition, our data strongly suggest that the enhanced levels of central noradrenaline in the brain of patients with schizophrenia gives rise to the impaired prepulse inhibition in these patients.

In this context it is important to note that the amount of central noradrenaline correlates not just with a single schizophrenic symptom, but with a whole set of positive and negative symptoms (van Kammen and Gelernter, 1987). This might imply that central noradrenaline plays not only a role in the impaired prepulse inhibition, but also in other schizophrenic symptoms. All these considerations together indicate that noradrenaline is much more important than hitherto thought: it is concluded that central noradrenaline needs to play an important role in the etiology of schizophrenia.

The present findings open new perspectives for the treatment of schizophrenia. First of all, our data indicate that it is stress that can have a negative effect upon the relapse of symptoms in patients with schizophrenia. This implies that both the patient with schizophrenia and the people dealing with the patient have to take into account that exposure to stressors should be prevented as much as possible. On the basis of these considerations we advise to create a stress-free environment for the patients. For example, it should be advised that the patient tries to maintain a stable day/night rhythm. Apart from this, people dealing with the patients should be advised to take care of the notion that stress can really worsen the symptoms of the patient.

Second, our data strongly indicate that the therapeutic efficacy of $alpha_1$-adrenergic antagonists should be investigated. In this context it is important to note that there is already evidence that agents with a strong noradrenergic antagonistic component such as clozapine have already proven to be effective in this respect (Kumari, Soni et al., 1999; Lee, Jayathilake et al., 1999). In sum, it is concluded that there is an urgent need for investigating in more detail the role of central noradrenaline in the etiology and therapy of schizophrenia.

Third, as already mentioned, impaired prepulse inhibition is not limited to schizophrenia: it also occurs in other neuropsychiatric diseases such as obsessive compulsive disorder (Braff, Geyer et al., 2001), Huntington's disease (Swerdlow, Paulsen et al., 1995), attention deficit disorder, Tourette's syndrome (Castellanos, Fine et al., 1996) and posttraumatic stress disorder (Grillon, Morgan et al., 1996). Although not only noradrenaline, but also other neurotransmitters can contribute to an impaired prepulse inhibition, like dopamine and glutamate and 5HT (Geyer, Krebs-Thomson et al., 2001), it becomes interesting to investigate whether or not abnormal noradrenaline activity and/or metabolism also contributes to the disturbed prepulse inhibition in these diseases. This appears to held true for patients with Tourette's syndrome, obsessive compulsive disorder (Leckman, Goodman et al., 1995), posttraumatic stress disorder (Geracioti, Baker et al., 2001) for, abnormal noradrenaline levels have been found in the brain of all these patients. To what extent noradrenaline plays also a role in the other symptoms of these diseases remains to be investigated in future studies. Anyhow, the role of noradrenaline in the etiology of these diseases needs far more attention that is has got in the past.

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Chapter 8

NEDERLANDSE SAMENVATTING

Het doel van deze studie was de rol van stress en noradrenaline bij schizofrenie te bestuderen en om het onderliggende neuronale mechanisme beter te begrijpen. Buiten andere symptomen is de reductie van prepuls inhibitie gevonden in patiënten. Het paradigma is een bijzonder waardevol hulpmiddel om de neurobiologie van schizofrenie te bestuderen, omdat de gedragsmatige respons in zowel dieren als mensen kan worden bestudeerd. Dit biedt de mogelijkheid om dieren te gebruiken in het schizofrenieonderzoek. In onze experimenten gebruiken we de zogenaamde APO-SUS en APO-UNSUS ratten, omdat APO-SUS ratten een groot aantal overeenkomsten hebben met schizofrene patiënten, zoals een minder goede reactie op stress en veranderingen in het noradrenerge en dopaminerge systeem en, nog belangrijker, een reductie van de prepuls inhibitie.

Het is bekend dat stress de symptomen van schizofrenie kan verergeren. Abrupte veranderingen in het leven kunnen bijvoorbeeld het risico op een terugval van schizofrene patiënten vergroten (Jones, Babington et al., 1993). We verwachtten daarom te vinden dat stress de prepuls inhibitie zou verslechteren in APO-SUS ratten (**hoofdstuk 2**). We hebben slechts isolatie van één enkele dag gebruikt als een stressor. In overeenkomst met onze verwachtingen vertoonden APO-SUS ratten een verslechterde prepuls inhibitie en bovendien vertoonden APO-UNSUS ratten een verhoging in prepuls inhibitie. Meer bewijs voor het effect van stress op prepuls inhibitie in APO-SUS en APO-UNSUS ratten is in **hoofdstuk 4** verkregen. We hebben bewijs gevonden dat een enkele injectie van fysiologisch zout twee weken voor het testen, waarvan bekend is dat het als een stressor werkt, een verlaging van prepuls inhibitie veroorzaak in APO-SUS ratten en een verhoging in APO-UNSUS ratten.

