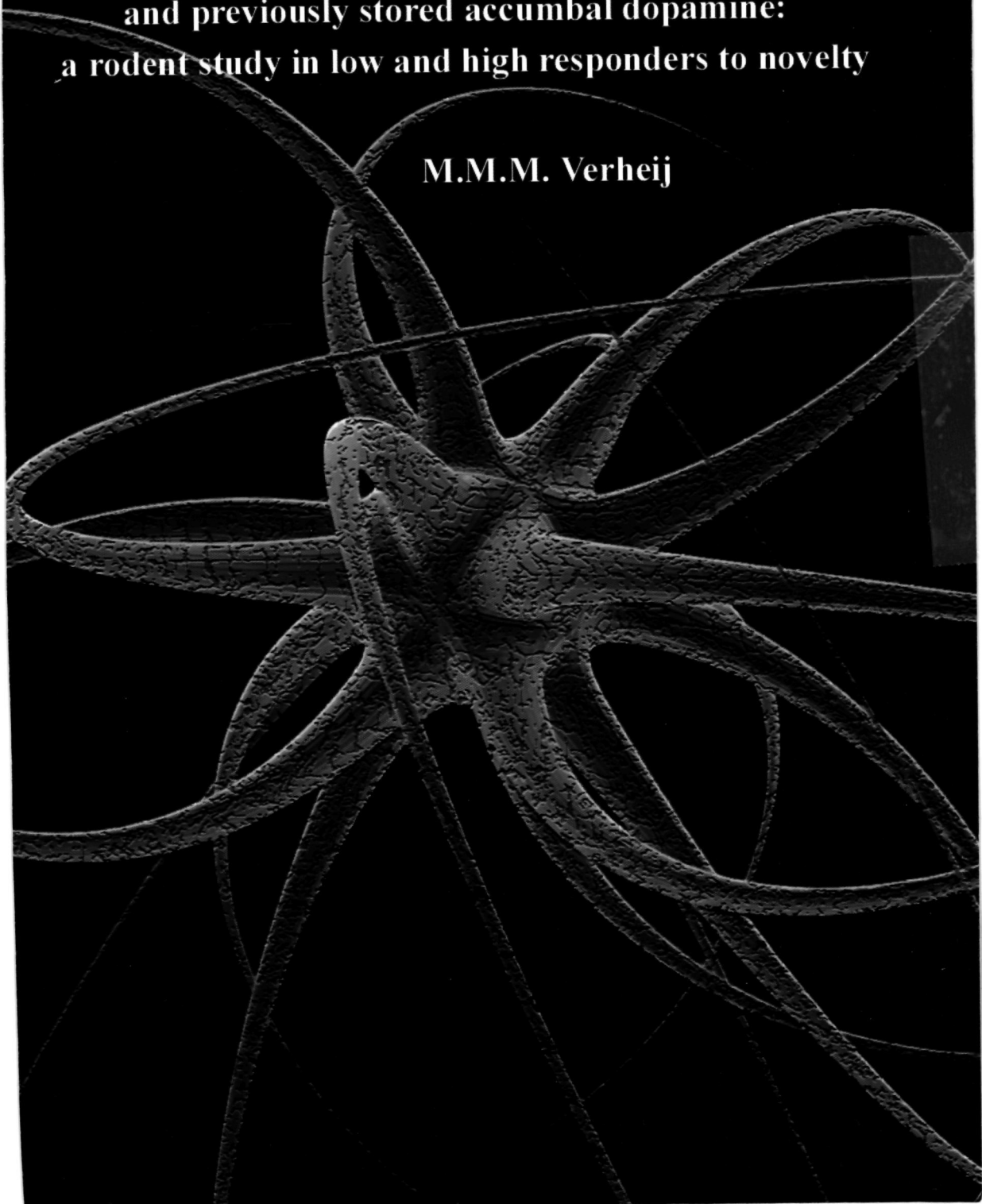
Individual differences in the release of newly-synthesised and previously stored accumbal dopamine: a rodent study in low and high responders to novelty

M.M.M. Verheij



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Voor mijn ouders.

De foto's in dit proefschrift beschrijven mijn promotietraject.
The pictures inside this thesis illustrate my journey to obtain a doctorate.

Contents

1/15: Aankomst op het centraal dierenlaboratorium te Nijmegen.
Arrival at the central animal house of Nijmegen.

Chapter 1

General introduction

M.M.M. Verheij and A.R. Cools (2008).

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1. Summary: The overall aim of this thesis is to provide evidence for individual differences in dopamine release from newly-synthesised pools and storage vesicles. This introductory chapter gives a short review of the synthesis and storage of dopamine, the release of dopamine from distinct types of pool, some behavioural effects of dopamine, the effects of psychostimulants on dopamine and the interaction between dopamine and noradrenaline. Special attention is directed towards dopamine release in the nucleus accumbens. This chapter ends with a short overview of the individual differences of the dopaminergic system of two different types of rat (high and low responders to novelty).

2.1 Catecholamines: synthesis and storage: Catecholamines belong to the wider group of neurotransmitters called monoamines (compounds that posses a single NH2 group). More specifically, catecholamines contain a nucleus of cathechol (a benzene ring possessing two adjacent OH groups) and a side chain of ethylamine (or one of its derivates). Two of the most important catecholamines are dopamine (3,4-dihydroxyphenylethylamine) and noradrenaline (norepinephrine). Both monoamines are found in the periphery as well as in the brain. Most of the pioneering work on the synthesis and storage of dopamine and noradrenaline was performed between 1960 and 1980 (see below).

Catecholamines are synthesised in several steps. The precursor for all catecholamines is L-tyrosine which is hydroxylated to L-dopa by the enzyme tyrosine hydroxylase (Nagatsu et al., 1964a; Nagatsu et al., 1964b). Tyrosine hydroxylase is the rate-limiting enzyme in the synthesis of catecholamines because its maximum activity is slower than that of any of the other enzymes in the synthetic pathway (Hess et al., 1961; Levitt et al., 1965). The second step in the synthesis of catecholamines is the decarboxylation of L-dopa to dopamine by the enzyme aromatic L-amino acid decarboxylase (Lovenberg et al., 1962; Christenson et al., 1972). In dopaminergic neurons, this is the final step in the biosynthetic pathway. Because of the localisation of tyrosine hydroxylase and aromatic L-amino acid decarboxylase in the cytoplasm, the

synthesis of dopamine is restricted to this cytoplasm (Musacchio, 1968; Laduron and Belpaire, 1968; Wurzburger and Musacchio, 1971; Christenson et al., 1972). In addition to its role as a neurotransmitter per se, dopamine also serves as the precursor of noradrenaline. Noradrenergic neurons contain an enzyme called dopamine-beta-hydroxylase that converts dopamine to noradrenaline (Levin et al., 1960; Kaufman and Friedman , 1965; Goldstein et al., 1965). Because dopamine-beta-hydroxylase is located within synaptic vesicles, dopamine must be transported from the cytoplasm into vesicles in order to be converted to noradrenaline (Kirshner , 1957; Oka et al., 1967; Laduron and Belpaire, 1968).

Like noradrenaline, dopamine may also be stored inside vesicles. These storage vesicles contain an ATP-ase that translocates protons inside vesicles (Johnson and Scarpa, 1976; Njus et al., 1978; Cidon and Nelson, 1983). The resulting electrochemical gradient is used by the vesicular-monoamine-transporter (VMAT) to take up dopamine from the cytoplasm into storage vesicles (Apps et al., 1980; Kanner et al., 1980). Vesicular-monoamine-transporters act as an antiporter (see Fig. 1), meaning that a proton is carried outside the vesicle for each molecule of dopamine that is carried inside (Winkler et al., 1986). Liu et al. (1992) have shown that vesicular-monoamine-transporters can be divided in at least two isoforms. Type I transporters are mainly located within the endocrine/paracrine cells of the adrenal gland whereas type II transporters are primarily found within the central nervous system (Liu et al., 1992; Weihe et al., 1994; Peter et al., 1995).

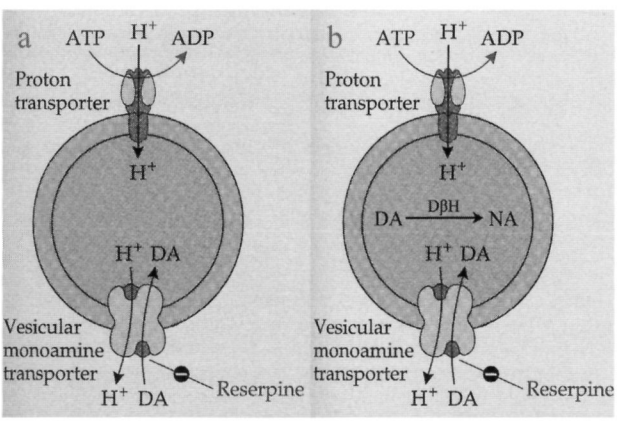


Figure 1: Dopaminergic (a) and noradrenergic (b) storage vesicle. Dopamine is accumulated inside storage vesicles by vesicular-monoamine-transporters (VMAT). These transporters act as an antiporter: a proton (H^+) is carried outside the vesicle for each molecule of dopamine (DA) that is carried inside. The vesicular proton gradient is maintained by an ATP-dependent proton transporter (V-ATPase). The enzyme dopamine-beta-hydroxylase (DβH) converts intravesicular dopamine (DA) to noradrenaline (NA) (Adapted from: Feldman et al., 1997).

Both the synthesis and the storage of catecholamines can be inhibited by several drugs The most frequently used inhibitor of the synthesis of catecholamines is a drug called alpha-methyl-para-tyrosine Alpha-methyl-para-tyrosine is known to inhibit tyrosine hydroxylase by competing with tyrosine at the tyrosine binding site (Nagatsu et al , 1964b, Sjoerdsma et al , 1965, Spector et al , 1965) Because alpha-methyl-para-tyrosine inhibits the synthesis of catecholamines, the newly synthesised pool of catecholamines is generally referred to as the alpha-methyl-para-tyrosine-sensitive pool The most frequently used inhibitors of the storage of catecholamines are reserpine and tetrabenazine Both drugs prevent the storage of newly synthesised transmitter molecules (Kirshner , 1962, Kirshner et al , 1963) by blocking vesicular monoamine transporters (Henry et al , 1994, Henry et al , 1998) Because reserpine inhibits the storage of catecholamines, the storage pool of catecholamines is generally referred to as the reserpine-sensitive pool

In addition to the above-mentioned biochemical studies, histochemical studies have also provided evidence in favour of two different catecholaminergic pools It has been demonstrated that dopaminergic neurons can be dived in neurons marked by a strong and dotted dopamine labelling, in addition to neurons marked by a weak and diffuse dopamine labelling (Olson et al , 1972, Fuxe et al , 1974, Descarries et al , 1980) Based on the level of dopamine fluorescence, the turn-over rate of dopamine and the sensitivity to dopamine depleting drugs, the diffuse compartment of dopamine was categorised as the alpha-methyl-para-tyrosine-sensitive, newly-synthesised pool whereas the dotted compartment of dopamine was categorised as the reserpine-sensitive, storage pool (Cools and Van Rossum, 1976, Cools, 1977, Cools and Van Rossum, 1980)

2.2 Catecholamines: release from distinct pools: Various models on dopamine release have been developed in the past twenty years (McMillen et al , 1980, Schoemaker and Nickolson, 1983, Justice et al , 1988, Leviel et al , 1989, Arbuthnott et al , 1990, Westerink, 2006) Numerous studies have demonstated that dopamine is released from both alpha-methyl-para-tyrosine-sensitive and from reserpine-sensitive pools The first evidence for the involvement of different pools in the release of

dopamine came from *in-vitro* studies demonstrating that the decline of spontaneous dopamine release consisted of two distinct phases (Besson et al., 1969; Javoy and Glowinski, 1971). The intracellular compartment associated with the first rapidly disappearing phase was supposed to be the newly-synthesised pool of dopamine whereas the compartment associated with the second slowly disappearing phase was supposed to be the dopaminergic storage pool (Besson et al., 1969; Javoy and Glowinski, 1971). Ewing et al. (1983) were the first to provide *in-vivo* evidence of dopamine release from either a newly-synthesised pool that was sensitive to alpha-methyl-para-tyrosine or a storage pool being resistant to this drug. Electrophysiological studies revealed that newly-synthesised dopamine is released after a single nerve stimulation whereas stored dopamine is progressively released after repeated stimulation at a short interval (Yavich, 1996; Yavich and MacDonald, 2000).

Dopamine is released by at least two intracellular mechanisms (see Fig. 2). First, dopamine may be released by an exocytotic process. This release depends on the influx of Ca^{2+} into the cell and appeared to be sensitive to blocking plasmalemmal Na^{+} channels (Katz and Miledi, 1967; Katz and Miledi, 1970; Raiteri et al., 1979; Westerink et al., 1987; Augustine et al., 1987; Lim et al., 1990). Because exocytotic release involves the fusion of vesicles with the plasmalemmal membrane, this type of release is supposed to be mediated by dopamine derived from reserpine-sensitive storage pools. Second, dopamine may be released by a process called reverse transport. This release is independent from the influx of Ca^{2+} into the cell and appeared to be sensitive to drugs that block plasmalemmal transporters (Heikkila et al., 1975; Raiteri et al., 1979; Fischer and Cho, 1979; Butcher et al., 1988). Because reverse transport involves cytoplasmatic dopamine to be transported out of the cell, this type of release is supposed to be mediated by dopamine derived from newly-synthesised pools. Schoemaker and Nickolson (1983) and Leviel et al. (1989) have independently proposed that the readily releasable pool of newly-synthesised dopamine is replenished by previously stored transmitters derived from a vesicular pool. Besides this mutual exchange between both types of pool, dopamine can also be released directly from the alpha-methyl-para-tyrosine-sensitive or reserpine-sensitive pool (Schoemaker and Nickolson, 1983; Leviel et al., 1989; Verheij and Cools, 2007).

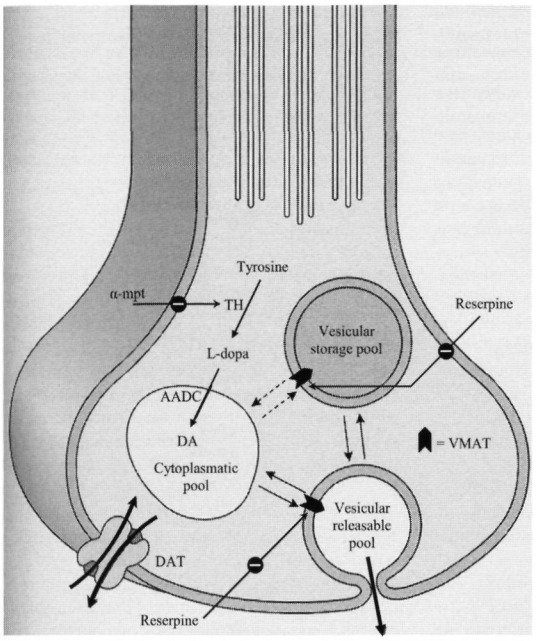


Figure 2: Dopaminergic nerve terminal. Tyrosine is converted to L-dopa, which is, in turn, converted to dopamine (DA), resulting in a cytoplasmatic pool of neurotransmitter. This newly-synthesised dopamine is taken-up by (large) storage vesicles and (small) releasable vesicles. The dopamine uptake into these vesicles is accomplished by vesicular-monoamine-transporters (VMAT). Newly-synthesised and cytoplasmatic dopamine is released into the extracellular space by reversal of dopamine transporters (DAT). Previously-stored and vesicular dopamine is released into the extracellular space by exocytosis. The drug alpha-methyl-para-tyrosine (α-mpt) reduces the amount of dopamine inside newly-synthesised pools by inhibiting tyrosine hydroxylase (TH). The drug reserpine reduces the amount of dopamine inside storage vesicles by inhibiting vesicular-monoamine-transporters. Treatment with either alpha-methyl-para-tyrosine or reserpine results in a decrease of the release of dopamine, which, in turn, results in a reduction of the amount of extracellular dopamine (Adapted from: Leviel et al., 1989).

2.3 Psychostimulants: release from distinct pools: It is generally accepted that cocaine and amphetamine increase the levels of dopamine in the brain (see section 2.8). This dopamine increase is commonly believed to be the result of the fact that cocaine blocks plasmalemmal dopamine transporters whereas amphetamine reverses the action of these transporters (Koob and Bloom, 1988). Recent findings that cocaine and amphetamine are still effective in mice lacking plasmalemmal transporters (Sora et al., 1998; Rocha et al., 1998; Carboni et al., 2001; Budygin et al., 2004), indicate that the effects of psychostimulants do not exclusively depend on these transporters. Both amphetamine and cocaine seem to have an additional mode of action that is different from their generally accepted mode of action on dopamine transporters. Scheel-Kruger and Braestrup were the first to report that psychostimulants also affect the presynaptic pools of monoamines (Scheel-Kruger, 1971; Scheel-Kruger, 1972; Braestrup, 1977; Scheel-Kruger et al., 1977). They demonstrated that the unconditioned locomotor response and stereotyped activity following amphetamine or methamphetamine were strongly inhibited by alpha-methyl-para-tyrosine, but not (or less) by reserpine. In contrast, the unconditioned locomotor response and stereotyped activity following

cocaine or methylphenidate were strongly inhibited by reserpine, but not (or less) by alpha-methyl-para-tyrosine These initial studies were subsequently confirmed by studies demonstrating that moderate doses of amphetamine release dopamine primarily from reserpine-resistant, alpha-methyl-para-tyrosine-sensitive, newly-synthesised pools (Davis et al , 1975, Chiueh and Moore, 1975b, McMillen et al , 1980, McMillen, 1983, Niddam et al , 1985, Parker and Cubeddu, 1986, Butcher et al , 1988, Mercuri et al , 1989, Callaway et al , 1989, Hiroi and White, 1990, Finn et al , 1990, Martin-Iverson et al , 1991, DiLullo and Martin-Iverson, 1992, Florin et al , 1995, Heeringa and Abercrombie, 1995, Cadoni et al , 1995, Sabol and Seiden, 1998) Recent studies, however, have revealed that high doses of amphetamine also release dopamine from storage pools High doses of amphetamine have been shown to redistribute dopamine from vesicles to the cytoplasm (Sulzer et al , 1995, Sulzer et al , 1996, Jones et al , 1998, Watanabe et al , 2005) It is suggested that all doses of amphetamine promote the efflux of cytoplasmatic dopamine by reverse transport (Fischer and Cho, 1979, Liang and Rutledge, 1982, Sulzer et al , 1993, Sulzer et al , 1995, Jones et al , 1998) The initial studies of Scheel-Kruger and Braestrup about the effect of cocaine were also confirmed by other studies demonstrating that the dopamine increase following cocaine depends on dopamine derived from alpha-methyl-para-tyrosine-resistant, reserpine-sensitive, storage pools (Jarbe, 1978, McMillen et al , 1980, McMillen, 1983, Davis, 1985, Einhorn et al , 1988, Hurd and Ungerstedt, 1989, Butcher et al , 1991, Florin et al , 1995, Pifl et al , 1995, Yan, 2003, Verheij et al , 2008)

2.4 Dopamine receptors: Dopamine that is released may bind to distinct types of dopamine binding sites The concept of two types of dopamine receptors, marked by their own properties, has first been introduced in the seventies (Cools and Van Rossum, 1976, Cools, 1977, Cools and Van Rossum, 1980) On the basis of a critical review of the available data at that time it was suggested that dopamine-loaded structures have to be marked by both inhibitory and excitatory binding sites These inhibitory binding sites were classified as dopamine DA1 receptors whereas the excitatory binding sites were classified as dopamine DAe receptors Shortly thereafter, biochemical evidence for two distinct dopamine receptors was provided by Garau et al (1978) and Titeler et al (1978)

These binding sites were labelled dopamine D_1 and dopamine D_2 receptors (Kebabian and Calne, 1979) Due to the development of new detection techniques, dopamine receptors can now be divided in 5 subtypes. Given that dopamine D_5 receptors share similarities with dopamine D_1 receptors this group of receptors is generally referred to as dopamine D_1-like receptors (Seeman and Van Tol, 1994) Because dopamine D_3 and D_4 receptors share similarities with dopamine D_2 receptors this group of receptors is generally referred to as dopamine D_2-like receptors (Seeman and Van Tol, 1994) Nowadays, the dopamine DA1/DAe receptor concept has been overruled by the dopamine D_1/D_2 receptor concept Over the years it became clear that dopamine DAe receptors are similar to dopamine D_2-like receptors (Cools et al , 1991, Cools and Tuinstra, 2003) It remains open for discussion whether the dopamine DA1 receptors are similar to dopamine D_1-like receptors At least, dopamine DA1 and D_1 receptors have been found to share some vital properties (Cools, 1986, Cools et al., 1989).

All known dopamine receptors are coupled to a membrane bound G-protein Activation of dopamine D_1-like receptors typically stimulates G-proteins which, in turn, stimulate adenylyl cyclase activity whereas activation of dopamine D_2-like receptors typically inhibits G-proteins which, in turn, inhibit the activity of adenylyl cyclase (Stoof and Kebabian, 1981, Clark and White, 1987). It is interesting to note that G-proteins exist in two distinct states one state with a low affinity and another state with a high affinity for dopamine and dopamine receptor agonists (Birnbaumer et al , 1990, Birnbaumer, 1990a, Birnbaumer, 1990b)

Dopaminergic receptors are present both pre- and post-synaptically Presynaptic receptors, which are found on axons, soma and dendrites, are generally referred to as auto receptors (Starke et al , 1989, Mercuri et al , 1992) Activation of dopaminergic auto receptors is known to inhibit 1) the firing of the dopaminergic cells (Aghajanian and Bunney, 1973, Aghajanian and Bunney, 1977a, Aghajanian and Bunney, 1977b), 2) the synthesis of dopamine (Kehr et al , 1972, Walters and Roth, 1976, Haubrich and Pflueger, 1982), and 3) the release of this neurotransmitter (Raiten et al , 1978, Starke et al , 1978, Jackisch et al , 1980) One may wonder how somatodendritic auto receptors have access to dopamine? In fact, there is strong biochemical evidence that dendrites of dopaminergic cells release dopamine similar to axons (Robertson et al , 1991, Santiago

and Westerink, 1992). Dendritic dopamine release is, therefore, supposed to control terminal dopamine release (Santiago and Westerink, 1991). Most studies have suggested that dopaminergic auto receptors belong to the D2-like family, instead of the D1-like family (Morelli et al., 1988; Starke et al., 1989; Santiago and Westerink, 1991; Bull et al., 1991; Fedele et al., 1993).

2.5 Noradrenaline receptors: Two types of noradrenaline receptors (adrenoceptors) exist. These types of receptors have initially been differentiated on the basis of their sensitivity to isoproterenol versus noradrenaline (Ahlquist, 1948; Ahlquist, 1976). Alpha adrenoceptors are defined as being more sensitive to noradrenaline than to isoproterenol and beta adrenoceptors are defined as being more sensitive to isoproterenol than to noradrenaline. Alpha adrenoceptors are divided in alpha-1 and alpha-2 subtypes whereas beta adrenoceptors are divided in beta-1, beta-2 and beta-3 subtypes (Bylund et al., 1994). Like the receptors for dopamine (see section 2.4), receptors for noradrenaline also belong to the G-protein-coupled family of receptors. It is generally accepted that activation of alpha adrenoceptors stimulates the second messenger guanylate cyclase (Greengard, 1979; Haidamous et al., 1980; Jaiswal and Sharma, 1986) whereas activation of beta adrenoceptors stimulates the second messenger adenylate cyclase (Greengard, 1979; Levitzki, 1988; Wallukat, 2002). In addition to dopaminergic receptors (see section 2.4), noradrenergic receptors also exist in two different states. One state in which the potency of noradrenaline and noradrenaline receptor agonists is stronger to stimulate second messengers than in the other (Hausdorff et al., 1990; Lefkowitz et al., 1990; Kobilka, 1992). Changes in the receptor state may be accompanied by phosphorylation of the receptor (Hausdorff et al., 1990; Lefkowitz et al., 1990; Kobilka, 1992) and are depending on the amount of endogenous noradrenaline that the receptor is exposed to (for ref. see: Cools et al., 1987; Cools et al., 1991; Tuinstra and Cools, 2000a; Cools and Tuinstra, 2003).

2.6 Dopaminergic pathways: Dopamine is estimated to constitute as much as 80% of the total brain catecholamine content. However, the total number of dopamine cells is relatively small. Dopamine-containing nerve cells are found in relatively rostral parts of

the brain compared with noradrenergic cells In their classical mapping studies Dahlstrom, Fuxe and co-workers designated the various catecholaminergic cell groups with a letter-number combination (Dahlstrom and Fuxe, 1964, Dahlstrom and Fuxe, 1965, Fuxe, 1965a, Fuxe, 1965b, Hillarp et al , 1966) Accordingly, cell groups A1-A7 were labelled noradrenergic whereas cell groups A8-A15 were labelled dopaminergic Based on the localisation of the dopaminergic fibres, four major dopaminergic pathways can be distinguished (for review Lindvall and Bjorklund, 1983, Fuxe et al , 1985, Feldman et al , 1997)

The mesostriatal pathway (dopaminergic pathway 1) This pathway originates from cells in the substantia nigra (A9 cell group), the ventral tegmental area (A10 cell group), and the retrorubral (A8) cell group The dorsal component of this system (nigrostriatal pathway) ascends via the medial forebrain bundle and the internal capsule to innervate the neostriatum (caudate-putamen) and globus pallidus The nigrostriatal pathway is vitally important in motor control, and degeneration of this system is the key feature of Parkinson's disease The mesostriatal pathway also possesses a ventral component that innervates the nucleus accumbens, olfactory tubercles and medial neostriatum Some authors include this pathway as part of the mesolimbic system

The mesolimbic pathway (dopaminergic pathway 2) This pathway originates primarily in the ventral tegmental area, with smaller contributions from the substantia nigra and retrorubral cell group In addition to the nucleus accumbens and olfactory tubercles, this system also projects to the septum, amygdala, hippocampus, anterior olfactory nucleus and limbic cortical areas The mesolimbic pathway is thought to play a critical role in the reinforcing properties of psychostimulants and is the system hypothesised to be hyperactive in the classic dopamine theory of schizophrenia

The mesothalamic pathway (dopaminergic pathway 3) and mesopontine pathway (dopaminergic pathway 4) The first pathway (also called the mesodiencephalic pathway) projects to the subthalamic nucleus and the habenula whereas the second pathway connects the A9 and A10 cell groups to the locus coeruleus

Besides the abovementioned major dopaminergic pathways, additional minor dopaminergic pathways also exist These minor dopaminergic pathway differ in the origin of the fibres Fibres arising from the A11 cell group form the periventricular and

diencephalonspinal system. The tuberohypophyseal system emanates from A12 cells whereas the incertohypothalamic system arises mainly in A13 cells. Of the dopaminergic cell groups, originally identified by Dahlström and Fuxe, the A15 cell group is located most rostral, i.e. inside the olfactory bulbs.

2.7 Noradrenergic pathways: Noradrenergic fibres mainly originate in the pons and medulla. Based on the distribution of these fibres, three major noradrenergic pathways can be distinguished (for review: Cotman and McGaugh, 1980; Lindvall and Bjorklund, 1983; Feldman et al., 1997).

The locus coeruleus pathways (noradrenergic pathway 1). The locus coeruleus (A6 cells) together with its caudal extensions (A4 cells) is the most important noradrenergic system in the brain. Its axons project dorsally into the cerebellum, caudally into the spinal cord and rostrally as the dorsal noradrenergic bundle. This bundle projects to virtually all parts of the tel- and di-encephalon, including the neocortex, hippocampus, amygdala, septum, thalamus and hypothalamus.

The lateral tegmental pathways. This system is divided into the pontine group (noradrenergic pathway 2: A5 and A7 cells, together called the subcoeruleus group) and the medullary group (noradrenergic pathway 3: A1, A2 and A3 cells). Like the coeruleus pathway, the subcoeruleus pathway also projects into the spinal cord. In addition, the rostral projections of the A1, A2, A3, A5 and A7 form the ventral noradrenergic bundle which terminates in the hypothalamus and a large number of other diencephalic structures.

2.8 Nucleus accumbens: organisation: The main focus of this thesis is on the dopaminergic and noradrenergic systems of the nucleus accumbens (see Fig. 3). The nucleus accumbens was first described by Ziehen (1901) as the structure ventral to the anterior commissure, comprising the anterior, ventromedial part of the striatum that surrounds the bottom of the anterior horn of the lateral ventricle and extends dorsally into the lateral part of the septum. The accumbens receives dopaminergic inputs from the ventral tegmental area and substantia nigra, glutamatergic inputs from the amygdala, hippocampus, prefrontal cortex, thalamus and ventral pallidum as well as serotonergic

inputs from the raphe nuclei (Groenewegen et al , 1999, Zahm, 2000, Morgane et al , 2005) These afferents are known to terminate on GABA-ergic, medium-sized and spiny projection neurons (Smith and Bolam, 1990) The nucleus accumbens also contains a relatively small amount of interneurons which are thought to connect both types of projection neurons (Emson et al , 1993) Interneurons are either GABA-ergic or cholinergic (Kawaguchi et al , 1995)

The accumbens can be subdivided into a central core, which is surrounded on its medial and ventral sides by the shell (Jongen-Relo et al , 1994) The (medial) shell region is predominantly innervated by the dopaminergic cells of the ventral tegmental area (A10 cell group), whereas the core region is predominantly innervated by the dopaminergic cells of the substantia nigra (A9 cell group) (Beckstead et al , 1979, Brog et al , 1993, Groenewegen et al , 1999) The nucleus accumbens also receives noradrenergic afferents from A6/A4 cells of the locus coeruleus group (Unemoto et al , 1985a, Unemoto et al , 1985b, Cole and Robbins, 1987, Lategan et al , 1990, Brog et al , 1993, Delfs et al , 1998) and from A1/A2/A3 cells of the medullary group (O'Donohue et al , 1979, Li et al , 1990, Zagon et al , 1994, Kirouac and Ciriello, 1997, Delfs et al , 1998) Noradrenergic neurons are known to terminate in the shell, but also in the core (Brog et al , 1993, Zagon et al , 1994, Ikemoto et al , 1996, Berridge et al , 1997, Delfs et al , 1998) Both the shell and core receive glutamatergic inputs from the amygdala and hippocampus (Groenewegen et al , 1987, Brog et al , 1993, Wright et al , 1996, Groenewegen et al , 1999) The (medial) shell of the accumbens projects mainly to ventral tegmental area and the ventromedial part of the ventral pallidum and to a lesser extent to the substantia nigra pars compacta whereas the core of the accumbens projects mainly to the substantia nigra pars reticulata and the dorsolateral part of the ventral pallidum and to a lesser extent to the substantia nigra pars compacta and retrorubral area (Heimer et al , 1991, Zahm and Brog, 1992, Pennartz et al , 1994, Groenewegen et al , 1996, Zahm, 1999, Groenewegen et al , 1999, Zahm, 2000) Recent studies have revealed a feed-forward connection between the shell and the core via the substantia nigra pars compacta (Haber et al , 2000, Haber, 2003) In addition, the shell has also been found to be directly connected to the core (van Dongen et al , 2005)

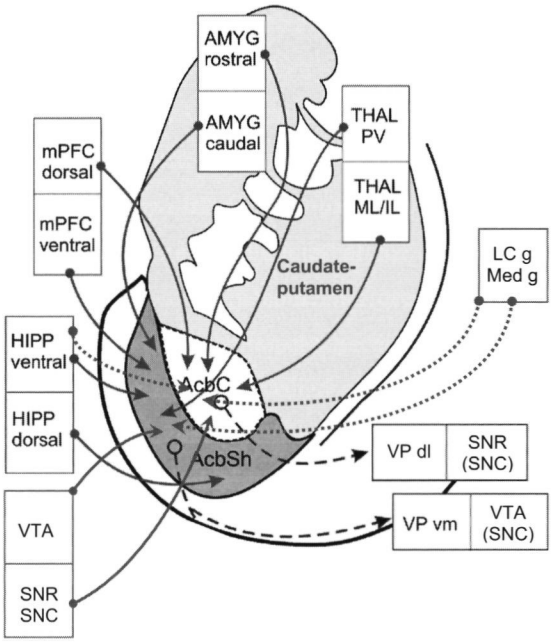


Figure 3: Afferent and efferent projections of the nucleus accumbens. The accumbens shell (AcbSh) receives dopaminergic fibres mainly from the ventral tegmental area (VTA), whereas the accumbens core (AcbC) receives dopaminergic fibres mainly from the substantia nigra (pars reticulata: SNR and pars compacta: SNC). Both the shell and the core receive noradrenergic fibres from the locus coeruleus (LC g) and the medullary (Med g) group. In addition, the amygdala (AMYG) and the hippocampus (HIPP) project to both the shell and the core. Moreover, the accumbens receives inputs from the medial prefrontal cortex (mPFC) and the thalamus (THAL).
The accumbens shell mainly projects to the ventral tegmental area (VTA) and the ventromedial part of the ventral pallidum (VP vm), whereas the accumbens core mainly projects to the substantia nigra pars reticulata (SNR) and the dorsolateral part of the ventral pallidum (VP dl). Note: not all projections are depicted. Figure adapted from: Groenewegen, 2007.

Because of its afferents from mesolimbic structures and its efferents to motor structures, the nucleus accumbens has been described as an interface between the limbic and motor system transferring emotion into motion (Mogenson et al., 1980; Cools, 1988; Groenewegen et al., 1996). Regarding the accumbens subdivisions, the shell is hypothesised to be part of the limbic loop and the core is hypothesised to be part of the motor loop (Deutch and Cameron, 1992; Deutch, 1993; Zahm, 2000).

Besides a clear anatomical distinction between the shell and core, both regions of the nucleus accumbens can also be distinguished on the basis of their dopaminergic reactivity to psychostimulants. Psychostimulants more strongly increase accumbal dopamine levels in the shell than in the core (Pontieri et al., 1995; Heidbreder and Feldon, 1998; Hedou et al., 1999; Lecca et al., 2004; Giorgi et al., 2005; Giorgi et al., 2007). Remarkably, the stronger psychostimulant-induced increase of dopamine in the shell than in the core is not accompanied by more or more active dopamine transporters in the shell than in the core (Sharpe et al., 1991; Jones et al., 1996; Nirenberg et al., 1997b; Budygin et al., 2002; Mateo et al., 2004). The finding that the region-specific

effects of psychostimulants do not match the region-specific distribution of dopamine transporters underlines the above-mentioned notion (see section 2 3) that alternative processes that do not necessarily involve an action at dopamine transporters may play an important role in the effects of cocaine and amphetamine

2.9 Behaviour and accumbal dopamine: The accumbens became focal point of interest among behavioural scientists with the discovery that dopamine injections into this brain structure strongly enhance locomotor activity (Pijnenburg and Van Rossum, 1973, Jackson et al , 1975, Costall and Naylor, 1975, Costall et al , 1976) This stimulating effect can be mimicked by intra-accumbens injections of the non-selective dopamine D_1/D_2 receptor agonist apomorphine (Jackson et al , 1975, Plaznik et al , 1985) and by a mixture of dopamine D_1 and D_2 receptor agonists (Plaznik et al , 1989, Essman et al , 1993, Koene et al , 1993) In addition to its role in mediating horizontal (locomotor) activity, dopamine in the nucleus accumbens is also known to mediate vertical (rearing) activity (Kalivas et al , 1984, Koene et al , 1993, Swanson et al , 1997) Later on, it became evident that dopamine in the nucleus accumbens is also involved in the regulation of forelimb rigidity (Ellenbroek et al , 1988) and the display of oral movements (Koene et al , 1993, Prinssen et al , 1994, Cools et al , 1995) Furthermore, accumbal dopamine is known to allow an organism to switch to cue-directed behaviour (Van den Bos et al , 1991, Cools et al , 1993a) In addition, accumbal dopamine is involved the acquisition and consolidation of memory tasks (Ploeger et al , 1991, Cools et al , 1993a, Ploeger et al , 1994, Setlow and McGaugh, 1998, Coccurello et al , 2000, Mele et al , 2004) In general, accumbal dopamine plays a role in the execution of goal-directed behaviour that is driven by appetitive or aversive stimuli (Salamone, 1994, Mitchell and Gratton, 1994, Baldo and Kelley, 2007, Grace et al , 2007) In particular, accumbal dopamine is involved in processes related to reward including the reinforcing effects of drugs of abuse (Sutton and Beninger, 1999, Carelli, 2002, Schultz, 2004, Salamone et al , 2005, Ikemoto, 2007, Di Chiara and Bassareo, 2007, Berridge, 2007) At present it is not clear whether the accumbens is involved in the regulation of (an)hedonic aspects (tendency to like), sensorimotor aspects (tendency to work) (Salamone, 1994, Salamone, 1996, Salamone and Correa, 2002) or perceptual aspects

(tendency to want) (Robinson and Berridge, 1993; Berridge and Robinson, 1998) of drugs of abuse.

As discussed below, exposure to novelty changes the dopamine levels in the nucleus accumbens in an individual-specific manner (see section 3.1).

2.10 Behaviour and accumbal noradrenaline: Sawaya and co-workers were the first to suggest that the nucleus accumbens contains two types of noradrenaline receptors (Sawaya et al., 1977). Nowadays, it is generally accepted that both alpha and beta adrenoceptors are present in the nucleus accumbens (Rainbow and Biegon, 1983; Nurse et al., 1984; Rainbow et al., 1984; Nurse et al., 1985; Jones et al., 1985; Boyajian et al., 1987; Russell et al., 1993; Tuinstra and Cools, 2000a; Tuinstra and Cools, 2000b; Cools and Tuinstra, 2003; Bhardwaj et al., 2004). For a long time it was believed that the accumbal alpha-adrenoceptors were not of the pre-synaptic type (Schoffelmeer et al., 1998). A recent study, however, providing evidence for alpha-adrenoceptor mediated auto-regulation of noradrenaline release in the nucleus accumbens indicated that, in addition to post-synaptic alpha-receptors, pre-synaptic alpha-receptors may also exist in this brain structure (Aono et al., 2007). The distribution of adrenoceptor types in the core and in the shell is still unknown.

Accumbal noradrenaline is involved in the regulation of various types of behaviour in which accumbal dopamine is also involved in (see section 2.9). Like intra-accumbens administration of dopamine, intra-accumbens administration of noradrenaline (Jackson et al., 1975; Pijnenburg et al., 1975; Costall et al., 1976; Plaznik et al., 1985) and intra-accumbens administration of noradrenergic drugs (Costall et al., 1976; Cools et al., 1987; Svensson et al., 1995) increase horizontal (locomotor) and vertical (rearing) activity. In addition, adrenergic alpha and beta receptor agents applied into the accumbens affect the acquisition and retrieval of memory tasks (Cools et al., 1991; Tuinstra et al., 2000; Tuinstra et al., 2002).

As discussed below, exposure to novelty not only changes accumbal dopamine levels, but also the levels of accumbal noradrenaline in an individual-specific manner (see section 3.2).

2.11 Nucleus accumbens: noradrenaline-dopamine interactions: The fact that dopamine and noradrenaline share the regulation of some processes (see sections 2 9 and 2 10) indicates an interaction between noradrenergic and dopaminergic systems (Antelman and Caggiula, 1977) Indeed, accumbal noradrenaline receptors play a dual role in modulating accumbal dopamine release Stimulation of accumbal alpha adrenoceptors decreases the (evoked) release of dopamine from accumbal slices whereas stimulation of beta adrenoceptors increases the (evoked) dopamine release from these slices (Nurse et al , 1984, Nurse et al , 1985, Russell et al , 1988, Russell et al , 1989, Russell et al , 1993) These *in-vitro* studies have subsequently been confirmed *in-vivo* The intra-accumbens injection of either the alpha adrenoceptor antagonist phentolamine or the beta adrenoceptor agonist isoproterenol was found to increase the levels of accumbal dopamine (Tuinstra and Cools, 2000a, Cools and Tuinstra, 2003) It was concluded that mesolimbic alpha adrenoceptors inhibit and that mesolimbic beta adrenoceptors facilitate the release of accumbal dopamine (Tuinstra and Cools, 2000a, Cools and Tuinstra, 2003) Evidence is provided that these alpha and beta adrenoceptors are located on the terminals of dopaminergic neurons (Tuinstra and Cools, 2000a, Cools and Tuinstra, 2003)

It was recently found that beta-, but not alpha-adrenoceptors, control the release of dopamine that is derived from newly-synthesised pools (Tuinstra and Cools, 2000b) The fact that the alpha-adrenoceptor mediated release of accumbal dopamine is not derived from alpha-methyl-para-tyrosine-sensitive pools suggest that alpha-adrenoceptors control the release of dopamine from reserpine-sensitive pools

3.1 Individual differences: accumbal dopamine: The experiments discussed in this thesis focus on two types of individuals that are known to differ in their dopaminergic neurotransmission within the nucleus accumbens These individuals, which are present in a normal outbred population of Wistar rats, are selected on the basis of their locomotor response to a novel open-field, and accordingly labelled High Responders to novelty (HR) and Low Responders to novelty (LR) Both ambulation and habituation are used to select these animals on the open-field Ambulation is defined as the distance travelled on the open-field during 30 min Habituation time is defined as the duration of

the period that starts as soon the rats begin to explore the open-field and ends as soon the locomotor activity stops for a period of at least 90 s Open-field behaviour is recorded with a computerised tracking system (Cools et al , 1990) Rats that habituate before 480 s and walk less than 4,800 cm are labelled LR whereas rats that habituate after 840 s and walk more than 6,000 cm are labelled HR (Cools et al , 1997, Saigusa et al , 1999)

Neurochemical studies have revealed that non-challenged HR are marked by a higher basal level of dopamine inside the nucleus accumbens than non-challenged LR (Hooks et al , 1992a) Exposure to novelty has been found to increase accumbal dopamine levels in both types of rat (Saigusa et al , 1999) However, the increase of accumbal dopamine is stronger in novelty-challenged HR than in novelty-challenged LR (Saigusa et al , 1999) The sensitivity of the accumbal dopamine levels to the monoamine synthesis inhibitor alpha-methyl-para-tyrosine has also been shown to be different in HR and LR Saigusa et al (1999) have demonstrated that the novelty-induced increase of accumbal dopamine in HR is inhibited by alpha-methyl-para-tyrosine, indicating that novelty-challenged HR release dopamine from newly-synthesised pools In contrast, alpha-methyl-para-tyrosine has been found not to reduce the novelty-induced increase of accumbal dopamine in LR (Saigusa et al , 1999), thereby opening the perspective that novelty-challenged LR release dopamine from reserpine-sensitive pools

3.2 Individual differences: accumbal noradrenaline: In the light of the interaction between mesolimbic dopamine and noradrenaline (see section 2 11), it is not surprisingly that, in addition to the functional activity of the accumbal dopamine system (see section 3 1), LR and HR were also found to differ in the functional activity of the noradrenergic system of the nucleus accumbens (Roozendaal and Cools, 1994, Tuinstra and Cools, 2000a, Cools and Tuinstra, 2003) Neurochemical studies have revealed that the noradrenergic activity at the level of alpha adrenoceptors is larger in non-challenged LR than in non-challenged HR (Tuinstra and Cools, 2000a, Cools and Tuinstra, 2003) Following exposure to novelty, the noradrenergic activity at the level of accumbal alpha adrenoceptors increases in HR and decreases in LR (Tuinstra and Cools, 2000a, Cools and Tuinstra, 2003) The noradrenergic activity at the level of accumbal beta

adrenoceptors was found to be equally low in non-challenged HR and non-challenged LR (Tuinstra and Cools, 2000a, Cools and Tuinstra, 2003) Following exposure to novelty, the noradrenergic activity at the level of accumbal beta adrenoceptors increases in HR, but does not change in LR (Tuinstra and Cools, 2000a, Cools and Tuinstra, 2003) The impact of these individual differences in accumbal noradrenaline release on the individual differences in accumbal dopamine release are explained in the general discussion of this thesis (see chapter 9)

3.3 Individual differences: psychostimulants: The fact that LR and HR differ in the functional activity of their accumbal dopamine system (see section 3 1) together with the fact that accumbal dopamine is involved in the reinforcing effect of drugs of abuse (see sections 2 8 and 2 9) indicates that LR and HR differ in their sensitivity to psychostimulants like amphetamine and cocaine Indeed, HR have been found to be more susceptible to the acute behavioural effects of moderate doses of amphetamine than LR (Piazza et al , 1989, Hooks et al , 1994, Gingras and Cools, 1997, Cools et al , 1997, Bevins et al , 1997) As discussed above, the acute response to moderate doses of amphetamine are depending on dopamine that is released from alpha-methyl-para-tyrosine-sensitive, and not, or less, from reserpine-sensitive pools (see section 2 3) The fact that HR are more sensitivity to moderate doses of amphetamine compared to LR, indicates that more accumbal dopamine can be derived from alpha-methyl-para-tyrosine-sensitive pools in HR than in LR This nicely fits in with the finding that the nucleus accumbens of HR contains more tyrosine hydroxylase than the nucleus accumbens of LR (see Fig 4)

Apart from the fact that HR are more sensitive to moderate doses of (dex)amphetamine than LR, HR are also more susceptible to the behavioural and accumbal dopamine increasing effects of cocaine than LR (Hooks et al , 1991b, Hooks et al , 1992a, Giorgi et al , 1997, Chefer et al , 2003) Cocaine is known to increase the amount of extracellular dopamine by blocking the neuronal re-uptake of this neurotransmitter after it has been released from reserpine-sensitive, instead of from alpha-methyl-para-tyrosine-sensitive pools (see section 2 3) The finding that HR are

more sensitive to cocaine than LR may suggest that HR release more dopamine from the reserpine-sensitive pools of the nucleus accumbens than LR.