Dus stress kan een verslechtering van de prepuls inhibitie bij APO-SUS ratten teweegbrengen, een effect dat vergelijkbaar is met het feit dat stress psychotische episodes kan teweegbrengen bij schizofrene patiënten. Daarom dragen deze data bij aan het verder valideren van APO-SUS ratten als een goed model voor schizofrenie.

Een eigenschap van APO-SUS en APO-UNSUS ratten is dat stress de functionele noradrenerge activiteit verhoogt in APO-SUS ratten, maar verlaagt in APO-UNSUS ratten (Cools, Brachten et al., 1990; Roozendaar en Cools, 1994). Het feit dat stress de prepuls inhibitie verlaagde in APO-

SUS ratten en verhoogde in APO-UNSUS ratten geeft een indicatie dat een verhoging in de noradrenerge activiteit de prepuls inhibitie verlaagt. Dit is in overeenstemming met verhoogde noradrenaline spiegels die gevonden zijn in schizofrene patiënten (Van Kammen, Peters et al., 1989). Verder, zoals genoemd in de Introductie, correleren deze spiegels met schizofrene symptomen (Van Kammen en Gelernter, 1987, Dajas, Barbeito et al., 1993). Deze data suggereren sterk dat er een functionele relatie bestaat tussen stress, noradrenaline spiegels en het verergeren van schizofrene symptomen. De situatie van de gestresste APO-SUS ratten is vergelijkbaar, omdat ze een hogere, door stress geïnduceerde, functionele noradrenerge activiteit hebben en een verlaagde prepuls inhibitie. De aard van de betrokken receptoren is niet in detail bestudeerd. Daarom hebben we de effecten van de $beta_2$-adrenoceptor agonist clenbuterol en de $alfa_1$-adrenoceptor agonist cirazoline op de prepuls inhibitie bestudeerd in niet gestresste APO-SUS en APO-UNSUS ratten **(hoofdstuk 3)**. In overeenstemming met onze verwachting hebben we gevonden dat de $alfa_1$-adrenoceptor agonist cirazoline de prepuls inhibitie verlaagde in niet gestresste APO-SUS ratten, een effect dat geantagoneerd kon worden met de $alfa_1$-adrenoceptor antagonist prazosine. Slechts een zwak, niet significant effect werd gezien in APO-UNSUS ratten. Bovendien had de $beta_2$-adrenoceptor agonist clenbuterol geen effect op de prepuls inhibitie. Deze bevindingen laten zien dat $alfa_1$-adrenoceptors betrokken zijn bij de modulatie van de prepuls inhibitie, een bevinding die overeen komt met voorgaande studies (Carasso, Bakshi et al., 1998). Nog interessanter is dat we laten zien dat alleen de APO-SUS ratten een reductie van de prepuls inhibitie vertonen na behandeling met prazosine en dit geeft aan dat APO-SUS ratten gevoeliger zijn voor de $alfa_1$-agonist dan APO-UNSUS ratten. Dit is ook weer in overeenkomst met het idee dat APO-SUS ratten veel gevoeliger zijn voor stress en dus een goed diermodel voor schizofrenie zijn. Voor zover onze kennis strekt, heeft tot nu toe niemand de gevoeligheid van schizofrene patiënten voor noradrenerge agonisten onderzocht.

Deze twee sets van data bij elkaar genomen (hoofdstukken 2 en 3), lijkt het zeer voor de hand liggend dat de verstorende effecten van een milde stressor op de prepuls inhibitie in een valide diermodel voor schizofrenie toe te schrijven zijn aan een verhoogde noradrenerge activiteit.

Op dit moment is het niet bekend welke neuronale structuren betrokken zijn bij de modulatie van prepuls inhibitie door stress of noradrenaline. Zoals genoemd in de Introductie innerveren de noradrenerge vezels uit de locus coeruleus het gedeelte van de PVN dat terminals bevat van CRH-afgevende neuronen, die, op hun beurt, de stress repons van de HPA-as beïnvloeden. Dus reguleren de nordrenerge terminals van de locus coeruleus de stress repons. Bovendien innerveert de locus coeruleus de nucleus accumbens, een structuur die betrokken is bij de regulatie van prepuls

inhibitie. Om die reden hypothetiseren wij dat de locus coeruleus ook betrokken is bij de regulatie van prepuls inhibitie.

Om deze hypothese the onderzoeken, hebben we de noradrenerge input geblokkeerd door selectief de terminals van de locus coeruleus te vernietigen door toediening van het neurotoxine DSP4. Wegens de omgekeerd evenredige relatie tussen noradrenaline en prepuls inhibitie, verwachtten we een verbetering in de prepulse inhibitie, omdat de vernietiging van de locus coeruleus terminals de noradrenerge activiteit verlaagt. De effecten van DSP4 op prepuls inhibitie in gestresste en niet gestresste APO-SUS en APO-UNSUS ratten zijn beschreven in **hoofdstuk 4**. We hebben gevonden dat DSP4 geen effect had op prepuls inhibitie in niet gestresste (niet geïsoleerde) APO-SUS ratten of APO-UNSUS ratten. Echter, in overeenstemming met de verwachtingen, gestresste DSP4-behandelde APO-UNSUS ratten vertoonden een verbeterde prepuls inhibitie. Interessant is dat de gestresste DSP4-behandelde APO-SUS ratten een verlaging van de prepuls inhibitie vertoonden, wat een hoge noradrenerge activiteit of een verhoogde adrenoceptor gevoeligheid suggereert. We hebben inderdaad aangetoond dat de behandeling met DSP4 supersensitisatie van de $alfa_1$-adrenoceptors in APO-SUS ratten veroorzaakt, maar niet in APO-UNSUS ratten. Hoewel de behandeling met DSP4 de noradrenerge activiteit reduceert, moet het opgemerkt worden dat deze behandeling de terminals van de locus coeruleus selectief vernietigt. De andere noradrenerge systemen in de hersenen, zoals de medullaire groep, zijn dus nog steeds actief. Zoals eerder genoemd, verhoogt stress de functionele noradrenerge activiteit in APO-SUS ratten. Dus, omdat de DSP4 behandelde APO-SUS ratten supersensitieve $alfa_1$-adrenoceptors hebben, kan een kleine verhoging in noradrenerge activiteit al voor een verlaging van de prepuls inhibitie zorgen. Op dit moment is het onduidelijk waarom alleen APO-SUS ratten supersensitieve $alfa_1$-receptoren hebben ontwikkeld. Een mogelijke verklaring kan zijn dat DSP4 een grotere reductie in noradrenaline spiegels veroorzaakt in APO-SUS dan in APO-UNSUS ratten. DSP4 wordt in wordt in de cel opgenomen via het noradrenaline heropname mechanisme (Jonsson, Hallman et al., 1981). Omdat het bekend is dat adrenoceptor gevoeligheid zich aanpast aan de noradrenerge activiteit, zou men zich kunnen voorstellen dat hetzelfde geldt voor het heropname mechanisme. Dus, ratten met een hoge noradrenerge activiteit zouden een hoge heropname hebben, terwijl ratten met een lage noradrenerge activiteit een lage heropname zouden hebben. Dit impliceert dat DSP4 meer toxisch is bij ratten met een hoge noradrenerge activiteit, omdat meer van deze stof opgenomen wordt in de cel. Zoals eerder genoemd, de injectie heeft de ratten gestresst en verhoogt daarom de noradrenerge activiteit in APO-SUS ratten, maar verlaagt het in APO-UNSUS ratten. Meer DSP4 wordt dus opgenomen in APO-SUS ratten en dit resulteert in meer vernietigde terminals en een grotere reductie in noradrenerge activiteit. Om deze reductie te compenseren ontwikkelen de

postsynaptische alfa$_1$-adrenoceptors supersensitiviteit. Omdat de injectie de noradrenerge activiteit in APO-UNSUS ratten reduceert, wordt er minder DSP4 opgenomen en worden er minder terminals vernietigd. Daarom is de noradrenerge activiteit minder gereduceerd in APO-UNSUS ratten en dus vindt er geen compensatie plaats door het ontwikkelen van supersensitieve alfa$_1$-adrenoceptors. Echter, todat noradrenaline spiegels gemeten zijn in APO-SUS en APO-UNSUS ratten blijft deze verklaring speculatief.