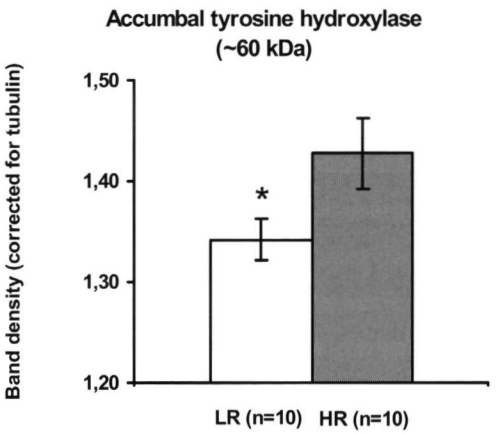


Figure 4: Individual differences in accumbal tyrosine hydroxylase levels. Western blot analysis was performed on punches of accumbal tissue. Immunoreactivity levels of the accumbal tyrosine hydroxylase protein of ~60 kDa were quantified using tubulin for normalisation (see: Verheij et al., 2008 for procedures). Data are expressed as mean ± S.E.M. *Significant differences between low responders (LR: n=10) and high responders (HR: n=10) to novelty rats (one-way ANOVA).

3.4 Individual differences: APO-SUS and UNSUS: Different research groups study individual differences using different types of rat. One has to be aware that not all features of the LR and HR that are studied by different research groups are identical between these groups. This is largely due to differences in the selection procedure assessed. Nevertheless, it is important to note that the LR and HR studied by Piazza et al. (Piazza et al., 1989; Piazza et al., 1990; Piazza et al., 1991a; Rouge-Pont et al., 1993; Dellu et al., 1996a) and by Hooks et al. (Hooks et al., 1991a; Hooks et al., 1991b; Hooks et al., 1992a; Hooks et al., 1992b; Hooks et al., 1994) share numerous features with the Nijmegen LR and HR (Saigusa et al., 1999).

Based on the susceptibility to a systemic injection of a dopamine D_1/ D_2 receptor agonist, two types of rat that share many similarities with the rats selected on the open-field can be identified. During this pharmacological selection procedure, the gnawing response to systemic apomorphine is measured (Cools et al., 1990; Ellenbroek and Cools, 2002). Rats with low gnawing scores are labelled apomorphine-unsusceptible

(APO-UNSUS) rats, whereas rats with high gnawing scores are labelled apomorphine-susceptible (APO-SUS) rats (Cools et al., 1990; Ellenbroek and Cools, 2002). After selection, these rats have successfully been incorporated in a breeding programme (Cools et al., 1990; Ellenbroek and Cools, 2002). The susceptibility to apomorphine has been found to correlate with the response to novelty. Similar to HR, APO-SUS rats travel a relatively large distance on the open-field and are marked by a long habituation time (Cools et al., 1990). Similar to LR, APO-UNSUS rats travel a relatively small distance on a novel open-field and are marked by a short habituation time (Cools et al., 1990). Moreover, the behavioural response to novelty correlates with the susceptibility to apomorphine as well. HR are marked by higher gnawing scores than LR (Fig. 5). Additional studies have demonstrated that HR and APO-SUS rats on one hand and LR and APO-UNSUS rats on the other hand share many similarities in their sensitivity to drugs of abuse [(APO-UNSUS/APO-SUS rats (alcohol): Sluyter et al., 2000; van der Kam et al., 2005a) vs (LR/HR (alcohol): Gingras and Cools, 1995)]. It is also important to note that the dopaminergic and noradrenergic reactivity and make-up of the nucleus accumbens of HR is very similar to the dopaminergic and noradrenergic reactivity and make-up of the nucleus accumbens of APO-SUS rats [(APO-SUS rats (dopamine): van der Elst et al., 2005b) vs (HR (dopamine): Verheij and Cools, 2007) and (APO-SUS rats (noradrenaline): Cools et al., 1990; Cools et al., 1994) vs (HR (noradrenaline): Roozendaal and Cools, 1994; Cools and Gingras, 1998) and (APO-SUS rats (tyrosine hydroxylase): van der Elst et al., 2005a) vs (HR (tyrosine hydroxylase): Fig. 4)]. In addition, the dopaminergic and noradrenergic make-up and reactivity of the nucleus accumbens of LR is very similar to the dopaminergic and noradrenergic make-up and reactivity of the nucleus accumbens of APO-UNSUS rats [(APO-UNSUS rats (dopamine): van der Elst et al., 2005b) vs (LR (dopamine): Verheij and Cools, 2007) and (APO-UNSUS rats (noradrenaline): Cools et al., 1990; Cools et al., 1994) vs (LR (noradrenaline): Roozendaal and Cools, 1994; Cools and Gingras, 1998) and (APO-UNSUS rats (tyrosine hydroxylase): van der Elst et al., 2005a) vs (LR (tyrosine hydroxylase): Fig. 4)]. It is beyond the scope of this thesis to point out all individual differences between HR and APO-SUS rats on one hand and LR and APO-UNSUS rats on the other hand. For references describing the 'hardware and software' of both types of

rat in a comprehensive and detailed way see: Cools et al., 1990; Cools et al., 1993b; Cools et al., 1994; Cools and Ellenbroek, 1996; Cools and Gingras, 1998; Cools and Tuinstra, 2003.

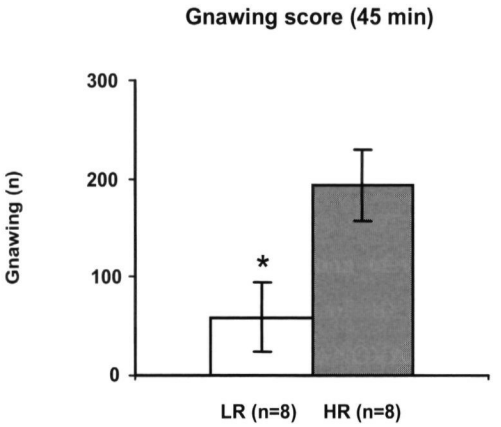


Figure 5: Individual differences in the gnawing response to 1.5 mg/kg (s.c.) of the D1/D2 agonist apomorphine. The total gnawing score during 45 min was calculated (see: Cools et al., 1990 for procedures). Data are expressed as mean ± S.E.M. *Significant differences between low responders (LR: n=8) and high responders (HR: n=8) to novelty (one-way ANOVA).

4. Microdialysis: The experiments described in this thesis mainly focus on individual differences in the extracellular levels of dopamine in the nucleus accumbens. One way to measure extracellular levels of accumbal dopamine in the brain is microdialysis (also termed intracerebral dialysis or intracranial dialysis). This *in-vivo* brain perfusion method was originally used by Ungerstedt and coworkers to measure the release of striatal dopamine in freely moving rats (Ungerstedt et al., 1982; Zetterstrom et al., 1983; Ungerstedt, 1984). Microdialysis is based on the principle of dialysis, in which a semi-permeable membrane separating two solutions allows diffusion to occur between these solutions. The diffusion takes place in a microdialysis probe, which is stereotaxically implanted into the brain. The microdialysis probe is continuously perfused with artificial cerebral fluid (Ringer solution) devoid of the compound of interest. Materials of interest diffuse down a concentration gradient from the interstitial fluid into the probe. The movement of the fluid through the probe ensures a constant concentration gradient between the fluid inside and the fluid outside the probe. The perfusate is subsequently directed to a collection site where it is injected into a system to

separate the compound of interest from the remaining compounds The most widely used technique to separate dopamine from the remaining neurotransmitters is high performance/pressure liquid chromatography (HPLC) During this technique the perfusate is mixed with a mobile phase (solution containing water and methanol) that is directed through a stationary phase (column of steel containing silica particles coated with C18 molecules) Separation is based on the fact that a-polar compounds of the perfusate spend more time in the a-polar stationary phase than in the polar mobile phase The HPLC technique is frequently combined with electrochemical detection (ECD) to measure the concentration of the compound of interest During electrochemical detection a potential is applied to the perfusate containing the separated compounds As a results of this potential dopamine molecules dissociate thereby delivering 2 electrons A recording device is used to visualise the changes of the current over time Based on the retention time dopamine can be identified whereas the peak height is used to calculate the amount of this compound

Microdialysis is also used to locally apply drugs The drug must be concentrated in the Ringer solution in higher concentrations than it occurs in the extracellular space, in order to diffuse into the brain Because microdialysis allows to monitor the levels of neurotransmitters in the brain of the conscious rat, microdialysis is an excellent tool to correlate changes in accumbal dopamine levels to changes in behaviour For more details on brain-microdialysis see Benveniste et al , 1989, Lindefors et al , 1989, Benveniste and Huttemeier, 1990, Westerink, 1992, Parsons and Justice, 1994, Westerink, 1995, Di Chiara et al , 1996, Elmquist and Sawchuk, 1997, Westerink et al , 1998, de Lange et al , 2000, Plock and Kloft, 2005, Cano-Cebrian et al , 2005, Chen, 2005a, Chen, 2005b, Chen, 2006

Aim and outline of this thesis

Individual differences in the dopaminergic system of the nucleus accumbens have extensively been reported These individual differences have frequently been used to explain individual differences in response to environmental and pharmacological challenges Remarkably, only little attention has been paid to the factors that underlie

these individual differences Subjects of the studies described in this thesis were Low Responders (LR) and High Responders (HR) to novelty (see section 3 of this chapter) These rats have previously been found to be marked by a different accumbal dopamine increase after both novelty (see section 3 1 of this chapter) and cocaine (see section 3 3 of this chapter) From the studies described in this introductory chapter it will be evident that individual differences in the dopamine release from pools that are sensitive to either alpha-methyl-para-tyrosine or reserpine may play an important role in the previously reported individual differences in the accumbal dopamine response to novelty (see section 3 1 of this chapter) and cocaine (see section 3 3 of this chapter) The overall aim of the studies described in this thesis was to provide evidence for individual differences in accumbal dopamine release from either newly-synthesised or storage pools of dopamine Given the interaction between the dopaminergic and noradrenergic systems of the nucleus accumbens (see section 2 11 of this chapter) special attention was directed to the question of how accumbal noradrenaline modulates the release of both newly-synthesised and stored dopamine

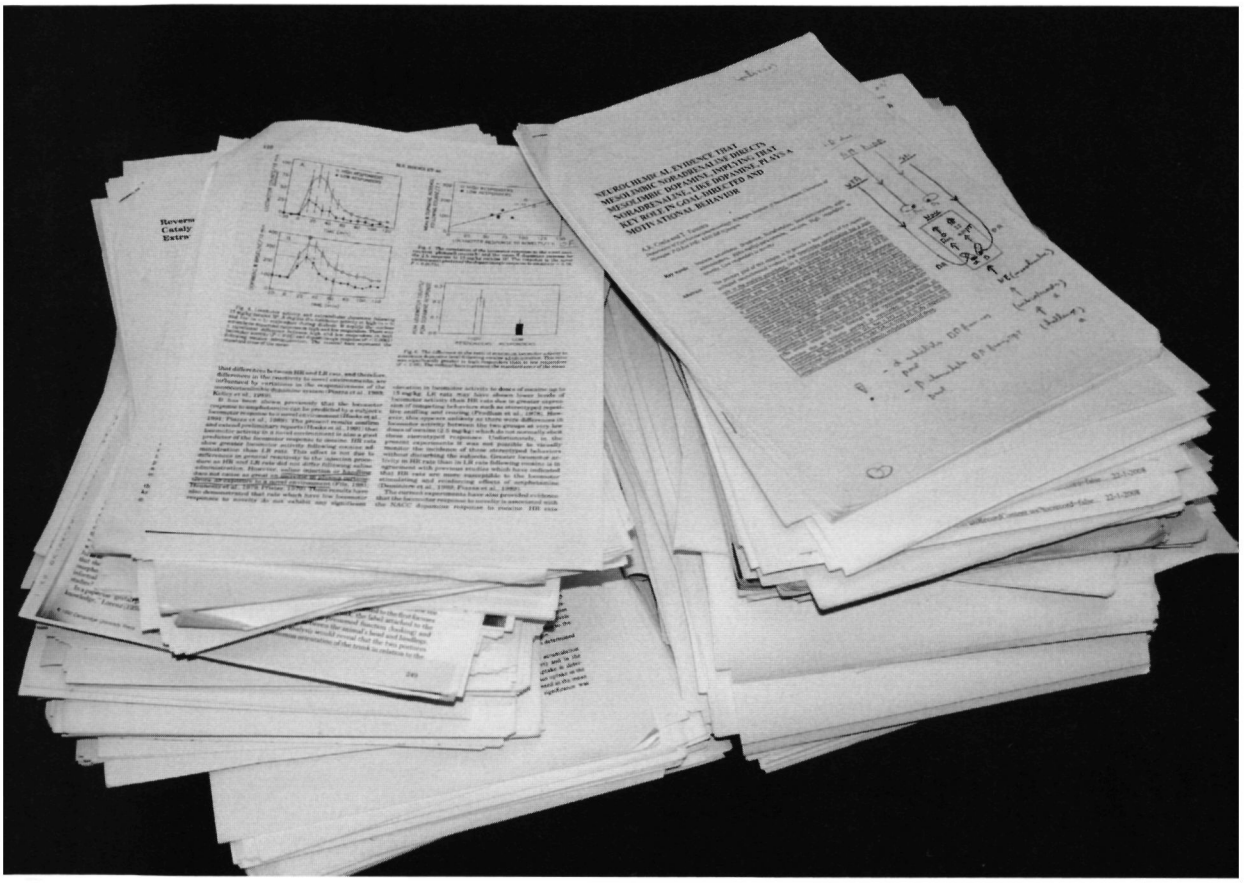
In **chapter 2** the previously reported finding that the novelty-induced increase of accumbal dopamine in HR could be inhibited by alpha-methyl-para-tyrosine (see section 3 1 of this chapter) was replicated Because alpha-methyl-para-tyrosine did not inhibit the novelty-induced increase of accumbal dopamine in LR (see section 3 1 of this chapter), we hypothesised that the novelty-induced accumbal dopamine increase in these rats could be inhibited by reserpine It was, indeed, found that the novelty-induced increase of accumbal dopamine is derived from storage pools in LR, but not in HR The effects of reserpine on the basal levels of dopamine in HR and LR under non-challenged conditions were analysed as well Non-challenged HR were found to release more accumbal dopamine from storage vesicles than non-challenged LR These results demonstrate that the effects of reserpine on accumbal dopamine release are depending on the condition the rats are exposed to In **chapter 3** we showed that cocaine increased accumbal dopamine levels more strongly in HR than in LR Because the effects of cocaine are known to depend on storage vesicles (see section 2 3 of this chapter), the effects of reserpine on the cocaine-induced increase of accumbal dopamine were

investigated in both types of rat In addition, the amount of dopamine inside the reserpine-sensitive storage vesicles of the nucleus accumbens and the amount of accumbal vesicular-monoamine-transporters (type 2) were measured It was demonstated that HR contain more accumbal vesicular-monoamine-transporters-2 and a larger amount of accumbal dopamine inside storage vesicles than LR It was hypothesised that HR are more sensitive to the dopamine increasing effects of cocaine than LR, because cocaine can release more accumbal dopamine from the storage vesicles in HR than from the storage vesicles in LR Finally, the dose of 1 mg/kg of reserpine completely prevented the cocaine-induced accumbal dopamine release in LR, but not in HR In fact, a higher dose of 2 mg/kg of reserpine was required to inhibit cocaine-induced accumbal dopamine release in HR **Chapter 4** describes the behavioural effects of cocaine and the effects of reserpine on cocaine-induced behaviour in HR and LR In agreement with the neurochemical data of chapter 3, the dose of 1 mg/kg of reserpine reduced behaviour in LR, but not in HR The dose of 2 mg/kg of reserpine was required to change behaviour in HR It was hypothesised that HR are more sensitive to the behavioural effects of cocaine than LR, because cocaine can release more accumbal dopamine from the storage vesicles in HR than from the storage vesicles in LR

The remaining part of this thesis concentrates on the noradrenaline-dopamine interactions in the nucleus accumbens In **chapter 5** we showed that the beta-adrenoceptor-mediated increase of accumbal dopamine could be inhibited by alpha-methyl-para-tyrosine Because alpha-methyl-para-tyrosine did not inhibit the alpha-adrenoceptor-mediated increase of accumbal dopamine (see section 2 11 of this chapter), the effects of reserpine to inhibit the alpha-adrenoceptor-mediated increase of accumbal dopamine were investigated in **chapter 6** In **chapter 7** a study is presented that investigates whether reserpine inhibits the alpha-adrenoceptor-mediated changes in behaviour It was, indeed, found that reserpine reduced the accumbal dopamine increase and the behavioural response to the alpha-adrenoceptor antagonist phentolamine, but not to the beta-adrenoceptor agonist isoproterenol The experiments described in chapter 6 also revealed that reserpine-sensitive pools of noradrenaline control the above-mentioned beta-adrenoceptor-mediated release of newly-synthesised dopamine

In **Chapter 8** it is shown that alpha-methyl-para-tyrosine-sensitive pools of noradrenaline control the above-mentioned alpha-adrenoceptor-mediated release of vesicular dopamine The data described in chapter 5-8 illustrate that the interaction between noradrenaline and dopamine in the nucleus accumbens is not only mediated by different adrenoceptors (alpha-receptors and beta-receptors), but also by different pools of neurotransmitter (AMPT-sensitive and RES-sensitive) in both dopaminergic and noradrenergic nerve terminals

Chapter 9 provides the general discussion of the results reported in this thesis A model is presented illustrating that individual differences in accumbal dopamine release are due to individual differences in 1) the functional reactivity of the noradrenergic system, 2) the accumbal concentration of vesicular-monoamine-transporters and tyrosine hydroxylase and 3) the size of the presynaptic pools of dopamine Our data are embedded in the available literature to create a model that illustrates the putative 'software and hardware' giving rise to the individual-specific release of accumbal dopamine An important role is contributed to individual differences in the reactivity of the hypothalamic-pituitary-adrenal-axes, the reactivity of second messenger systems as well as in the aminergic reactivity of the accumbens shell and core The experiments described in this thesis indicate that, apart from agents that interact with the dopaminergic system, noradrenergic agents as well as agents that interact with vesicular-monoamine-transporters (reserpine) or tyrosine hydroxylase (alpha-methyl-para-tyrosine) may have therapeutic effects in subjects that are suffering from diseases in which the dopaminergic system is disturbed Special attention is directed to the putative therapeutic effects of these agents to treat cocaine and amphetamine abuse, Parkinson's disease and schizophrenia Finally, the results of this thesis are summarised in **chapter 10**



2/15: Lezen van de beschikbare wetenschappelijke literatuur.
Reading the available scientific literature.

Chapter 2

Differential contribution of newly-synthesised and storage pools to the extracellular amount of accumbal dopamine in high and low responders to novelty: effects of alpha-methyl-para-tyrosine and reserpine

M.M.M. Verheij and A.R. Cools (2007).

Journal of Neurochemistry 100: 810-821.

Abstract

The present study examined the effects of reserpine and alpha-methyl-para-tyrosine on the extracellular concentration of accumbal dopamine in High Responder to novelty rats (HR) and Low Responder to novelty rats (LR).

Reserpine reduced the baseline concentration of extracellular accumbal dopamine more in HR than in LR, indicating that non-challenged HR release more dopamine from reserpine-sensitive vesicles in the Nucleus Accumbens than non-challenged LR. In addition, reserpine reduced the novelty-induced increase of the extracellular concentration of accumbal dopamine in LR, but not in HR, indicating that only novelty-challenged LR, but not novelty-challenged HR, release dopamine primarily from accumbal reserpine-sensitive storage pools.

The previously reported effects of alpha-methyl-para-tyrosine on the extracellular concentration of accumbal dopamine in novelty-challenged HR and LR (Saigusa et al., 1999) were replicated. Alpha-methyl-para-tyrosine reduced the novelty-induced increase of the extracellular concentration of accumbal dopamine in HR, but not in LR, indicating that only novelty-challenged HR, but not novelty-challenged LR, release dopamine primarily from accumbal alpha-methyl-para-tyrosine-sensitive newly-synthesised pools.

The present data, together with earlier published data (Tuinstra and Cools, 2000) were used to develop a model that explains how novelty differentially affects the release of dopamine from accumbal reserpine-sensitive pools and accumbal alpha-methyl-para-tyrosine-sensitive pools in LR and HR, respectively.

The present data demonstrate that HR and LR differ in processes that are associated with the function of 1) vesicular mono-amino transporters and 2) tyrosine hydroxylase.

Introduction

The main goal of the present study was to examine individual-specific differences in the release of dopamine from storage vesicles located in the nerve terminals of the Nucleus Accumbens. The drug reserpine (RES) is frequently used to study the neurochemistry of vesicular storage pools (Arbuthnott et al., 1990). RES, which blocks type II vesicular monoamine transporters in the brain, prevents the sequestering of dopamine inside storage vesicles (Henry et al., 1994). After treatment with RES, storage vesicles are known to become empty (Dahlstrom et al., 1965; Wagner, 1985; Pothos et al., 1998; Colliver et al., 2000; Gong et al., 2003). As a result, the extracellular concentration of dopamine in brain areas containing dopaminergic nerve terminals strongly decreases (Imperato and Di Chiara, 1984; Kannari et al., 2000). Dopamine can also be released from cytosolic pools in which dopamine is continuously synthesised. The drug alpha-methyl-para-tyrosine (AMPT) is frequently used to study the neurochemistry of these newly-synthesised pools (Arbuthnott et al., 1990). AMPT reduces the synthesis of dopamine due to the inhibition of tyrosine hydroxylase (Corrodi and Hanson, 1966). As a result, the extracellular concentration of dopamine in brain areas containing dopaminergic nerve terminals strongly decreases (Stahle and Ungerstedt, 1990; Tuinstra and Cools, 2000b). Both types of pool are not fully independent: a redistribution of dopamine from vesicles to the cytosol, and *vice-versa*, may occur (Schoemaker and Nickolson, 1983; Justice et al., 1988; Leviel et al., 1989; Arbuthnott et al., 1990; Watanabe et al., 2005).

Various studies have indicated that presynaptic pools of dopamine are essential for psycho-stimulants to be effective. For instance, the behavioural and neurochemical response to the transporter blocker cocaine (Lee et al., 2001) depends mainly on dopamine inside storage vesicles (Scheel-Kruger et al., 1977; McMillen et al., 1980; McMillen, 1983; Davis, 1985; Einhorn et al., 1988; Hurd and Ungerstedt, 1989; Florin et al., 1995; Pifl et al., 1995; Yan, 2003; Venton et al., 2006). Although individual differences in sensitivity to cocaine have extensively been reported (Hooks et al., 1991b; Hooks et al., 1992a; Giorgi et al., 1997; Piazza et al., 2000; Ranaldi et al., 2001; Mantsch et al., 2001; Chefer et al., 2003; van der Kam et al., 2005b), the underlying mechanisms,

such as individual differences in the characteristics of storage vesicles, have not been studied so far. Therefore, we investigated the effects of RES on accumbal dopamine levels in two types of individual that are known to differ in their sensitivity to cocaine. These individuals, which co-exist in a normal population of Wistar rats, are selected on the basis of their locomotor response to a novel open-field and accordingly labelled High Responder to novelty (HR) rats and Low Responder to novelty (LR) rats (Dellu et al., 1996b; Bevins et al., 1997; Cools and Gingras, 1998; Cools and Tuinstra, 2003; Kabbaj, 2004).

Recent studies have revealed that intra-accumbens infusion of AMPT equally reduces the accumbal extracellular dopamine levels in non-challenged HR and LR (Tuinstra and Cools, 2000b), indicating that non-challenged HR and LR release an equal amount of dopamine from newly-synthesised pools. In contrast, it has been demonstrated that intra-accumbens infusion of AMPT reduces the increase of accumbal extracellular dopamine in novelty-challenged HR, but not in novelty-challenged LR (Saigusa et al., 1999). Therefore, it has been concluded that novelty-challenged HR primarily release dopamine from newly-synthesised pools. Because AMPT was not effective in reducing the novelty-induced increase of accumbal dopamine in LR, we hypothesised that novelty-challenged LR primarily release dopamine from RES-sensitive pools.

Using microdialysis, we investigated whether novelty-challenged LR are more sensitive to the dopamine decreasing effects of RES than novelty-challenged HR are. Because the sequestering of dopamine inside RES-sensitive storage vesicles is dependent on the translocation of protons into these storage vesicles (Winkler et al., 1986; Johnson, 1987), we also investigated whether novelty-challenged HR and LR were differentially sensitive to systemic acidosis. The effects of both RES and acidosis were also tested in non-challenged rats. The study by Saigusa et al. (1999) on the effects of AMPT in HR and LR was replicated for reasons described in the section experimental procedures.

Experimental procedures

Animal care: All experiments were performed in accordance with institutional, national and international guidelines for animal care and welfare.

Subjects: Adult male LR and HR (LR: n=92, HR: n=93; weight=180-220 g) that were selected from the outbred strain of Nijmegen Wistar rats, were used throughout the study. All rats were reared and housed in macrolon cages (42 x 26 x 15 cm; n=3-4 per cage) under a fixed 12/12 h light/dark cycle (lights on: 07.00 a.m.) in a temperature-controlled room (21 ± 1.7 °C). Water and food pellets were available *ad libitum*.

Open-field selection: Rats were individually housed 3 days before the open-field selection procedure (Tuinstra and Cools, 2000b). Testing took place between 09.00 h and 17.00 h in a room illuminated by white light of 170 Lux. The rat was placed on a black, square table (160 x 160 cm) made of Perspex. This open-field is 95 cm elevated above the floor and surrounded by a white background (270 x 270 x 270 cm). As described by Cools et al., (1990) behaviour was recorded with a computerised tracking system for a period of 30 min. Both ambulation and habituation time were used to select LR and HR. Ambulation was defined as the overall distance (cm) travelled in 30 min. Habituation time was defined as the duration of the period (s) that started as soon as the rat began to explore the open-field and ended as soon as the locomotor activity stopped for at least 90 s. Rats that habituated in less than 480 s and walked less than 4,800 cm in 30 min were labelled LR, whereas rats that habituated after 840 s and walked more than 6,000 cm in 30 min were labelled HR (Cools et al., 1997).

Surgery: One day after the open-field selection, LR and HR were unilaterally implanted with a stainless steel guide cannula (length: 5.5 mm, outer diameter: 0.65 mm, inner diameter: 0.3 mm) directed at the right Nucleus Accumbens according to previously described procedures (Tuinstra and Cools, 2000b). Under sodium pentobarbital anaesthesia (60 mg/kg, i.p.) rats were placed in a stereotactic apparatus and the following coordinates were used according to the atlas of Paxinos and Watson

(1986): anterior: +10.6 mm (relative to the interaural line) and lateral: -1.5 mm (relative to the midline suture). The guide cannula was lowered 5.5 mm relative to the dura surface resulting in a vertical coordinate of +3.5 mm for the cannula tip. Finally, the cannula was angled 10° laterally to the right side. Screws and cement were used to fixate the cannula to the skull. Because our novel data on the effects of RES showed that these effects were limited to novelty-challenged LR, it was necessary to replicate the finding by Saigusa et al. (1999) that the effects of AMPT were, in contrast, limited to novelty-challenged HR. The present study was a follow-up study of the experiments performed by Saigusa and of the experiments performed by Tuinstra and Cools (2000b). The angle at which the dialysis probe was implanted into the brain slightly differed between these two studies. We, therefore, replicated the study of Saigusa using the coordinates of Tuinstra. In order to ensure that all rats throughout the present study were equipped with a cannula directed at the same location, the coordinates of Tuinstra were also used for the new experiments on the effects of RES and acidosis.

The rats were allowed to recover from surgery for the next 7 to 10 days in Plexiglas dialysis cages (25 x 25 x 35 cm) covered with sawdust on the floor. These cages became the animal's home cage for the rest of the experiment. On 3 consecutive days just prior to the start of the microdialysis experiment, each rat was gently picked up in order to habituate to the procedure assessed on the 2 days when the accumbal dopamine was measured. This handling procedure was repeated 3 times per day (Tuinstra and Cools, 2000b).

Microdialysis: A dialysis probe (outer diameter: 0.22 mm, 50,000-molecular-weight cut-off) was carefully inserted into the brain of a conscious rat. The tip of the dialysis probe protruded 2 mm below the distal end of the guide cannula. The inlet and outlet of the probe were connected to a swivel allowing the rat to move undisturbed. Accumbal dialysates were analysed for dopamine (pg/40 µl) according to previously described procedures (Tuinstra and Cools, 2000b). Briefly, the probe was perfused at a rate of 2.0 µl/min with modified Ringer solution and the outflow was collected in a sample loop and injected automatically once every 20 min into a high performance liquid chromatography (HPLC) system. Dopamine was separated by means of reversed

phase, ion-paring liquid chromatography and the concentration was measured using electrochemical detection (ECD). The probes had an *in vitro* recovery of 10-12% for dopamine. The microdialysis system was calibrated with a standard dopamine solution before and after each experiment. The detection limit was 500 fg per sample.

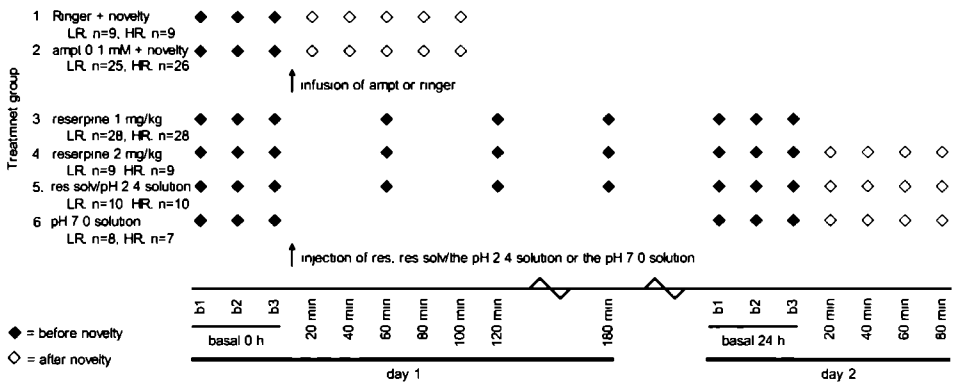


Figure 1. Schematic illustration of the experiments of the present study. Each square represents an accumbal dopamine sample taken at a particular time point. Filled squares represent accumbal dopamine samples taken in non-challenged rats whereas open squares represent accumbal dopamine samples taken when these rats were exposed to novelty (new cage). Arrows indicate at which time point AMPT was locally infused in the Nucleus Accumbens or RES was intraperitoneally administered.

Baseline concentration of accumbal dopamine: 4 H following probe insertion the extracellular accumbal concentration of dopamine (pg/sample) is known to reach a stable baseline ± 10% (Tuinstra and Cools, 2000b). As soon as a stable baseline concentration of dopamine was reached, 3 consecutive samples were taken in order to assess the accumbal extracellular concentration of dopamine in fully habituated LR and HR. Because the baseline concentration of accumbal dopamine served as the starting point of the experiment, it was labelled 'basal 0 h'. Immediately after basal 0 h was measured, LR and HR were randomly divided over 6 treatment groups according to figure 1.

Effects of novelty and alpha-methyl-para-tyrosine in novelty-challenged rats (Exp. 1): The aim of experiment 1 was to replicate the original study by Saigusa et al (1999) on the effects of AMPT in novelty-challenged LR and HR. Immediately after basal 0 h was established, LR and HR were exposed to novelty by removing these rats from their home cage and transferring them into a new environment, which consisted of a Plexiglas box that was slightly larger compared to the home cage (new dimensions: 30 x 30 x 35 cm) and lacked sawdust on the floor. Rats that continuously received modified Ringer solution (Fig. 1, group 1) served as control group for the AMPT-treated rats (Fig. 1, group 2). The intra-accumbens infusion of 0.1 mM of AMPT (dissolved in Ringer) started just before the rats were transferred into the new cage and lasted for exactly 40 min. 5 Dopamine samples were taken at 20 min intervals for a total period of 100 min after exposure to novelty (see Fig. 1). The animals treated with AMPT were also used in a follow-up study (Verheij and Cools, chapter 5), which explains the relatively large number of animals used in the present study.

Effects of reserpine in non-challenged rats (Exp. 2): The first aim of experiment 2 was to establish the effects of RES on the extracellular accumbal dopamine levels in non-challenged LR and HR. Immediately after basal 0 h was established, LR and HR were treated with 1 mg/kg of RES (Fig. 1, group 3), 2 mg/kg of RES (Fig. 1, group 4) or its solvent (Fig. 1, group 5). These drugs were administered in a volume of 1 ml/kg (i.p.), after which the rats were returned to their home cage. LR and HR were further not disturbed and 3 dopamine samples of 20 min were collected once every 60 min for a total period of 3 h (see Fig. 1). At 24 h after RES or its solvent, 3 additional dopamine samples of 20 min were taken in order to establish the basal levels of dopamine before exposure to novelty (Fig. 1, basal 24 h). The animals treated with 1 mg/kg of RES were also used in a follow-up study (Verheij and Cools, chapter 6), which explains the relatively large number of animals used in the present study.

Effects of reserpine in novelty-challenged rats (Exp. 2): The second aim of experiment 2 was to establish the effects of RES on the extracellular accumbal dopamine levels in novelty-challenged LR and HR. Because 2 mg/kg of RES was more effective

than 1 mg/kg of RES in reducing dopamine levels under non-challenged conditions, only the higher dose of RES was used to study its effects under novelty-challenged conditions. LR and HR were transferred to the novel cage (see Exp. 1) at 24 h after treatment with either 2 mg/kg of RES (Fig. 1: group 4) or its solvent (Fig. 1: group 5). 4 Dopamine samples were collected at 20 min intervals for a total period of 80 min after exposure to novelty (see Fig. 1).

Effects of systemic acidosis (Exp. 3): The aim of experiment 3 was to establish the putative after-effects of pH on the extracellular accumbal dopamine levels in LR and HR. Because the control solution of the RES experiment (RES solvent) had a pH 2.4, a new experiment on the effects of a solution with pH 7.0 was performed. To allow direct comparison between the effects of these solutions with a different pH, both solutions contained the same adjuvants, apart from phosphoric acid that was used to get a pH 2.4 (see section solutions). Directly after basal 0 h was measured, LR and HR were injected with the pH 7.0 solution (Fig. 1: group 6). The solution was administered in a volume of 1 ml/kg (i.p.), after which the rats were returned to their home cage. At 24 h after the pH 7.0 solution, 3 dopamine samples of 20 min were taken in order to establish the basal levels of dopamine before exposure to novelty (Fig. 1: basal 24 h). Immediately after basal 24 h was established, LR and HR were transferred to the novel cage (see Exp. 1). 4 Dopamine samples were collected at 20 min intervals for a total period of 80 min after exposure to novelty (see Fig. 1).

Behavioural observation: The duration (s) of walking behaviour (displacement of all 4 paws over a minimum distance of 1 cm for a period of at least 3 s) was analysed for all animals that were exposed to novelty on either the first day or the second day of the experiment (see Fig. 1). Recordings were made for a period of 20 min directly before the transfer to the new environment as well as during a period of 40 min starting directly after exposure to the new environment. Behaviour was scored by an observer blind to the treatment and type of the rat with the help of a computer programme (KEYS^{R}) developed at our institute (Saigusa et al., 1999).

Histology: At the end of each experiment the rat was deeply anaesthetised with an overdose of sodium-pentobarbital and, after breathing was stopped, intracardially perfused with 60 ml 4% paraformaldehyde solution. The rats were decapitated, the brains were removed and *post* fixated in the same solution for at least 24 h. Vibratome sections (100 µm) were cut to determine the exact location of the microdialysis probe.

Solutions: 1) RES: ampoules containing 1 or 2 mg of RES per ml solvent. 2) RES solvent = the pH 2.4 solution: 30 mg dl-methionine dissolved in 10 ml aquadest containing 6.75% propylene glycol. The pH of the RES solution and that of its solvent was adjusted to 2.4 using phosphoric acid. 3) The pH 7.0 solution: 30 mg dl-methionine dissolved in 10 ml aquadest containing 6.75% propylene glycol.

Expression of the data: The effects of RES, the pH 2.4 solution and the pH 7.0 solution on accumbal dopamine under non-challenged conditions are expressed as percentage of the basal level 0 h (Fig. 1). The same holds true for the effects of AMPT or its solvent on accumbal dopamine under novelty-challenged conditions. For evident reasons, the effects of RES, the pH 2.4 solution and the pH 7.0 solution on accumbal dopamine under novelty-challenged conditions are expressed as percentage relative of the basal level 24 h (Fig. 1).

Analysis of the data: The baseline concentrations of extracellular accumbal dopamine (basal 0 h) before exposure to the treatments of figure 1 were statistically compared using a two-way ANOVA with the factors type of rat and treatment. The neurochemical effects of the various treatments were statistically compared, using a three-way ANOVA for repeated measures with the factors type of rat, treatment and time. Where appropriate, this test was followed by a two-way ANOVA for repeated measures. In case more than 2 doses of a drug were used, a post-hoc least significant difference (LSD) pairwise multiple comparison test (factor: dose) was also performed. To analyse at which time points the effects of the various doses significantly differed, a Student's t-test was assessed. In addition, a one-sample t-test was used to evaluate

whether a specific treatment significantly changed accumbal dopamine levels from baseline.

The behavioural effects of the various treatments were statistically compared, using a two-way ANOVA with the factors type of rat and treatment. Where appropriate, this test was followed by a one-way ANOVA. In case LR and HR significantly differed in their novelty-induced increase of walking behaviour after a particular treatment, the relationship between the mean novelty-induced increase of accumbal dopamine in the first 40 min after novelty and the total time spent on walking behaviour in that period was evaluated by means of Pearson's 2-tailed correlation analysis in a pooled group of LR and HR. A probability level of $p<0.05$ was taken as significant in every test. SPSS for Windows (Release 12.0) was used to statistically analyse the data. All data are expressed as the mean ± SEM per treatment group.

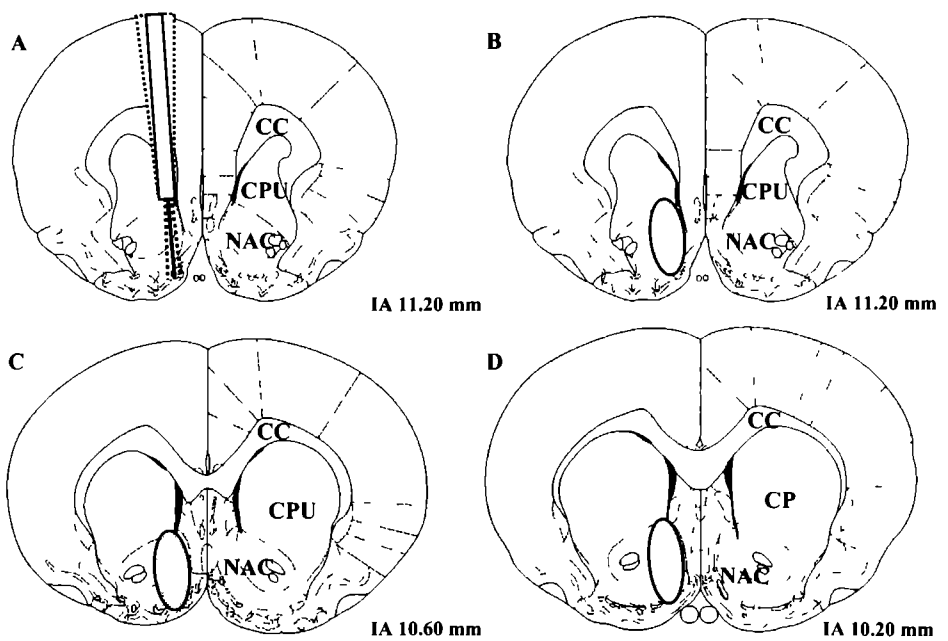


Figure 2. Example of 3 unilateral microdialysis probe tracks located in the right Nucleus Accumbens (A). The probe protrudes 2 mm below the distal end of the guide cannula **Schematic illustration of coronal brain sections containing the Nucleus Accumbens (B-D).** The brain region in which correctly placed probes were found is indicated in grey IA corresponds to the distance (mm) from the interaural line according to Paxinos and Watson (1986), NAC=Nucleus Accumbens, CPU=caudate putamen, CC=corpus callosum

Results

Open-field selection: The open-field selection procedure revealed 22% LR and 31% HR. The average distance travelled in 30 min (± SEM) was 3,500 ± 90.6 cm and 8,255 ± 139.4 cm in LR and HR, respectively. The average habituation time (± SEM) was 358 ± 12.4 s in LR and 1,286 ± 35.8 s in HR.

Histology: Histological verification revealed that 1 LR and 1 HR had to be excluded from analysis because of incorrect placement of the microdialysis guide cannula. Because of obstruction of the microdialysis probe 2 LR and 3 HR were also excluded from analysis. The coronal region of the Nucleus Accumbens in which all correctly placed microdialysis probe tracks were located is shown in figure 2.

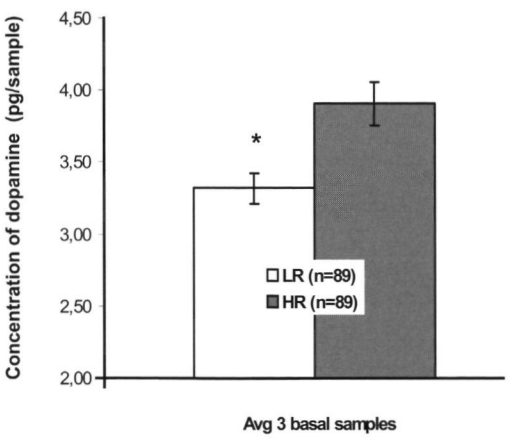


Figure 3. Baseline concentration (pg/sample = pg/40 µl) of extracellular dopamine in the Nucleus Accumbens at 4 h following probe insertion (basal 0 h) in non-challenged LR and non-challenged HR. Values are expressed as mean of the dopamine concentration of the 3 consecutive samples taken immediately before the rats were exposed to a manipulation (Fig. 1). Avg = average. *significant difference between HR and LR (treatments pooled).

Baseline concentration of accumbal dopamine: All rats were either sitting or sleeping during the 20 min preceding their exposure to novelty. Walking behaviour was simply absent in this period. The baseline extracellular concentration of accumbal dopamine (basal 0 h) did not differ between the treatment groups before exposure to any treatment (Fig. 1: two-way ANOVA: no treatment effect). Pooling the animals of the

various treatment groups revealed that non-challenged LR were marked by a smaller baseline concentration of accumbal dopamine than non-challenged HR (Fig. 3: two-way ANOVA: type effect: $F_{(1,166)} = 10.183$, $p = 0.002$).

Effects of novelty and alpha-methyl-para-tyrosine in novelty-challenged rats (Exp. 1): Figure 4 clearly illustrates that the novelty-induced increase of accumbal dopamine over time was smaller in LR than in HR (two-way ANOVA: type x time effect: $F_{(7,112)} = 3.246$, $p = 0.004$). Exposure to novelty increased accumbal dopamine levels during the first 60 min in LR (one sample t-test), whereas exposure to novelty increased accumbal dopamine levels during the first 80 min in HR (one sample t-test). Novelty-challenged LR displayed a small amount of walking behaviour compared to novelty-challenged HR (one-way ANOVA: type effect: $F_{(1,16)} = 13{,}393$, $p = 0.002$).

Figure 4 also demonstrates that AMPT affected the novelty-induced increase of accumbal dopamine over time to a smaller degree in LR than in HR (three-way ANOVA: type x treatment x time effect: $F_{(7,455)} = 5.418$, $p \leq 0.001$). AMPT reduced the accumbal dopamine levels in novelty-challenged LR only at t = 80 min (two-way ANOVA: treatment x time effect: $F_{(7,224)} = 4.062$, $p \leq 0.001$; post-hoc Student's t-test), whereas it reduced the accumbal dopamine levels in novelty-challenged HR at all time points (two-way ANOVA: treatment x time effect: $F_{(7,231)} = 16.093$, $p \leq 0.001$; post-hoc Student's t-test). A two-way ANOVA revealed that AMPT differentially affected the amount of novelty-induced walking behaviour in LR and HR (type x treatment effect: $F_{(1,65)} = 7.099$, $p = 0.010$). Analysis per type of rat showed that AMPT did not alter the amount of walking behaviour in LR (one-way ANOVA: no treatment effect), whereas it reduced the amount of walking behaviour in HR (one-way ANOVA: treatment effect: $F_{(1,33)} = 5.949$, $p = 0.020$).