De data laten zien dat de noradrenerge terminals van de locus coeruleus een belangrijke modulator zijn van de prepuls inhibitie. De rol van de medullaire groep moet hierin nog worden onderzocht.

De locus coeruleus, onder andere, projecteert vezels naar de nucleus accumbens (Speciale, Crowley et al., 1978), een structuur die bekend is betrokken te zijn bij de modulatie van de prepuls inhibitie. Het is gevonden dat de prepuls inhibitie verlaagd kan worden door bijvoorbeeld de dopaminerge activiteit in deze nucleus te verhogen (Swerdlow, Geyer et al., 2001). Dit is in overeenstemming met het feit dat amfetamine en andere drugs die de afgifte van dopamine stimuleren een toestand kunnen veroorzaken in normale individuen die lijkt op paranoïde schizofrenie (Connell, 1958) en een reductie in prepuls inhibitie in zowel mensen (Hutchison en Swift, 1999) als ratten (Mansbach, Geyer et al., 1998) kunnen veroorzaken. Binnen de nucleus accumbens is het bekend dat noradrenaline de afgifte van dopamine stuurt (Tuinstra en Cools, 2000). Dit leidt tot de hypothese dat noradrenaline de prepuls inhibitie moduleert door de dopaminerge activiteit in de nucleus accumbens te veranderen. Om deze hypothese te testen hebben we verschillende noradrenerge farmaca in de nucleus accumbens toegediend en het effect hiervan op de prepuls inhibitie in ratten getest.

Dit experiment werd gebaseerd op de microdialyse studie van Tuinstra en collega's (Tuinstra en Cools, 2000). De auteurs laten zien dat zowel de alfa$_1$-adrenoceptor agonist phenylephrine als de beta$_2$-adrenoceptor agonist isoproterenol de dopamine afgifte in de nucleus accumbens verhogen. Ook de alfa$_1$-adrenoceptor antagonist phentolamine verhoogde de afgifte van dopamine. De auteurs hebben geconcludeerd dat de agonist presynaptisch werkt en de afgifte van noradrenaline in de terminals blokkeert en vervolgens de inhibitie van de dopamine afgifte opheft. De antagonist, daarentegen, werkt door direct de postsynaptische receptoren op de dopaminerge terminals te blokkeren en daarmede de dopamine afgifte te stimuleren. Op deze feiten baseren wij onze verwachting om een reductie van de prepuls inhibitie te vinden in ratten na de toediening van phentolamine, isoproterenol en phenylephrine (**hoofdstuk 5**).

Injecties in de nucleus accumbens van noch de alfa$_1$-adrenoceptor agonist phenylephrine, noch de beta$_2$-adrenoceptor agonist proterenol veranderden de prepuls inhibitie in ratten. De alfa$_1$-adrenoceptor antagonist phentolamine was ook ineffectief. Wanneer samen gegeven, echter, reduceerden isoproterenol en phentolamine de prepuls inhibitie significant. Dit suggereert een cumulatief effect van deze twee drugs op dopamine. Zoals aangetoond door Zhang en collega's moet de verhoging van dopamine minstens 100% zijn om de prepuls inhibitie te verstoren (Zhang, Pouzet et al., 2000). Nog belangrijker is om te realiseren dat er twee te onderscheiden noradrenerge-dopaminerge systemen in de nucleus accumbens aanwezig zijn. Volgens de gating theorie van Cools en collega's (Cools, Van den Bos et al., 1991) verhogen beta$_2$-adrenoceptor agonisten de dopaminerge activiteit op het niveau van de D_2-receptoren, terwijl alfa$_1$-adrenoceptor antagonisten de dopaminerge activiteit verhogen op het niveau van een subtype dopamine receptoren, dat verschilt van het klassieke D_1 en D_2 type van receptoren. Omdat noch isoproterenol, noch phentolamine alleen de prepuls inhibitie veranderden, suggereren deze data sterk dat activering van beide typen dopamine receptoren nodig is om een reductie in prepuls inhibitie te veroorzaken. Verder is, volgens de gating theorie, een verhoging van dopamine in de nucleus accumbens geassocieerd met de regulatie van informatie input naar de nucleus accumbens. De verhoging van de dopaminerge activiteit op het niveau van de D_2-receptoren, door activering van beta$_2$-adrenoceptors in de nucleus accumbens, blokkeert de informatie input van de hippocampus. De verhoging van de dopaminerge activiteit op het niveau van het niet-klassieke D_1/D_2 receptor subtype, aan de andere kant, blokkeert de informatie input van de amygdala. Omdat het gevonden is dat de prepuls inhibitie alleen gereduceerd werd wanneer isoproterenol en phentolamine samen toegediend werden, suggereren deze data sterk dat de reductie prepuls inhibitie slechts optreedt wanneer de informatiestroom vanuit beide kernen geblokkeerd is. Dit is in overeenstemming met data over neofobie, waarbij slechts een verstoring werd gevonden wanneer zowel de hippocampus als de amygdala gate gesloten waren (Roozendaal en Cools, 1994). Deze bevinding suggereert dat de informatie input uit zowel de hippocampus als de amygdala nodig zijn voor een correcte prepuls inhibitie. Het was al gevonden dat de hippocampus en de amygdala de prepuls inhibitie moduleren. Bijvoorbeeld, de infusie van de NMDA antagonist dizocilipine in de basolaterale amygdala reduceert de prepuls inhibitie (Fendt, Schwienbacher et al., 2000). Bovendien reduceert de infusie van de glutamaat agonist NMDA in de ventrale hippocampus de prepuls inhibitie (Wan, Caine et al., 1996). Lesies van de entorhinale cortex (welke de primaire output connectie vormt van de hippocampus naar de nucleus accumbens) reduceert de prepuls inhibitie (Goto, Ueki et al., 2002). Dit effect kan opgeheven worden door systemische toediening van de D_2 antagonist haloperidol. Hoewel het niet duidelijk is of deze opheffing plaats vindt op het niveau van