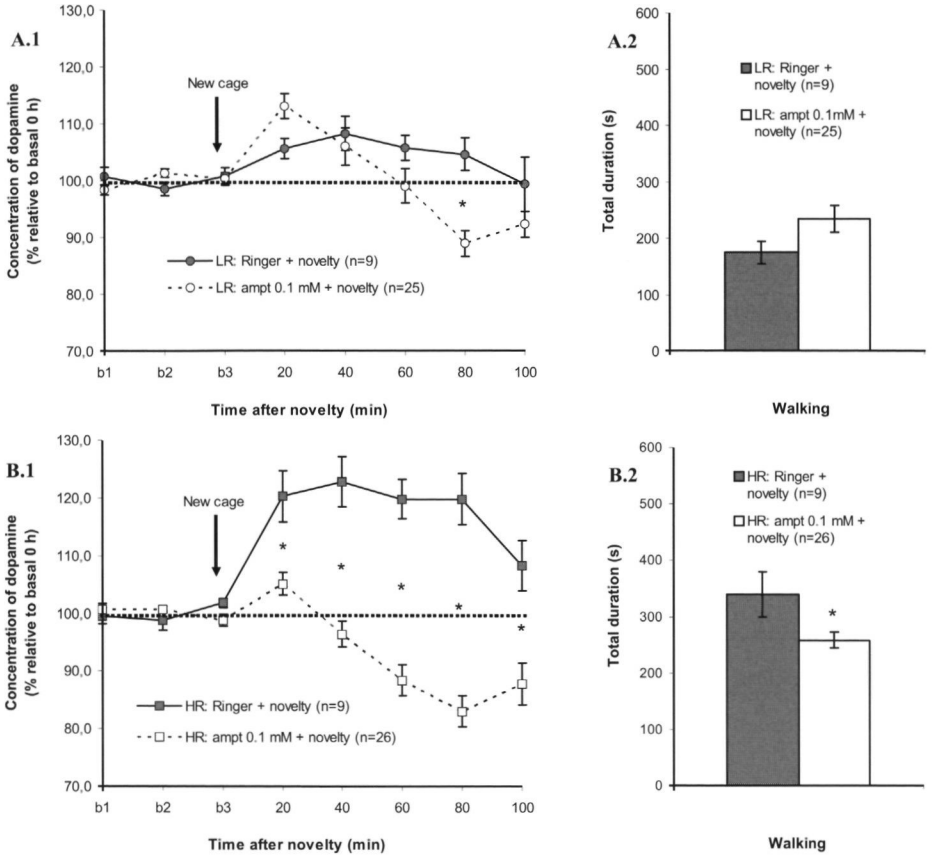


Figure 4. Effects of intra-accumbens infusion of AMPT (0.1 mM, 2 µl/min, 40 min) on the extracellular concentration of dopamine in the Nucleus Accumbens (left) and on the total amount of walking behaviour (right) in novelty-challenged LR (A) and novelty-challenged HR (B). Accumbal dopamine levels after novelty are expressed as percentage of baseline accumbal dopamine levels (basal 0 h in Fig. 1). The dotted line represents basal 0 h (= 100%). Walking behaviour is expressed as the total time (s) performing novelty-induced locomotor activity during a period of 40 min after AMPT or its Ringer solvent. *Significant effect of 0.1 mM of AMPT. All data are expressed as mean ± SEM.

Effects of reserpine in non-challenged rats (Exp. 2): A three-way ANOVA revealed that RES differentially affected the extracellular concentration of accumbal dopamine in LR and HR that were not challenged by novelty (Fig. 5: three-way ANOVA: type x treatment x time effect: $F_{(6,264)} = 2.341$, $p = 0.032$). RES reduced the accumbal dopamine levels in both non-challenged LR (two-way ANOVA: treatment

effect: $F_{(2,44)} = 4.281$, $p = 0.020$) and non-challenged HR (two-way ANOVA: treatment effect: $F_{(2,44)} = 52.661$, $p \leq 0.001$). However, the maximum RES-induced reduction of accumbal dopamine in LR was already reached after 1 hour and remained stable over time (two-way ANOVA: no treatment x time effect), whereas the RES-induced reduction of accumbal dopamine in HR increased for a period of at least 24 h (two-way ANOVA: treatment x time effect: $F_{(6,132)} = 9.142$, $p \leq 0.001$). The maximum RES-induced reduction of the concentration of accumbal dopamine was less in non-challenged LR (reduction: 35%) compared to non-challenged HR (reduction: 75%). Post-hoc analysis demonstrated that 1 mg/kg and 2 mg/kg of RES were equally effective in reducing dopamine in LR (LSD: no dose effect), whereas 2 mg/kg of RES was more effective than 1 mg/kg of RES in reducing dopamine in HR (LSD: dose effect: $p = 0.045$). Analysis per time point revealed that 2 mg/kg of RES was more effective than 1 mg/kg of RES at t=24 h in HR (Student's t-test). All rats, regardless of their treatment, were either sitting or sleeping 24 h after they received their injection.

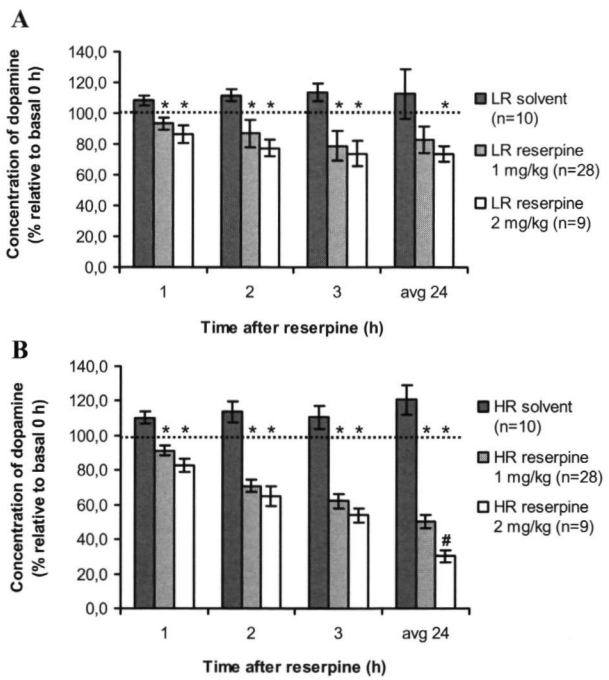


Figure 5. Effects of systemic RES (1 and 2 mg/kg, i.p.) on the extracellular concentration of accumbal dopamine in non-challenged LR (A) and non-challenged HR (B). Accumbal dopamine levels after RES or its solvent (= the pH 2.4 solution) are expressed as percentage of baseline accumbal dopamine levels (basal 0 h in Fig. 1). The dotted line represents basal 0 h (= 100%). Avg 24 = average of 3 consecutive samples taken at t = 24 h (Fig. 1). Rats were either sitting or sleeping at t = 24 h. *Significant difference between 1 or 2 mg/kg of RES and RES solvent. #Significant difference between 1 and 2 mg/kg of RES. Data are expressed as mean percentage ± SEM.

Effects of reserpine in novelty-challenged rats (Exp. 2): A three-way ANOVA revealed that RES differentially affected the novelty-induced increase of the extracellular concentration of accumbal dopamine in LR and HR (Fig 6 three-way ANOVA type x treatment x time effect $F_{(6\ 204)} = 3\ 510$, $p = 0\ 003$) Analysis per type of rat demonstrated that 2 mg/kg of RES inhibited the increase of accumbal dopamine over time in novelty-challenged LR (two-way ANOVA treatment x time effect $F_{(6\ 102)} = 11\ 255$, $p \leq 0\ 001$) In fact, the novelty-induced increase of the concentration of accumbal dopamine was completely abolished in all LR treated with 2 mg/kg of RES (one-sample t-test) In contrast, 2 mg/kg of RES did not alter the increase of accumbal dopamine over time in novelty-challenged HR (two-way ANOVA no treatment x time effect) In fact, the concentration of accumbal dopamine increased in all HR treated with 2 mg/kg of RES (one-sample t-test) RES equally reduced the amount of walking behaviour in LR and HR (two-way ANOVA treatment effect $F_{(1\ 34)} = 49\ 389$, $p \leq 0\ 001$, no type x treatment effect)

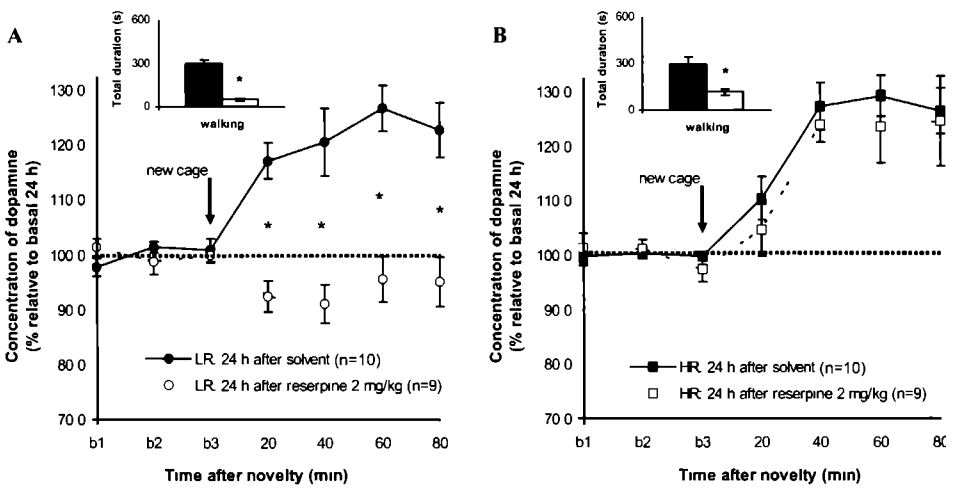


Figure 6. Effects of systemic RES (2 mg/kg, i.p.) on the extracellular concentration of dopamine in the Nucleus Accumbens (main graph) and on the total amount of walking behaviour (inlay) in novelty-challenged LR (A) and novelty-challenged HR (B). Accumbal dopamine levels after novelty are expressed as percentage of the accumbal dopamine levels that were obtained 24 h after the injection of RES or its solvent (= the pH 2 4 solution) The dotted line represents basal 24 h (= 100%) Walking behaviour is expressed as the total time (s) performing locomotor activity during a period of 40 min after novelty *Significant effect of 2 mg/kg of RES All data are expressed as mean ± SEM

Effects of systemic acidosis (Exp. 3): Figure 7 reveals that the extracellular levels of accumbal dopamine 24 h after the injection of the pH 7.0 solution were not different from baseline dopamine levels immediately before the injection (one sample t-test). In addition, figure 8 shows that both the neurochemical and the behavioural response to novelty in rats injected with the pH 7.0 solution did not differ from the neurochemical and the behavioural response to novelty in rats that did not receive this injection (three-way ANOVA: dopamine: no treatment x time effect, walking: no treatment effect).

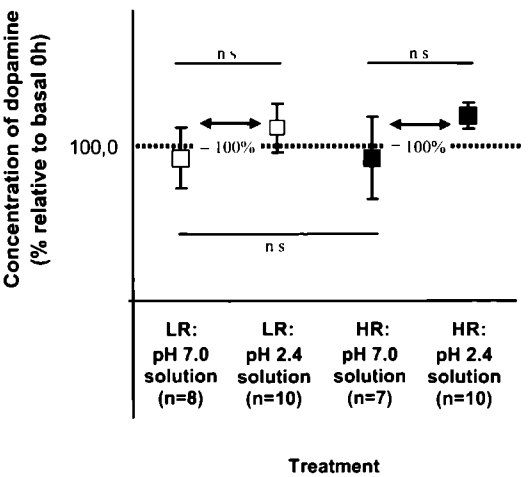


Figure 7. No effects of systemic acidosis (1 ml/kg, i.p.) on the extracellular concentration of accumbal dopamine measured in non-challenged LR (left) and non-challenged HR (right). Accumbal dopamine levels after the pH 2.4 solution (RES solvent) or the pH 7.0 solution (RES solvent without phosphoric acid) are expressed as percentage of baseline accumbal dopamine levels that were obtained 24 h earlier (basal 0 h in Fig. 1). The dotted line represents basal 0 h (= 100%). The systemic injection of both the pH 7.0 solution and the pH 2.4 solution did not change accumbal dopamine levels 24 h later. Therefore, no significant differences were found between the pH 2.4 solution and the pH 7.0 solution. All rats were sitting or sleeping 24 h after the injection. Data are expressed as mean ± SEM. n.s. = not significant.

Figure 7 also reveals that non-challenged LR and HR were not differentially sensitive to systemic acidosis (two-way ANOVA: no type effect). In fact, systemic acidosis had no effect at all in these rats. Namely, dopamine levels 24 h after the pH 2.4 solution were not different from dopamine levels 24 h after the pH 7.0 solution (two-way ANOVA: no treatment effect). In addition, one sample t-tests revealed that the extracellular levels of accumbal dopamine 24 h after systemic acidosis were not different from baseline dopamine levels immediately before systemic acidosis (no change from 100%). Figure 9 illustrates that systemic acidosis differentially affected the increase of accumbal dopamine in novelty-challenged LR and HR (three-way ANOVA: type x treatment x time effect: $F_{(6,186)} = 2.177$, $p = 0.047$). Analysis per type of rat revealed that

the administration of the pH 2.4 solution enhanced the increase of accumbal dopamine over time in novelty-challenged LR (two-way ANOVA: treatment x time effect: $F_{(6,96)} = 4.917$, $p \leq 0.001$), whereas it did not alter this increase in novelty-challenged HR (two-way ANOVA: no treatment x time effect). Systemic acidosis differentially affected the novelty-induced increase of walking behaviour in LR and HR (two-way ANOVA: type x treatment: $F_{(1,31)} = 7.302$, $p = 0.011$). Analysis per type of rat revealed that the administration of the pH 2.4 solution increased the amount of walking behaviour in novelty-challenged LR (one-way ANOVA: treatment effect: $F_{(1,16)} = 16.229$, $p = 0.001$), whereas it did not alter the amount of walking behaviour in novelty-challenged HR (one-way ANOVA: no treatment effect).

Correlation: Figure 10 shows that the mean increase of the extracellular levels of accumbal dopamine in the first 40 min after novelty positively correlated with the novelty-induced increase of walking behaviour in both the control rats of the AMPT experiment (Fig. left: Pearson's analysis: $R = 0.650$, $p = 0.003$; LR and HR pooled: n=18) and the control rats of the pH 2.4 experiment (Fig. right: Pearson's analysis: $R = 0.552$, $p = 0.033$; LR and HR pooled: n=15). Walking behaviour was most prominent during the first 20 min after novelty and diminished during the next 20 min. LR and HR were either sitting or sleeping at t=40 min after novelty.

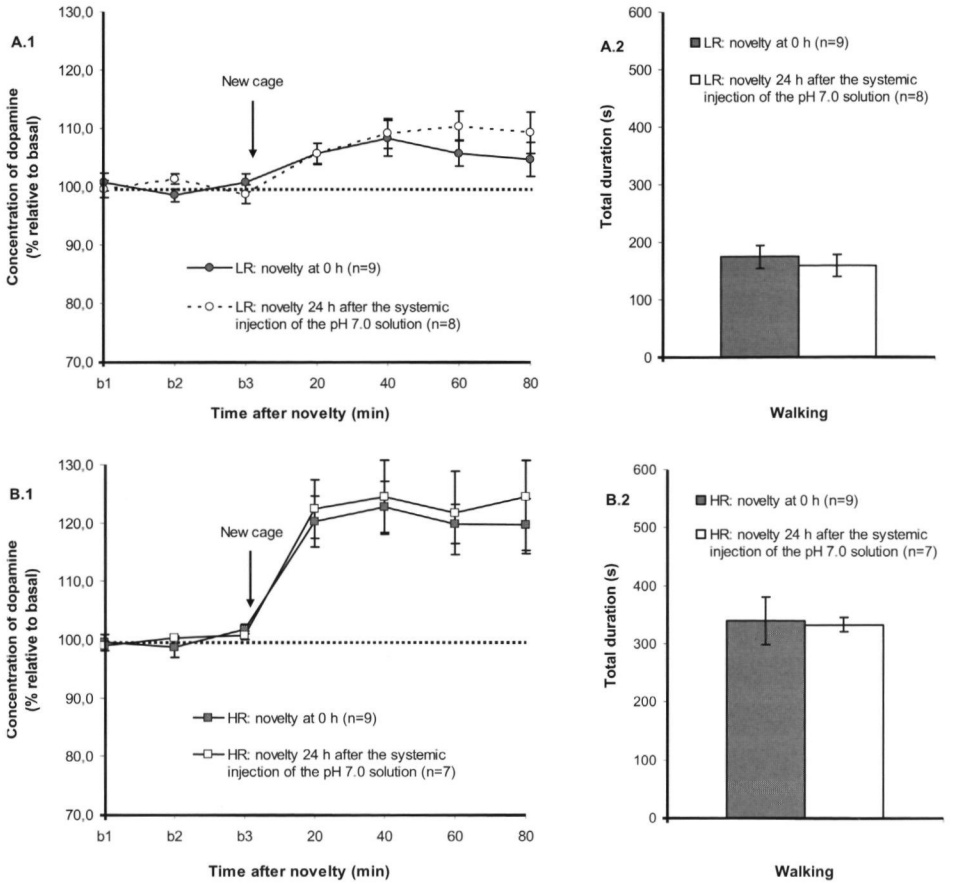


Figure 8. No effects of the systemic injection of the pH 7.0 solution on the dopamine response (left) and the walking response (right) to novelty. This figure incorporates the data of the control rats of the AMPT experiment depicted in figure 4 (Ringer) and of the control rats of the acidosis experiment depicted in figure 9 (the pH 7.0 solution). The accumbal dopamine response to novelty at 0 h is expressed as percentage of basal 0 h. The accumbal dopamine response to novelty 24 h after the injection of the pH 7.0 solution is expressed as percentage of basal 24 h. Walking behaviour is expressed as the total time (s) performing locomotor activity during a period of 40 min after novelty. No significant differences were found between these treatment groups. All data are expressed as mean ± SEM.

Discussion

The effects of AMPT and RES were investigated in LR and HR under both non-challenged and novelty-challenged conditions. The neurochemical and behavioural changes after these drugs are discussed below.

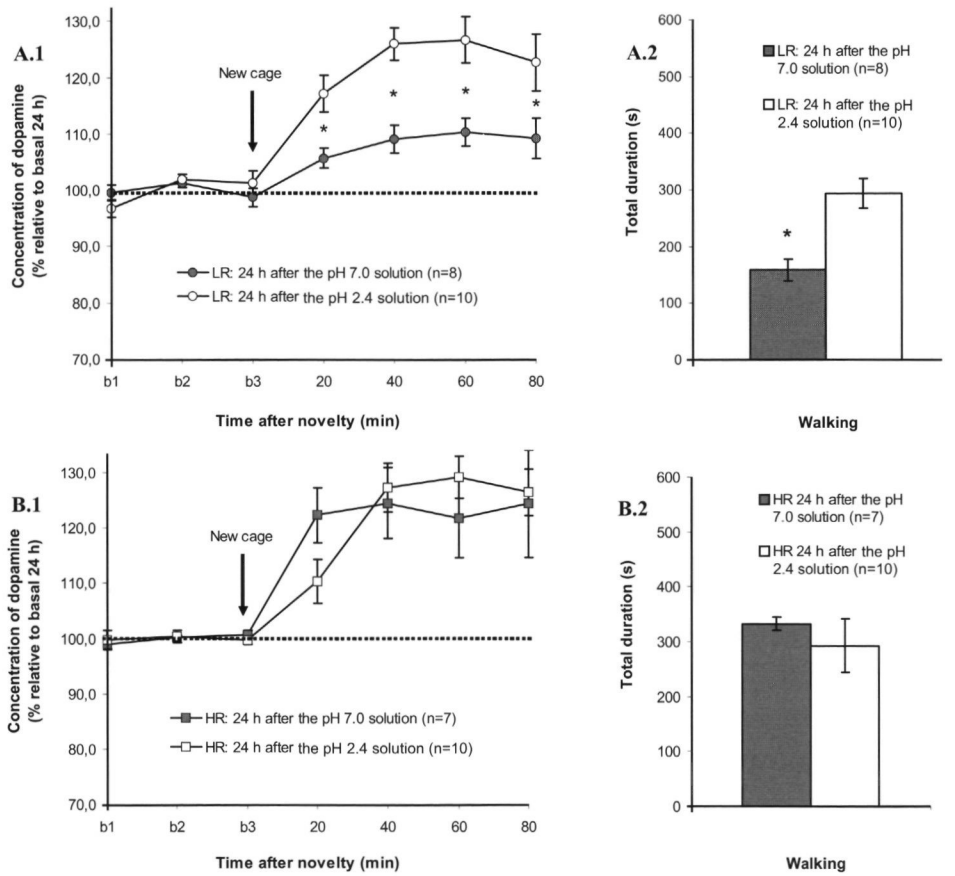


Figure 9. Effects of systemic acidosis (1 ml/kg, i.p) on the extracellular concentration of dopamine in the Nucleus Accumbens (left) and on the total amount of walking behaviour (right) in novelty-challenged LR (A) and novelty-challenged HR (B). Accumbal dopamine levels after novelty are expressed as percentage of accumbal dopamine levels that were obtained 24 h after the injection of the pH 2.4 solution (RES solvent) or the pH 7.0 solution (RES solvent without phosphoric acid). The dotted line represents basal 24 h (= 100%). Walking behaviour is expressed as the total time (s) performing locomotor activity during a period of 40 min after novelty. *Significant effect of 1 ml/kg of the pH 2.4 solution. All data are expressed as mean ± SEM.

Baseline concentration of accumbal dopamine: This study provided direct evidence (Fig. 3) that non-challenged HR are marked by a higher baseline extracellular concentration of accumbal dopamine than non-challenged LR are. These individual differences in absolute levels of accumbal dopamine are relatively small and have previously been detected only by quantitative (Hooks et al., 1992a) instead of semi-quantitative (Hooks et al., 1991b; Rouge-Pont et al., 1993) microdialysis. However, the present study demonstrated that, by using relatively large numbers of rats, even semi-quantitative microdialysis is able to reveal small differences between HR and LR.

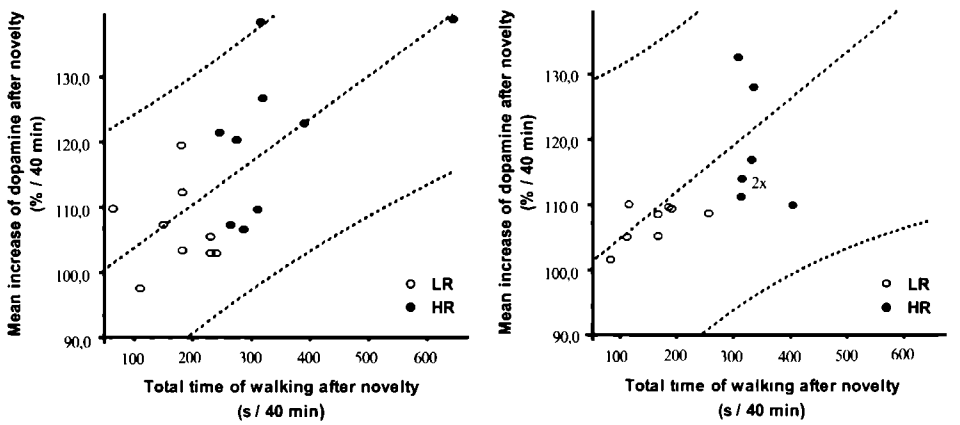


Figure 10. Correlation of novelty-induced walking behaviour and novelty-induced increase of accumbal dopamine in drug-naive rats. The left figure incorporates data of the control rats of the AMPT experiment depicted in figure 4 (Ringer: LR. n=9, HR. n=9), whereas the right figure incorporates the data of the control rats of the acidosis experiment depicted in figure 9 (the pH 7.0 solution LR n=8, HR n=7) Walking behaviour is expressed as the total time (s) performing locomotor activity during a period of 40 min after novelty Accumbal dopamine levels are expressed as mean increase from baseline in the first 40 min after novelty The dotted lines represent the regression based fit line ± the prediction interval at a confidence level of 95% One single dot represents one single rat

Effects of novelty and alpha-methyl-para-tyrosine (Exp. 1): The present study confirmed the outcome of a study by Saigusa et al. (1999) that both the extracellular levels of accumbal dopamine and the total amount of walking behaviour increased more in novelty-challenged HR (Fig. 8B) than in novelty-challenged LR (Fig. 8A).

Interestingly, Pearson's analysis revealed that the novelty-induced increase of accumbal dopamine positively correlated with the increase of walking behaviour after novelty (Fig 10) The present data, therefore, indicate that novelty-induced walking in both HR and LR is regulated by dopamine in the Nucleus Accumbens Apparently, this correlation between walking behaviour and accumbal dopamine exists not only in cocaine-treated rats (Hooks et al , 1991b), but also in drug-naive rats (present study)

Although AMPT has been shown to equally reduce accumbal dopamine levels in HR and LR that were non-challenged (Tuinstra and Cools, 2000b), the present study revealed that AMPT differentially affected HR and LR that were novelty-challenged AMPT reduced the novelty-induced increase of accumbal dopamine levels and the novelty-induced increase of walking behaviour in HR (Fig 4B), whereas both were not affected in LR (Fig 4A) First, these data demonstrate that the novelty-induced accumbal dopamine increase in HR, but not in LR, is primarily derived from AMPT-sensitive pools (Saigusa et al , 1999) Second, these data indicate that walking behaviour in novelty-challenged HR is regulated by newly-synthesised accumbal dopamine, whereas walking behaviour in novelty-challenged LR is not (Saigusa et al , 1999) As a final remark, the time curves shown in figure 4 slightly differed quantitatively, but not qualitatively, from those presented in the original study of Saigusa et al (1999) Subtle differences in location of the probes between both studies (see experimental procedures) may have contributed to these small variation in time curves

Effects of reserpine in non-challenged rats (Exp. 2): Figure 7 demonstrates that the solvent of RES (the pH 2 4 solution) did not produce any after effect in non-challenged rats The fact that the dopamine levels measured 24 h after both the pH 2 4 solution and the pH 7 0 solution were identical to the basal levels of dopamine measured before these solutions were given (Fig 7) together with the fact that all rats were either sitting or sleeping 24 h after administration of these solutions (Fig 7), demonstrate that non-challenged HR and LR were fully habituated 24 h following a systemic injection Consequently, we were able to establish to what extent the extracellular accumbal dopamine levels in habituated rats are dependent on RES-sensitive storage pools The present study showed that RES decreased the baseline accumbal dopamine levels

stronger in HR at 24 h (Fig. 5B) than in LR at that time (Fig. 5A). Therefore, we conclude that the accumbal dopamine levels in non-challenged HR are more dependent on RES-sensitive vesicles than the accumbal dopamine levels in non-challenged LR are.

Effects of reserpine in novelty-challenged rats (Exp. 2): The present study revealed that RES not only reduced accumbal dopamine levels in non-challenged LR (Fig. 5A), but also completely abolished the increase of accumbal dopamine in novelty-challenged LR (Fig. 6A). In contrast, RES did not reduce the increase of accumbal dopamine in novelty-challenged HR (Fig. 6B), in spite of the fact that accumbal dopamine levels were drastically reduced by RES before these rats were exposed to novelty (Fig. 5B). These data provide direct evidence in favour of our hypothesis (see introduction) that the novelty-induced accumbal dopamine increase in LR, but not in HR, is primarily derived from RES-sensitive storage pools.

Surprisingly, RES equally reduced the novelty-induced increase of walking behaviour in both types of rat (Fig. 6A vs 6B). Because this behavioural reduction also occurred in HR in which RES was unable to lower the dopamine levels, the reduction of walking behaviour in RES-treated rats could not be the result of RES-induced changes of the amount of dopamine in the Nucleus Accumbens. The fact that RES is also known to reduce monoamines in other brain structures than the Nucleus Accumbens (Pan et al., 1993) may well explain the lack of correlation between accumbal dopamine levels and walking behaviour in the RES-treated rats of the present study.

Effects of systemic acidosis (Exp. 3): The pH 2.4 solution clearly enhanced the novelty-induced increase of accumbal dopamine levels in LR (Fig. 9A), but not in HR (Fig. 9B). Indeed, it has been reported that systemic acidosis can have long-lasting and irreversible effects on neurotransmission in the brain (Rentero et al., 1998). This phenomenon can easily be explained. Lowering of the systemic pH can reduce the extracellular brain pH (Eldridge et al., 1984; Somjen et al., 1987; Shapiro et al., 1989; Eleff et al., 1995; Cappellen et al., 2003; Hodges et al., 2004) which, subsequently, results in a decrease of the intraneuronal pH (Drapeau and Nachshen, 1988; Nachshen and Drapeau, 1988). This intracellular acidosis leads to intravesicular acidosis (Russell

and Holz, 1981, Hara et al , 1989, Tabb et al , 1992) Because intravesicular acidosis results in the accumulation of neurotransmitters inside storage vesicles (Tsudzuki, 1984, Tabb et al , 1992, Pothos, 2002) it will be evident that systemic acidosis can ultimately enlarge the novelty-induced release of neurotransmitters from these vesicles (Fujiwara et al , 1994, Cannizzaro et al , 2003) The finding that the effects of acidosis were only evident in novelty-challenged LR, and not in novelty-challenged HR, can simply be explained by the fact that only novelty-challenged LR release dopamine from RES-sensitive vesicles whereas novelty-challenged HR do not (see Exp 2) In addition, the novelty-induced increase of accumbal dopamine that is released from RES-sensitive pools was paralleled by an increase of walking behaviour only in LR rats (Fig 9A), but not in HR rats (Fig 9B) These data indicate that walking behaviour in novelty-challenged LR is regulated by previously stored accumbal dopamine, whereas walking behaviour in novelty-challenged HR is not The finding that systemic acidosis did not increase accumbal dopamine levels in non-challenged rats can be explained by the fact that the effects of acidosis on vesicular dopamine release in the striatum are limited to the evoked release, and not to the release during rest (Guan and McBride, 1988)

Conclusions

The present study investigated the individual differences in sensitivity to AMPT and RES The effects of intra-accumbens infusion of AMPT were measured at t=0 h (Fig 1) whereas the effects of systemic RES were measured at t=24 h (Fig 1) The fact that both the baseline concentration of dopamine (Fig 7) as well as the neurochemical (dopamine) and behavioural (walking) response to novelty (Fig 8) were not altered by the systemic injection 24 h earlier, allowed us to compare the data of the RES experiment with those of the AMPT experiment

The results of the present study are summarised in figure 11 The experiments in non-challenged rats revealed that non-challenged HR release more accumbal dopamine from RES-sensitive storage pools than non-challenged LR do In contrast, non-challenged HR and LR are known to release equal amounts of accumbal dopamine from AMPT-sensitive newly-synthesised pools (Tuinstra and Cools, 2000b) These data result

in the notion that the overall release of accumbal dopamine should be stronger in non-challenged HR than in non-challenged LR (Fig. 11). As discussed above, this was indeed the case. The experiments in novelty-challenged rats revealed that the accumbal dopamine release derived from AMPT-sensitive pools increases in novelty-challenged HR, whereas the dopamine release derived from RES-sensitive pools in these rats does not. Whether the dopamine release from RES-sensitive pools is not changed, or even decreases, remains to be investigated. Moreover, our data indicate that the increase of newly-synthesised dopamine in the Nucleus Accumbens of novelty-challenged HR regulates the relatively large novelty-induced increase of locomotor activity in these rats. It was also demonstrated that the release of accumbal dopamine derived from RES-sensitive pools increases in novelty-challenged LR, whereas the dopamine release derived from AMPT-sensitive pools in these rats does not. Whether the release from AMPT-sensitive pools is not changed, or even decreases, remains to be investigated. Furthermore, our data indicate that the increase of previously stored dopamine in the Nucleus Accumbens of novelty-challenged LR regulates the relatively small novelty-induced increase of locomotor activity in these rats. The finding that the novelty-induced increase of accumbal dopamine levels is larger in HR than in LR suggests that the increase of dopamine released from AMPT-sensitive pools in novelty-challenged HR is larger compared to the increase of dopamine released from RES-sensitive pools in novelty-challenged LR (Fig. 11). Because AMPT inhibits tyrosine hydroxylase and because RES blocks vesicular mono-amino transporters (see introduction), the present data clearly show that HR and LR differ in processes that are associated with the function of 1) tyrosine hydroxylase and 2) vesicular mono-amino transporters.

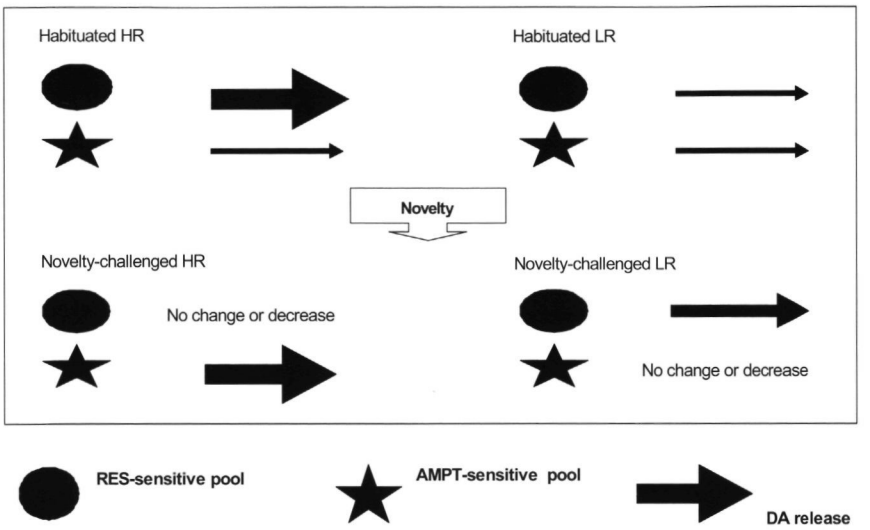


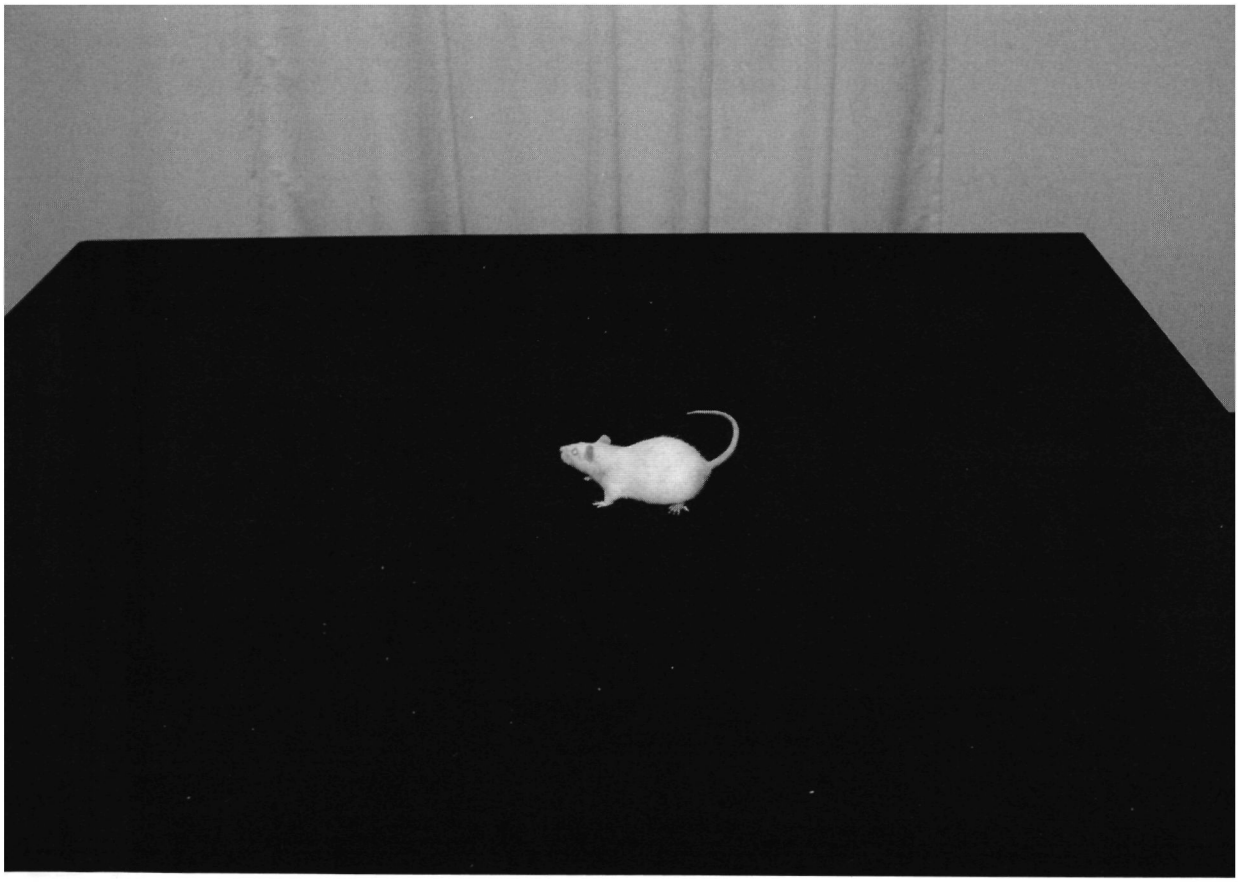
Figure 11. Effects of novelty on dopamine release from accumbal RES-sensitive and accumbal AMPT-sensitive pools in HR and LR. Arrows indicate accumbal dopamine release from AMPT-sensitive newly synthesised pools and from RES-sensitive storage pools in non-challenged and in novelty-challenged HR and LR, circles indicate reserpine (RES)-sensitive dopamine pools in the Nucleus Accumbens, stars indicate alpha-methyl-para-tyrosine (AMPT)-sensitive dopamine pools in the Nucleus Accumbens. The dimension of the arrows represents the amount of dopamine being released from a particular pool.

Impact

A mutual exchange of dopamine between the two types of presynaptic pool has frequently been suggested (Schoemaker and Nickolson, 1983; Justice et al., 1988; Leviel et al., 1989; Arbuthnott et al., 1990; Sulzer et al., 1995; Watanabe et al., 2005). The present study revealed that only AMPT, and not RES, reduced the novelty-induced increase of accumbal dopamine in HR whereas only RES, and not AMPT, reduced the novelty-induced increase of accumbal dopamine in LR. These data imply that the currently used low doses of AMPT and RES selectively affected the two distinct types of dopamine pool. The results of our study, therefore, indicate that dopamine can also be released directly from RES-sensitive pools without affecting AMPT-sensitive pools, and *vice versa*.

The present findings have far-reaching consequences for understanding individual differences between HR and LR in sensitivity to drugs that interact with RES-sensitive and/or AMPT-sensitive pools. Because the effects of psychostimulants have been found

to depend on dopamine inside these pools (for ref. on cocaine see: introduction, for ref. on amphetamine see: Scheel-Kruger, 1971; Butcher et al., 1988; Hiroi and White, 1990; Finn et al., 1990; DiLullo and Martin-Iverson, 1992; Heeringa and Abercrombie, 1995; Sulzer et al., 1995; Sabol and Seiden, 1998; Watanabe et al., 2005), our findings open the perspective that the individual specific susceptibility to cocaine (for ref. see introduction) and amphetamine (Piazza et al., 1989; Hooks et al., 1994; Gingras and Cools, 1997; Cools et al., 1997; Bevins et al., 1997) is, at least in part, due to individual differences in the release of dopamine from presynaptic pools. Studies in this respect are currently in progress.



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Chapter 3

Rats that differentially respond to cocaine differ in their dopaminergic storage capacity of the nucleus accumbens

M.M.M. Verheij, E.L.W. de Mulder, E. De Leonibus, K.M.J. van Loo and A.R. Cools (2008).

Journal of Neurochemistry 105: 2122-2133.

Abstract

Cocaine (COC) inhibits the re-uptake of dopamine. However, the dopamine response to COC also depends on dopamine inside storage vesicles. The aim of this study was to investigate whether rats that differentially respond to COC differ in their dopaminergic storage capacity of the nucleus accumbens. Total and vesicular levels of accumbal dopamine as well as accumbal vesicular-monoamine-transporter-2 levels were established in high (HR) and low responders (LR) to novelty rats. Moreover, the effects of reserpine (RES) on the COC-induced increase of extracellular accumbal dopamine were investigated. HR displayed higher accumbal levels of total and vesicular dopamine than LR. Moreover, HR displayed more accumbal vesicular-monoamine-transporters-2 than LR. COC increased extracellular accumbal dopamine more strongly in HR than in LR. A low dose of RES prevented the COC-induced increase of accumbal dopamine in LR, but not in HR. A higher dose of RES was required to inhibit the COC-induced increase of accumbal dopamine in HR. These data demonstrate that HR were marked by a larger accumbal dopaminergic storage pool than LR. It is hypothesised that HR are more sensitive to COC than LR, because COC can release more dopamine from accumbal storage vesicles in HR than in LR.

Introduction

Individual differences in the susceptibility to psychostimulants have extensively been reported, both in humans (Jaffe and Archer, 1987; Ball et al., 1994a; Gynther et al., 1995; van den Bree et al., 1998) and in animals (Piazza et al., 1989; Piazza et al., 2000; Mantsch et al., 2001). This study focused on two types of rat that differ in their acute response to cocaine (COC). These individuals are selected on the basis of their locomotor response to a novel open-field and, accordingly, labelled high (HR) and low responders (LR) to novelty (Piazza et al., 1989; Piazza et al., 1991b; Rouge-Pont et al., 1993; Dellu et al., 1996b; Bevins et al., 1997; Cools and Gingras, 1998; Cools and Tuinstra, 2003; Kabbaj, 2004). Previous studies have demonstrated that COC increases the locomotor response and the extracellular levels of accumbal dopamine more strongly in HR than in LR (Hooks et al., 1991b; Chefer et al., 2003).

COC inhibits the re-uptake of monoamines by blocking plasmalemmal monoamine transporters (Lee et al., 2001). Several studies have suggested that individual differences in the re-uptake of dopamine may explain individual differences in the response to COC (Sabeti et al., 2002; Sabeti et al., 2003; Chefer et al., 2003; Briegleb et al., 2004; Zahniser and Sorkin, 2004). However, behavioural and neurochemical studies have demonstrated that the response to COC depends on storage vesicles as well (Scheel-Kruger et al., 1977; McMillen et al., 1980; McMillen, 1983; Davis, 1985; Hurd and Ungerstedt, 1989; Sulzer and Rayport, 1990; Florin et al., 1995; Pifl et al., 1995; Venton et al., 2006). It is unknown to what extent individual differences in the dopaminergic storage capacity contribute to individual differences in response to COC. The above-mentioned finding that HR are marked by a larger COC-induced increase of accumbal dopamine than LR suggests that HR store more accumbal dopamine inside storage vesicles than LR. Accordingly, total and vesicular levels of accumbal dopamine were measured in both types of rat. Given that vesicular monoamine transporters-2 (VMAT-2) control the amount of dopamine inside storage vesicles (Pothos et al., 2000; Pothos, 2002), the levels of the accumbal VMAT-2 were also measured. Based on the notion that LR store less accumbal dopamine inside vesicles than HR, it was

hypothesised that the nucleus accumbens of LR contains less VMAT than the nucleus accumbens of HR.

The drug reserpine (RES) inhibits the VMAT-mediated uptake of cytoplasmatic monoamines into storage vesicles (Kirshner et al., 1963; Henry et al., 1998). As the extracellular levels of monoamines strongly depend on an intact shuttle between cytoplasmatic and vesicular monoamines (Schoemaker and Nickolson, 1983; Leviel et al., 1989; Arbuthnott et al., 1990), RES decreases the extracellular levels of accumbal dopamine (Verheij and Cools, 2007). The present study also investigated the effects of RES on the COC-induced increase of extracellular accumbal dopamine. It was hypothesised that COC-treated LR, which are supposed to be marked by a relatively small storage pool containing low amounts of VMAT, are more vulnerable to the RES-induced dopamine depletion than COC-treated HR, which are supposed to be marked by a relatively large storage pool containing high amounts of VMAT.

Experimental procedures

Animal care: All experiments were performed in accordance with institutional, national and international guidelines for animal care and welfare.

Subjects: Adult male LR and HR (LR: n=48, HR: n=59, weight=180-220 g) that were selected from the outbred strain of Nijmegen Wistar rats, were used throughout the study. All rats were reared and housed in macrolon cages (42 x 26 x 15 cm; n=3-4 per cage) under a fixed 12/12 h light/dark cycle (lights on: 07.00 a.m.) in a temperature-controlled room (21 ± 1.7 °C). Water and food pellets were available *ad libitum*.

Open-field selection: Rats were individually housed 3 days before the open-field selection procedure (Saigusa et al., 1999; Verheij and Cools, 2007). Testing took place between 09.00 h and 17.00 h in a room illuminated by white light of 170 Lux. The rat was placed on a black, square table (160 x 160 cm) made of Perspex. This open-field is 95 cm elevated above the floor and surrounded by a white neutral background (270 x 270 x 270 cm). As described by Cools et al. (1990), behaviour was recorded with a

computerised automated tracking system for a period of 30 min. Both ambulation and habituation time were used to select LR and HR. Ambulation was defined as the overall distance (cm) travelled in 30 min. Habituation time was defined as the duration of the period (s) that started as soon as the rat began to explore the open-field and ended as soon as the locomotor activity stopped for at least 90 s. Rats that habituated in less than 480 s and walked less than 4800 cm in 30 min were labelled LR, whereas rats that habituated after 840 s and walked more than 6000 cm in 30 min were labelled HR. Habituation time in addition to ambulation was used as selection criterion, because travelled distance *per se* is not always a reliable criterion (Cools et al., 1997; Saigusa et al., 1999). Directly after the open-field selection, LR and HR were assigned to one of the treatment groups.

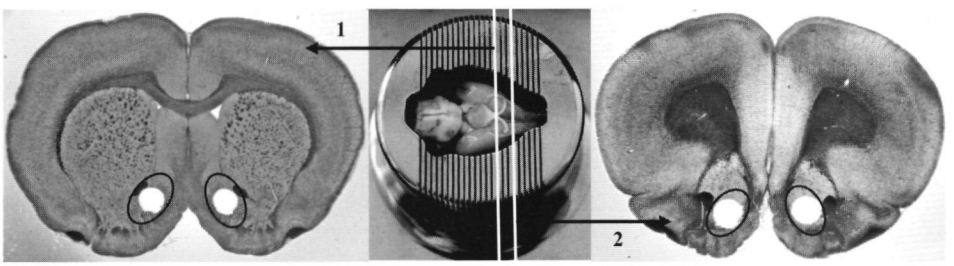


Figure 1. Representative placement of accumbal punches. The brain was placed in a brain matrix. The first incision was made at the position where the two optic nerves fuse with the optic chiasm. The second incision was made 3 mm rostral to this point. Coronal incisions were similar to anterior/posterior 9.0 and 12.0 mm of Paxinos and Watson (1986). The anterior commissure was used as a landmark to reliably punch out accumbal tissue. For each rat, tissue of the left and right punch was pooled. Correctly placed punches were located within the area of the black oval.

Vesicular levels of accumbal dopamine (exp 1)

Accumbal punches: Seven days after the open-field selection, a group of 10 LR and 10 HR were sacrificed by decapitation. Their brains were quickly removed, immediately placed in a stainless steel brain matrix (Electron Microscopy Sciences: Hatfield, USA), frozen on dry ice and subsequently sectioned at 3.0 mm intervals (Mong et al., 2003). Coronal incisions were made at anterior/posterior 9.0 and 12.0 mm (Paxinos and Watson, 1986). The most caudal incision was made at the position where

the two optic nerves fuse with the optic chiasm (Fig. 1). From the identified slice, one punch of accumbal tissue was obtained from either side of the brain using a 1.22 mm i.d. stainless steel needle (Szczypka et al., 2001). The anterior commissure was used as a landmark to reliably punch out accumbal tissue (see Fig. 1). For each rat, tissue of the left and right punch was pooled (total volume: 2 x 3.5 mm^3 = 7.0 mm^3) and stored at -70 °C until vesicular dopamine levels were measured (see below). The remaining tissue was fixated in 4% paraformaldehyde solution in order to allow histological verification of the exact position of the punch needle.

Vesicular dopamine: Purified accumbal vesicles were prepared as described by Staal et al. (2000). All steps of the isolation procedure were performed at 4 °C. For each rat, tissue of the left and right accumbal punch was pooled and homogenised in 0.32 M sucrose buffer (pH 7.3) containing 1 mM PMSF and 0.1 mg/ml soybean trypsin inhibitor. The homogenate was centrifuged at 2000g for 10 min. The resulting supernatant (S1) was divided over two aliquots. The first aliquot was used as a measure to quantify the total amount of general protein (Bradford assay). The second aliquot was centrifuged at 10000g for 30 min. The resulting synaptosomal pellet (P2) was resuspended by swirling in 0.32 M sucrose buffer. The crude suspension was subjected to osmotic shock by the addition of cold, distilled, deionised water. The osmolarity was restored by the immediate addition of 0.25 M HEPES and 1.0 M potassium tartrate buffer (pH 7.5). The sample was centrifuged at 20000g for 20 min. The resulting supernatant (S3) was centrifuged at 55000g for 60 min. $MgSO_4$ was added to the supernatant (S4) to bring the final magnesium concentration to 0.9 mM. Purified accumbal vesicles were obtained from the pellet (P5) after ultracentrifugation of supernatant (S4) at 100000g for 45 min.

Vesicular levels of dopamine were obtained according to the procedures described by Sandoval et al. (2003). The vesicular pellet (P5) was sonicated (Branson cell disruptor: Danbury, US) for approximately 5s in cold tissue buffer (0.05 M sodium phosphate, 0.03 M citric acid buffer with 15% methanol (v/v), pH 2.5) and centrifuged for 15 min at 22000g. The final supernatant (S6) was injected into a HPLC-ECD system (see below) for separation and quantification of vesicular dopamine. All centrifugation

steps were performed in a Sorvall Micro ultracentrifuge, type RC-M150GX, rotor: S120AT2 (Kendro Laboratory Products, Newton, US).

Vesicular dopamine levels were normalised for variation in protein loading using the total protein concentration of the first supernatant (Sandoval et al., 2003).

Accumbal VMAT-2 levels and total levels of accumbal dopamine (exp 2)

Western blot analysis: Seven days after the open-field selection, a group of 12 LR and 12 HR were sacrificed by decapitation and accumbal punches were obtained as described above. Accumbal punches were homogenised in phosphate buffered saline (pH 7.3) containing 6 M Urea, 1% SDS, 1% β-mercaptoethanol, 1 mM PMSF and 0.1 mg/ml soybean trypsin inhibitor (Jensen et al., 1998). For each rat, 15 µg of accumbal protein was size fractioned on an 8% SDS-PAGE gel and transferred to nitrocellulose membranes (Protran, Schleicher & Schuell, Keene, US). Following blocking in 5% skimmed milk / 1% Tween-20 / PBS solution for 1 hr at RT, blots were incubated with an anti VMAT-2 (1:1000; AB1767, Chemicon: Hampshire, UK) and an anti β-tubulin (1:3000, E7, Chu and Klymkowsky, 1989) antibody overnight at 4°C. After extensively washing with 1% skimmed milk / 1% Tween-20 / PBS solution, blots were incubated with a peroxidase conjugated secondary antibody (1:5000) for 45 min at RT, and subsequently washed with 1% skimmed milk / 1% Tween-20 / PBS. Proteins on Western blots were immunodetected using Lumi-Light (plus) substrate (Roche Diagnostics, Mannheim, Germany) and subsequently exposed to a bioimaging system. Hybridization signals were analysed using the Labworks 4.0 programme (UVP bioimaging systems, Cambridge, UK). Band intensities were corrected for the background.

Total dopamine: In addition to the vesicular levels of accumbal dopamine, the total levels of accumbal dopamine were assessed. For quantification of the total levels of accumbal dopamine, the samples that were used to asses the amount of VMAT (see above) were diluted in 0.1 M HCl (1:100) and immediately injected into the HPLC-ECD system (see below).

VMAT and total dopamine levels were normalised for variation in protein loading using the levels of tubulin (Hedtjarn et al , 2002)

Effects of reserpine on the cocaine-induced increase of extracellular accumbal dopamine (exp 3):

Surgery: One day after the open-field selection, a group of 26 LR and 37 HR were unilaterally implanted with stainless steel guide cannulas (length 5 5 mm, outer diameter 0 65 mm, inner diameter 0 3 mm) directed at the right nucleus accumbens according to previously described procedures (Tuinstra and Cools, 2000b, Verheij and Cools, 2007) Under sodium pentobarbital anaesthesia (60 mg/kg, volume 1 ml/kg, i p) rats were placed in a stereotaxic apparatus and the following coordinates were used according to the atlas of Paxinos and Watson (1986) anterior +10 6 mm (relative to the interaural line) and lateral -1 5 mm (relative to the midline suture) The guide cannula was lowered 5 5 mm relative to the dura surface resulting in a vertical coordinate of +3 5 mm for the cannula tip Finally, the cannula was angled 10^{0} laterally to the right side Screws and cement were used to fixate the cannula to the skull The guide cannula contained an inner cannula to prevent infections and occlusions

The rats were allowed to recover from surgery for the next 7 to 10 days in Plexiglas dialysis cages (25 x 25 x 35 cm) covered with sawdust on the floor (Saigusa et al , 1999, Verheij and Cools, 2007) On 3 consecutive days just prior to the start of the experiment, each rat was gently picked up This handling procedure was repeated 3 times per day (Saigusa et al , 1999, Verheij and Cools, 2007)

Reserpine treatment: At the first day of the experiment, a dialysis probe (type A-I-8-02, outer diameter 0 22 mm, 50000-molecular-weight cut-off, Eicom, Tokyo, Japan) was carefully inserted into the brain of a conscious rat The tip of the dialysis probe protruded 2 mm below the distal end of the guide cannula The probe had an *in vitro* recovery of 10-12% for dopamine (Saigusa et al , 1999, Verheij and Cools, 2007) 4 Hours following probe insertion, HR and LR were injected with RES (1 or 2 mg/kg see below) or its solvent (volume 1 ml/kg, i p) Because the RES-induced decrease of

the accumbal extracellular dopamine levels observed in HR and LR on day 1 have already been reported (Verheij and Cools, 2007), we now report only the effects of RES on the accumbal extracellular dopamine levels of day 2

Microdialysis: At the second day of the experiment, the inlet and outlet of the dialysis probe were connected to a swivel and accumbal dialysates were analysed for dopamine according to previously described procedures (De Leonibus et al , 2006) The probe was perfused at a rate of 2 0 µl/min with modified Ringer solution and the outflow was collected into a stand-alone HPLC-ECD system (HTEC-500, software version 1 02) of Eicom (Tokyo, Japan) Dopamine was separated from the remaining neurotransmitters by means of reversed phase, ion-paring liquid chromatography using an Eicompak PP-ODS column (particle size 2 µm, 4 6 x 30 mm, Eicom, Tokyo, Japan) in combination with a mobile phase containing 1% of methanol (flow rate 500 µl/min, temp 25 °C) The concentration of dopamine was measured by setting the working electrode of the electrochemical detector at + 400 mV against a silver/silver-chloride reference electrode The HPLC-ECD unit was calibrated with a standard dopamine solution twice before each experiment The detection limit was about 30 fg per sample (– 10 µl)

At 4 h following the start of the microdialysis, the extracellular accumbal concentration of dopamine (pg/sample) is known to reach a stable baseline ± 10% (Saigusa et al , 1999, Tuinstra and Cools, 2000b, van der Elst et al , 2005b, De Leonibus et al , 2006, Verheij and Cools, 2007) As soon as 4 h had past and successive samples differed less than 10%, 3 baseline samples were taken The average of these 3 samples served as control value (100%) to study the drug-induced changes of accumbal dopamine

Cocaine treatment: Immediately after the third baseline sample was taken, rats that were treated with RES or its solvent on day 1 were injected with COC (15 mg/kg) or saline (volume 1 ml/kg, i p) Because the effects of COC on the accumbal dopamine levels in LR and HR under non-challenged conditions have already been reported (Hooks et al , 1991b, Chefer et al , 2003), the present study focused only on the effects of COC on the accumbal dopamine levels in LR and HR under novelty-challenged

conditions. Immediately after the injection of COC or saline, rats were exposed to a cage that was slightly larger than the home cage (new dimensions: 30 x 30 x 35 cm) and lacked sawdust on the floor (Verheij and Cools, 2007). LR and HR were left undisturbed in their new environment and the accumbal extracellular concentration of dopamine was recorded (at 5 min intervals) for an additional period of 90 min.

Treatment strategy: COC was given at 24 h after RES because the dopamine depleting effects of RES are known to be maximal at this time (Verheij and Cools, 2007). Both LR and HR were injected with 1 mg/kg of RES. Because 1 mg/kg of RES had no effect on the COC-induced increase of accumbal dopamine in HR, a new group of HR was pre-treated with 2 mg/kg of RES. The relatively low doses of 1 and 2 mg/kg of RES were chosen because it was previously shown that these doses selectively affect RES-sensitive dopaminergic storage vesicles (Cools and Verheij, 2002).

Histology: At the end of the microdialysis experiments, rats were given an overdose of sodium-pentobarbital (250 mg/kg, i.p.) and were intracardially perfused with 60 ml 4% paraformaldehyde solution. Vibratome sections (100 μm) were cut to determine the exact location of the microdialysis probe.

Solutions: The following solutions were used: 1) Modified Ringer solution: 147 mM NaCl, 4 mM KCl, 1.1 mM $CaCl_2\,2H_2O$ and 1.1 mM $MgCl_2\,6H_2O$ were dissolved in ultra pure water (pH 7.4), 2) Mobile phase (pH = 6.0): 0.1 M phosphate buffer ($NaH_2PO_4\,2H_2O$: $Na_2HPO_4\,12H_2O$, ratio 25:4), 2.0 mM sodium 1-decanesulphonate and 0.1 mM di-sodium EDTA were dissolved in ultra pure water (>18 MΩ) containing 1% methanol, 3) Dopamine standard solution: 50 pg in 10 μl of 0.1 M HCl solution, 4) Reserpine: ampoules containing 1 mg or 2 mg of RES per ml solvent (Verheij and Cools, 2007), 5) Reserpine solvent: 30 mg dl-methionine dissolved in 10 ml aquadest containing 6.75% propylene glycol (Verheij and Cools, 2007). The pH of the RES solution and that of its solvent was adjusted to 2.4 using phosphoric acid.

Analysis of the data (exp 1-3): Data were statistically analysed using an ANOVA with the factor type of rat (experiment 1 and 2) or the factors type of rat, treatment and time for repeated measures (experiment 3). In case HR and LR were differentially sensitive to COC, the effects of RES on the effects of COC were statistically analysed per type of rat. One sample t-tests were used to evaluate whether a specific treatment significantly changed accumbal dopamine levels from baseline. The relationship between the mean COC-induced increase of accumbal extracellular dopamine during 90 min and the response to novelty on the open-field (travelled distance and habituation time) were evaluated by means of Pearson's 2-tailed correlation analysis in a pooled group of LR and HR. All data are expressed as mean ± SEM. A probability level of $p<0.05$ was taken as significant in every test. SPSS for Windows (Release 12.0) was used to statistically analyse the data.

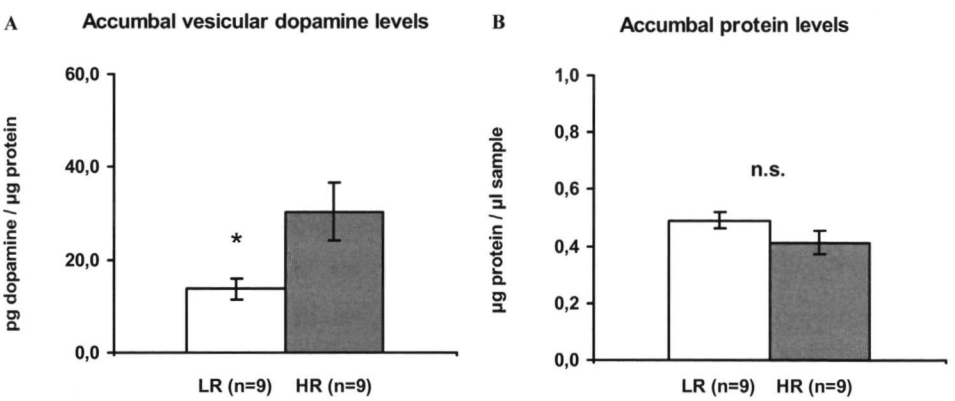


Figure 2. Vesicular levels of accumbal dopamine (A). The amount of vesicular dopamine (pg) was determined in punches of accumbal tissue. Vesicular dopamine levels were normalised against the protein levels of supernatant 1. *Significant difference between LR (n=9) and HR (n=9) rats (one-way ANOVA). **Accumbal protein levels (B).** The total levels of general protein of supernatant 1 did not differ between LR (n=9) and HR (n=9). n.s: no significant differences (one-way ANOVA). All data are expressed as mean ± SEM.

Results

Open-field selection: The open-field selection procedure revealed 24% LR and 30% HR. The average distance travelled in 30 min was 3493 ± 191 cm and 8643 ± 373 cm in LR and HR, respectively. The average habituation time was 324 ± 28 s in LR and 1340 ± 66 s in HR. Rats that did not fulfil the criteria (46%), were not included in this study.

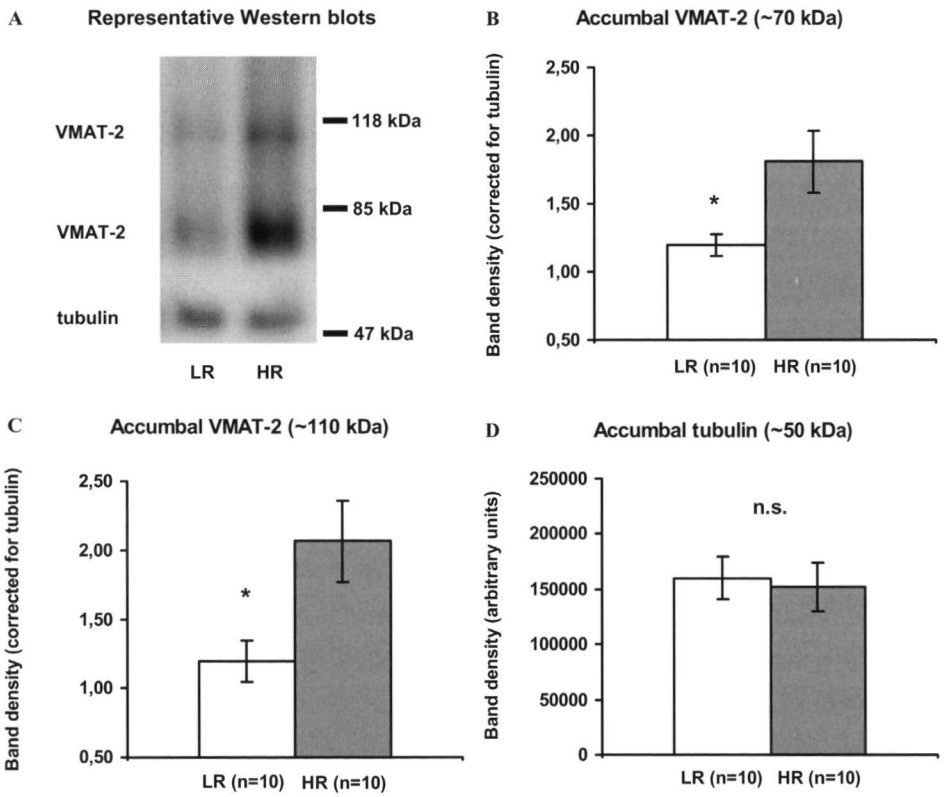


Figure 3. Representative Western blots of one LR (left) and one HR (right) using anti β-tubulin and anti vesicular monoamine transporter-2 (VMAT-2) antibodies (A). Western blot analysis was performed on punches of accumbal tissue. One band for tubulin and two bands for VMAT-2 were observed. **Immunoreactivity levels of the relatively small accumbal VMAT-2 protein of ~70 kDa and the relatively large accumbal VMAT-2 protein of ~110 kDa in LR and HR (B-C).** VMAT-2 levels were quantified using tubulin for normalization. *Significant differences between LR (n=10) and HR (n=10) rats (one-way ANOVA). **Immunoreactivity levels of the accumbal tubulin protein in LR and HR (D).** Tubulin levels did not differ between LR (n=10) and HR (n=10). n.s, no significant differences (one-way ANOVA). All data are expressed as mean ± SEM.

Vesicular levels of accumbal dopamine (exp 1): Histological verification revealed that three LR and three HR had to be excluded because incorrect placement of the punch needle. Figure 1 shows the coronal region of the nucleus accumbens in which all correctly placed punches were located.

The vesicular levels of accumbal dopamine are depicted in figure 2 (LR: n=9 and HR: n=9). The nucleus accumbens of LR was marked by smaller levels of dopamine inside storage vesicles than the nucleus accumbens of HR (Fig. 2A: one-way ANOVA: type effect $F_{(1,16)} = 6.100$, $p = 0.025$). The accumbal levels of general protein were equal in LR and HR (Fig. 2B: one-way ANOVA: type effect: n.s.), demonstrating that similar pieces of tissue were punched from the nucleus accumbens of both types of rat.

Accumbal VMAT-2 levels and total levels of accumbal dopamine (exp 2): The accumbal VMAT-2 immunoreactivity levels are depicted in figure 3 (LR: n=10 and HR: n=10). The anti-VMAT-2 antibody labelled both a relatively small protein of ~70 kDa and a relatively large protein of ~110 kDa (Fig. 3A: Yao et al., 2004; Yao and Hersh, 2007). A one-way ANOVA revealed that the levels of both the small and the large VMAT-2 protein were significant less in the nucleus accumbens of LR than of HR (Fig. 3B (VMAT-2 ~70 kDa): type-effect: $F_{(1,18)} = 6.490$, $p = 0.020$; Fig. 3C (VMAT-2 ~110 kDa): type effect: $F_{(1,18)} = 6.822$, $p = 0.018$). The antibody raised against the loading control tubulin selectively labelled a protein of ~50 kDa (Fig. 3A: Kong et al., 1999). The accumbal tubulin levels were equal in LR and HR (Fig. 3D: one-way ANOVA: type effect: n.s.), demonstrating that similar pieces of tissue were punched from the nucleus accumbens of both types of rat (Hedtjarn et al., 2002). The total levels of accumbal dopamine are depicted in figure 4 (LR: n=10 and HR: n=10). The total levels of dopamine were significant less in the nucleus accumbens of LR than in the nucleus accumbens of HR (Fig.4: one-way ANOVA: type-effect: $F_{(1,18)} = 6.000$, $p = 0.023$).

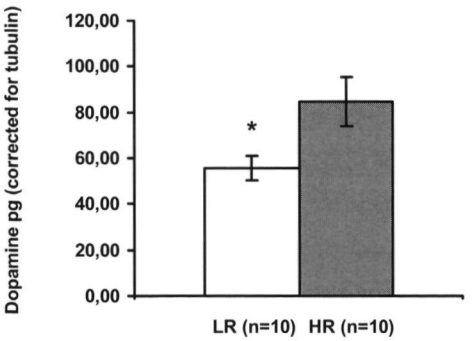


Figure 4. Total levels of accumbal dopamine. The total amount of dopamine (pg) was determined in punches of accumbal tissue. Dopamine levels were quantified using tubulin for normalization (Fig. 3D). *Significant difference between LR (n=10) and HR (n=10) rats (one-way ANOVA). All data are expressed as mean ± SEM.

Effects of reserpine on the cocaine-induced increase of extracellular accumbal dopamine (exp 3): The dialysis probes of the present study were located in the same region of the nucleus accumbens as the dialysis probes of a previous study (see Fig. 2 of chapter 2). Histological verification revealed that two HR and two LR had to be excluded because of incorrect placement of the dialysis probe. One additional HR had to be excluded from analysis because of obstruction of the microdialysis probe.

The baseline absolute concentration of extracellular accumbal dopamine was 0.68 ± 0.11 pg / 10 µl in LR (mean ± SEM of rats belonging to the pooled groups of solvent-treated LR: n=8+8=16, rats of Fig. 6A) and 0.84 ± 0.15 pg /10 µl in HR (mean ± SEM of rats belonging to the pooled groups of solvent-treated HR: n=9+8=17, rats of Fig. 6A). Extracellular dopamine levels after RES were 0.58 ± 0.07 pg / 10 µl in LR (1 mg/kg: n=8, rats of Fig. 7) and 0.31 ± 0.05 pg / 10 µl in HR (1 and 2 mg/kg pooled: n=8+9=17, rats of Fig. 7). The RES-induced decrease of the basal levels of dopamine in LR (100% - (0.58 pg/0.68 pg) = 15%) and HR (100% - (0.31 pg/0.84 pg) = 63%) are very similar to the previously reported RES-induced decrease of the basal levels of dopamine in these rats (Verheij and Cools, 2007). No rat had to be excluded because of undetectable dopamine levels.

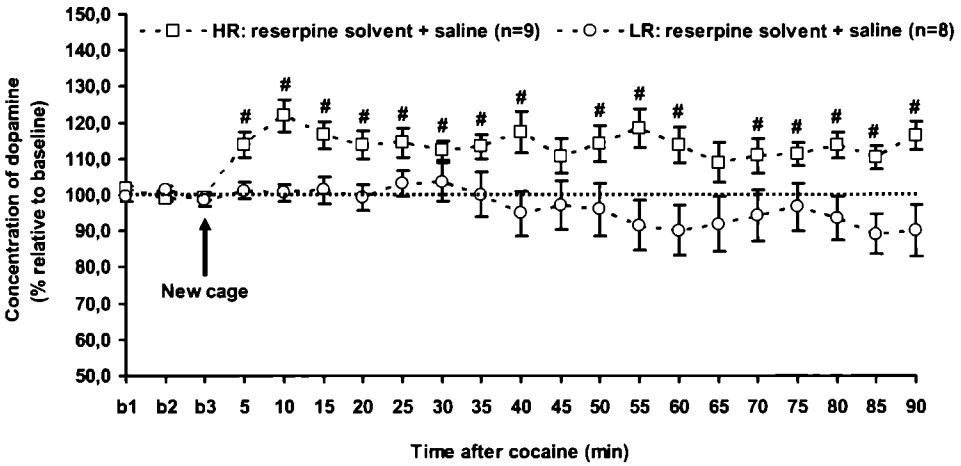


Figure 5. Effects of novelty (new cage) on the extracellular levels of dopamine in the nucleus accumbens in reserpine-solvent and saline-treated LR (circles) and reserpine-solvent and saline-treated HR (squares). Reserpine-solvent (1 ml/kg, i p) was administered 24 h before saline (1 ml/kg, i p) Rats (LR n=8, HR n=9) were exposed to novelty immediately after the saline injection. Accumbal dopamine levels after novelty are expressed as percentage of baseline accumbal dopamine levels The horizontal line represents basal dopamine levels (= 100%) #Significant increase relative to baseline (one sample t-test) All data are expressed as mean ± SEM

The effects of saline (= solvent of COC) on the extracellular amount of accumbal dopamine in novelty-challenged LR and HR are depicted in figure 5 (LR: saline: n=8 and HR saline: n=9). The accumbal dopamine response to novelty was larger in saline-treated HR than in saline-treated LR (Fig. 5: two-way ANOVA: type x time effect: n.s; type effect: $F_{(1,15)} = 12{,}733$, $p = 0.003$). One sample t-tests revealed that the extracellular levels of dopamine significantly increased from baseline at 16 out of the 18 time points in control HR, whereas the extracellular levels of dopamine did not differ from baseline at any time point in control LR (Fig. 5).

The effects of 15 mg/kg of COC on the extracellular amount of accumbal dopamine in novelty-challenged rats are depicted in figure 6A (LR: COC: n=8 and HR: COC: n=8). COC increased the extracellular dopamine levels in both HR (Fig. 6A: two-way ANOVA: treat x time effect: $F_{(18,270)} = 11.832$, $p < 0.001$) and LR (Fig. 6A: two-way ANOVA: treat x time effect: $F_{(18,252)} = 5.163$, $p < 0.001$). However, the COC-

induced increase of dopamine was stronger in HR than in LR (Fig. 6A: three-way ANOVA: type x treat x time effect: $F_{(18,522)} = 2.889$, $p < 0.001$). Accumbal dopamine levels increased during the first 70 min in COC-treated HR (Fig. 6A: Student's t-tests), whereas accumbal dopamine levels increased only during the first 25 min in COC-treated LR (Fig. 6A: Student's t-tests). Pearson's analysis revealed that both travelled distance and habituation time on the open-field positively correlated with the mean COC-induced increase of accumbal extracellular dopamine (Fig. 6B: LR and HR pooled (n=16): distance (left): R = 0.553, p = 0.02; habituation time (right): R = 0.572, p = 0.02).

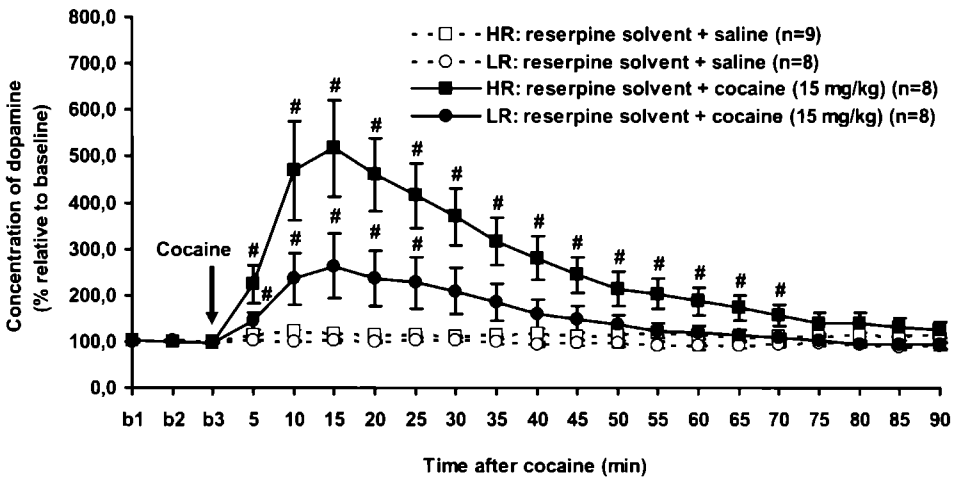


Figure 6 (A) Effects of 15 mg/kg of cocaine on the extracellular levels of dopamine in the nucleus accumbens of novelty-challenged LR (circles) and novelty-challenged HR (squares). Accumbal dopamine levels after cocaine (filled, LR n=8, HR n=8) or saline (open, LR n=8, HR n=9) are expressed as percentage of baseline accumbal dopamine levels. #Significant increase relative to saline (Student's t-test). All data are expressed as mean + SEM

The effects of 1 mg/kg of RES on the COC-induced increase of extracellular accumbal dopamine are depicted in figure 7 (LR: RES 1 mg/kg + COC: n=8 and HR: RES 1 mg/kg + COC: n=8). The dose of 1 mg/kg of RES strongly reduced the COC-induced increase of extracellular dopamine in LR (Fig. 7 (left): two-way ANOVA: treat x time effect: $F_{(18,252)} = 5.263$, $p < 0.001$). In fact, one sample t-tests revealed that

accumbal dopamine did not anymore increase from baseline at any time point in these rats (Fig. 7: left). The dose of 1 mg/kg of RES did not at all affect the COC-induced increase of extracellular dopamine levels in HR (Fig. 7 (right): two-way ANOVA: treat x time effect: n.s; treat effect: n.s.). One sample t-tests revealed that accumbal dopamine significantly increased from baseline in HR during the first 65 min (Fig. 7: right).

The effects of 2 mg/kg of RES on the extracellular amount of accumbal dopamine in COC-treated HR are also depicted in figure 7 (HR: RES 2 mg/kg + COC: n=9). A two-way ANOVA revealed that the effects of RES were dose-dependent (Fig. 7 (right): dose x time effect: $F_{(36,396)} = 3.135$, $p < 0.001$). The dose of 2 mg/kg of RES strongly reduced the COC-induced increase of accumbal dopamine in HR (Fig. 7 (right): two-way ANOVA: treat x time effect: $F_{(18,270)} = 8.272$, $p < 0.001$). One sample t-tests revealed that dopamine significantly increased from baseline only during the first 35 min (Fig. 7: right).

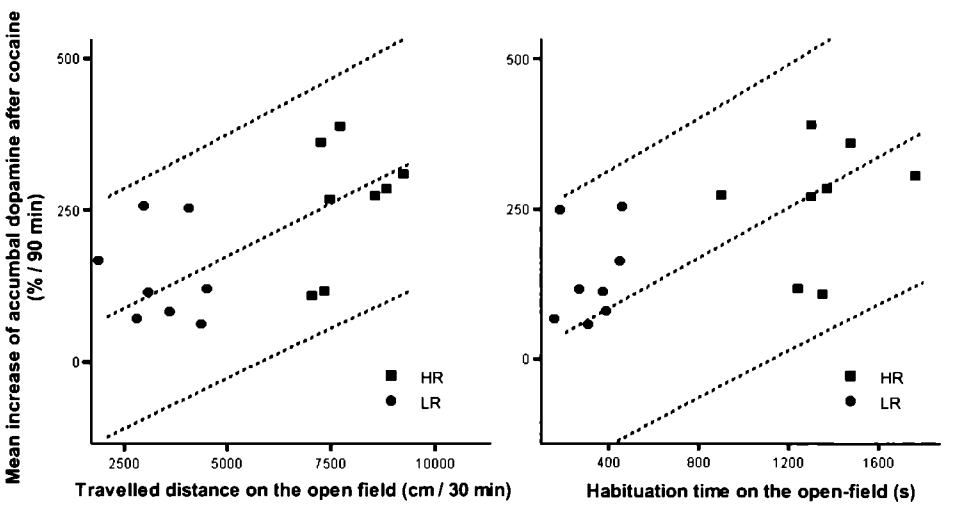


Figure 6 (B). Correlation of travelled distance (left) / habituation time (right) on the open-field and accumbal dopamine increase after cocaine. Travelled distance is expressed as locomotor activity (cm) during 30 min on the open-field. Habituation time is expressed as the duration of the period (s) that started as soon as the rat began to explore the open-field and ended as soon as the locomotor activity stopped for at least 90 s. Accumbal dopamine levels are expressed as mean increase from baseline during 90 min after cocaine (LR (circles) n=8, HR (squares) n=8) The dotted lines represent the regression based fit line ± the prediction interval at a confidence level of 95%. One single dot represents one single rat.

Discussion

Accumbal levels of vesicular dopamine and VMAT-2 (exp 1 + 2): Low responders to novelty displayed smaller amounts of dopamine inside the storage vesicles of the nucleus accumbens than HR (see Fig. 2). These results can be explained by the finding that LR had less accumbal VMAT-2 than HR (see Fig. 3). It must be noted that two VMAT-2 proteins of different molecular size were identified. It has previously been shown that VMAT-2 proteins are expressed in two morphological distinct types of storage vesicles (Nirenberg et al., 1995; Nirenberg et al., 1996; Nirenberg et al., 1997a). The small molecular size of VMAT-2 (~70 kDa) has been found to be localised on small synaptic vesicles, whereas the large molecular size of VMAT-2 (~110 kDa) has been found to be localised on large dense core vesicles, depending on the glycosylation of the protein (Yao et al., 2004; Yao and Hersh, 2007). The present finding that LR displayed lower levels of both types of VMAT-2 than HR, indicates that LR are marked by smaller amounts of accumbal dopamine in both small and large vesicles than HR. These smaller levels of vesicular dopamine in LR than in HR may well account for the finding that the total levels of dopamine were smaller in LR than in HR (see Fig. 4).

Effects of reserpine on cocaine-induced accumbal dopamine levels (exp 3): Cocaine increased the extracellular accumbal dopamine levels more strongly in HR than in LR (see Fig. 6A). These results in novelty-challenged rats are very similar to the previous reported results in non-novelty-challenged rats (Hooks et al., 1991b; Chefer et al., 2003). In fact, the dopamine increase after novelty hardly contributed to the dopamine increase after COC (see Fig 6A). It was also demonstrated that both behavioural criteria to select HR and LR on the open-field (travelled distance and habituation time) positively correlated with the COC-induced increase of accumbal dopamine (see Fig. 6B). These data were in agreement with the previously reported notion that the response to novelty can predict the individual-specific response to drugs of abuse (Piazza et al., 1989; Hooks et al., 1991a; Piazza et al., 1991a; Hooks et al., 1991b; Cools and Gingras, 1998). The relatively low dose of 1 mg/kg of RES reduced the COC-induced increase of extracellular accumbal dopamine in LR, but not in HR (see

Fig. 7). A higher dose of 2 mg/kg of RES was required to inhibit the COC-induced increase of accumbal dopamine in HR (see Fig. 7). These data confirm the hypothesis that COC-treated LR are more vulnerable to the RES-induced dopamine depletion than COC-treated HR.

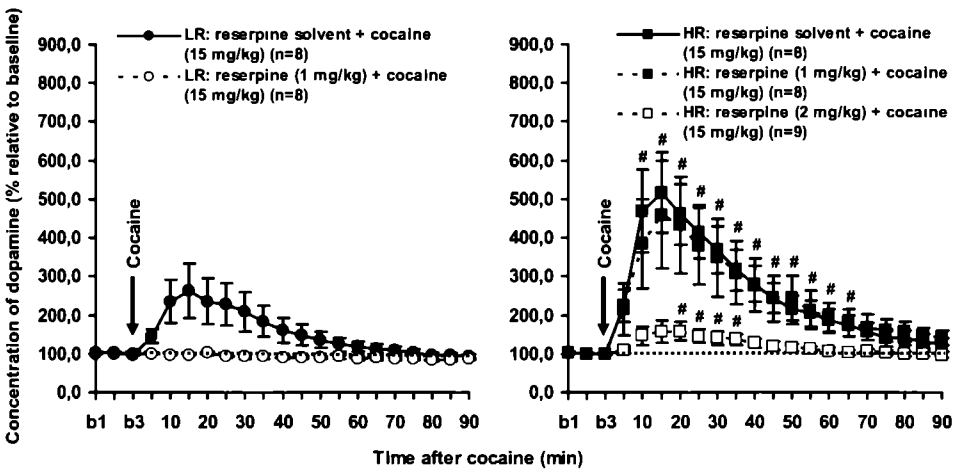


Figure 7. Effects of 1 and 2 mg/kg of reserpine (RES) on the cocaine-induced increase of accumbal extracellular dopamine levels in novelty-challenged LR (left) and novelty-challenged HR (right). Cocaine-induced accumbal dopamine levels in rats treated with solvent (LR n=8, HR n=8) or reserpine (1 mg/kg LR n=8, HR n=8, 2 mg/kg HR n=9) are expressed as percentage of baseline accumbal dopamine levels. The horizontal line represents basal dopamine levels (= 100%). Reserpine reduced baseline levels of dopamine in LR (solvent vs RES (1 mg/kg): reduction: 100% - (0.58 pg/0.68 pg) = 15%) and HR (solvent vs RES (1 and 2 mg/kg pooled) reduction 100% - (0.31 pg/0.84 pg) = 63%). #Significant dopamine increase after reserpine (one sample t-test). All data are expressed as mean ± SEM.

Noradrenaline and serotonin are both known to control the release of dopamine (Kilpatrick et al., 1996; Cools and Tuinstra, 2003). Because RES ultimately depletes dopamine, noradrenaline and serotonin, the observed effects of RES may be the result of drug-induced changes in the levels of each of these neurotransmitters. However, the fact that the vesicular levels of dopamine differed between LR and HR, suggests that the observed individual differences in the effects of RES are, most likely, because of individual differences in the RES-induced decrease of the levels of dopamine inside storage vesicles.

One could argue that RES reduced the dopamine response to COC for the reason that RES diminished the basal levels of dopamine. Under the condition that COC blocks the re-uptake of neurotransmitters, low basal levels of extracellular dopamine result in a reduced COC-induced increase of dopamine. This explanation, however, is not supported by the present data. In HR, in which 1 mg/kg of RES strongly reduced the basal levels of dopamine (reduction: 63%), the dopamine increase following COC was not inhibited at all (Fig. 7). Moreover, in LR, in which 1 mg/kg of RES only slightly reduced the basal levels of dopamine (reduction: 15%), the dopamine increase following COC was completely inhibited (Fig. 7). The fact that the results in RES-treated rats can not exclusively be explained by the generally accepted mode of action that COC inhibits dopamine re-uptake implies that COC must have an additional mode of action. The finding that RES reduced the extracellular dopamine increase to COC (Fig. 7) indicates that COC facilitates the release of dopamine that is derived from storage vesicles (Sulzer and Rayport, 1990).

Dopamine releasing action of cocaine: The present data were in agreement with previously reported data demonstrating that the COC-induced release of dopamine is caused by exocytosis (Carboni et al., 1989; Yan, 2003; Venton et al., 2006) and not by the reversal of plasmalemmal transporters (Fischer and Cho, 1979; Butcher et al., 1988; Sulzer et al., 1993; Pifl et al., 1995; Scarponi et al., 1999). The exact mechanism of action for COC to release dopamine from vesicles is currently unknown Superfusion studies have demonstrated that the dopamine and noradrenaline releasing action of COC depends not only on storage pools, but also requires the inhibition of plasmalemmal transporters (Pifl et al., 1995; Pifl et al., 1999). These data indicate that the COC-induced inhibition of dopamine transporters (DATs), somehow, promotes the dopamine release from vesicles. The notion that both DATs and dopaminergic storage vesicles are involved in the effects of COC in animals is confirmed by the outcome of studies in humans demonstrating that the chronic use of COC produces changes not only in DAT-binding, but also in VMAT-binding (Wilson et al., 1996; Little et al., 1999; Little et al., 2003).

A dopamine releasing action of COC involves that dopaminergic storage vesicles become empty after this drug (Pothos and Sulzer, 1998; Pothos, 2002). Under the condition that COC depletes dopaminergic storage vesicles, replenishment of these vesicles is required. In fact, it has recently been demonstrated that stimulation of D_1 and D_2 receptors promotes the refill of vesicles after dopamine has been released by transporter blockers like COC (Brown et al., 2001a; Brown et al., 2001b; Sandoval et al., 2002). Given the observed individual differences in COC-induced dopamine release, this D1/D2-receptor mediated refill of vesicles is expected to be larger in COC-treated HR than in COC-treated LR. This nicely fits in with the finding that HR express more VMAT than LR.

Effects of novelty on accumbal dopamine levels: The finding that HR that were exposed to saline and novelty were marked by a larger increase of extracellular accumbal dopamine than LR that were exposed to saline and novelty (see Fig. 5) fits in with the available literature reporting that challenged HR are marked by a larger accumbal dopamine response than challenged LR (Piazza et al., 1991b; Rouge-Pont et al., 1993; Saigusa et al., 1999; Verheij and Cools, 2007). The finding, however, that the extracellular levels of dopamine did not at all increase in novelty-challenged LR is not in agreement with a previous study (Verheij and Cools, 2007). It is important to note that the saline-treated and novelty-challenged rats of the present study were also treated with RES-solvent (see experimental procedures). It has been shown that repeated exposure to the same stressor reduces, or even prevents, the stress-induced increase of dopamine in the nucleus accumbens (Imperato et al., 1992; Imperato et al., 1993; Cabib and Puglisi-Allegra, 1996a; Cabib and Puglisi-Allegra, 1996b). Accordingly, the most likely explanation for the present finding that accumbal dopamine levels did not increase in novelty-challenged LR, is that the rats of the present study were stressed twice by a systemic injection (RES solvent on day 1 and saline on day 2), whereas the rats of the previous study were stressed only once (RES solvent on day 1 and no saline on day 2). The previously reported finding that RES blocked the accumbal dopamine increase in novelty-challenged LR (Verheij and Cools, 2007), suggest that the long-term processes that are triggered by multiple exposure to stressors (anticipation/adaptation) might be

related to dopamine stored in RES-sensitive vesicles. The fact that RES did not at all inhibit the accumbal dopamine increase in novelty-challenged HR (Verheij and Cools, 2007) may explain why the dopamine decreasing effects of repeated exposure to injection stress did not occur in these rats.

It has previously been reported that RES strongly reduces the baseline levels of dopamine in HR, but not LR (Verheij and Cools, 2007). As discussed above, RES strongly reduces the dopamine response to novelty in LR, but not HR. The present study shows that RES decreased the dopamine response to COC in both types of rat. It is, therefore, suggested that the individual differences in (i) the basal dopamine response, (ii) the dopamine response to novelty and (iii) the dopamine response to COC are regulated by three distinct neuronal substrates (for details see: Verheij and Cools, 2008).