de nucleus accumbens, is het toch in overeenstemming met de gating theorie, welke aangeeft dat de hippocampus gate gecontroleerd wordt door D_2 receptoren in de nucleus accumbens.

Hoe dan ook, deze data laten zien dat noradrenaline in de nucleus accumbens de prepuls inhibitie moduleren. Het feit dat noradrenaline de afgifte van dopamine in de nucleus accumbens verhoogt, samen met het feit dat een verhoogde dopaminerge activiteit in de nucleus accumbens de prepuls inhibitie reduceert, suggereert dat noradrenaline de prepuls inhibitie reduceert door de verhoging van dopamine. Bovendien duiden deze data erop dat de informatie input van zowel de hippocampus als de amygdala nodig zijn voor een correcte prepuls inhibitie.

Samenvattend, de gepresenteerde data in dit proefschrift laten zien dat stress de prepuls inhibitie moduleert. Bovendien hebben we laten zien dat dit effect gebaseerd is op de verhoging in noradrenerge activiteit, meest waarschijnlijk door activatie van de locus coeruleus. Ten slotte hebben we bewijs gevonden dat noradrenaline de prepuls inhibitie moduleert in de nucleus accumbens, waarschijnlijk door dopamine in de nucleus accumbens te beïnvloeden, en dientengevolge de informatie input van de amydala en de hippocampus naar de accumbens te reguleren.

In **hoofdstuk 7** bediscussiëren we de consequenties van deze bevindingen voor de modulatie van prepuls inhibitie en de behandeling van schizofrenie.

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3th Forum of European Neuroscience Paris 13-17 July 2002
T. Sontag, A.R. Cools & B.A. Ellenbroek

CURRICULUM VITAE

Thomas A. Sontag was born on the 5th of September in Göttingen, Germany. He completed his high-school education in 1988. After finishing his military service in 1989 he went to the "Medizinische Hochschule Hannover" to become a Technical Assistant in Biology. In 1992 he successful finished this training period. In the same year he started to study Biology at the "Georg-August University in Göttingen. In 1998 he successful finished his studies. During the studies at the University he worked as an assistant in a research project of the Max-Planck-Institut for Experimental Medicine,Göttingen, and of the Biologicaly and Veterinary Lab in Northeim with studies and registration of behaviour data of animals after cerebral lesions following mild cerebral oligemia and oxidative stress. In addition he joined training courses at the Nuffield Laboratory of Ophthalmology Oxford, Great Britain in 1992 and at the Institute of Pharmacology in Krakau, Poland in 1995. In 1999 he started as a PhD student at the University of Nijmegen under the supervision of Prof.Dr.Cools and Dr. Ellenbroek. The research conducted during his PhD-period resulted in the writing of this Thesis.

Thomas A. Sontag is currently working as a scientific assistant at the Institute of Psychology at the University of Regensburg, Germany.