Conclusions

The results of the present study indicate that the search for individual differences in the susceptibility to COC should focus not only on individual differences in the re-uptake mechanisms of dopamine, but also on individual differences in the capacity to store dopamine inside vesicles. The present data give rise to the conclusion that LR contain less dopamine inside accumbal storage vesicles than HR because the nucleus accumbens of LR display lower levels VMAT-2 than the nucleus accumbens of HR. The fact that LR are marked by a relatively small storage pool containing low amounts of VMAT, whereas HR are marked by a relatively large storage pool containing high amounts of VMAT may well explain why COC-treated LR are more vulnerable to the dopamine depleting effects of RES than COC-treated HR.

Although it is likely that several mechanisms contribute to individual differences in the sensitivity to COC, the results of the present study indicate that HR are more sensitive to COC than LR because COC can release more dopamine from accumbal storage vesicles in HR than in LR. Given that this release of vesicular dopamine may be mediated by dopamine re-uptake transporters, it is hypothesised that the individual differences in the COC-induced dopamine increase in HR and LR are due to a

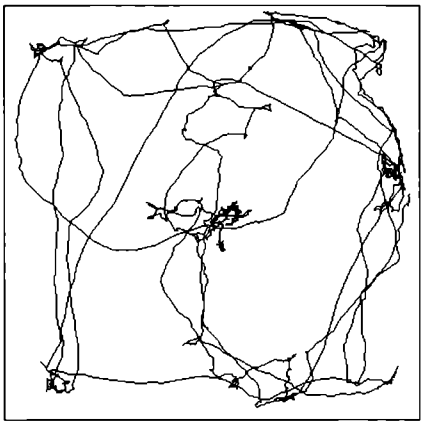
combination of individual differences in both dopamine re-uptake (Chefer et al., 2003) and vesicular dopamine release (present study).

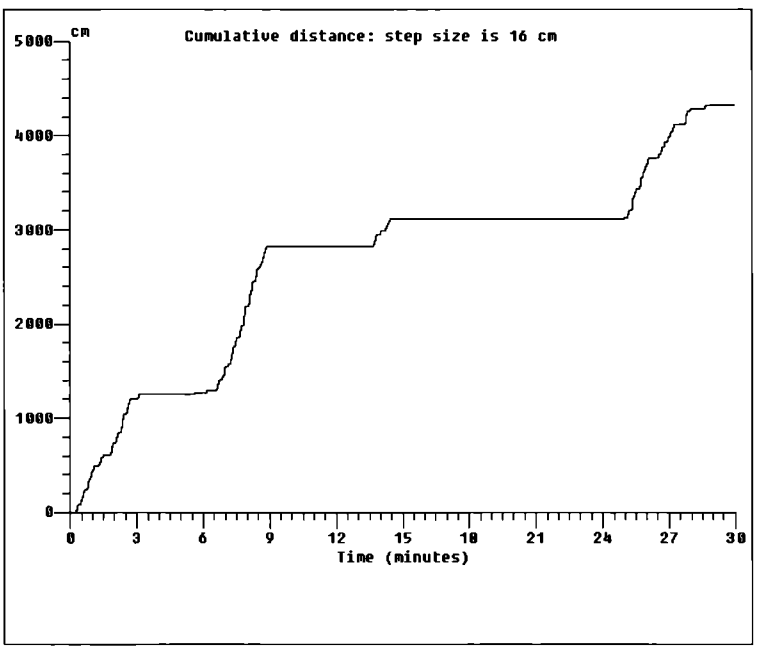
Impact

The present findings open the intriguing possibility that drugs that deplete dopaminergic storage vesicles of the mesolimbic system (e.g. RES, Ro 4-1284 and tetrabenazine) might become the drugs of choice for the treatment of COC abuse. Interestingly, recent clinical screening trials on the effects of RES in COC-addicted subjects have already revealed promising results in this respect (Gorelick et al., 2004; Berger et al., 2005). RES-like agents have also been found to be effective in the treatment of hyperkinetic movements disorders like Huntington's chorea (Kenney and Jankovic, 2006; Huntington study group, 2006). These results suggest that hyperkinesia may, at least in part, be mediated by dopamine derived from storage vesicles.

In addition to the effects of COC, the effects of methylphenidate (Ritalin) are also known to depend on RES-sensitive storage pools (Scheel-Kruger, 1971; Chiueh and Moore, 1975a; Braestrup, 1977; McMillen et al., 1980; McMillen, 1983; Butcher et al., 1991). Methylphenidate is used to treat patients suffering from attention deficit hyperactivity disorder (ADHD). Studies in ADHD patients have revealed large individual differences in the clinical response to methylphenidate (Volkow et al., 2002; Volkow and Swanson, 2003). Given that HR and LR differ in the size of the storage pools that are affected by these drugs, HR and LR may well be a used as an animal model to study the individual-specific variability in the treatment of ADHD (Wooters et al., 2006).

Finally, vesicular uptake is suggested to protect a neuron against the toxic effects of high levels of cytoplasmatic dopamine (Truong et al., 2003; Truong et al., 2004). In this respect it important to note that HR, which are marked by a large number of VMAT, are less sensitive to the neurotoxic effects of 6-hydroxydopamine than LR, which are marked by a small number of VMAT (van Oosten and Cools, 2002).





4/15: Analyseren van de open-veld data: low responder to novelty.
Analysis of the open-field data: low responder to novelty.

Chapter 4

Reserpine differentially affects the behavioural response to cocaine in low and high responders to novelty

M.M.M. Verheij and A.R. Cools.

Submitted.

Abstract

Numerous studies have indicated that the behavioural response to cocaine is not only the result of monoamine uptake-inhibition, but depends also on monoamines inside storage vesicles The present study examined whether rats that are known to differ in their behavioural response to cocaine, differ in their sensitivity to a drug that is known to deplete vesicles The effects of reserpine (1 and 2 mg/kg) on cocaine-induced behaviour (10 and 15 mg/kg) were investigated in Low Responders (LR) and High Responders (HR) to novelty

LR displayed less cocaine-induced walking, wall rearing, free rearing and stereotyped behaviour than HR did The dose of 1 mg/kg of reserpine decreased cocaine-induced walking, wall rearing, free rearing and stereotyped behaviour in LR, but not in HR The dose of 2 mg/kg of reserpine inhibited cocaine-induced behaviour in HR These behavioural data fit in with our previously reported neurochemical finding that higher doses of reserpine are required to inhibit the accumbal dopamine response in HR than in LR Combining the previously reported neurochemical data with the present behavioural data results in the hypothesis that LR are less sensitive to the behavioural effects of cocaine than HR because cocaine can release less monoamines from storage vesicles in LR than in HR It is suggested that distinct types of behaviour are mediated by reserpine-sensitive storage vesicles in distinct brain structures

Introduction

Individual differences in the susceptibility to psychostimulants have extensively been reported, both in humans (Jaffe and Archer, 1987, Ball et al , 1994a, Gynther et al , 1995, van den Bree et al , 1998) and in animals (Piazza et al , 1989, Piazza et al , 2000, Mantsch et al , 2001) In this study we focused on two types of rat that are known to differ in their acute response to cocaine (COC) These individuals, which co-exist in a normal outbred population of Wistar rats, are selected on the basis of their locomotor response to a novel open-field and, accordingly, labelled Low Responders (LR) and High Responders (HR) to novelty (Piazza et al , 1989, Piazza et al , 1991b, Rouge-Pont et al , 1993, Dellu et al , 1996b, Bevins et al , 1997, Cools and Gingras, 1998, Cools and Tuinstra, 2003, Kabbaj, 2004, Verheij and Cools, 2008) Previous studies have demonstrated that COC increases monoamine levels in the nucleus accumbens to a smaller degree in LR than in HR (Hooks et al , 1991b, Chefer et al , 2003, Verheij et al , 2008) In addition, the behavioural response to COC has been shown to be smaller in LR than in HR (Hooks et al , 1991b, Chefer et al , 2003)

COC is known to inhibit the re-uptake of monoamines by blocking plasmalemmal monoamine transporters (Lee et al , 2001) However, both neurochemical and behavioural studies have demonstrated that the response to COC depends also on the release of monoamines from storage vesicles (Scheel-Kruger et al , 1977, McMillen et al , 1980, McMillen, 1983, Davis, 1985, Hurd and Ungerstedt, 1989, Florin et al , 1995, Pifl et al , 1995, Venton et al , 2006, Verheij et al , 2008) We have recently demonstrated that the monoaminergic storage pool of the nucleus accumbens of LR is smaller than the monoaminergic storage pool of HR (Cools and Verheij, 2002, Verheij et al , 2008) Accordingly, we have hypothesised that LR are less sensitive to the neurochemical effects of COC than HR because COC can release less monoamines from storage vesicles in LR than in HR (Verheij and Cools, 2008, Verheij et al , 2008)

Reserpine (RES) binds to vesicular monoamine transporters (Kirshner et al , 1963, Henry et al , 1998) After RES treatment, monoaminergic storage vesicles are known to become empty (Dahlstrom et al , 1965, Wagner, 1985, Pothos et al , 1998, Colliver et al , 2000, Gong et al , 2003) Because RES has been used to deplete

monoaminergic storage pools in our previous studies (Cools and Verheij, 2002, Verheij et al , 2008), we also used RES in the present study After 1 mg/kg of RES, COC could still increase accumbal dopamine release from storage vesicles in HR, but not in LR (Verheij et al , 2008) The dose of 2 mg/kg of RES was needed to inhibit COC-induced accumbal dopamine release in HR (Verheij et al , 2008) Based on these previously reported neurochemical findings, we hypothesised that the dose of RES required to inhibit the behavioural response to COC is higher in HR than in LR

Experimental procedures

Subjects: Adult male LR (n=41) and HR (n=64) that were selected from the outbred strain of Nijmegen Wistar rats were used throughout the study All rats (weight=180-220 g) were reared and housed in macrolon cages (42 x 26 x 15 cm, n–3-4 per cage) under a fixed 12/12 h light/dark cycle (lights on 07 00 a m) in a temperature-controlled room (21 ± 1 7 °C) Water and food pellets were available *ad libitum* The experiments were performed in accordance with institutional, national and international policies on animal care and welfare All procedures were in agreement with the NRC (2003) guidelines for the care and use of mammals in neuroscience and behavioural research

Open-field selection: Rats were individually housed 3 days before the open-field selection procedure (Verheij et al , 2008) The housing conditions before the open-field selection were similar to the housing conditions stated above (see section subjects) Testing took place between 09 00 h and 17 00 h in a room illuminated by white light of 170 Lux The rat was placed on a black, square table (160 x 160 cm) made of Perspex This open-field is 95 cm elevated above the floor and surrounded by a white neutral background (270 x 270 x 270 cm) As described by Cools et al (1990), behaviour was recorded with a computerised automated tracking system for a period of 30 min The objective parameters of ambulation and habituation time were used to select LR and HR (see also Cools et al , 1990, Ellenbroek and Cools, 2002) Ambulation was defined as the overall distance (cm) travelled in 30 min Habituation time was defined as the

duration of the period (s) that started as soon as the rat began to explore the open-field and ended as soon as the locomotor activity stopped for at least 90 s. Rats that habituated in less than 480 s and walked less than 4 800 cm in 30 min were labelled LR, whereas rats that habituated after 840 s and walked more than 6 000 cm in 30 min were labelled HR (see also: Verheij et al., 2008). Habituation time in addition to ambulation was used as selection criterion, because travelled distance per se is not always a reliable criterion (Cools et al., 1997; Saigusa et al., 1999).

Surgery: Apart from the assessment of the behavioural response to COC, the rats treated with 15 mg/kg of this drug (see below) were also used to measure accumbal dopamine release (see introduction). The results of this neurochemical analysis have been published in a separate paper (see: Verheij et al., 2008). The rats treated with 10 mg/kg of COC (see below) were only used to measure behaviour. In order to allow direct comparison of the behavioural effects of both doses of COC, not only rats treated with 15 mg/kg of COC, but also rats treated with 10 mg/kg of this drug were unilaterally implanted with a guide cannula directed at the right nucleus accumbens (Verheij et al., 2008). Surgery was performed one day after the open-field selection and rats were allowed to recover for the next 7 to 10 days in Plexiglas cages (25 x 25 x 35 cm) covered with sawdust on the floor. These cages became the animal's home cage for the rest of the experiment. On 3 consecutive days just prior to the start of the experiment, each rat was gently picked up in order to habituate to the procedure assessed on the day when behaviour was measured. This handling procedure was repeated 3 times per day.

Reserpine and cocaine treatment: In order to measure the neurochemical response in the nucleus accumbens, a dialysis probe was carefully inserted into the brain of all rats (see: Verheij et al., 2008). The inlet and outlet of the probe were connected to a swivel allowing the animal to move freely. At 12.00 h on the first day of the experiment, LR and HR were injected with RES or its solvent (see section solutions). After this systemic injection (volume: 1 ml/kg, i.p.), rats were returned to their home cage and left undisturbed. At 12.00 h on the second day of the experiment, a systemic injection (volume 1 ml/kg, i.p.) of COC or its solvent (saline) was given. Because the

COC-induced changes of behaviour in LR and HR under non-challenged conditions have already been reported (Hooks et al., 1991a; Hooks et al., 1991b; Hooks et al., 1992a), the present study focused only on the COC-induced changes of behaviour in LR and HR under novelty-challenged conditions (see also: Verheij et al., 2008). All rats were exposed to a novel cage immediately after the injection of COC or its solvent. The novel cage was slightly larger than the home cage (new dimensions: 30 x 30 x 35 cm) and lacked saw-dust on the floor (Verheij and Cools, 2007; Verheij et al., 2008).

Both LR and HR were injected with 1 mg/kg of RES on day 1 and COC on day 2. Because 1 mg/kg of RES had no effect on COC-induced behaviour in HR, a new group of HR was pretreated with 2 mg/kg of RES on day 1. It has been demonstrated that the individual differences in the behavioural response to psychostimulants like amphetamine become smaller, and eventually disappear, with increasing doses of amphetamine (Gingras and Cools, 1997; Cools et al., 1997). Because the dose at which LR and HR do not anymore differ in their behavioural response to COC was unknown at the start of the present study, the behavioural effects of the moderate dose of 10 mg/kg as well as the relatively high doses of 15 mg/kg of COC were tested.

Solutions: The following solutions were used: 1) COC (Brocacef, Amsterdam, The Netherlands), 2) RES (Daiichi, Tokyo, Japan): ampoules containing 1 mg or 2 mg of RES per ml solvent, 3) RES solvent: 30 mg dl-methionine (Sigma, St. Louis, USA), dissolved in 10 ml aquadest containing 6.75% propylene glycol (Sigma). The pH of the RES solution and that of its solvent was adjusted to 2.4 using phosphoric acid (Sigma). The relatively low doses of 1 and 2 mg/kg of RES were chosen because it has previously been demonstrated that these doses selectively affect RES-sensitive monoaminergic storage vesicles (Cools and Verheij, 2002; Verheij and Cools, 2007).

Behaviour: Behaviour was recorded on video tape and analysed offline by an observer blind to the type of rat and its treatment, using a computer programme (KEYS®) developed at our institute (Saigusa et al., 1999). Recordings were made for a period of 20 min directly before the administration of COC as well as during a period of 90 min starting directly after the administration of COC. This period of 90 min was

chosen because accumbal dopamine levels after COC did not anymore differ from baseline after this period (Verheij et al., 2008). The presence or absence of a particular type of behaviour was analysed using a standard ethogram (Clifford et al., 1998; Clifford and Waddington, 2000). Only the duration of those behavioural items that were increased by COC are given (Saigusa et al., 1999): walking (displacement of all 4 paws over a minimum distance of 1 cm for a period of at least 3 s), free rearing (front paw(s) raised off the cage floor without touching a side wall of the cage), wall rearing (front paw(s) raised against the side wall(s) of the cage) and grooming (washing any part of the body). The frequency of the two types of rearing was also calculated.

Analysis and expression of the data: Behaviour was expressed as the mean duration ± SEM per block of 10 min. The behavioural effects were statistically compared, using a three-way ANOVA with the factors type of rat, treatment and time for repeated measures. Preferentially, (type x) treatment x time interactions are shown. In case this interaction was not found, (type x) treatment effects are displayed. To identify normal and repetitive rearing in COC-treated rats (see results), z-score analysis (Larsen and Marx, 2000) was performed on the frequency of rearing (frequency of rearing in a subject that received COC - average frequency of rearing in saline-treated rats / standard deviation of the average frequency of rearing in saline treated rats). A probability level of $p<0.05$ was taken as significant in every test. A z-score > 1.96 reflects a frequency of rearing that is significantly different ($p<0.05$) from the frequency of rearing observed in saline treated rats (Larsen and Marx, 2000). SPSS for Windows (Release 12.0) was used to statistically analyse the data.

Results

Histology: Although 1 HR had to be excluded from the previously reported neurochemical analysis because of obstruction of the microdialysis probe (see: Verheij et al., 2008), the behavioural data of this HR were included in the present analysis.

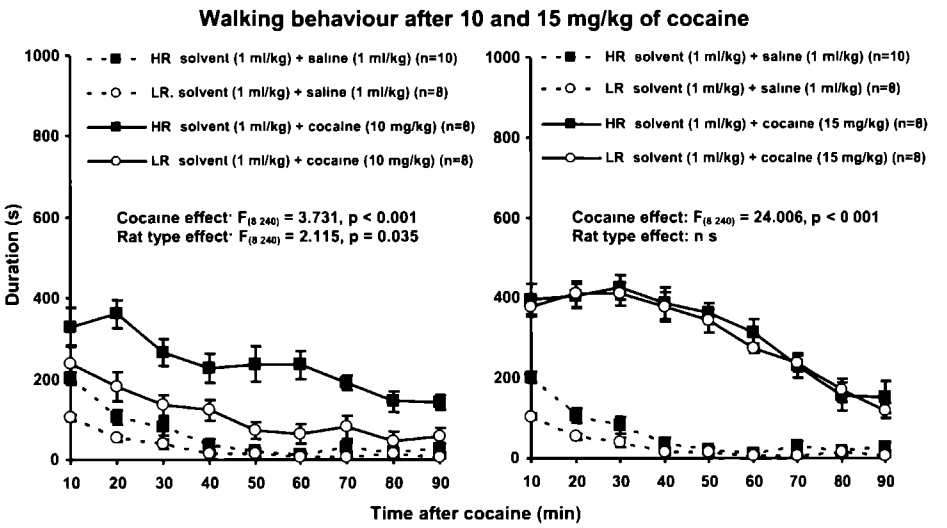


Figure 1: Effects of saline (left/right), 10 mg/kg (left) and 15 mg/kg (right) of cocaine on the duration (s) of walking behaviour in novelty-challenged LR (circle) and novelty-challenged HR (square). Cocaine-treated rats are represented by a filled line, saline-treated rats are represented by a dotted line. All rats were pretreated with the solvent of reserpine (= solvent) 24 hours before saline or cocaine was given. Data are expressed as mean ± SEM. The results of an ANOVA are displayed. LR = Low Responders to novelty, HR = High Responders to novelty.

Open-field selection: The open-field selection procedure provided 21% LR and 35% HR. The average distance travelled in 30 min (± SEM) was 3,757 ± 152 cm and 8,260 ± 269 cm in LR and HR respectively. The average habituation time (± SEM) was 392 ± 20 s in LR and 1,323 ± 52 s in HR. Rats that did not fulfil the criteria (44%) were not included in this study.

Baseline behaviour: All rats were either sitting or sleeping in the 20 min lasting period before COC or its solvent were given. Walking, rearing and grooming were simply absent in this period during which the rats remained in their home cage.

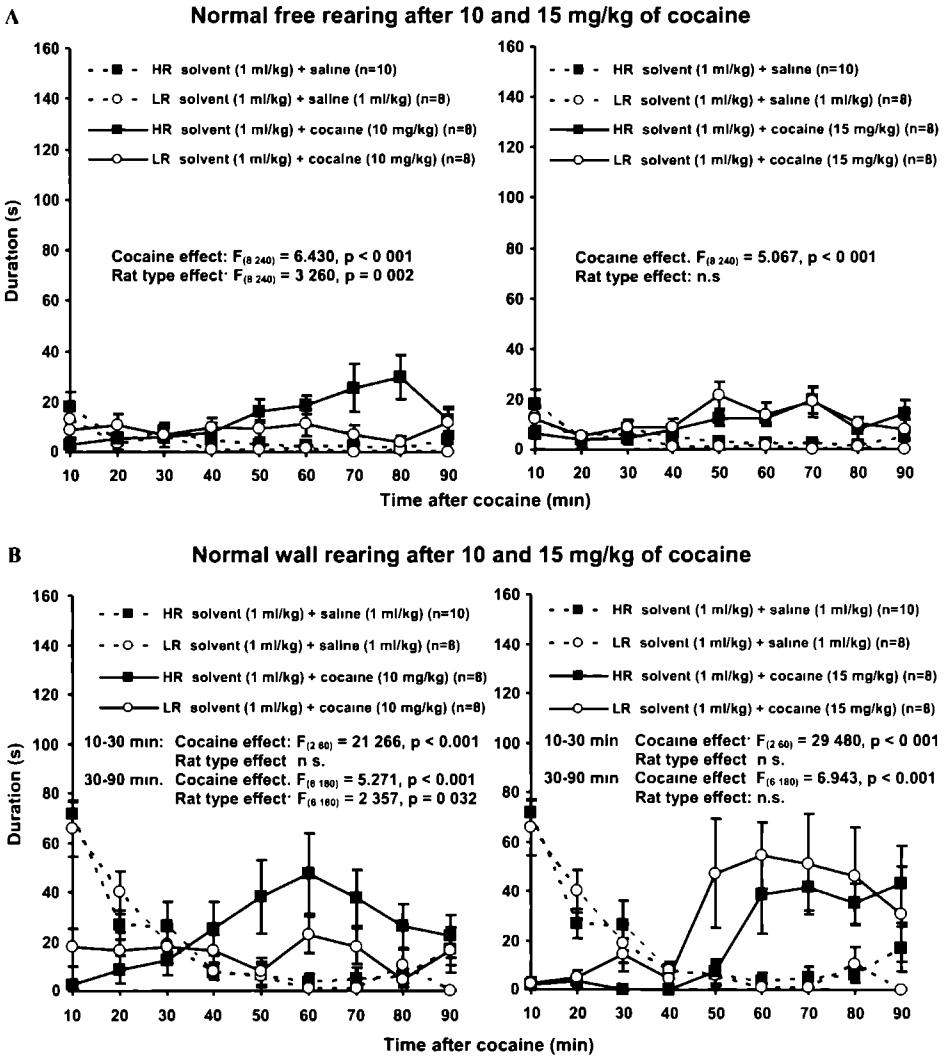


Figure 2: Effects of saline (left/right), 10 mg/kg (left) and 15 mg/kg (right) of cocaine on the duration (s) of normal free rearing (a) and normal wall rearing (b) in novelty-challenged LR (circle) and novelty-challenged HR (square). Cocaine-treated rats are represented by a filled line, saline-treated rats are represented by a dotted line. All rats were pretreated with the solvent of reserpine (= solvent) 24 hours before saline or cocaine was given. Data are expressed as mean ± SEM. The results of an ANOVA are displayed. LR = Low Responders to novelty, HR = High Responders to novelty.

Normal and repetitive rearing: The mean duration of a single rearing spell after saline was 5 0 ± 0 2 s In case the duration of a rearing spell after COC was not different from the duration of a rearing spell after saline (z-score < 1 96), this type of rearing was labelled 'normal' All rats displayed normal rearing However, normal rearing was mostly pronounced in rats treated with 15 mg/kg of COC Rearing of this type was directed both towards and not towards the wall of the cage The maximum frequency of these long-lasting rearing spells was 16 ± 3 rearing acts per 10 min, the average duration of a single rearing spell in this period was 4 6 ± 0 4 s and rats spent a total time of 213 ± 34 s rearing of this type (15 mg/kg of COC HR and LR pooled free + wall rearing)

Rats treated with 15 mg/kg of COC displayed significantly faster rearing spells compared to saline treated rats (z-score > 1 96) Rearing of this type was directed towards the wall of the cage only The maximum frequency of these short-lasting rearing spells was 94 ± 12 rearing acts per 10 min, the average duration of a single rearing spell in this period was 1 3 ± 0 1 s and rats spent a total time of 388 ± 68 s rearing of this type (15 mg/kg of COC HR and LR pooled wall rearing) Because the frequency of these rearing spells was relatively large, this type of rearing was labelled 'repetitive'

Behavioural changes after cocaine:

Treatment effects of saline and cocaine: The behavioural effects of COC are depicted in figures 1-4 (solvent (upper/lower panel) LR n=8, HR n=10, COC 10 mg/kg (upper panel) LR n=8, HR n=8 and COC 15 mg/kg (lower panel) LR n=8, HR n=8) Walking (Fig 1 time effect $F_{(8\ 128)} = 46\ 253$, $p<0\ 001$), normal free rearing (Fig 2 time effect $F_{(8\ 128)} = 6\ 766$, $p<0\ 001$), normal wall rearing (Fig 2 time effect $F_{(8\ 128)} = 34\ 257$), $p<0\ 001$) and grooming (Fig 3 time effect $F_{(8\ 128)} = 9\ 111$, $p<0\ 001$) were all increased in rats treated with the solvent of COC (saline) As discussed above, rats treated with saline did not show repetitive rearing

Figure 1 and 2 show that both the dose of 10 mg/kg and the dose of 15 mg/kg of COC increased walking and normal free rearing (treatment x time effect $p<0\ 05$) The effects of both doses of COC on normal wall rearing (Fig 2) and grooming (Fig 3) were

biphasic. COC decreased normal wall rearing between 10 and 30 min (Fig. 2: treatment x time effect (10-30 min): $p<0.05$) and grooming between 10 and 40 min (Fig. 3: treatment x time effect (10-40 min): $p<0.05$) and increased normal wall rearing between 30 and 90 min (Fig. 2: treatment x time effect (30-90 min): $p<0.05$) and grooming between 40 and 90 min (Fig. 3: treatment x time effect (40-90 min): $p<0.05$). As discussed above, rat treated with 10 mg/kg of COC did not display repetitive wall rearing. Figure 4 illustrates that this type of rearing increased only after 15 mg/kg of COC (time effect: $p<0.05$).

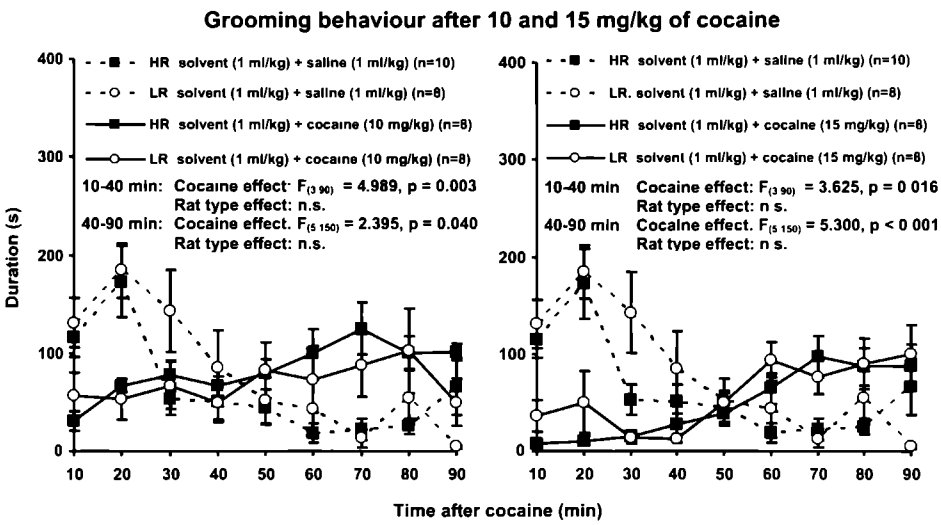


Figure 3: Effects of saline (left/right), 10 mg/kg (left) and 15 mg/kg (right) of cocaine on the duration (s) of grooming behaviour in novelty-challenged LR (circle) and novelty-challenged HR (square). Cocaine-treated rats are represented by a filled line, saline-treated rats are represented by a dotted line. All rats were pretreated with the solvent of reserpine (= solvent) 24 hours before saline or cocaine was given. Data are expressed as mean ± SEM. The results of an ANOVA are displayed. LR = Low Responders to novelty, HR = High Responders to novelty.

Type effects of saline: In rats treated with the solvent of COC (saline), type-effects were found only for walking behaviour. Figure 1 illustrates that walking increased not as much in control LR as in control HR (type x time effect: $F_{(8,128)} = 4.766$, $p<0.001$).

Type effects of 10 mg/kg of cocaine: In rats treated with the moderate dose of 10 mg/kg of COC, type-effects were found for walking, normal free rearing and normal wall rearing. Figures 1 and 2 demonstrate that walking and normal free rearing increased not as much in LR as in HR (walking and normal free rearing: type x treatment x time effect: $p<0.05$). Analysis of the first period of normal wall rearing (Fig. 2: 10-30 min) revealed that the decrease of this behaviour did not differ between the two types of rat (Fig. 2: type x treatment (x time) effect (10-30 min): n.s.) whereas the increase of normal wall rearing in the second period (Fig. 2: 30-90 min) was less in LR compared to HR (type x treatment x time effect (30-90 min): $p<0.05$). No type-specific differences were found for both the decrease (Fig. 3: type x treatment (x time) effect (10-40 min): n.s.) and the increase (Fig. 3: type x treatment (x time) effect (40-90 min): n.s.) of grooming.

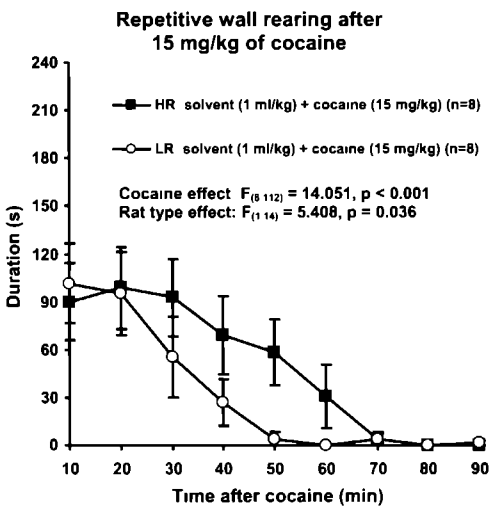


Figure 4: Effects of 15 mg/kg of cocaine on the duration (s) of repetitive wall rearing in novelty-challenged LR (circle) and novelty-challenged HR (square). Rats treated with saline or 10 mg/kg of cocaine did not show repetitive wall rearing. All rats were pretreated with the solvent of reserpine (= solvent) 24 hours before cocaine was given. Data are expressed as mean ± SEM. The results of an ANOVA are displayed. LR = Low Responders to novelty, HR = High Responders to novelty.

Type effects of 15 mg/kg of cocaine: In rats treated with the relatively high dose of 15 mg/kg of COC, type-effects were found only for repetitive wall rearing (Fig 1: walking, Fig. 2A: normal free rearing, Fig 2B: normal cage rearing, Fig. 3: grooming: type x treatment x (time) effect: n.s.). Figure 4 illustrates that 15 mg/kg of COC increased repetitive wall rearing not as much in LR as in HR (type x time effect: n.s; type effect: $p<0.05$).

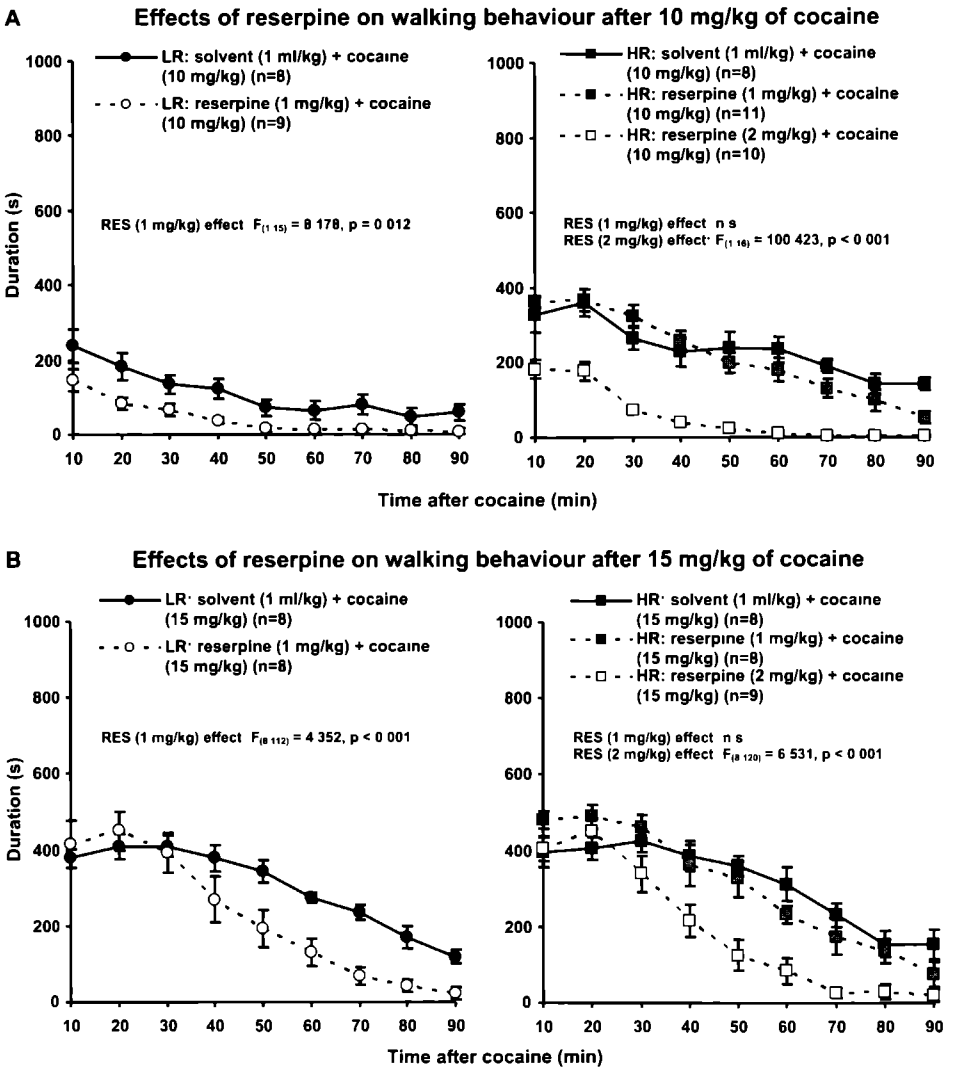


Figure 5: Effects of reserpine on the duration (s) of walking elicited by 10 mg/kg (a) and 15 mg/kg (b) of cocaine in novelty-challenged LR (left) and novelty-challenged HR (right). Rats treated with reserpine-solvent and cocaine are represented by a filled line, rats treated with reserpine and cocaine are represented by a dotted line. The effective dose of reserpine (1 mg/kg in LR and 2 mg/kg in HR) is displayed in white symbols whereas the non-effective dose of reserpine (1 mg/kg in HR) is displayed in grey symbols Data are expressed as mean ± SEM The results of an ANOVA are displayed LR = Low Responders to novelty, HR High Responders to novelty

Effects of 1 mg/kg of reserpine on cocaine-induced behaviour

Figures 5-8 show the effects of 1 mg/kg of RES on COC-induced behaviour (LR (upper panel) and HR (lower panel): RES 1 mg/kg + COC 10 mg/kg: LR: n=9, HR: n=11, RES 1 mg/kg + COC 15 mg/kg: LR: n=8, HR: n=8).

Effects of 1 mg/kg of reserpine in LR: 1 mg/kg of RES changed all behavioural items in COC-treated LR. Figures 5-8 illustrate that this low dose of RES reduced walking (10 mg/kg of COC: treatment x time effect: n.s., treatment effect: $p<0.05$ and 15 mg/kg of COC: treatment x time effect: $p<0.05$), normal free rearing (10 and 15 mg/kg of COC: treatment x time effect: n.s., treatment effect: $p<0.05$), normal wall rearing (10 mg/kg of COC: treatment x time effect: $p<0.05$ and 15 mg/kg of COC: treatment x time effect: n.s; treatment effect: $p<0.05$) and repetitive wall rearing (15 mg/kg of COC: treatment x time effect: $p<0.05$) in these rats, but could increase grooming (10 and 15 mg/kg of COC: treatment x time effect: $p<0.05$).

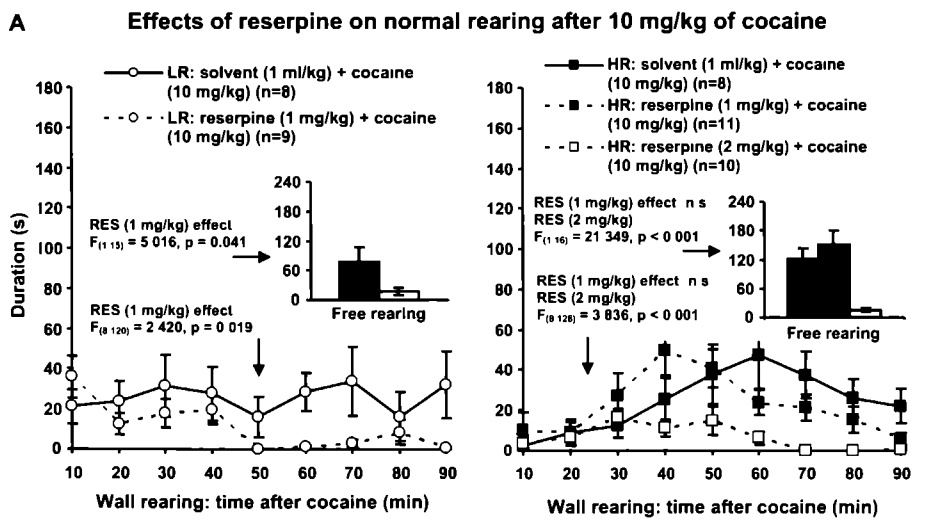


Figure 6a: Effects of reserpine on the duration (s) of normal free rearing (inlay) and normal wall rearing (main graph) elicited by 10 mg/kg of cocaine in novelty-challenged LR (left) and novelty-challenged HR (right). Rats treated with reserpine-solvent and cocaine are represented by a filled line, rats treated with reserpine and cocaine are represented by a dotted line The effective dose of reserpine (1 mg/kg in LR and 2 mg/kg in HR) is displayed in white symbols whereas the non-effective dose of reserpine (1 mg/kg in HR) is displayed in grey symbols Data are expressed as mean ± SEM The results of an ANOVA are displayed LR = Low Responders to novelty, HR = High Responders to novelty

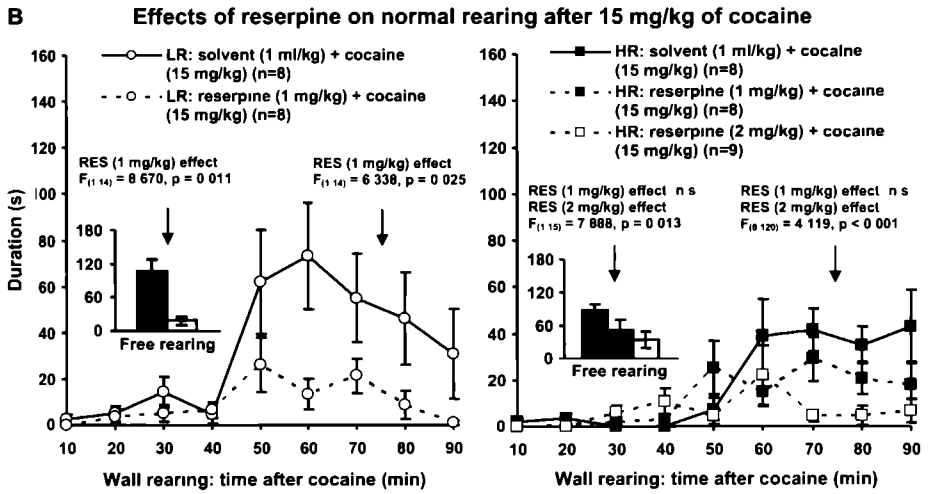


Figure 6b: Effects of reserpine on the duration (s) of normal free rearing (inlay) and normal wall rearing (main graph) elicited by 15 mg/kg of cocaine in novelty-challenged LR (left) and novelty-challenged HR (right). Rats treated with reserpine-solvent and cocaine are represented by a filled line, rats treated with reserpine and cocaine are represented by a dotted line The effective dose of reserpine (1 mg/kg in LR and 2 mg/kg in HR) is displayed in white symbols whereas the non-effective dose of reserpine (1 mg/kg in HR) is displayed in grey symbols Data are expressed as mean ± SEM The results of an ANOVA are displayed LR = Low Responders to novelty, HR = High Responders to novelty

Effects of 1 mg/kg of reserpine in HR: Figures 5-8 revealed that none of the behavioural items were affected by the relatively low dose of 1 mg/kg of RES in COC-treated HR.

Effects of 2 mg/kg of reserpine on cocaine-induced behaviour in HR

Because 1 mg/kg of RES had no effect on COC-induced behaviour in HR, a new group of HR was pretreated with 2 mg/kg of RES on day 1. The effects of 2 mg/kg of RES on COC-induced behaviour in HR are also depicted in figures 5-8 (lower panel) (HR: RES 2 mg/kg + COC 10 mg/kg: n=10, RES 2 mg/kg + COC 15 mg/kg: n=9). The dose of 2 mg/kg of RES changed all behavioural items in COC-treated HR, apart from repetitive wall rearing (Fig. 8). Figures 5-7 illustrate that this high dose of RES reduced walking (10 mg/kg of COC: treatment x time effect: n.s., treatment effect: $p<0.05$ and 15 mg/kg of COC: treatment x time effect: $p<0.05$), normal free rearing (10 and 15 mg/kg

of COC: treatment x time effect: n.s., treatment effect: $p<0.05$) and normal wall rearing (10 and 15 mg/kg of COC: treatment x time effect: $p<0.05$) in these rats, but could increase grooming (10 and 15 mg/kg of COC: treatment x time effect: $p<0.05$).

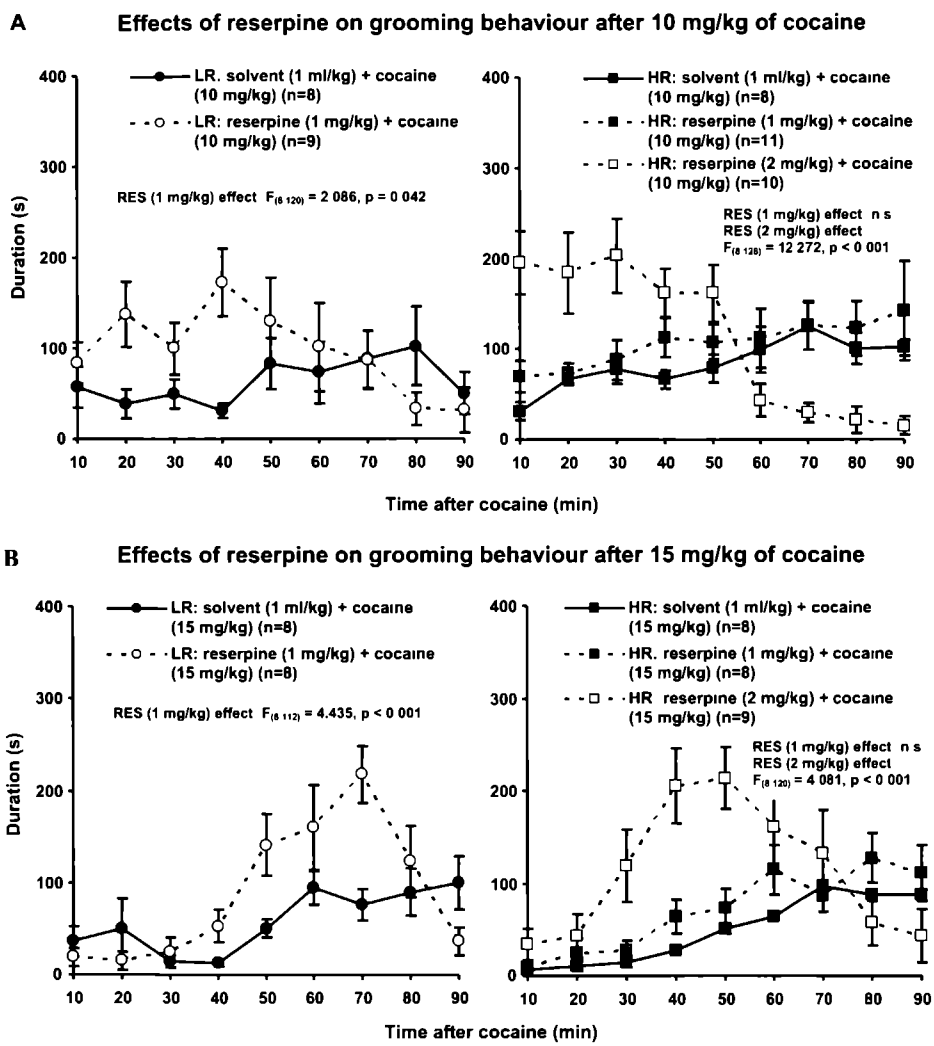


Figure 7: Effects of reserpine on the duration (s) of grooming behaviour elicited by 10 mg/kg (a) and 15 mg/kg (b) of cocaine in novelty-challenged LR (left) and novelty-challenged HR (right). Rats treated with reserpine-solvent and cocaine are represented by a filled line, rats treated with reserpine and cocaine are represented by a dotted line. The effective dose of reserpine (1 mg/kg in LR and 2 mg/kg in HR) is displayed in white symbols whereas the non-effective dose of reserpine (1 mg/kg in HR) is displayed in grey symbols. Data are expressed as mean ± SEM The results of an ANOVA are displayed LR = Low Responders to novelty, HR = High Responders to novelty

Discussion

The aim of this study was to examine whether the dose of RES required to change the behavioural response to COC is higher in HR than in LR (see introduction).

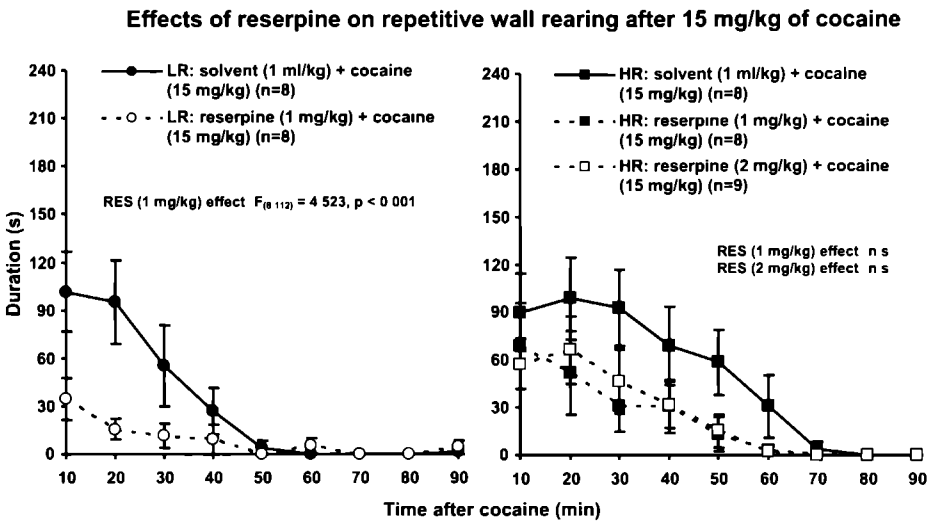


Figure 8: Effects of reserpine on the duration (s) of repetitive wall rearing elicited by 15 mg/kg of cocaine in novelty-challenged LR (left) and novelty-challenged HR (right). Rats treated with reserpine-solvent and cocaine are represented by a filled line, rats treated with reserpine and cocaine are represented by a dotted line Data are expressed as mean ± SEM The results of an ANOVA are displayed LR – Low Responders to novelty, HR – High Responders to novelty

Treatment effects of cocaine: Both doses of COC increased walking (Fig. 1), normal free rearing (Fig. 2) and repetitive wall rearing (Fig. 4). The effects of the two doses of COC on normal wall rearing (Fig. 2) and grooming (Fig. 3) were biphasic. These two behavioural items initially decreased and subsequently increased. Given that especially walking and repetitive wall rearing dominated behaviour immediately after the administration of COC, the execution of these behavioural items might initially have prevented normal wall rearing and grooming to take place (behavioural competition). Consequently, normal wall rearing and grooming appeared once walking and repetitive wall rearing started to decrease.

Type effects of cocaine: COC or its solvent (saline) resulted in individual differences across all behavioural items, apart from grooming LR treated with saline displayed less walking than HR treated with saline (Fig 1) whereas the duration of the remaining behavioural items did not differ between these control rats (Fig 2-4) The moderate dose of 10 mg/kg of COC increased walking, normal wall rearing and normal free rearing to a smaller degree in LR than in HR (Fig 1-2) Similar to saline, the dose of 15 mg/kg of COC resulted in individual differences in one behavioural item only The higher dose of COC increased repetitive wall rearing not as much in LR as in HR (Fig 4) These results in novelty-challenged HR and LR nicely fit in with the finding that COC more strongly increases general activity (photocell counts) in non-challenged HR than in non-challenged LR (Hooks et al , 1991a, Hooks et al , 1991b, Hooks et al , 1992a) Our data indicate that the previously reported individual differences in photocell counts following 10 mg/kg of COC (Hooks et al , 1991a, Hooks et al , 1991b) are due to individual differences in walking and normal rearing (Fig 1 and 2) whereas the previously reported individual differences in photocell counts following 15 mg/kg of COC (Hooks et al , 1991b, Hooks et al , 1992a) may be the result of individual differences in repetitive rearing (Fig 4)

The present findings confirm our previously reported notion (Cools et al , 1997) that the behavioural differences between LR and HR are particularly evident in case these rats are exposed to intermediate (pharmacological) challenges (injection of 10 mg/kg of COC) and become less when the challenge is either too small (saline injection) or too large (injection of 15 mg/kg of COC) This phenomenon may well be explained by the fact that the degree in which a particular neuronal substrate is involved in regulating behaviour depends on the dose of COC that is given In other words moderate doses of psychostimulants seem to affect those neuronal substrates that neurochemically differ between LR and HR In case the dose of COC is altered, these specific substrates may only slightly be affected or may be overruled by the action of COC on other substrates (see also below)

Effects of reserpine on cocaine-induced walking and rearing: The dose of 1 mg/kg of RES strongly reduced COC-induced walking, normal wall rearing and normal

free rearing in LR (Fig. 5-6), but had no effect on these behaviours in HR (Fig. 5-6). Only the higher dose of 2 mg/kg of RES was able to reduce COC-induced walking, normal wall rearing and normal free rearing in HR (Fig. 5-6). These data provide the first piece of evidence in favour of our hypothesis that higher doses of RES are required to change the behavioural response to COC in HR than in LR. In addition, the present study revealed that the low dose of RES strongly decreased COC-induced repetitive wall rearing in LR, whereas no dose of RES affected this behaviour in HR (Fig. 8).

The fact that RES, which depletes vesicular monoamines, decreased COC-induced walking, normal rearing and repetitive rearing suggests that these COC-induced behaviours are all mediated by an increased release of monoamines from storage vesicles. However, the present data indicate that COC-induced walking and normal rearing are mediated by a substrate that is different from the substrate involved in COC-induced repetitive rearing. First, 10 mg/kg of COC did not increase the duration of repetitive rearing in LR or HR (see results). In contrast, this dose of COC strongly increased the duration of walking and normal rearing in both types of rat (Fig. 1-2). Second, the dose of 15 mg/kg of COC resulted in individual differences in repetitive rearing (Fig. 4), but this dose of COC did not result in individual differences in walking and normal rearing (Fig. 1-2). Third, 2 mg/kg of RES did not alter COC-induced repetitive rearing in HR (Fig. 8). In contrast, this dose of RES strongly reduced COC-induced walking and normal rearing in these rats (Fig. 5-6).

Putative substrates for walking and rearing: Evidence demonstrating that the exploration response to psychostimulants is accompanied by an increase of dopamine in the nucleus accumbens (Kelly et al., 1975; Sharp et al., 1987; Delfs et al., 1990; Amalric and Koob, 1993) opens the perspective that walking and normal rearing following 10 mg/kg of COC is mediated by dopamine in the nucleus accumbens. Furthermore, it is known that LR are marked by a smaller novelty-induced as well as COC-induced increase of accumbal dopamine when compared with HR (Hooks et al., 1991b; Saigusa et al., 1999; Verheij and Cools, 2007; Verheij et al., 2008). Combining these data results in the notion that both novelty-induced walking after saline and walking/normal rearing following 10 mg/kg of COC increased less in LR than in HR (see section type effects of

cocaine), because the novelty-induced as well as the COC-induced increase of dopamine in the nucleus accumbens is smaller in LR than in HR

In contrast to normal rearing that was marked by a low frequency and a short total duration, repetitive rearing was marked by a high frequency and a long total duration (see results) Because stereotyped behaviour has been defined as behaviour that is continuously repeated and lasts for a long period of time (Ellenbroek and Cools, 1993), COC-induced repetitive wall rearing is considered to be stereotypic Evidence demonstrating that psychostimulant-induced stereotyped behaviour is accompanied by an increase of neostriatal dopamine (Kelly et al , 1975, Sharp et al , 1987, Delfs et al , 1990, Amalric and Koob, 1993) opens the perspective that the repetitive wall rearing following 15 mg/kg of COC is mediated by dopamine in the neostriatum Accordingly, large doses of COC may increase striatal dopamine to a smaller degree in LR than in HR

The dose of 15 mg/kg of COC increases accumbal dopamine levels less in LR than in HR (Verheij et al , 2008) The fact that this dose of COC did not result in individual differences in walking and normal rearing whereas it did result in individual differences in repetitive rearing, indicates that individual differences in striatal dopamine overrule individual differences in accumbal dopamine in terms of mediating behaviour after a large dose of COC (see also section type effects of cocaine)

Effects of reserpine on cocaine-induced grooming The dose of 1 mg/kg of RES strongly increased COC-induced grooming in LR, but had no effect on this behaviour in HR (Fig 7) Only the higher dose of 2 mg/kg of RES was able to increase COC-induced grooming in HR (Fig 7) These data provide the second piece of evidence in favour of our hypothesis that higher doses of RES are required to change the behavioural response to COC in HR than in LR

The fact that RES, which depletes vesicular monoamines, increases COC-induced grooming indicates that COC-induced grooming is accompanied by a decreased release of monoamines from storage vesicles In contrast, COC-induced walking, normal rearing and stereotyped rearing were all accompanied by a monoamine increase (see section effects of reserpine on cocaine-induced walking and rearing) These data result in

the suggestion that COC-induced grooming is mediated by a substrate that differs from the substrates involved in either COC-induced walking/normal rearing (nucleus accumbens) or COC-induced stereotyped rearing (neostriatum)

Putative substrate for grooming: Given that rats with a prefrontal cortex lesion display excessive grooming (Dunn et al , 1984, Tzschentke and Schmidt, 1998, Flores et al , 2005), grooming could well be mediated by a decrease of monoamines in the prefrontal cortex Because COC is known to (indirectly) reduce the amount of dopamine in particular subregions of the prefrontal cortex (Peterson et al , 1990, Pan et al , 1996, Hedou et al , 1999, van der Elst, 2006), we hypothesise that the grooming response following COC is mediated by a dopamine decrease in the prefrontal cortex Our suggestion that changes of dopamine in the prefrontal cortex, but not in the nucleus accumbens or neostriatum, give rise to grooming is supported by two sets of data First, rats marked by a strong grooming response to novelty (high grooming rats) display lower dopamine levels in the prefrontal cortex than rats marked by a weak grooming response to novelty (low grooming rats) (Homberg et al , 2002, Homberg et al , 2003) Second, dopamine levels in the nucleus accumbens or in the neostriatum do not differ between low and high grooming rats (Homberg et al , 2002, Homberg et al , 2003)

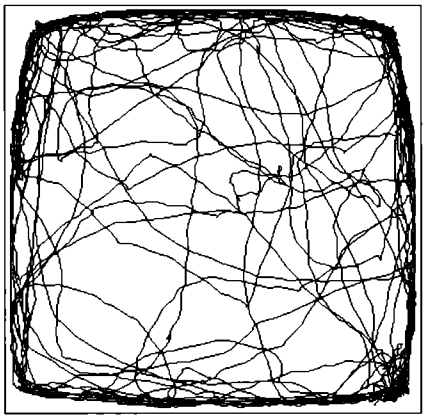
Conclusions

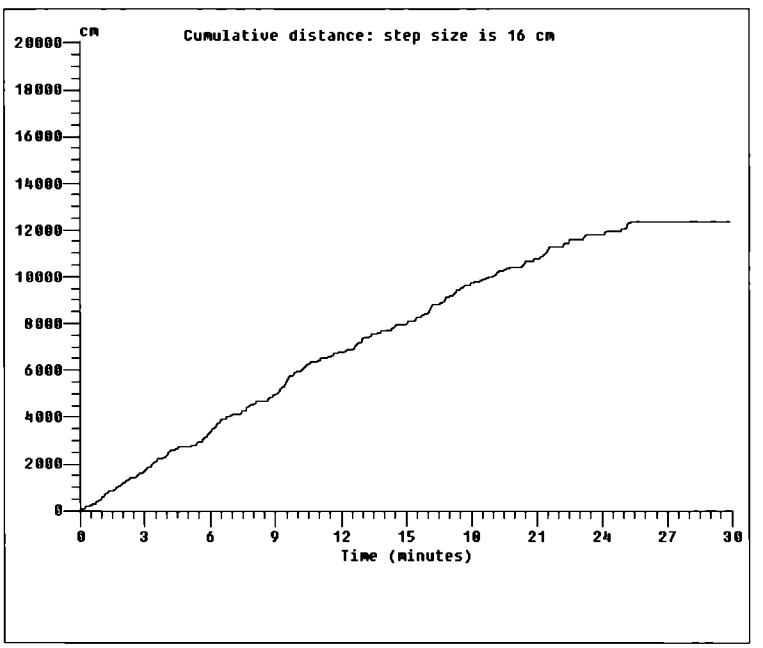
The present data demonstrate that higher doses of RES were required to affect COC-induced walking and normal wall and free rearing in HR than in LR This finding may well be explained by the previously reported finding that the nucleus accumbens of LR is marked by a smaller RES-sensitive monoaminergic storage pool than the nucleus accumbens of HR (Cools and Verheij, 2002, Verheij et al , 2008) Consequently, we hypothesise that COC increased walking and normal rearing less in LR than in HR, because COC can release less monoamines from accumbal storage vesicles in LR than in HR Because RES treatment not only resulted in individual differences in COC-induced walking/normal rearing, but also in COC-induced stereotyped rearing as well as in COC-induced grooming, we speculate that the individual differences in monoaminergic

storage capacity are not only limited to the dopaminergic nerve terminals of the nucleus accumbens, but may also be present in the neostriatum and the prefrontal cortex Although the present results can fully be explained by individual differences in dopaminergic storage capacity, it can not be excluded that individual differences in the storage capacity of noradrenaline and/or serotonin play a role as well

Impact

RES has been shown to strongly reduce the behavioural response to COC This opens the intriguing possibility that drugs that deplete monoaminergic storage vesicles might become the drugs of choice for the treatment of COC abuse Clinical trials in COC-addicted subjects have revealed both promising (Gorelick et al , 2004, Berger et al , 2005) and disappointing (Winhusen et al , 2007) results of RES In these studies one single dose of RES was tested The fact that no differences were found between RES and placebo treated cocaine users in the second study may be explained by our present finding that not all subject are sensitive to the same dose of RES High and low responders to novelty rats are generally referred to as an animal model for high and low sensation seeking in man (Dellu et al , 1996b, Cools and Ellenbroek, 2002, Ballaz et al , 2007a, Ballaz et al , 2007b) On the basis of our animal studies, it is hypothesised that COC-addicted individuals that are marked by high sensation seeking scores need higher doses of RES in order to elicit therapeutic effects compared to COC-addicted individual that are marked by low sensation seeking scores It is evident that the clinical safety of RES-like agents needs to be established as well





5/15: Analyseren van de open-veld data: high responder to novelty.
Analysis of the open-field data: high responder to novelty.

Chapter 5

Mesolimbic beta-, but not alpha-adrenoceptors control accumbal dopamine release from alpha-methyl-para-tyrosine-sensitive pools of newly-synthesised dopamine

M.M.M. Verheij and A.R. Cools

Submitted

Abstract

We have previously demonstrated that intra-accumbens infusion of both the beta-adrenoceptor-agonist isoproterenol and the alpha-adrenoceptor-agonist phenylephrine increase the release of accumbal dopamine. It has been shown that the isoproterenol-induced, but not the phenylephrine-induced, release of dopamine is derived from pools of newly-synthesised dopamine. The first aim of this study was to replicate the previous finding that isoproterenol releases dopamine from alpha-methyl-para-tyrosine-sensitive pools. In addition to the alpha-adrenoceptor-agonist phenylephrine, the alpha-adrenoceptor-antagonist phentolamine is known to increase accumbal dopamine release as well. The second aim of this study was to investigate whether the dopamine-increasing effects of phentolamine were, in addition to the dopamine-increasing effects of phenylephrine, resistant to alpha-methyl-para-tyrosine.

Rats were divided in low- and high-responders to novelty. The original finding that isoproterenol and phentolamine increase accumbal dopamine levels in both types of rat was replicated. Alpha-methyl-para-tyrosine reduced the isoproterenol-induced increase of accumbal dopamine in both low- and high-responders. Alpha-methyl-para-tyrosine did not reduce the accumbal dopamine increase after phentolamine in any type of rat. These results confirm that mesolimbic beta-, but not alpha-adrenoceptors control the release of accumbal dopamine from pools of newly-synthesised dopamine. The dopamine-increasing effects of phenylephrine have previously been ascribed to stimulation of presynaptic receptors whereas the dopamine-increasing effects of phentolamine have been ascribed to inhibition of postsynaptic receptors. Combining the present and previous data results in the notion that the presynaptic alpha-adrenoceptors of the nucleus accumbens are located on the terminals of those noradrenergic neurons that impinge on dopaminergic nerve terminals equipped with postsynaptic alpha-, but not beta-receptors.

Introduction

It has previously been demonstrated that the release of mesolimbic noradrenaline directs the release of mesolimbic dopamine (for review: Cools and Tuinstra, 2003). For instance, both the stimulation of the postsynaptic beta-adrenoceptors in the nucleus accumbens by means of the beta-adrenoceptor-agonist isoproterenol (ISO) and the inhibition of the postsynaptic alpha-adrenoceptors in the nucleus accumbens by means of the alpha-adrenoceptor-antagonist phentolamine (PA) have been found to facilitate accumbal dopamine release (Tuinstra and Cools, 2000a; Verheij and Cools, chapter 6). These postsynaptic receptors are suggested to be located on dopaminergic nerve terminals (Tuinstra and Cools, 2000a). In addition to postsynaptic adrenoceptors, the accumbens contains presynaptic adrenoceptors as well. The presynaptic adrenoceptors of the nucleus accumbens have been found to be alpha-, but not beta-receptors (Tuinstra and Cools, 2000a; Aono et al., 2007). These presynaptic receptors are suggested to be located on noradrenergic nerve terminals (Tuinstra and Cools, 2000a; Aono et al., 2007). Stimulation of the presynaptic alpha-adrenoceptors of the nucleus accumbens by means of the alpha-adrenoceptor-agonist phenylephrine (PE) has been reported to decrease accumbal noradrenaline release (Aono et al., 2007). This PE-induced inhibition of the release of noradrenaline is associated with an increase of the extracellular levels of accumbal dopamine (Tuinstra and Cools, 2000a; Tuinstra and Cools, 2000b; Verheij and Cools, 2009). There is evidence that these presynaptic alpha-adrenoceptors are located on those noradrenergic neurons that impinge on dopaminergic nerve terminals that are equipped with postsynaptic alpha-, but not beta-adrenoceptors (Tuinstra and Cools, 2000a; Tuinstra and Cools, 2000b).

It has previously been shown that the ISO-induced dopamine release is derived from pools of newly-synthesised dopamine (Tuinstra and Cools, 2000b). The first aim of the present study was to replicate the previous finding that ISO releases dopamine from alpha-methyl-para-tyrosine-sensitive pools. It has also been shown that the PE-induced dopamine release is not derived from pools of newly-synthesised dopamine (Tuinstra and Cools, 2000b). The second aim of this study was to investigate whether the

accumbal dopamine increasing effects of PA are, in addition to the accumbal dopamine increasing effects of PE, resistant to AMPT.

Subjects of the present study were low responders (LR) and high responders (HR) to novelty (Piazza et al., 1989; Dellu et al., 1996b; Bevins et al., 1997; Cools and Gingras, 1998; Cools and Tuinstra, 2003; Kabbaj, 2004). These rats were incorporated, because they are differentially sensitive to intra-accumbens administration of noradrenergic drugs. Although non-challenged LR are equally sensitive to ISO as non-challenged HR, the former are more sensitive to PA than the latter (Tuinstra and Cools, 2000a). Accordingly, LR and HR were pre-treated with AMPT before ISO and PA were locally infused into the nucleus accumbens. On the basis of the above-mentioned data, it was hypothesised that ISO equally increases dopamine in non-challenged HR and non-challenged LR, and that these dopamine-increasing effects are inhibited by AMPT. It was also hypothesised that PA stronger increases dopamine release in non-challenged LR than in non-challenged HR, and that these dopamine-increasing effects are not inhibited by AMPT.

Experimental procedures

Subjects: The AMPT-treated rats of the present study were also subject of a previous study. The AMPT-induced changes of accumbal dopamine release measured before the infusion of the adrenergic drugs have also been published elsewhere (Verheij and Cools, chapter 2). Adult male LR (n=51) and HR (n=51) of 180-220 g were selected from the outbred strain of Nijmegen Wistar rats (see section open-field selection). All rats were reared and housed in macrolon cages (42 x 26 x 15 cm; n=3-4 per cage) under a fixed 12/12 h light/dark cycle (lights on: 07.00 a.m.) in a temperature-controlled room (21 ± 1.7 °C). Water and food pellets (Ssniff, Soest, Germany) were available *ad libitum*, except during the testing periods. All experiments were performed in accordance with institutional, national and international guidelines for animal care and welfare.

Selection of LR and HR to novelty: Rats were individually housed 3 days before the open-field selection procedure (Tuinstra and Cools, 2000a; Tuinstra and

Cools, 2000b; Verheij and Cools, 2007). Testing took place between 09.00 h and 17.00 h in a room illuminated by white light of 170 Lux at the middle of the open-field. Rats were placed on a black square table (160 x 160 cm) for a period of 30 min. This open-field was 95 cm elevated above the floor and surrounded by a white neutral background (270 x 270 x 270 cm). As described by Cools et al. (1990), behaviour was recorded with a computerised tracking system. Both ambulation and habituation time were used to select LR and HR. Ambulation was defined as the overall distance (cm) travelled in 30 min. Habituation time was defined as the duration of the period (s) that started as soon as the rat began to explore the open-field and ended as soon as the locomotor activity stopped for at least 90 s. Rats that habituated in less than 480 s and walked less than 4,800 cm in 30 min were labelled LR, whereas rats that habituated after 840 s and walked more than 6,000 cm in 30 min were labelled HR (Tuinstra and Cools, 2000a; Tuinstra and Cools, 2000b; Verheij and Cools, 2007). Habituation time in addition to ambulation was used as selection criterion, because travelled distance *per se* is not always a reliable criterion (Cools et al., 1997; Saigusa et al., 1999). Rats that did not fulfil the criteria were excluded from this study. Efforts were made to use these rats in other studies (Verheij et al., 2007).

Surgery: One day after the open-field selection procedure took place, LR and HR were unilaterally implanted with a stainless steel guide cannula (length: 5.5 mm, outer diameter: 0.65 mm, inner diameter: 0.3 mm) directed to the right nucleus accumbens according to previously described procedures (Tuinstra and Cools, 2000a; Tuinstra and Cools, 2000b; Verheij and Cools, 2007). Rats were placed in a stereotactic apparatus and the following coordinates were used according to the atlas of Paxinos and Watson (1986): anterior: +10.6 mm (relative to the interaural line) and lateral: -1.5 mm (relative to the midline suture). The guide cannula was lowered 5.5 mm relative to the dura surface resulting in a vertical coordinate of +3.5 mm for the cannula tip. Finally, the cannula was angled 10° laterally to the right side. The rats were allowed to recover from surgery for the next 7 to 10 days in Plexiglas dialysis cages (25 x 25 x 35 cm) covered with sawdust on the floor. On 3 consecutive days just prior to the start of the microdialysis experiment, each rat was gently picked up in order to habituate to the

procedure assessed on the day when the concentration of accumbal dopamine was measured This handling procedure was repeated 3 times per day

Microdialysis: As previously described, a dialysis probe (type A-I-8-02, outer diameter 0 22 mm, 50,000-molecular-weight cut-off, Eicom, Tokyo, Japan) was carefully inserted into the brain of a conscious rat and secured to the guide cannula with a screw (Tuinstra and Cools, 2000a, Tuinstra and Cools, 2000b, Verheij and Cools, 2007) The tip of the dialysis probe protruded 2 mm below the distal end of the guide cannula into the nucleus accumbens The probes had an *in vitro* recovery of 10-12% for dopamine The inlet and outlet of the probe were connected to a swivel allowing the rat to move undisturbed Accumbal dialysates were analysed for dopamine (pg/40 µl) according to previously described procedures (Tuinstra and Cools, 2000a, Tuinstra and Cools, 2000b, Verheij and Cools, 2007) Briefly, the probe was perfused at a rate of 2 0 µl/min with modified Ringer solution (see section compounds) and the outflow was collected in a sample loop and injected, once every 20 min, into a high performance liquid chromatography (HPLC) system Dopamine was separated from the remaining neurotransmitters by means of reversed phase, ion-paring, liquid chromatography and the concentration was measured using electrochemical detection (ECD) The HPLC-ECD system was calibrated with a standard dopamine solution before and after each experiment

Effects of phentolamine and isoproterenol in rats treated with reserpine or alpha-methyl-para-tyrosine: At 4 h following probe insertion, the extracellular accumbal concentration of dopamine is known to reach a stable baseline ± 10% (Saigusa et al , 1999, Tuinstra and Cools, 2000a, Tuinstra and Cools, 2000b, De Leonibus et al , 2006, Verheij and Cools, 2007, Verheij et al , 2008) As soon as a stable baseline concentration of dopamine was reached, rats were treated with AMPT according to previously described procedures (Saigusa et al , 1999) In short, 0 1 mM of AMPT or its solvent were locally infused into the nucleus accumbens (40 min 2 µl/min) where after the rats were immediately exposed to novelty (Saigusa et al , 1999) The novel cage was slightly larger than the home cage (new dimensions 30 x 30 x 35 cm) and lacked saw-

dust on the floor (Saigusa et al., 1999). At 100 min after AMPT or its solvent (see also section compounds), rats were subjected to a 40-min-lasting intra-accumbens infusion of 0.001 mM of the beta-adrenoceptor-agonist ISO (solvent AMPT: LR: n=8, HR: n=8; AMPT: LR: n=8, HR: n=8) or 0.01 mM (2 µl/min) of the alpha-adrenoceptor-antagonist PA (solvent AMPT: LR: n=8, HR: n=8; AMPT: LR: n=9, HR: n=10). Infusion of modified Ringer solution (2 µl/min, 40 min) served as control for PA and ISO treatment (solvent AMPT: LR: n=9, HR: n=9, AMPT: LR: n=8, HR: n=8).

The adrenergic drugs were administered 100 min after AMPT, because at this time the rats were found to be at rest (see results). Moreover, AMPT reduced the levels of dopamine at t = 100 min (see results) whereas the amount of noradrenaline has previously been found to be returned to baseline levels at this time (Verheij and Cools, 2009). The dose of PA and ISO were chosen because these doses have been shown to increase extracellular levels of dopamine in the nucleus accumbens (Tuinstra and Cools, 2000a; Tuinstra and Cools, 2000b; Verheij and Cools, chapter 6).

Histology: At the end of each experiment the rat was deeply anaesthetised with an overdose of sodium-pentobarbital (60 mg, i.p.) and intracranially perfused with 60 ml 4% paraformaldehyde solution. Vibratome sections (100 µm, Leica VT1000F; Leica, Rijswijk, The Netherlands) were cut to determine the exact location of the microdialysis probe.

Compounds: The following compounds and solutions were used (Tuinstra and Cools, 2000a; Tuinstra and Cools, 2000b; Verheij and Cools, chapter 6): 1) Isoproterenol-hydrochloride and phentolamine-hydrochloride (Sigma, St Louis, USA), 2) dl-Alpha-methyl-para-tyrosine-hydrochloride (Axel Kistner AB Fack, Göteborg, Sweden), 3) Modified Ringer solution: 147 mM NaCl, 4 mM KCl, 1.1 mM $CaCl_2 2H_2O$ and 1.1 mM $MgCl_2 6H_2O$ were dissolved in ultra pure water (pH 6.0). The Ringer solution served as solvent for all compounds.

Statistical analysis: The drug-induced changes of accumbal dopamine were expressed as a percentage of the concentration of dopamine that was measured in the 20-

min-lasting period just before these drugs were infused. All data are expressed as the mean ± SEM. A three-way ANOVA with the factors rat type, treatment and time (for repeated measures) was assessed, followed by a post-hoc Student's t-test were appropriate. A probability level of $p<0.05$ was taken as significant. SPSS for Windows (Release 12) was used to statistically analyse the data.

Results

Open-field selection procedure: The open-field selection procedure revealed 25% LR and 26% HR. The average distance travelled in 30 min (± SEM) was 3,425 ± 123.6 cm and 8,273 ± 233.0 cm in LR and HR, respectively. The average habituation time (± SEM) was 353 ± 20.8 s in LR and 1,322 ± 58.2 s in HR.

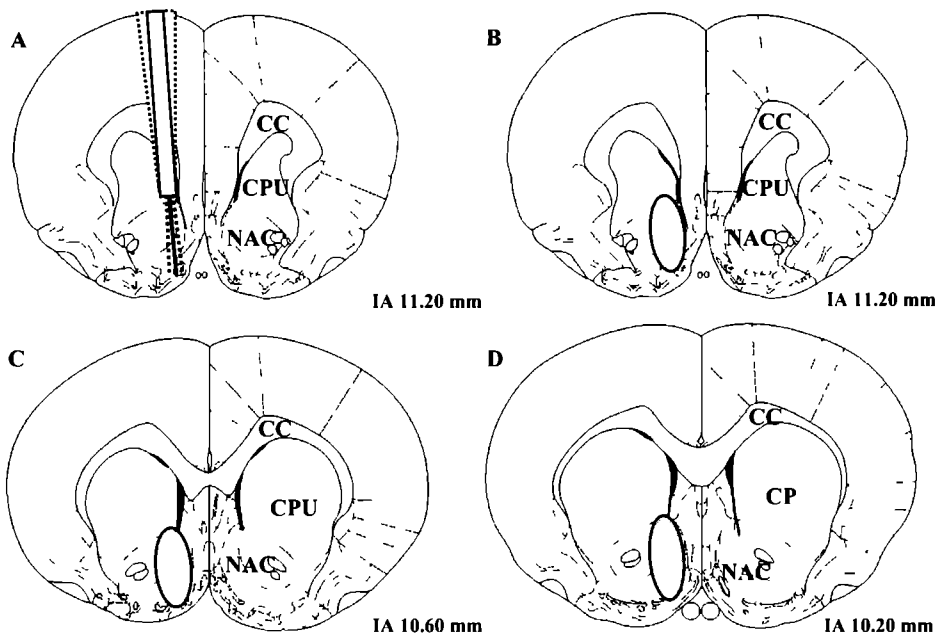


Figure 1. A: Example of 3 unilateral microdialysis probe tracks located in the right nucleus accumbens. The probe protrudes 2 mm below the distal end of the guide cannula **B-D: Schematic illustration of coronal brain sections containing the nucleus accumbens.** The brain region in which correctly placed probes were found is indicated as a grey oval IA corresponds to the distance (mm) from the interaural line according to Paxinos and Watson (1986), NAC=Nucleus Accumbens, CPU=caudate putamen, CC=corpus callosum

Histology: Histological verification revealed that 1 LR that was treated with the solvent of AMPT and PA was excluded from analysis because of incorrect placement of the microdialysis probe. The coronal region of the nucleus accumbens in which all correctly placed microdialysis probe tracks were located is shown in Figure 1 (see also: Verheij and Cools, 2007).

Basal levels of dopamine: Baseline extracellular levels of accumbal dopamine were 3.8 ± 0.15 pg/sample in LR and 4.40 pg ± 0.31 pg/sample in HR (rat type effect: $F_{(1,99)} = 3.219$, $p = 0.076$).

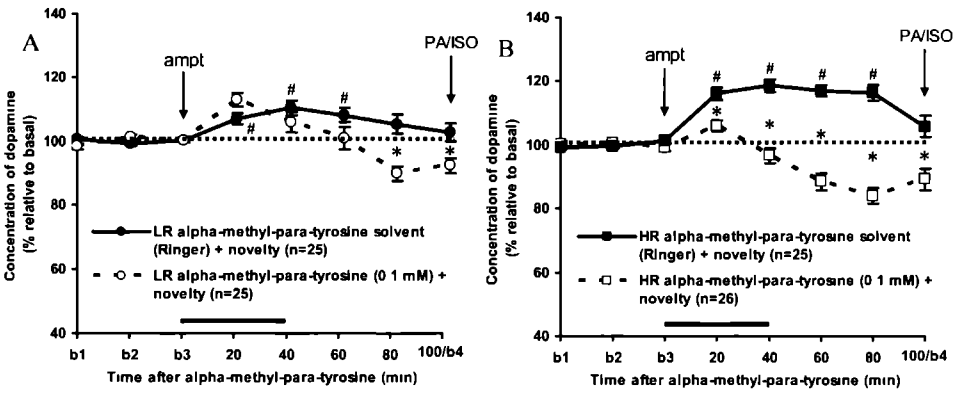


Figure 2: Effects of intra-accumbens infusion of alpha-methyl-para-tyrosine (ampt: 0.1 mM, 2 µl/min, 40 min) on the dopamine levels in novelty-challenged low responders (LR: Fig. A) and novelty-challenged high responders (HR: Fig. B). Values are accumbal dopamine levels expressed as percentage of the average of the 3 baseline samples collected before alpha-methyl-para-tyrosine was infused (basal samples b1-b3). Both phentolamine (PA) and isoproterenol (ISO) were infused 100 min after alpha-methyl-para-tyrosine was given. The point t = 100 min was labelled basal sample b4. Data are expressed as mean percentage ± SEM. The solid horizontal line represents the infusion time of alpha-methyl-para-tyrosine. # Significant dopamine increase after novelty (one sample t-test). * Significant dopamine decrease after alpha-methyl-para-tyrosine (student's t-test).

Effects of alpha-methyl-para-tyrosine: As previously reported (Saigusa et al., 1999; Verheij and Cools, 2007; Verheij et al., 2008), accumbal dopamine levels increased less in novelty-challenged LR than in novelty-challenged HR (Fig. 2: type x time effect: $F_{(7,336)} = 4.304$, $p < 0.001$). The novelty-induced increase of dopamine lasted

60 min in LR (Fig. 2A: one sample t-test: t = 20-60 min: $p < 0.05$ and t = 80-100 min: ns) and 80 min in HR (Fig. 2B: one sample t-test: t = 20-80 min: $p < 0.05$ and t = 100 min: ns). AMPT strongly decreased the extracellular levels of accumbal dopamine in these rats (Fig. 2: treatment x time effect (t = 0-100 min): $F_{(7,679)} = 33.437$, $p < 0.001$, type x treatment x time effect (t = 0-100 min): $F_{(7,679)} = 6.705$, $p < 0.001$, see also: Verheij and Cools, chapter 2).

It has to be mentioned that AMPT did not reduce the increase of accumbal dopamine in the 60-min-lasting-period that dopamine increased in novelty-challenged LR (Fig. 2A: Student's t-test: t (20-60 min): ns, t (80-100 min): $p < 0.05$) whereas it did reduce the dopamine increase during the 80-min-lasting-period that dopamine increased in novelty-challenged HR (Fig. 2B: Student's t-test: t (20-100 min): $p < 0.05$). The present findings confirm our previous findings that the relative large novelty-induced increase of accumbal dopamine in HR is derived from AMPT-sensitive pools whereas the relative small novelty-induced increase of accumbal dopamine in LR is derived from AMPT-resistant pools (Saigusa et al., 1999; Verheij and Cools, 2007; Verheij and Cools, chapter 2).

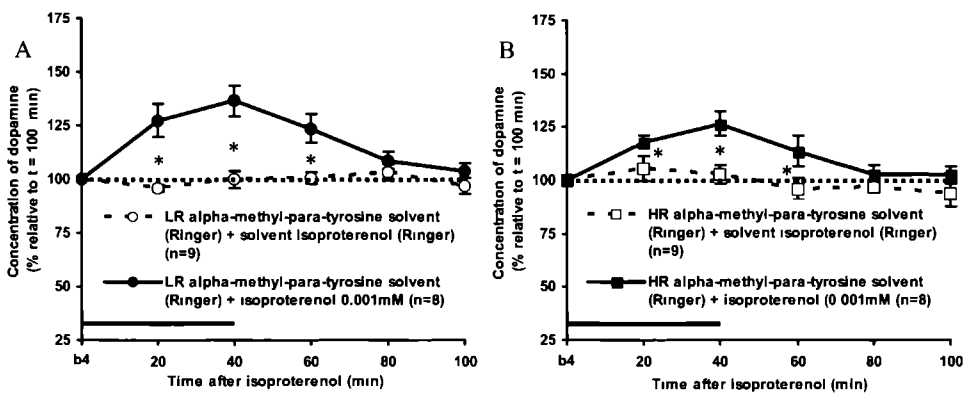


Figure 3. Effects of the intra-accumbens infusion of the beta-adrenoceptor-agonist isoproterenol (0.001 mM, 2 µl/min, 40 min) on the extracellular concentration of dopamine in the nucleus accumbens of low responders (LR: Fig. A) and high responders (HR: Fig. B) under non-novelty-challenged conditions. Rats were treated with the solvent op alpha-methyl-para-tyrosine. Values are accumbal dopamine levels expressed as percentage of the sample collected just before phentolamine was infused (basal sample b4 of Fig 2) Data are expressed as mean percentage ± SEM The solid horizontal line represents the infusion time of phentolamine * Significant dopamine increase after isoproterenol (student's t- test)

Rats are at rest during the infusion of isoproterenol and phentolamine: As mentioned above, the dopamine levels of the control rats did not anymore differ from the baseline levels of dopamine at t = 100 min after novelty (Fig. 2: one sample t-test at t = 100 min: LR: ns, HR: ns). In addition, the accumbal dopamine levels of these rats did not change over time during the period after t = 100 min (Fig. 3 and 5: time effect: LR: ns, HR: ns). These data demonstrate that both LR and HR were not anymore challenged (at rest) at the time of the infusion of the adrenergic drugs.

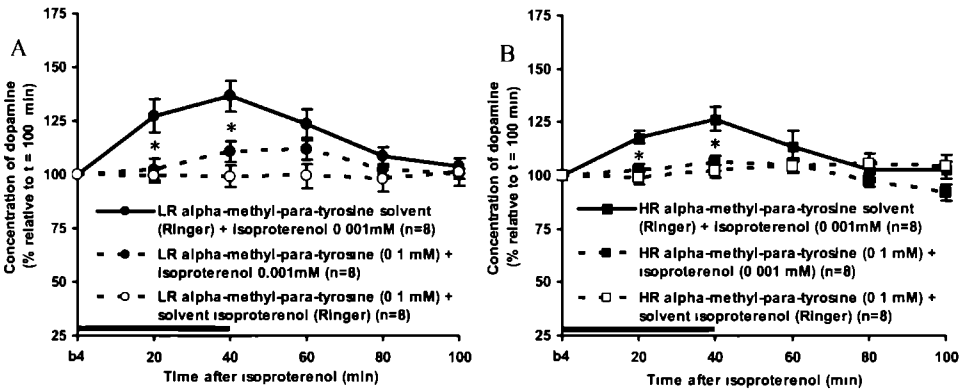


Figure 4. Effects of alpha-methyl-para-tyrosine (0.1 mM, 2 µl/min, 40 min) on the isoproterenol-induced increase of the extracellular concentration of dopamine in the nucleus accumbens of non-challenged low responders (LR: Fig. A) and non-challenged high responders (HR: Fig. B). Values are accumbal dopamine levels expressed as percentage of the sample collected just before isoproterenol was infused (basal sample b4 of Fig 2) Data are expressed as mean percentage ± SEM The solid horizontal line represents the infusion time of isoproterenol * Alpha-methyl-para-tyrosine-induced reduction of the isoproterenol-induced dopamine increase (student's t- test)

Effects of isoproterenol in alpha-methyl-para-tyrosine-treated rats: The dose of 0.001 mM of the beta-adrenoceptor-agonist ISO equally increased accumbal dopamine levels in non-challenged LR and non-challenged HR that were treated with the solvent of AMPT (Fig. 3: treatment x time effect: $F_{(5\ 150)} = 12.477$, $p < 0.001$; type x treatment (x time) effect: ns). AMPT equally reduced this ISO-induced dopamine increase in both types of rat (Fig. 4: treatment x time effect: $F_{(5\ 140)} = 9.045$, $p < 0.001$; type x treatment (x time) effect: ns). ISO did not anymore increase accumbal dopamine

levels in either AMPT-treated LR or AMPT-treated HR (Fig. 4: treatment (x time) effect: ns).

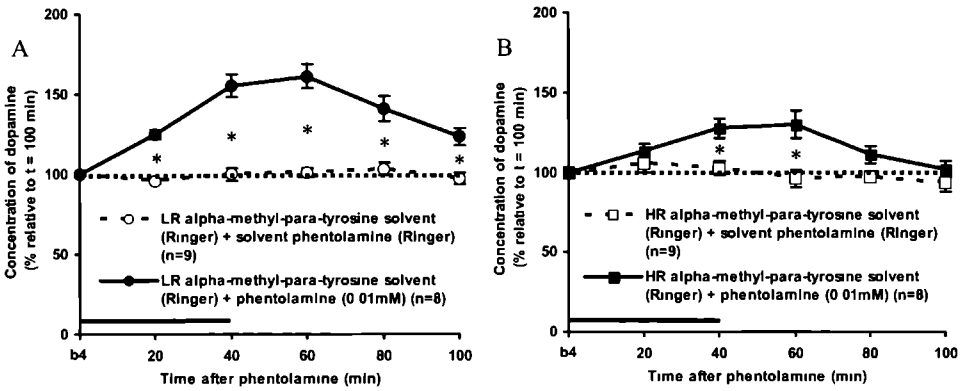


Figure 5. Effects of the intra-accumbens infusion of the alpha-adrenoceptor-antagonist phentolamine (0.01 mM, 2 µl/min, 40 min) on the extracellular concentration of dopamine in the nucleus accumbens of low responders (LR: Fig. A) and high responders (HR: Fig. B) under non-novelty-challenged conditions. Rats were treated with the solvent op alpha-methyl-para-tyrosine Values are accumbal dopamine levels expressed as percentage of the sample collected just before phentolamine was infused (basal sample b4 of Fig 2) Data are expressed as mean percentage ± SEM The solid horizontal line represents the infusion time of phentolamine * Significant dopamine increase after phentolamine (student's t- test)

Effects of phentolamine in alpha-methyl-para-tyrosine-treated rats: The dose of 0.01 mM of the alpha-adrenoceptor-antagonist PA increased accumbal dopamine levels in both LR and HR that were treated with the solvent of AMPT (Fig. 5). The PA-induced increase of accumbal dopamine was larger in non-challenged LR than in non-challenged HR (Fig. 5: type x treatment x time effect: $F_{(5,150)} = 8.911$, $p < 0.006$; LR (Fig. 5A): treatment x time effect: $F_{(5,75)} = 21.436$, $p < 0.001$; HR (Fig. 5B): treatment x time effect: $F_{(5,75)} = 4.929$, $p = 0.001$). As in solvent treated rats, PA increased accumbal dopamine levels more strongly in AMPT-treated LR than in AMPT-treated HR (Fig. 6: type x treatment x time effect: $F_{(5,155)} = 3.474$, $p = 0.005$; LR (Fig. 6A): treatment x time effect: $F_{(5,75)} = 20.643$, $p < 0.001$; HR (Fig. 6B): treatment x time effect: $F_{(5,80)} = 4.842$, $p = 0.001$). In fact, the dopamine-increasing effects of PA in AMPT-treated rats was not different from the dopamine-increasing effects of PA in rats that were treated with its solvent (Fig. 6: treatment (x time) effect: ns).

Discussion

We have previously demonstrated that both the postsynaptic acting beta-adrenoceptor-agonist ISO and the presynaptic acting alpha-adrenoceptor-agonist PE increase accumbal dopamine release. It has been shown that the ISO-induced, but not PE-induced, release of accumbal dopamine is derived from pools of newly-synthesised dopamine (Tuinstra and Cools, 2000b). The first aim of the present study was to replicate our previous finding that ISO releases dopamine from AMPT-sensitive pools. In addition to the presynaptic acting alpha-adrenoceptor-agonist PE, the postsynaptic acting alpha-adrenoceptor-antagonist PA mediates accumbal dopamine release as well. The second aim of this study was to investigate whether the dopamine increasing effects of PA were, in addition to the dopamine increasing effects of PE, resistant to AMPT. The experiments showed that ISO releases dopamine from pools of newly-synthesised dopamine whereas PA does not.

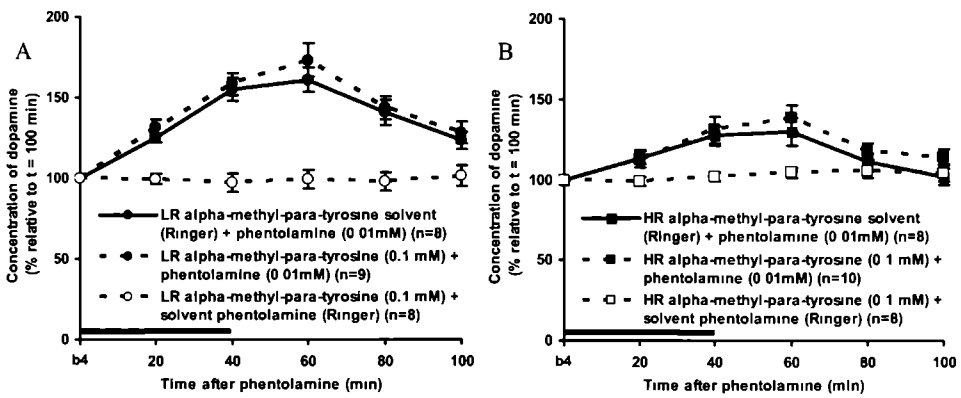


Figure 6. Effects of alpha-methyl-para-tyrosine (0.1 mM, 2 µl/min, 40 min) on the phentolamine-induced increase of the extracellular concentration of dopamine in the nucleus accumbens of the non-challenged low responders (LR: Fig. A) and non-challenged high responders (HR: Fig. B). Values are accumbal dopamine levels expressed as percentage of the sample collected just before phentolamine was infused (basal sample b4 of Fig 2) Data are expressed as mean percentage ± SEM The solid horizontal line represents the infusion time of phentolamine No alpha-methyl-para-tyrosine-induced reduction of the phentolamine-induced dopamine increase (student's t- test)

Dual role of noradrenaline in mediating dopamine release: The present study shows that intra-accumbens infusion of both the beta-agonist ISO and the alpha-antagonist PA increased accumbal dopamine levels in rats that were treated with the solvent of AMPT (Fig. 3 and 5). These data confirm the outcome of our previous studies that stimulation of postsynaptic beta-adrenoceptors facilitates accumbal dopamine release, and that inhibition of postsynaptic alpha-adrenoceptors inhibits the release of accumbal dopamine (Tuinstra and Cools, 2000a; Verheij and Cools, chapter 6). It has been demonstrated that the dopamine increasing effects after the intra-accumbens administration of beta- and alpha-adrenergic agents is dose-dependent and receptor-specific (Tuinstra and Cools, 2000a). The present study also confirmed the previous reported findings that the effects of ISO do not differ between non-challenged LR and non-challenged HR (Tuinstra and Cools, 2000a), whereas the effects of PA are greater in the former rats than in the latter rats (Tuinstra and Cools, 2000a). These results have previously been ascribed to individual differences in the adrenergic activity at the postsynaptic alpha-adrenoceptors, but not at the postsynaptic beta-adrenoceptors of the nucleus accumbens of non-challenged LR and non-challenged HR (for details: Tuinstra and Cools, 2000a).

Effects of alpha-methyl-para-tyrosine on the dopamine release after isoproterenol or phentolamine: The finding that AMPT completely prevented the ISO-induced increased accumbal dopamine levels in both LR (Fig. 4A) and HR (Fig. 4B) confirms our previous finding that ISO increases dopamine release from AMPT-sensitive pools (Tuinstra and Cools, 2000b). Apparently, the dose of 0.1 mM of AMPT completely depleted the accumbal pools of newly-synthesised dopamine in these rats.

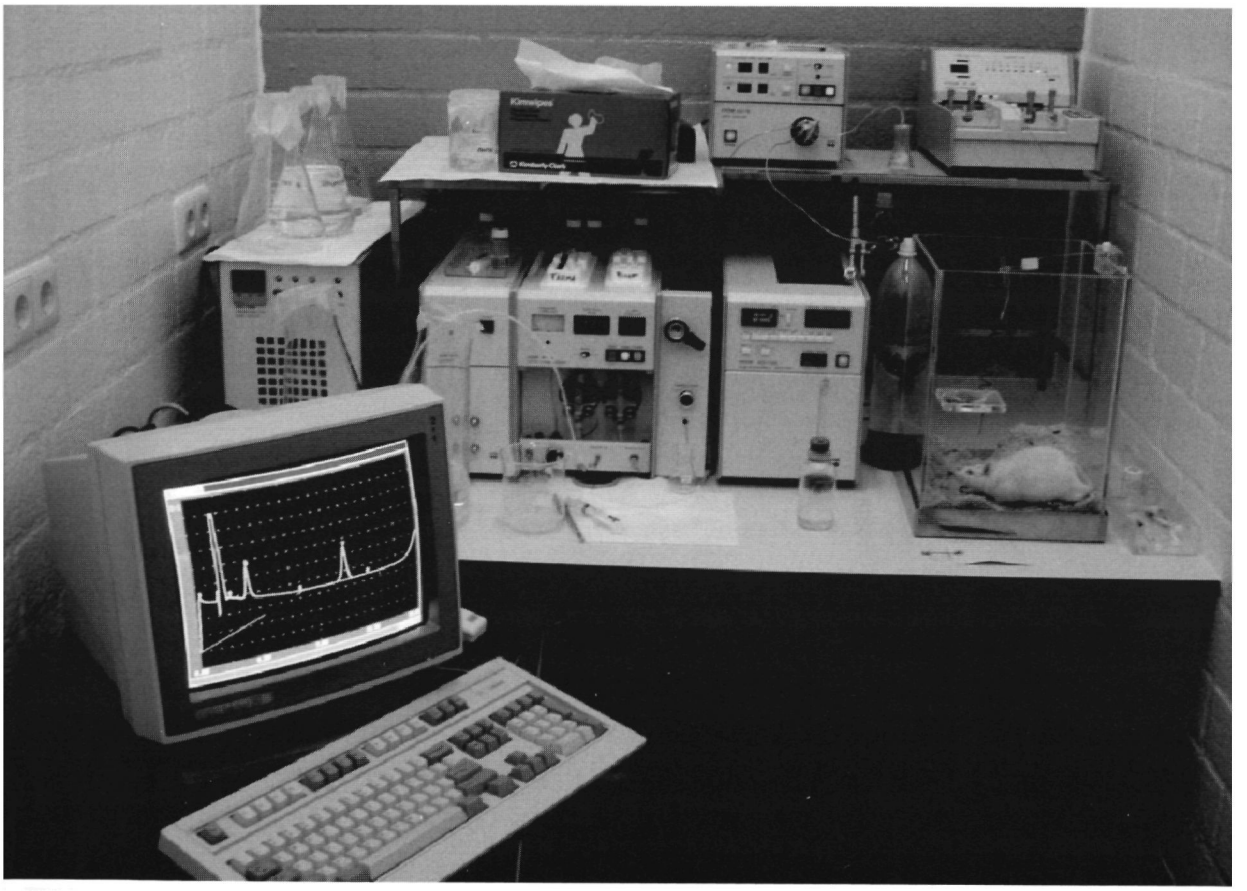
PA increased accumbal dopamine levels in both AMPT-treated LR (Fig. 6A) and AMPT-treated HR (Fig. 6B), despite of the fact that the AMPT-sensitive pools of newly-synthesised dopamine were completely empty in these rats. In fact, the PA-induced dopamine release in AMPT-treated rats did not differ from the PA-induced dopamine release in rats treated with its solvent (Fig. 6). These data demonstrate that postsynaptic alpha-adrenoceptors do not control dopamine release from AMPT-sensitive pools. We have recently provided evidence that the postsynaptic alpha-adrenoceptor

mediate the release of accumbal dopamine that is derived from AMPT-resistant, RES-sensitive storage pools (Verheij and Cools, chapter 6).

Conclusions and impact

The present finding that ISO increased dopamine release from AMPT-sensitive pools confirms our previously reported finding that postsynaptic beta adrenoceptors mediate (stimulate) accumbal dopamine release from pools of newly-synthesised dopamine (Tuinstra and Cools, 2000b). The finding that PA increase accumbal dopamine release that was not derived from AMPT-sensitive pools demonstrates that postsynaptic alpha-adrenoceptors do not control dopamine release from pools of newly-synthesised dopamine. The fact that the dopamine release that is mediated by post-synaptic beta-adrenoceptors is sensitive to AMPT whereas the dopamine release that is mediated by pre-synaptic alpha-adrenoceptors is not, indicates that the presynaptic alpha-adrenoceptors of the nucleus accumbens are not located on the terminals of adrenergic neurons that impinge upon accumbal neurons equipped with postsynaptic beta-receptors. The finding that both the dopamine release that is mediated by presynaptic alpha-adrenoceptors and the dopamine release that is mediated by postsynaptic alpha-adrenoceptors are insensitive to AMPT indicates that presynaptic alpha-adrenoceptors in the nucleus accumbens are located on the terminals of those adrenergic neurons that impinge upon accumbal neurons equipped with post-synaptic alpha-receptors.

The present study underlines our notion that mesolimbic noradrenaline fulfils many functions that are, up to now, primarily ascribed to mesolimbic dopamine (Cools and Tuinstra, 2003). This insight opens interesting perspectives for the development of a new class of therapeutic drugs. Drugs that interact with mesolimbic beta- and alpha-adrenoceptors may, in addition to drugs that affect newly-synthesised and storage pools, have therapeutic effects that are usually ascribed to drugs that interact with mesolimbic dopaminergic receptors. The therapeutic effects of these noradrenergic and dopaminergic agents are elaborated elsewhere in detail (noradrenaline: Aono et al., 2007; Ikeda et al., 2007, dopamine: Verheij and Cools, 2007; Verheij et al., 2008).



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Collecting dopamine samples (microdialysis system 1/2).

Chapter 6

Mesolimbic alpha-, but not beta-adrenoceptors control accumbal dopamine release from reserpine-sensitive storage vesicles

M.M.M. Verheij and A.R. Cools

In revision.

Abstract

It has previously been shown that mesolimbic beta-, but not alpha-adrenoceptors control the release of accumbal dopamine from alpha-methyl-para-tyrosine-sensitive pools of newly-synthesised dopamine. The aim of this study was to investigate which of these adrenoceptors control the release of accumbal dopamine from reserpine-sensitive, storage pools. Rats, that were divided in low-responders and high-responders to novelty, were pretreated with 1 mg/kg of reserpine before the alpha-adrenergic-agent phentolamine or the beta-adrenergic-agent isoproterenol was locally applied into the nucleus accumbens. The original finding that phentolamine and isoproterenol increased accumbal dopamine levels in low-responders and high-responders was replicated. Reserpine reduced the phentolamine-induced increase of accumbal dopamine in both types of rat. However, phentolamine could still increase accumbal dopamine levels in reserpine-treated high-responders, but not anymore in reserpine-treated low-responders. Reserpine did not reduce the isoproterenol-induced increase of accumbal dopamine in any type of rat.

This study provides the original evidence that mesolimbic alpha-, but not beta-adrenoceptors control accumbal dopamine release from reserpine-sensitive storage vesicles. Moreover, these data confirm our previously reported finding that 1 mg/kg of reserpine results in empty storage pools in the nucleus accumbens of low-responders, but not of high-responders. The present study also suggests that the accumbal noradrenaline that contributes to the beta-adrenoceptor-mediated release of dopamine from alpha-methyl-para-tyrosine-sensitive pools of newly-synthesised dopamine, is derived from reserpine-sensitive storage vesicles. The collected data underline our previously reported notion that alpha- and beta-adrenergic drugs, in addition to reserpine and alpha-methyl-para-tyrosine, may have therapeutic effects in patients suffering from diseases in which accumbal dopamine is involved.

Introduction

There is an important relationship between the noradrenergic and dopaminergic systems of the striatum (Antelman and Caggiula, 1977; Cools et al., 1991; Ikeda et al., 2007). Both noradrenergic (Berridge et al., 1997; Delfs et al., 1998) and dopaminergic fibres (Swanson, 1982; Oades and Halliday, 1987) are known to innervate the nucleus accumbens. *In vitro* studies have revealed that both accumbal alpha-adrenoceptors and accumbal beta-adrenoceptors control the release of accumbal dopamine (Nurse et al., 1984; Nurse et al., 1985; Russell et al., 1988; Russell et al., 1989; Russell et al., 1993). We have recently investigated whether these adrenoceptors control the release of accumbal dopamine *in vivo* as well. These studies have revealed that intra-accumbens infusion of either the beta-adrenergic agonist isoproterenol (ISO) or the alpha-adrenergic antagonist phentolamine (PA) increases dopamine release within the nucleus accumbens (Tuinstra and Cools, 2000a; Cools and Tuinstra, 2003; Verheij and Cools, chapter 5). Given the finding that the dopamine-increasing effects after the intra-accumbens administration of beta- and alpha-adrenergic agents are dose-dependent and receptor-specific, it has been concluded that stimulation of mesolimbic beta-adrenoceptors facilitates accumbal dopamine release in contrast to stimulation of mesolimbic alpha-adrenoceptors that inhibits accumbal dopamine release (Tuinstra and Cools, 2000a; Cools and Tuinstra, 2003; Verheij and Cools, chapter 5).

Dopaminergic nerve terminals contain two types of pool from which dopamine can be released. Alpha-methyl-para-tyrosine (AMPT) and reserpine (RES) are frequently used to discriminate these pools (Cools and Van Rossum, 1980; Leviel et al., 1989; Arbuthnott et al., 1990; Yavich and MacDonald, 2000). AMPT, which inhibits tyrosine hydroxylase (Corrodi and Hanson, 1966), only affects RES-resistant pools (Schoemaker and Nickolson, 1983; Parker and Cubeddu, 1986) in which dopamine is continuously synthesised (Ewing et al., 1983; Leviel et al., 1989; Arbuthnott et al., 1990; Yavich, 1996). As a result of a diminished release from these pools of newly-synthesised dopamine, the extracellular concentration of dopamine in the nucleus accumbens is significantly reduced after AMPT treatment (Tuinstra and Cools, 2000b; Verheij and Cools, chapter 5). RES, which depletes dopaminergic vesicles (Dahlstrom et al., 1965;

Margotta et al , 1997, Colliver et al , 2000), only affects AMPT-resistant pools (Ewing et al , 1983, Yavich, 1996) in which dopamine is stored (Schoemaker and Nickolson, 1983, Leviel et al , 1989, Arbuthnott et al , 1990, Yavich and MacDonald, 2000) As a result of a diminished release from these storage pools, the extracellular concentration of dopamine in the nucleus accumbens is significantly reduced after RES treatment (Verheij and Cools, 2007, Verheij et al , 2008) Apart from a direct dopamine release from both types of pool (Verheij and Cools, chapter 2), a redistribution of dopamine from vesicles to the cytosol, and *vice versa*, may also occur (Schoemaker and Nickolson, 1983, Justice et al , 1988, Leviel et al , 1989, Arbuthnott et al , 1990)

Recent studies have revealed that the accumbal dopamine release that is derived from AMPT-sensitive pools in the nucleus accumbens is controlled by beta-, but not alpha-adrenoceptors (Tuinstra and Cools, 2000b, Verheij and Cools, chapter 5) The aim of the present study was to investigate to what extent these adrenoceptors control accumbal dopamine release that is derived from RES-sensitive pools On the basis of previously reported pharmacological (Cools et al , 1991) and neurochemical (Saigusa et al , 1999) data, Cools and Saigusa have hypothesised that mesolimbic alpha-, but not beta-adrenoceptors control the dopamine release from storage vesicles To test whether alpha- but not beta-adrenoceptors indeed control the release of dopamine from storage vesicles, rats were pretreated with RES before the accumbal dopamine response to PA or ISO was established

Subjects of the present study were low responders (LR) and high responders (HR) to novelty (Piazza et al , 1989, Dellu et al , 1996b, Bevins et al , 1997, Cools and Gingras, 1998, Cools and Tuinstra, 2003, Kabbaj, 2004) These rats were incorporated because they are known to differ in their sensitivity to RES (Verheij and Cools, 2007, Verheij et al , 2008) Previous studies have demonstrated that 1 mg/kg of RES completely depletes the dopaminergic storage pools of the nucleus accumbens of LR (Verheij et al , 2008), but only partially these pools of the nucleus accumbens of HR (Verheij et al , 2008) On the basis of these data, we hypothesised that this dose of RES fully blocks the PA-induced increase of dopamine in LR, but only partially in HR

Experimental procedures

Subjects: The RES-treated rats of the present study were also subject of a previous study. The RES-induced changes of accumbal dopamine release measured before the infusion of the adrenergic drugs have also been published elsewhere (Verheij and Cools, 2007). Adult male LR (n=54) and HR (n=54) of 180-220 g were selected from the outbred strain of Nijmegen Wistar rats (see section open-field selection). All rats were reared and housed in macrolon cages (42 x 26 x 15 cm; n=3-4 per cage) under a fixed 12/12 h light/dark cycle (lights on: 07.00 a.m.) in a temperature-controlled room (21 ± 1.7 °C). Water and food pellets (Ssniff, Soest, Germany) were available *ad libitum*, except during the testing periods. All experiments were performed in accordance with institutional, national and international guidelines for animal care and welfare.

Open-field selection: Rats were individually housed 3 days before the open-field selection procedure (Tuinstra and Cools, 2000a; Tuinstra and Cools, 2000b; Verheij and Cools, 2007; Verheij and Cools, chapter 5). Testing took place between 09.00 h and 17.00 h in a room illuminated by white light of 170 Lux at the middle of the open-field. Rats were placed on a black square table (160 x 160 cm) for a period of 30 min. This open-field was 95 cm elevated above the floor and surrounded by a white neutral background (270 x 270 x 270 cm). As described by Cools et al. (1990), behaviour was recorded with a computerised tracking system. Both ambulation and habituation time were used to select LR and HR. Ambulation was defined as the overall distance (cm) travelled in 30 min. Habituation time was defined as the duration of the period (s) that started as soon as the rat began to explore the open-field and ended as soon as the locomotor activity stopped for at least 90 s. Rats that habituated in less than 480 s and walked less than 4,800 cm in 30 min were labelled LR, whereas rats that habituated after 840 s and walked more than 6,000 cm in 30 min were labelled HR (Tuinstra and Cools, 2000a; Tuinstra and Cools, 2000b; Verheij and Cools, 2007; Verheij and Cools, chapter 5). Habituation time in addition to ambulation was used as selection criterion, because travelled distance *per se* is not always a reliable criterion (Cools et al., 1997; Saigusa et

al , 1999) Rats that did not fulfil the criteria were excluded from this study Efforts were made to use these rats in other studies (Verheij et al , 2007)

Surgery: One day after the open-field selection procedure took place, LR and HR were unilaterally implanted with a stainless steel guide cannula (length 5 5 mm, outer diameter 0 65 mm, inner diameter 0 3 mm) directed to the right nucleus accumbens according to previously described procedures (Tuinstra and Cools, 2000a, Tuinstra and Cools, 2000b, Verheij and Cools, 2007, Verheij and Cools, chapter 5) Rats were placed in a stereotactic apparatus and the following coordinates were used according to the atlas of Paxinos and Watson (1986) anterior +10 6 mm (relative to the interaural line) and lateral -1 5 mm (relative to the midline suture) The guide cannula was lowered 5 5 mm relative to the dura surface resulting in a vertical coordinate of +3 5 mm for the cannula tip Finally, the cannula was angled 10^{o} laterally to the right side The rats were allowed to recover from surgery for the next 7 to 10 days in Plexiglas dialysis cages (25 x 25 x 35 cm) covered with sawdust on the floor On 3 consecutive days just prior to the start of the microdialysis experiment, each rat was gently picked up in order to habituate to the procedure assessed on the day when the concentration of accumbal dopamine was measured This handling procedure was repeated 3 times per day

Microdialysis: As previously described, a dialysis probe (type A-I-8-02, outer diameter 0 22 mm, 50,000-molecular-weight cut-off, Eicom, Tokyo, Japan) was carefully inserted into the brain of a conscious rat and secured to the guide cannula with a screw (Verheij and Cools, 2007) The tip of the dialysis probe protruded 2 mm below the distal end of the guide cannula into the nucleus accumbens The probes had an *in vitro* recovery of 10-12% for dopamine The inlet and outlet of the probe were connected to a swivel allowing the rat to move undisturbed Accumbal dialysates were analysed for dopamine (pg/40 µl) according to previously described procedures (Tuinstra and Cools, 2000a, Tuinstra and Cools, 2000b, Verheij and Cools, 2007, Verheij and Cools, chapter 5) Briefly, the probe was perfused at a rate of 2 0 µl/min with modified Ringer solution (see section compounds) and the outflow was collected in a sample loop and injected,

once every 20 min, into a high performance liquid chromatography (HPLC) system. Dopamine was separated from the remaining neurotransmitters by means of reversed phase, ion-paring, liquid chromatography and the concentration was measured using electrochemical detection (ECD). The HPLC-ECD system was calibrated with a standard dopamine solution before and after each experiment.

Effects of phentolamine and isoproterenol in rats treated with reserpine or alpha-methyl-para-tyrosine: At 4 h following probe insertion, the extracellular accumbal concentration of dopamine is known to reach a stable baseline ± 10% (Saigusa et al., 1999; Tuinstra and Cools, 2000a; Tuinstra and Cools, 2000b; De Leonibus et al., 2006; Verheij and Cools, 2007; Verheij et al., 2008; Verheij and Cools, chapter 5). As soon as a stable concentration of dopamine was reached, 3 baseline samples were taken (t = 0 hours) and rats were treated with RES (1 mg/kg, i.p) or its solvent (Verheij and Cools, 2007). At 24 hours after RES or its solvent (see section compounds), rats were subjected to a 40-min-lasting intra-accumbens infusion of 0.01 mM (2 µl/min) of the alpha-adrenoceptor antagonist PA (solvent RES: LR: n=8, HR: n=8; RES: LR: n=11, HR: n=9) or 0.001 mM of the beta-adrenoceptor agonist ISO (solvent RES: LR: n=8, HR: n=7; RES: LR: n=9, HR: n=9). Infusion of modified Ringer solution (2 µl/min, 40 min) served as control for PA and ISO treatment (solvent RES: LR: n=10, HR: n=10, RES: LR: n=8, HR: n=9). These adrenergic drugs were administered 24 hours after RES, because the drug-induced effects on the levels of accumbal dopamine were found to be maximal and stable at this time (Verheij and Cools, 2007). The doses of PA and ISO were chosen because these doses have been shown to increase extracellular levels of dopamine in the nucleus accumbens in a receptor specific manner (Tuinstra and Cools, 2000a; Tuinstra and Cools, 2000b; Verheij and Cools, chapter 5).

PA has been found to increase accumbal dopamine levels more strongly in rats that are exposed to novelty than in rats that are not (Tuinstra and Cools, 2000a). In view of this finding, all rats were exposed to a novel cage immediately after the start of the infusion of the noradrenergic drugs. The novel cage was slightly larger than the home-cage (new dimensions: 30 x 30 x 35 cm) and lacked sawdust on the floor (Tuinstra and Cools, 2000a; Verheij and Cools, 2007).

Histology: At the end of each experiment the rat was deeply anaesthetised with an overdose of sodium-pentobarbital (60 mg, i p) and intracranially perfused with 60 ml 4% paraformaldehyde solution Vibratome sections (100 µm, Leica VT1000F, Leica, Rijswijk, The Netherlands) were cut to determine the exact location of the microdialysis probe

Compounds: The following compounds and solutions were used (Tuinstra and Cools, 2000a, Verheij and Cools, 2007, Verheij and Cools, chapter 5) 1) Isoproterenol-hydrochloride and phentolamine-hydrochloride (Sigma, St Louis, USA), 2) Modified Ringer solution 147 mM NaCl, 4 mM KCl, 1 1 mM $CaCl_2 2H_2O$ and 1 1 mM $MgCl_2 6H_2O$ were dissolved in ultra pure water (pH 6 0) This Ringer solution served as solvent for all compounds except RES 3) RES ampoules containing 1 mg reserpine per ml solvent (Daiichi, Tokyo, Japan), and 4) RES solvent 30 mg dl-methionine dissolved in 10 ml aquadest containing 6 75% propylene glycol The pH of the RES solution and that of its solvent was adjusted to 2 4 using phosphoric acid

Statistical analysis: The drug-induced changes of accumbal dopamine were expressed as a percentage of the concentration of dopamine that was measured in the 20-min-lasting period just before these drugs were infused All data are expressed as the mean ± SEM Because RES differentially altered the basal levels of accumbal dopamine in HR compared to LR (Verheij and Cools, 2007, see also results), the neurochemical effects of ISO and of PA were analysed per type of rat A two-way ANOVA with the factors treatment and time (for repeated measures) was assessed A probability level of $p<0\ 05$ was taken as significant SPSS for Windows (Release 12) was used to statistically analyse the data

Results

Open-field selection procedure: The open-field selection procedure resulted in 19% LR and 37% HR The average distance travelled in 30 min (± SEM) was 3,575 ± 128 5 cm and 8,237 ± 231 9 cm in LR and HR, respectively The average habituation time (± SEM) was 363 ± 21 4 s in LR and 1,250 ± 55 3 s in HR

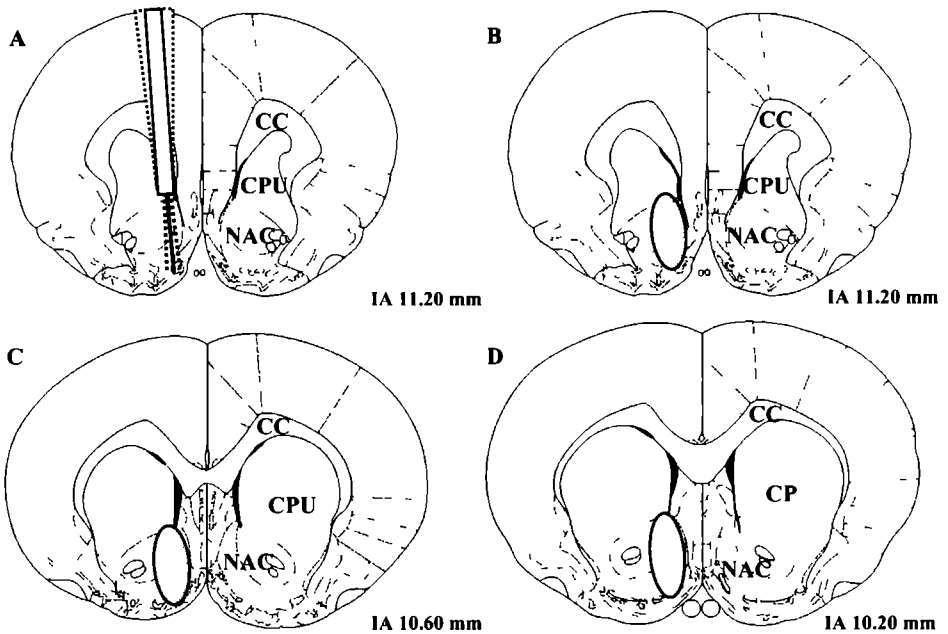


Figure 1. Example of 3 unilateral microdialysis probe tracks located in the right nucleus accumbens (A). The probe protrudes 2 mm below the distal end of the guide cannula **Schematic illustration of coronal brain sections containing the nucleus accumbens (B-D).** The brain region in which correctly placed probes were found is indicated as a grey oval IA corresponds to the distance (mm) from the interaural line according to Paxinos and Watson (1986), NAC=Nucleus Accumbens, CPU=caudate putamen, CC=corpus callosum

Histology: Histological verification revealed that 1 HR that was treated with RES solvent and ISO had to be excluded from analysis because of incorrect placement of the microdialysis guide cannula. One additional HR that was treated with RES was excluded from analysis because of obstruction of the microdialysis probe after PA was given. This rat was included in the analysis of the effects of RES before PA. The coronal region of the nucleus accumbens in which all correctly placed microdialysis probe tracks were located is shown in figure 1 (see also: Verheij and Cools, 2007).

Basal levels of dopamine: Baseline extracellular levels of accumbal dopamine were 3.2 ± 0.12 pg/sample in LR and 3.8 pg ± 0.21 pg/sample in HR (rat type effect: $F_{(1,105)} = 6.511$, $p = 0.012$). The dose of 1 mg/kg of RES decreased the extracellular levels of accumbal dopamine before PA and ISO were given (Fig. 2: treatment effect (at t = 24 h): $F_{(1,103)} = 24.416$, $p < 0.001$, see also: Verheij and Cools, 2007). RES reduced accumbal dopamine levels with 17% in LR and 58% in HR (Fig. 2: rat type x treatment effect (at t = 24 h): $F_{(1,103)} = 7.852$, $p = 0.006$, see also: Verheij and Cools, 2007).

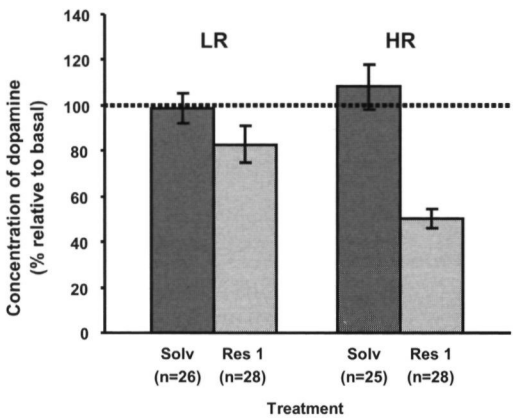


Figure 2: Effects of 1 mg/kg of reserpine (Res 1) or its solvent (Solv) on the basal levels of accumbal dopamine in non-challenged low responders (left) and non-challenged high responders (right). Values are accumbal dopamine levels 24 h after reserpine treatment. The levels of dopamine are expressed as percentage of the average of the 3 baseline samples that were taken at t = 0 h. Both phentolamine and isoproterenol were infused 24 h after reserpine was given. Data are expressed as mean percentage ± SEM.

Novelty conditions: Exposure to novelty resulted in an increase of the levels of accumbal dopamine (Fig. 3 and 5: time effect: $F_{(5,90)} = 21,952$, $p < 0.001$). The dopamine response to novelty did not differ between LR and HR that were treated with the solvent of RES (Fig. 3 and 5: type (x time) effect: n.s). It has previously been demonstated that the lack of individual differences in the accumbal dopamine response to novelty in these rats is due to the low pH of the RES solvent (for details: Verheij and Cools, 2007).

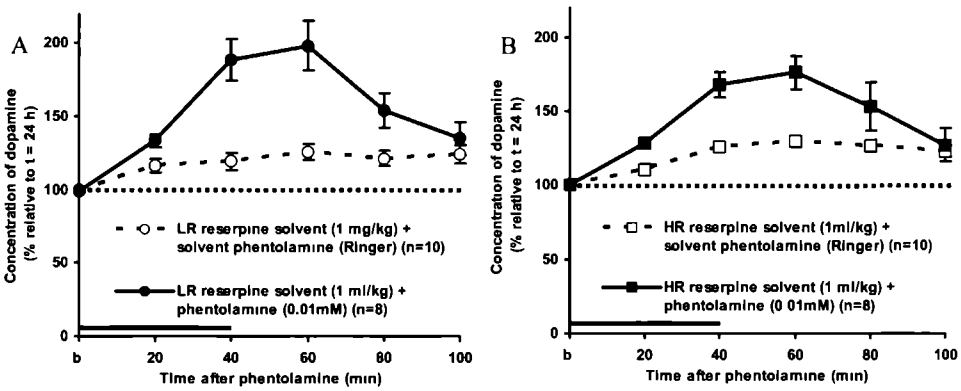


Figure 3. Effects of the intra-accumbens infusion of the alpha-adrenoceptor antagonist phentolamine (0.01 mM, 2 µl/min, 40 min) on the extracellular concentration of dopamine in the nucleus accumbens of low responders (A) and high responders (B) that were exposed to novelty. All rats were treated with the solvent op reserpine Values are accumbal dopamine levels expressed as percentage of the sample collected just before phentolamine was infused (basal sample=b) Data are expressed as mean percentage ± SEM The solid horizontal line represents the infusion time of phentolamine

Effects of phentolamine in reserpine-treated rats: The dose of 0.01 mM of the alpha-adrenoceptor antagonist PA increased accumbal dopamine levels in novelty-challenged LR and novelty-challenged HR that were treated with the solvent of reserpine (LR (Fig. 3A): treatment x time effect: $F_{(5,80)} = 14.873$, $p < 0.001$; HR (Fig. 3B): treatment x time effect: $F_{(5,80)} = 35.637$, $p < 0.001$). RES differentially decreased this PA-induced dopamine increase in both types of rat (LR (Fig. 4A): treatment x time effect: $F_{(5,85)} = 6.571$, $p < 0.001$; HR (Fig. 4B): treatment x time effect: $F_{(5,75)} = 5.616$, $p < 0.001$). PA did not anymore increase accumbal dopamine levels in RES-treated LR (Fig. 4A: treatment (x time) effect: ns) whereas it still did in RES-treated HR (Fig. 4B: treatment x time effect: $F_{(5,80)} = 3.590$, $p = 0.006$).

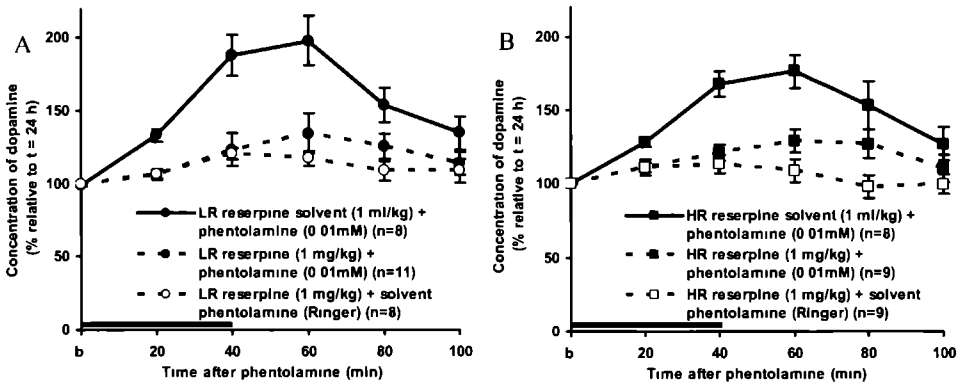


Figure 4. Effects of reserpine (1 mg/kg, i.p.) on the phentolamine-induced increase of the extracellular concentration of dopamine in the nucleus accumbens of novelty-challenged low responders (A) and novelty-challenged high responders (B). Values are accumbal dopamine levels expressed as percentage of the sample collected just before phentolamine was infused (basal sample=b) Data are expressed as mean percentage ± SEM The solid horizontal line represents the infusion time of phentolamine

Effects of isoproterenol in reserpine-treated rats: The dose of 0.001 mM of the beta-adrenoceptor agonist ISO increased accumbal dopamine levels in novelty-challenged LR and novelty-challenged HR that were treated with the solvent of RES (LR (Fig. 5A): treatment x time effect: $F_{(5\,80)} = 7.322$, $p < 0.001$; HR (Fig. 5B): treatment x time effect: $F_{(5\,75)} = 9.151$, $p < 0.001$). ISO strongly increased accumbal dopamine levels in both RES-treated LR (Fig. 6A: treatment x time effect: $F_{(5\,75)} = 5.735$, $p < 0.001$) and RES-treated HR (Fig. 6B: treatment x time effect: $F_{(5,80)} = 11.352$, $p < 0.001$). In fact, the dopamine-increasing effects of ISO were larger in RES-treated LR and HR than in LR and HR that were treated with its solvent (LR (Fig. 6A): treatment x time effect: $F_{(5\,75)} = 4.072$, $p = 0.002$; HR (Fig. 6B): treatment x time effect: $F_{(5\,70)} = 5.095$, $p < 0.001$).

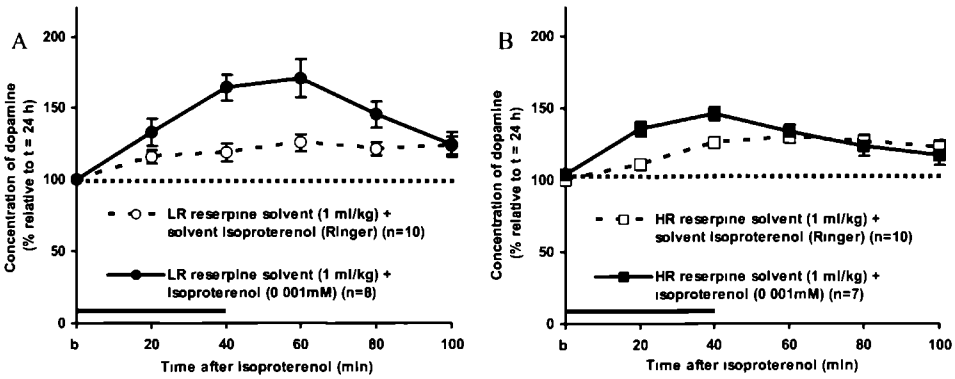


Figure 5. Effects of the intra-accumbens infusion of the beta-adrenoceptor agonist isoproterenol (0.001 mM, 2 µl/min, 40 min) on the extracellular concentration of dopamine in the nucleus accumbens of low responders (A) and high responders (B) that were exposed to novelty. Rats were treated with the solvent or reserpine. Values are accumbal dopamine levels expressed as percentage of the sample collected just before phentolamine was infused (basal sample=b). Data are expressed as mean percentage ± SEM. The solid horizontal line represents the infusion time of phentolamine.

Discussion

We have previously demonstrated that accumbal beta-, but not alpha-adrenoceptors control the accumbal dopamine release that is derived from pools of newly-synthesised dopamine (Tuinstra and Cools, 2000b; Verheij and Cools, chapter 5). The aim of the present study was to investigate which of these adrenoceptors control the accumbal dopamine release that is derived from storage pools. For that reason, LR and HR were treated with RES 24 h before the accumbal dopamine release in response to intra-accumbens infusion of the alpha-adrenergic agent PA or the beta-adrenergic agent ISO was measured. The present study confirmed our hypothesis (see introduction) that alpha-, but not beta-adrenoceptors regulate accumbal dopamine release that is derived from reserpine-sensitive storage vesicles.

Dual role of noradrenaline in mediating dopamine release: The present study showed that intra-accumbens infusion of both the alpha-antagonist PA and the beta-agonist ISO increased accumbal dopamine levels in rats that were treated with the

solvent of RES (Fig. 3 and 5). These data confirm the outcome of our previous studies that stimulation of beta-adrenoceptors facilitates accumbal dopamine release in contrast to stimulation of alpha-adrenoceptors that inhibits accumbal dopamine release (Tuinstra and Cools, 2000a; Verheij and Cools, chapter 5). It has been demonstrated that the dopamine increasing effects after the intra-accumbens administration of beta- and alpha-adrenergic agents are dose-dependent and receptor-specific (Tuinstra and Cools, 2000a). These previous studies have also revealed that the receptors at which PA and ISO act upon are located postsynaptically on dopaminergic nerve terminals (Tuinstra and Cools, 2000a; Verheij and Cools, chapter 5).

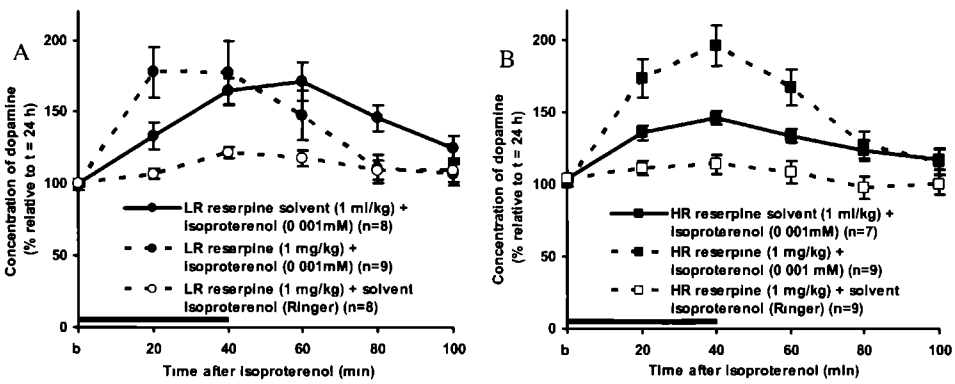


Figure 6. Effects of reserpine (1 mg/kg, i.p.) on the isoproterenol-induced increase of the extracellular concentration of dopamine in the nucleus accumbens of novelty-challenged low responders (A) and novelty-challenged high responders (B). Values are accumbal dopamine levels expressed as percentage of the sample collected just before isoproterenol was infused (basal sample=b) Data are expressed as mean percentage ± SEM The solid horizontal line represents the infusion time of isoproterenol.

Effects of reserpine on the phentolamine-induced dopamine release: The finding that RES significantly reduced the PA-induced dopamine increase in both LR (Fig. 4A) and HR (Fig. 4B) demonstrates that PA increases dopamine release from RES-sensitive storage vesicles. The finding that PA did not anymore increase the vesicular levels of accumbal dopamine in RES-treated LR confirms our previous finding that 1 mg/kg of RES completely depleted the accumbal dopaminergic storage pools of these rats (Verheij and Cools, 2007; Verheij et al., 2008). The finding that PA still increased

the vesicular levels of accumbal dopamine in RES-treated HR (Fig. 4B) underlines our previously reported finding that 1 mg/kg of RES only partially depleted the accumbal dopaminergic storage vesicles in these rats (Verheij et al., 2008; Verheij and Cools, 2009).

Effects of reserpine on the isoproterenol-induced dopamine release: ISO strongly increased accumbal dopamine levels in both RES-treated HR (Fig. 6B) and RES-treated LR (Fig. 6A), despite the fact that the RES-sensitive dopaminergic storage pools were partially empty in HR and completely empty in LR (see above). These data demonstrate that beta-adrenoceptors do not control dopamine release from RES-sensitive storage vesicles. The finding that ISO could still increase the levels of accumbal dopamine in RES-treated rats becomes understandable in view of the fact that the ISO-induced dopamine increase is derived from RES-resistant, AMPT-sensitive pools (see above).

Interestingly, the ISO-induced dopamine increase was larger in RES-treated rats than in rats treated with its solvent (Fig. 6). Agents that are known to reduce the levels of noradrenaline in the synapse have been found to make adrenoceptors more sensitive to their agonists (Verheij and Cools, 2009). Because our data indicate that RES resulted in an increased sensitivity of accumbal beta-adrenoceptors to ISO, we suggest that RES reduced the noradrenaline release from the storage vesicles of those noradrenergic neurons that impinge upon the dopaminergic nerve terminals that contain beta-adrenoceptors. It is therefore suggested that RES-sensitive pools of noradrenaline control the beta-adrenoceptor-mediated release of dopamine from pools of newly-synthesised dopamine. In this context it is interesting to note that there is evidence that RES-insensitive pools of noradrenaline control the alpha-adrenoceptor-mediated release of dopamine from storage vesicles (Verheij and Cools, 2009). Our data indicate that the interaction between noradrenaline and dopamine in the nucleus accumbens is not only mediated by different receptors (alpha-adrenoceptors and beta-adrenoceptors), but also by different pools of neurotransmitter (AMPT-(in)sensitive and RES-(in)sensitive) in both dopaminergic and noradrenergic nerve terminals (for details on these dopaminergic and noradrenergic neurons see: Cools and Tuinstra, 2003).

Conclusions

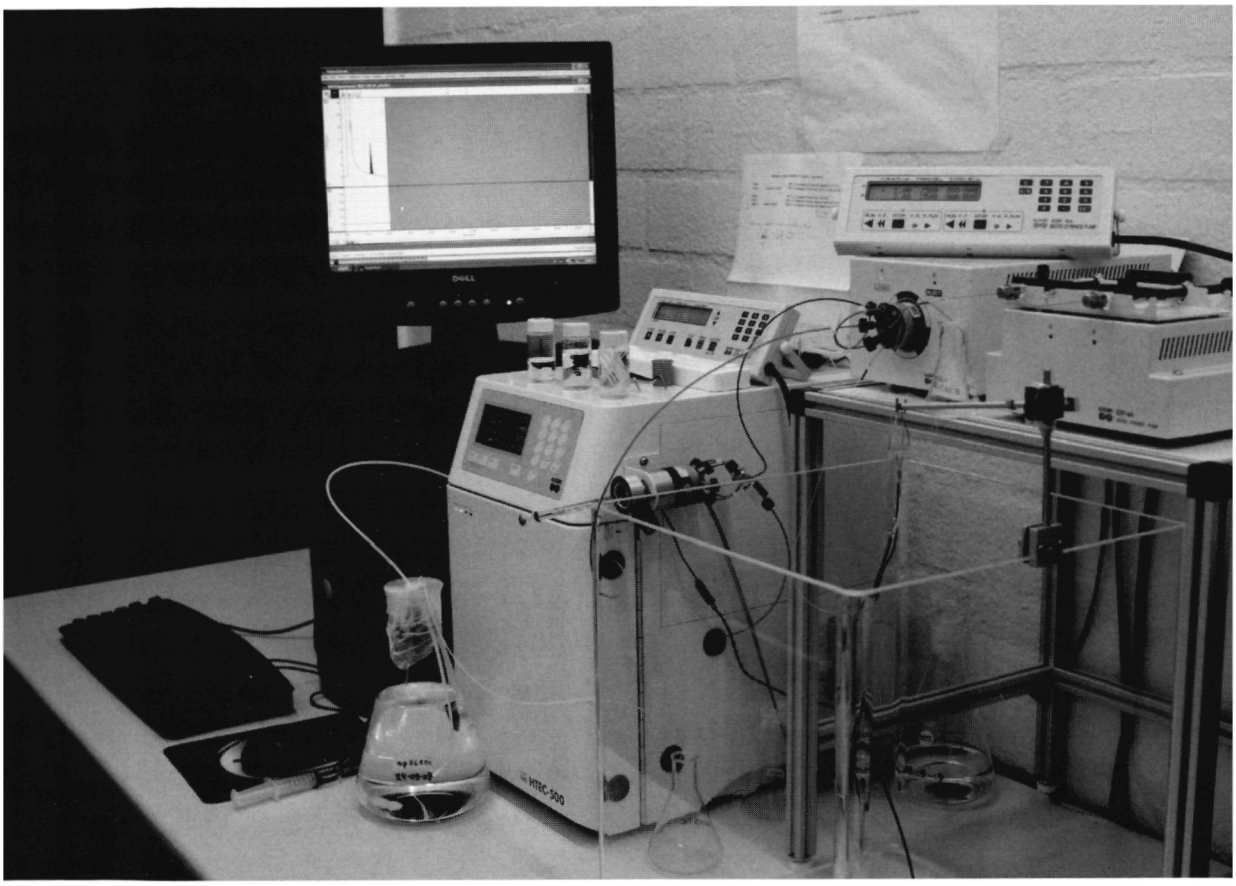
The finding that PA increased dopamine release from RES-sensitive pools, whereas ISO did not, confirms our hypothesis (see introduction) that alpha-, but not beta-adrenoceptors mediate (inhibit) accumbal dopamine release from vesicular storage pools The present data indicate that RES-sensitive pools of noradrenaline control the beta-adrenoceptor-induced release of dopamine from AMPT-sensitive pools

Combing the above-mentioned data results in the notion that RES directly affects the alpha-adrenoceptor-mediated dopamine release by acting at the storage vesicles of dopaminergic neurons whereas RES indirectly affects the beta-adrenoceptor-mediated dopamine release by acting at the storage vesicles of the noradrenergic neurons

Impact

The present study underlines our notion that mesolimbic noradrenaline fulfils many functions that are, up to now, primarily ascribed to mesolimbic dopamine (Cools and Tuinstra, 2003) This insight opens interesting perspectives for the development of a new class of therapeutic drugs Drugs that interact with mesolimbic alpha- and beta-adrenoceptors may, in addition to drugs that affect newly-synthesised and storage pools, have therapeutic effects that are usually ascribed to drugs that interact with mesolimbic dopaminergic receptors The therapeutic effects of these noradrenergic and dopaminergic agents are elaborated elsewhere in detail (noradrenaline Aono et al , 2007, Ikeda et al , 2007, dopamine Verheij and Cools, 2007, Verheij et al , 2008)

Finally, it is important to realise that the direct and indirect therapeutic effects of noradrenergic agents strongly depend on the amount of endogenous noradrenaline in the synapse In fact, the present study indicates that a beta-adrenoceptor agonist may be more effective in increasing dopamine in individuals with low levels of endogenous noradrenaline than in individuals with high levels of endogenous noradrenaline (see above)



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Collecting dopamine samples (microdialysis system 2/2).

Chapter 7

Mesolimbic alpha-, but not beta-adrenoceptors regulate behaviour that is mediated by reserpine-sensitive storage vesicles

M.M.M. Verheij, J.M. Elferink and A.R. Cools

Submitted.

Abstract

It has previously been demonstated that mesolimbic alpha-, but not beta-adrenoceptors control the dopamine release from accumbal reserpine-sensitive storage vesicles The aim of the present study was to investigate whether mesolimbic alpha-, but not beta-adrenoceptors regulate behaviour that is mediated by these storage pools Accordingly, rats were pre-treated with reserpine before the alpha-adrenergic agent phentolamine or the beta-adrenergic agent isoproterenol were locally applied into the nucleus accumbens Both phentolamine and isoproterenol increased the duration of rearing, walking, and grooming and decreased the duration of sitting Reserpine counteracted the behavioural response elicited by phentolamine, but not by isoproterenol The results of the present study demonstrate that mesolimbic alpha-adrenoceptors, but not mesolimbic beta-adrenoceptors, regulate behaviour that is mediated by reserpine-sensitive storage pools Combing the results of the previous neurochemical study with the results of present behavioural study results in the notion that the alpha-adrenoceptor-mediated changes in behaviour may well be the result of changes in the release of previously stored dopamine

Introduction

Neurochemical studies have revealed that intra-accumbens administration of either alpha-adrenoceptor antagonists or beta-adrenoceptor agonists increase accumbal dopamine release (Nurse et al., 1984; Nurse et al., 1985; Tuinstra and Cools, 2000a; Verheij and Cools, chapter 6; Verheij and Cools, chapter 5). The dopamine increasing effects of these noradrenergic agents have been found to be dose-dependent and receptor specific (Tuinstra and Cools, 2000a). Based on these data it has been concluded that mesolimbic alpha-adrenoceptors inhibit dopamine release in the nucleus accumbens in contrast to mesolimbic beta-adrenoceptors that stimulate accumbal dopamine release (Nurse et al., 1984; Nurse et al., 1985; Tuinstra and Cools, 2000a; Verheij and Cools, chapter 6; Verheij and Cools, chapter 5). In a follow-up study we have provided evidence that the accumbal dopamine release evoked by inhibition of mesolimbic alpha-adrenoceptors is derived from reserpine (RES)-sensitive storage pools, whereas the accumbal dopamine release evoked by stimulation of mesolimbic beta-adrenoceptors is not (Verheij and Cools, chapter 6).

The first aim of the present study was to investigate whether the intra-accumbens administration of either the alpha-adrenoceptor antagonist phentolamine (PA) or the beta-adrenoceptor agonist isoproterenol (ISO) changes the expression of behaviour. Because both noradrenergic drugs increase accumbal dopamine release (see above) and because accumbal dopamine mediates horizontal (Pijnenburg and Van Rossum, 1973; Jackson et al., 1975; Costall and Naylor, 1975; Costall et al., 1976) as well as vertical (Kalivas et al., 1984; Swanson et al., 1997) activity, it was expected that both PA and ISO increase the duration of walking and rearing behaviour. The second aim of the present study was to investigate whether the putative behavioural response to PA and ISO is mediated by RES-sensitive storage pools. Based on the above-mentioned neurochemical data, it was hypothesised that RES counteracts the behavioural response elicited by the intra-accumbens administration of the alpha-antagonist PA , but not by the intra-accumbens administration of the beta-agonist ISO.

Experimental procedures

Subjects: The rats of this behavioural study were also used to measure the neurochemical response to PA and ISO Accordingly, the rats have been selected on their behavioural response to a novel open-field, resulting in high responders (HR) and low responders (LR) to novelty The animals of the present study are the HR and LR described in Verheij and Cools, chapter 6 (RES solvent + solvent PA/ISO HR n=10, LR n=10, RES solvent + 0 01 mM PA HR n=8, LR n=8, 1 mg/kg of RES + 0 01 mM of PA HR n=9, LR n=11, RES solvent + 0 001 mM ISO HR n=7, LR n=8, 1 mg/kg of RES + 0 001 mM ISO HR n=9, LR n=9, 1 mg/kg of RES + solvent LR n=8, HR n=9) All rats (weight=180-220 g) were reared and housed in macrolon cages (42 x 26 x 15 cm, n=3-4 per cage) under a fixed 12/12 h light/dark cycle (lights on 07 00 a m) in a temperature-controlled room (21 ± 1 7 °C) Water and food pellets were available *ad libitum* All experiments were performed in accordance with institutional, national and international guidelines for animal care and welfare

Surgery: As previously described (Verheij and Cools, chapter 6), rats were unilaterally implanted with a guide cannula directed at the right nucleus accumbens Subsequently, rats were allowed to recover from surgery for the next 7 to 10 days in Plexiglas cages (25 x 25 x 35 cm) covered with sawdust on the floor On 3 consecutive days just prior to the start of the experiment, each rat was gently picked up in order to habituate to the procedure assessed on the day when behaviour was measured This handling procedure was repeated 3 times per day

Drug treatment: A dialysis probe (type A-I-8-02, outer diameter 0 22 mm, 50,000-molecular-weight cut-off, Eicom, Tokyo, Japan) was carefully inserted into the brain of a conscious rat (Verheij and Cools, chapter 6) The tip of the dialysis probe protruded 2 mm below the distal end of the guide cannula The inlet and outlet of the probe were connected to a swivel allowing the animal to move freely At 12 00 h on the first day of the experiment, LR and HR were injected with RES (dose 1 mg/kg) or its solvent (Verheij and Cools, chapter 6) After this systemic injection (i p), rats were

returned to their home cage. At 12.00 h of the second day of the experiment, 0.01 mM of PA (2 µl/min for 40 min) or 0.001 mM of ISO (2 µl/min for 40 min) was locally infused into the nucleus accumbens and behaviour was recorded (Verheij and Cools, chapter 6). The relatively low dose of 1 mg/kg of RES was chosen because it has previously been demonstrated that this dose selectively affect RES-sensitive storage vesicles (Verheij and Cools, 2007; Verheij and Cools, chapter 6). The dose of 0.01 mM of PA (2 µl/min, 40 min) and that of 0.001 mM of ISO (2 µl/min, 40 min) were chosen because these doses have been shown to increase accumbal dopamine release (Tuinstra and Cools, 2000a; Verheij and Cools, chapter 6; Verheij and Cools, chapter 5). Immediately after the infusion of the noradrenergic drugs, rats were exposed to a novel cage because intra-accumbal administration of noradrenergic agents has been found to change behaviour only of rats that are challenged (Pijnenburg et al., 1975; Cools et al., 1987; Ikeda et al., 2007). This novel cage was slightly larger than the home-cage (new dimensions: 30 x 30 x 35 cm) and lacked sawdust on the floor (Verheij and Cools, chapter 6).

Behaviour: Behaviour was recorded on video tape and analysed offline by an observer blind to the treatment and the type of rat using a computer programme (KEYS®) developed at our institute (Saigusa et al., 1999; Verheij and Cools, chapter 4). Recordings were made for a period of 15 min directly before the administration of PA or ISO as well as during a period of 30 min starting directly after the administration of these noradrenergic agents (Verheij and Cools, chapter 2). The presence or absence of a particular type of behaviour was analysed using a standard ethogram (Clifford et al., 1998; Clifford and Waddington, 2000). The duration (s) of the following items was scored (Saigusa et al., 1999; Verheij and Cools, chapter 4): walking (displacement of all 4 paws over a minimum distance of 1 cm for a period of at least 3 s), rearing (front paw(s) raised off the cage floor), grooming (washing any part of the body) and sitting (sitting motionless without grooming for a period of at least 3 s).

Solutions: The following solutions were used (Verheij and Cools, chapter 6): 1) Phentolamine-hydrochloride and Isoproterenol-hydrochloride (Sigma, St Louis, USA), 2) Reserpine (Daiichi, Tokyo, Japan): ampoules containing 1 mg of reserpine per ml

solvent, 2) Reserpine solvent 30 mg dl-methionine, dissolved in 10 ml aquadest containing 6 75% propylene glycol The pH of the reserpine solution and that of its solvent was adjusted to 2 4 using phosphoric acid (Verheij and Cools, 2007)

Analysis and expression of the data: The behavioural response to PA and ISO lasted for 30 min Given that the neurochemical response to PA and ISO and the effects of RES on the neurochemical response to these noradrenergic agents have previously been found not to differ between LR and HR during this period (Verheij and Cools, chapter 6), the behavioural data of both types of rat were pooled Behaviour was scored in blocks of 15 min and expressed as the mean duration ± SEM The effects of PA and ISO and the effect of RES on behaviour induced by these agents were statistically analysed using a one-way ANOVA with the factor treatment SPSS for Windows (Release 12 0) was used to statistically analyse the data A p-value of 0 05 or smaller was considered to be significant

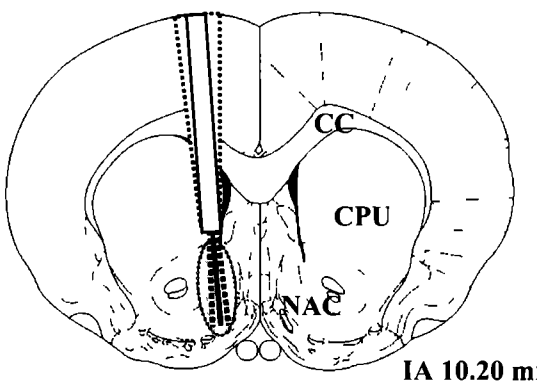


Figure 1. Example of 3 unilateral microdialysis probe tracks located in the right nucleus accumbens (see also: Verheij and Cools, chapter 6) The probe protrudes 2 mm below the distal end of the guide cannula The brain region in which correctly placed probes were found is indicated in grey IA corresponds to the distance (mm) from the interaural line according to Paxinos and Watson (1986), NAC Nucleus Accumbens, CPU caudate putamen, CC corpus callosum

Results

Histology: The coronal region of the nucleus accumbens in which all correctly placed microdialysis probe tracks were located is shown in figure 1 (see also Verheij and Cools, chapter 6)

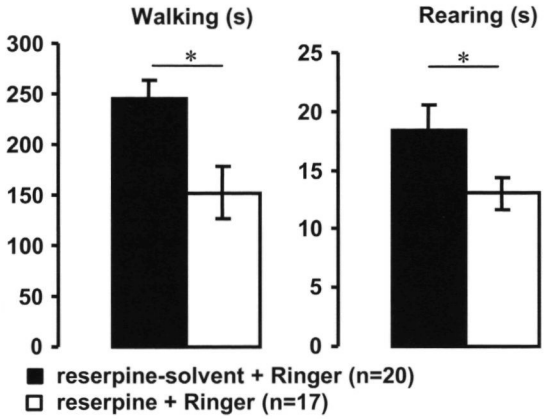


Figure 2: Effects of reserpine (1 mg/kg, i.p.) on the duration of walking and rearing behaviour during the first 15 min. Data are expressed as mean duration of walking and rearing ± SEM. * $p < 0.05$.

Baseline behaviour: All rats were either sitting or sleeping in the 15 min lasting period preceding the exposure to PA or ISO. Walking, rearing and grooming were simply absent in this control period (see also: Saigusa et al., 1999; Verheij and Cools, chapter 2; Verheij and Cools, chapter 4).

Effect of reserpine: RES itself reduced walking and rearing during the first 15 min of the test (treatment effect: walking (Fig. 2): $F_{(1,35)} = 7.992$, $p = 0.008$, rearing (Fig. 2): $F_{(1,35)} = 4.658$, $p = 0.038$), but not during the second 15 min of the test (treatment effect: walking (Fig. 3): ns, rearing (Fig. 4): ns). Accordingly, the effects of RES on the PA- and ISO-induced changes of behaviour were analysed only during this second period of 15 min.

Effects of reserpine on phentolamine-induced behaviour: PA increased the duration of walking (Fig. 3A: treatment effect: $F_{(1,34)} = 4.763$, $p = 0.036$), rearing (Fig. 4A: treatment effect: $F_{(1,34)} = 9.163$, $p = 0.005$) and grooming (Fig. 5A: treatment effect: $F_{(1,34)} = 13.141$, $p<0.001$) during the second 15 min of the test. In addition, PA decreased the duration of sitting (Fig. 6A: treatment effect: $F_{(1,34)} = 13.110$, $p<0.001$) in this period.

RES counteracted all behavioural effects of PA (treatment effect: walking (Fig. 3A): $F_{(1,34)} = 5.089$, $p = 0.031$, rearing (Fig. 4A): $F_{(1,34)} = 4.993$, $p = 0.032$, grooming

(Fig. 5A): $F_{(1,34)} = 7.605$, $p = 0.009$, sitting (Fig. 6A): $F_{(1,34)} = 11.822$, $p = 0.002$). PA did not anymore change behaviour in RES-treated rats (walking (Fig. 3A), rearing (Fig. 4A), grooming (Fig. 5A) and sitting (Fig. 6A): treatment effect: ns).

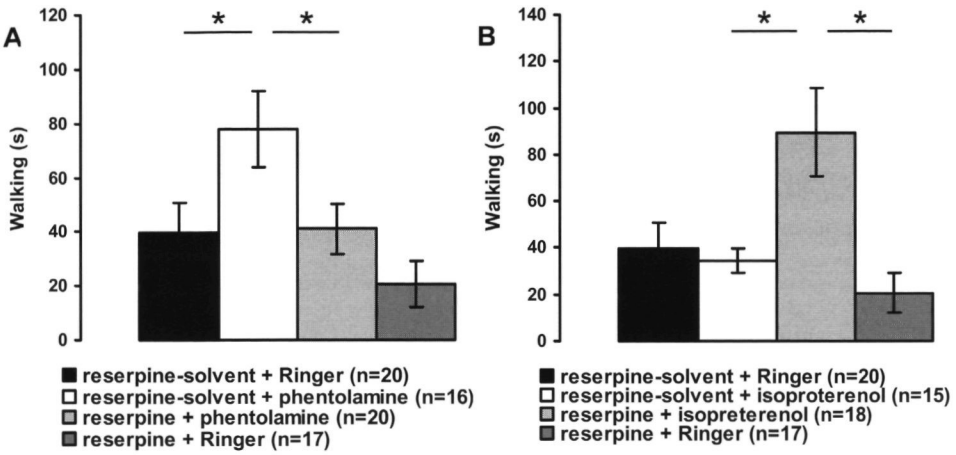


Figure 3. Effects of reserpine (1 mg/kg, i.p.) on the duration of walking behaviour elicited by intra-accumbens infusion (2 µl/min, 40 min) of 0.01 mM of the alpha-adrenoceptor antagonist phentolamine (Fig. A) or 0.001 mM of the beta-adrenoceptor agonist isoproterenol (Fig. B). Data are expressed as mean duration of walking ± SEM. * $p < 0.05$, # $p = 0.065$.

Effects of reserpine on isoproterenol-induced behaviour: ISO increased the duration of rearing (Fig. 4B: treatment effect: $F_{(1,33)} = 4.740$, $p = 0.037$) and grooming (Fig. 5B: treatment effect: $F_{(1,33)} = 10.172$, $p = 0.003$), but not of walking (Fig. 3B: treatment effect: ns) during the second 15 min of the test. Moreover, ISO decreased the duration of sitting (Fig. 6B: treatment effect: $F_{(1,33)} = 5.486$, $p = 0.025$) in this period.

ISO could still change behaviour in RES-treated rats (treatment effect: walking (Fig. 3B): $F_{(1,33)} = 10.571$, $p = 0.003$), rearing (Fig. 4B): $F_{(1,33)} = 3.631$, $p = 0.065$, grooming (Fig. 5B): $F_{(1,33)} = 4.218$, $p = 0.048$ and sitting (Fig. 6B): $F_{(1,33)} = 4.535$, $p = 0.041$). In fact, the duration of ISO-induced rearing, grooming and sitting was similar in RES-treated rats as in rats treated with its solvent (treatment effect: rearing (Fig. 4B), grooming (Fig. 5B) and sitting (Fig. 6B): ns). It is interestingly to note that the effects of ISO on the duration of walking behaviour were even larger in rats treated with RES as in rats treated with its solvent (Fig. 3B: treatment effect: $F_{(1,31)} = 6.699$, $p = 0.015$).

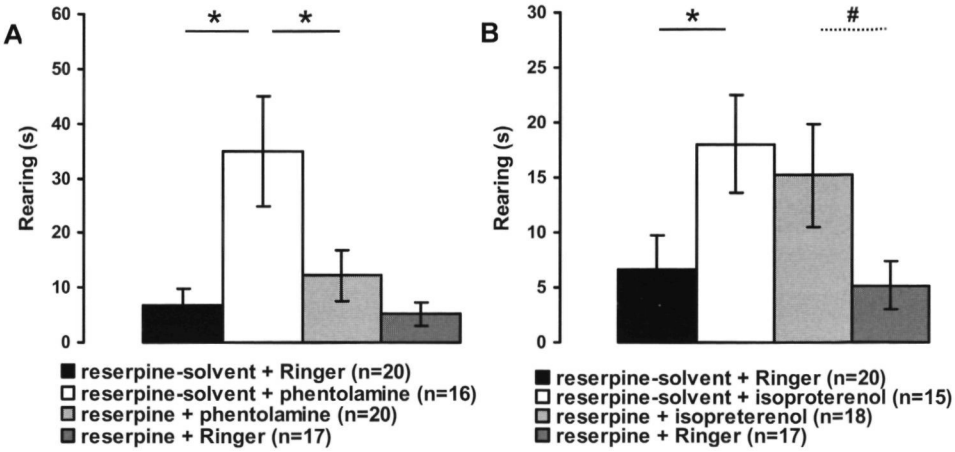


Figure 4. Effects of reserpine (1 mg/kg, i.p.) on the duration of rearing behaviour elicited by intra-accumbens infusion (2 µl/min, 40 min) of 0.01 mM of the alpha-adrenoceptor antagonist phentolamine (Fig. A) or 0.001 mM of the beta-adrenoceptor agonist isoproterenol (Fig. B). Data are expressed as mean duration of rearing ± SEM. * $p < 0.05$

Discussion

We have previously provided evidence that the accumbal dopamine release evoked by inhibition of mesolimbic alpha-adrenoceptors is derived from RES-sensitive storage pools whereas the accumbal dopamine release evoked by stimulation of mesolimbic beta-adrenoceptors is not (Verheij and Cools, chapter 6). The first aim of the present study was to investigate whether the intra-accumbens administration of either the alpha-adrenoceptor antagonist PA or the beta-adrenoceptor agonist ISO changes the expression of behaviour. The second aim of the present study was to investigate whether the behavioural response to PA or to ISO is mediated by RES-sensitive storage pools. The experiments demonstated that intra-accumbens administration of both types of noradrenergic drug change the expression of behaviour and that RES counteracts the behavioural response elicited by PA, but not by ISO.

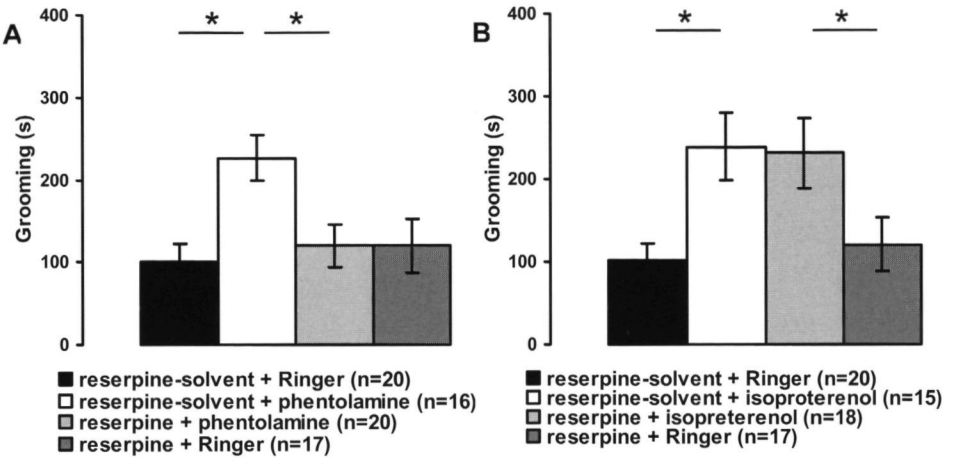


Figure 5. Effects of reserpine (1 mg/kg, i.p.) on the duration of grooming behaviour after intra-accumbens infusion (2 µl/min, 40 min) of 0.01 mM of the alpha-adrenoceptor antagonist phentolamine (Fig. A) or 0.001 mM of the beta-adrenoceptor agonist isoproterenol (Fig. B). Data are expressed as mean duration of grooming ± SEM. * $p < 0.05$

Effects of phentolamine and isoproterenol: According to our hypothesis, both intra-accumbens PA and intra-accumbens ISO increased the duration of walking (Fig. 3) and rearing (Fig. 4) behaviour. In addition, administration of either type of drug into the nucleus accumbens increased grooming (Fig.5) and reduced sitting (Fig. 6). These data demonstrate that both inhibition of mesolimbic alpha-adrenoceptors and stimulation of mesolimbic beta-adrenoceptors increases the overall activity of the rat. It has to be mentioned that ISO increased walking behaviour only under the condition that the rats were treated with RES (see also below).

Effects of reserpine on the phentolamine- and isoproterenol-induced changes of behaviour: The present study confirmed our hypothesis that RES counteracts the behavioural response to PA, but not to ISO (Fig. 3-6). The present study, therefore, demonstrates that mesolimbic alpha, but not beta, adrenoceptors regulate behaviour that is mediated by RES-sensitive storage pools. The fact that intra-accumbens PA increases accumbal dopamine release from RES-sensitive storage vesicles (Verheij

and Cools, chapter 6) suggests that the observed behavioural response to PA is due to the alpha-adrenoceptor-mediated release of previously stored accumbal dopamine. In contrast, the fact that intra-accumbens ISO increases dopamine release from alpha-methyl-para-sensitive pools (Tuinstra and Cools, 2000b; Verheij and Cools, chapter 5) indicates that the observed behavioural response to ISO is due to the beta-adrenoceptor-induced stimulation of the release of accumbal newly-synthesised dopamine.

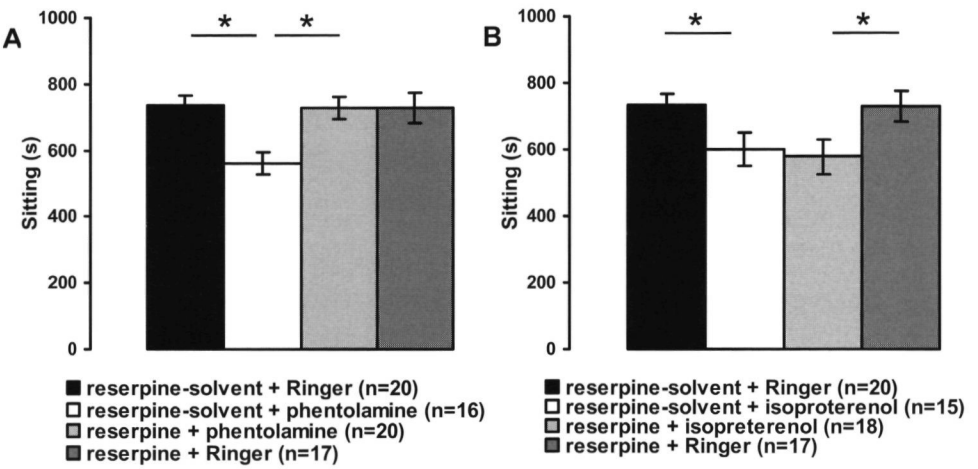


Figure 6. Effects of reserpine (1 mg/kg, i.p.) on the duration of sitting behaviour elicited by intra-accumbens infusion (2 µl/min, 40 min) of 0.01 mM of the alpha-adrenoceptor antagonist phentolamine (Fig. A) or 0.001 mM of the beta-adrenoceptor agonist isoproterenol (Fig. B). Data are expressed as mean duration of sitting ± SEM. * $p < 0.05$.

The present behavioural finding that RES increased ISO-induced walking behaviour (Fig. 3) fits in with the previously reported neurochemical finding that the ISO-induced accumbal dopamine release was larger in rats treated with RES than in rats treated with its solvent (Verheij and Cools, chapter 6). Drugs that are known to reduce the levels of noradrenaline in the synapse are known to make adrenoceptors more sensitive to their agonists (Cools et al., 1987; Cools et al., 1991; Verheij and Cools, 2009). It is, therefore, concluded that RES resulted in an increased behavioural response

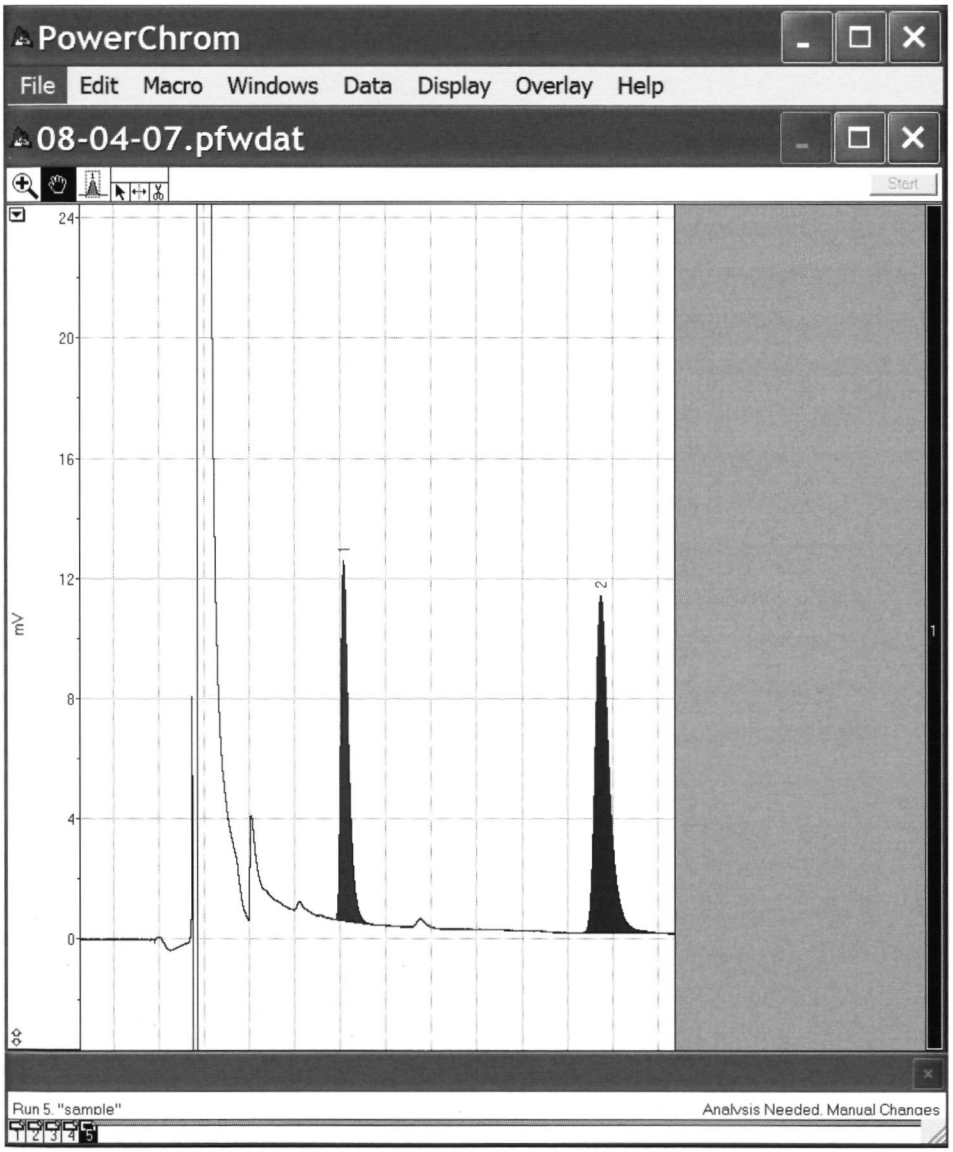
to ISO because RES reduced the amount of accumbal noradrenaline at the level of the beta-adrenoceptors of the nucleus accumbens.

Although ISO increased walking in RES-treated rats, it did not in rats treated with solvent. (Fig. 3). The fact that the ISO-induced increase of accumbal dopamine was stronger in RES-treated rats than in rats treated with its solvent (Verheij and Cools, chapter 6) indicates that beta-adrenoceptor-mediated walking only takes place in case the increase of newly-synthesised dopamine in the nucleus accumbens is relatively high. The fact that ISO did increase rearing and grooming in solvent treated rats (Fig. 4 and 5) indicates that beta-adrenoceptor-mediated rearing and grooming already appear when the increase of accumbal newly-synthesised dopamine is relatively low.

Two substrates for grooming: Not only walking and rearing after PA, but also walking and rearing after cocaine have previously been hypothesised to be the result of a drug-induced increase of the release of vesicular dopamine in the nucleus accumbens (Verheij and Cools, chapter 4). In contrast to the present study, this previous study has indicated that cocaine-induced grooming was not due to changes of accumbal vesicular dopamine release (Verheij and Cools, chapter 4). These data underline the previously reported notion that grooming is mediated by at least two distinct neuronal substrates (Cools et al., 1978; Cools et al., 1988).

Conclusions and impact

The results of the present study show that both inhibition of accumbal alpha-adrenoceptors and stimulation of accumbal beta-adrenoceptors change the expression of behaviour. Behaviour mediated by mesolimbic alpha-adrenoceptors depends on monoamines inside storage vesicles whereas behaviour mediated by mesolimbic beta-adrenoceptors does not.



8/15: Berekenen van de hoeveelheid dopamine per sample.
Calculation of the concentration of dopamine per sample.

Chapter 8

Accumbal noradrenaline that contributes to the alpha-adrenoceptor-mediated release of dopamine from reserpine-sensitive storage vesicles in the nucleus accumbens is derived from alpha-methyl-para-tyrosine-sensitive pools

M.M.M. Verheij and A.R. Cools (2009).

Journal of Neural Transmission: in press.

Abstract

Alpha-adrenoceptors in the nucleus accumbens are known to inhibit accumbal dopamine release from reserpine-sensitive pools The aim of this study was to test our previously reported hypothesis that accumbal noradrenaline that controls the dopamine release from these storage vesicles is derived from alpha-methyl-para-tyrosine-sensitive pools The sensitivity of accumbal alpha-adrenoceptors to noradrenergic agents depends on the amount of noradrenaline that is available in the synapse In case the synaptic noradrenaline levels decrease, the conformation of alpha-adrenoceptors changes into a state that makes these receptors more sensitive to its agonists The effects of alpha-methyl-para-tyrosine, respectively reserpine, on the alpha-adrenoceptor-agonist-induced changes of accumbal dopamine release were investigated Alpha-methyl-para-tyrosine, but not reserpine, made accumbal postsynaptic alpha-adrenoceptors more sensitive to phenylephrine These results indicate that noradrenaline that inhibits the release of dopamine from reserpine-sensitive storage vesicles *via* stimulation of accumbal postsynaptic alpha-adrenoceptors, is derived from alpha-methyl-para-tyrosine-sensitive pools The clinical impact of these data is discussed

Introduction

It has been found that the sensitivity of alpha-adrenoceptors to noradrenergic agents depends on the amount of noradrenaline that is available in the synapse. In case the synaptic noradrenaline levels increase the conformation of alpha-adrenoceptors change into a state that makes them less sensitive to agonists and more sensitive to antagonists (Cools et al., 1987; Cools et al., 1991; Tuinstra and Cools, 2000a; Cools and Tuinstra, 2003; Aono et al., 2007). The opposite holds also true: in case the synaptic noradrenaline levels decrease the conformation of alpha-adrenoceptors change into a state that makes them more sensitive to agonists and less sensitive to antagonists (Cools et al., 1987; Cools et al., 1991; Tuinstra and Cools, 2000a; Cools and Tuinstra, 2003). It is evident that these processes provide a homeostatic mechanism, controlling the adrenergic activity at postsynaptic receptors.

Alpha-adrenoceptors are known to control the release of dopamine. Both *in-vitro* and *in-vivo* experiments have revealed that stimulation of postsynaptic alpha-adrenoceptors that are located on dopaminergic neurons inhibits the release of dopamine in the nucleus accumbens whereas inhibition of these receptors stimulates the release of dopamine in this brain structure (Nurse et al., 1984; Nurse et al., 1985; Russell et al., 1988; Russell et al., 1993; Tuinstra and Cools, 2000a; Cools and Tuinstra, 2003; Verheij and Cools, chapters 5 and 6; see also: Verheij and Cools, 2008). It is generally accepted that presynaptic alpha-adrenoceptors regulate the amount of noradrenaline in the synapse and, accordingly, regulate the amount of noradrenaline at the level of postsynaptic alpha-adrenoceptors. Interestingly, the noradrenaline agonist phenylephrine (PE) has been found to act at accumbal presynaptic, but not postsynaptic, alpha-adrenoceptors of otherwise untreated rats (Tuinstra and Cools, 2000a; Tuinstra and Cools, 2000b; Cools and Tuinstra, 2003; Aono et al., 2007; see also below). This action of PE has been found to reduce the synaptic noradrenaline levels (Aono et al., 2007), which, in turn, results in an increased release of accumbal dopamine in these rats (Tuinstra and Cools, 2000a; Tuinstra and Cools, 2000b; Cools and Tuinstra, 2003). It has recently been demonstrated that the alpha-adrenoceptor mediated release of accumbal dopamine is not derived from alpha-methyl-para-tyrosine (AMPT)-sensitive dopamine pools (Tuinstra and Cools,

2000b, Verheij and Cools, chapter 5, see also Verheij and Cools, 2008) In fact, the alpha-adrenoceptor-mediated dopamine release is derived from reserpine (RES)-sensitive pools of dopamine (Cools and Verheij, 2002, Verheij and Cools, chapter 6, see also Verheij and Cools, 2008) It is currently unknown which type of noradrenergic pool releases noradrenaline that inhibits accumbal dopamine release from these storage pools On the basis of previously reported neurochemical data, however, it has been hypothesised that accumbal noradrenaline derived from AMPT-sensitive pools controls the alpha-adrenoceptor-mediated release of accumbal dopamine from RES-sensitive vesicles (Saigusa et al , 1999)

Given the fact that stimulation of postsynaptic alpha-adrenoceptors in the nucleus accumbens inhibits the release of accumbal dopamine (see above), the inability of the alpha-adrenoceptor agonist PE to decrease the amount of accumbal dopamine in otherwise untreated rats (Tuinstra and Cools, 2000a, Tuinstra and Cools, 2000b, see also above) has been ascribed to the presence of a relatively high amount of noradrenaline in the synapses of these rats (Tuinstra and Cools, 2000a) Under these conditions it can be predicted that an experimentally-induced decrease of the amount of synaptic noradrenaline makes the accumbal postsynaptic alpha-adrenoceptors sensitive to PE AMPT is known to reduce the alpha-adrenoceptor mediated release of noradrenaline (Brannan et al , 1991, McTavish et al , 1999)

In order to test whether the noradrenaline release at the level of postsynaptic alpha-adrenoceptors in the nucleus accumbens is derived from AMPT-sensitive pools, the effects of AMPT on the PE-induced accumbal dopamine release were established Combining the above-mentioned considerations results in the hypothesis that AMPT makes the accumbal postsynaptic alpha-adrenoceptors sensitive to PE It is hypothesised that PE decreases the amount of accumbal dopamine in AMPT-treated rats (postsynaptic action) in contrast to PE that will increase the amount of accumbal dopamine in control rats (presynaptic action) In order to establish whether RES-sensitive pools contribute to the release of accumbal noradrenaline at postsynaptic alpha-adrenoceptors as well, the effects of RES on the PE-induced accumbal dopamine release were also investigated

Materials and methods

Rats were unilaterally implanted with a guide cannula directed at the right nucleus accumbens (for procedures see Verheij and Cools, 2007) The rats were allowed to recover from surgery for the next 7-10 days in dialysis cages On 3 days prior to the start of the experiment, each rat was gently picked up (3 times per day) in order to habituate to the procedure assessed on the day when accumbal dopamine was measured At the start of the experiment, a dialysis probe was connected to a swivel and accumbal dialysates were analysed for dopamine according to previously described procedures (see Verheij and Cools, 2007) The probes had an in vitro recovery of 10 12% for dopamine (Verheij and Cools, 2007) The dopamine sensitivity was 500 fg per sample (Verheij and Cools, 2007) This sensitivity has repeatedly been shown to be sufficient for detecting the currently observed changes in the amount of dopamine (De Leonibus et al , 2006, Verheij and Cools, 2007, Verheij et al , 2008)

As soon as the dopamine samples differed less than 10%, 3 baseline samples were taken and one group of rats was treated with 0 1 mM (2 µl/min for 40 min, intra-accumbens) of AMPT or its solvent (Ringer) whereas another group of rats was treated with 1 mg/kg (1 ml/kg, i p) of RES or its solvent (pH = 2 4) At 40 and 100 min after AMPT or its solvent and at 24 h after RES or its solvent, rats were subjected to a 40 min lasting intra-accumbens infusion of 0 01 mM (2 µl/min) of the alpha-1 adrenoceptor agonist PE

The AMPT- and RES-induced changes of accumbal dopamine over time have already been published elsewhere (time effects of AMPT Tuinstra and Cools, 2000b, time effects of RES Verheij and Cools, 2007) The dopamine decreasing effects of these agents were found to be maximal and stable throughout the period that PE was given (see Tuinstra and Cools, 2000b, Verheij and Cools, 2007) Modified Ringer solution served as control for PE At the end of the microdialysis experiments, rats were given an overdose of pentobarbital and were intracardially perfused with paraformaldehyde Vibratome sections were cut to verify the location of the microdialysis probe

It is important to realise that one can only measure a PE-induced increase of accumbal dopamine under the condition that the presynaptic pools of dopamine are not

empty For this reason, experiments were performed in High Responders to novelty (HR) that were selected from the Nijmegen outbred strain of Wistar rats (Cools et al , 1990) It has previously been reported that an environmental or pharmacological challenge could still increase accumbal dopamine levels in these animals after the chosen treatment with AMPT (see Saigusa et al , 1999) or RES (see· Verheij et al., 2008). HR were selected on the basis of their locomotor response to a novel open-field and defined as animals that travelled more than 6,000 cm in 30 min and habituated (no locomotor activity for a period of at least 90 s) after 840 s (Verheij and Cools, 2007) All experiments were performed in accordance with institutional, national and international guidelines on animal care and welfare Data were statistically analysed using a two-way ANOVA with the factors treatment and time (for repeated measures) Post-hoc t-tests were performed where appropriate All values are expressed as mean ± SEM

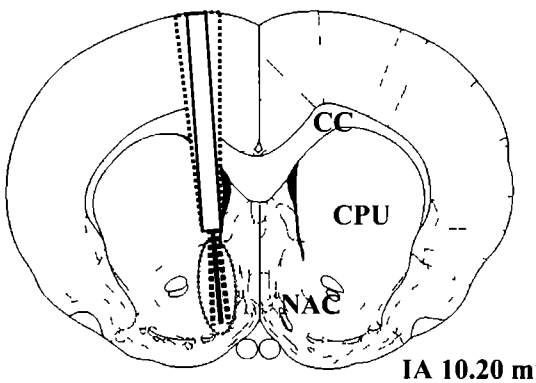


Figure 1: Example of 3 unilateral microdialysis probe tracks located in the right nucleus accumbens (see also: Verheij and Cools, 2007). The probe protrudes 2 mm below the distal end of the guide cannula The brain region in which correctly placed probes were found is indicated in grey IA corresponds to the distance (mm) from the interaural line according to Paxinos and Watson (1986), NAC Nucleus Accumbens, CPU caudate putamen, CC corpus callosum

Results

Thirty-seven per cent of the selected rats fulfilled the criteria for HR (n=61). The average distance travelled on the open-field was 8 976 ± 261 cm in 30 min The average habituation time of the animals was 1 384 ± 48 s Representative placements of the dialysis probes are depicted in Fig 1 (interaural level 10 20 mm) Probes were found between interaural levels 10 2 and 11 2 mm (Paxinos and Watson, 1986, Verheij and Cools, 2007) Only data of rats with correctly placed probes were incorporated in the

study (n=59). Basal levels of dopamine were 3.8 ± 0.5 pg / 20 min in HR treated with the solvent of AMPT (Ringer). The dose of 0.1 mM of AMPT reduced these levels of dopamine to 79 ± 3.1% at t = 40 min and to 81 ± 5.9% at t = 100 min (one sample t-test: 40 min: $t_{(7)}$ = -6.840, p≤0.001, 100 min: $t_{(7)}$ = -3.209, p=0.015; reductions similar to: Tuinstra and Cools, 2000b). Basal levels of dopamine were 2.9 ± 0.33 pg / 20 min in HR treated with the solvent of RES. The basal concentration of dopamine did not differ between rats that were treated with the solvent of AMPT or the solvent of RES (Student's t-test: ns). The dose of 1 mg/kg of RES reduced the basal levels of dopamine to 56 ± 8.3% at 24h (one sample t-test: 24 h: $t_{(17)}$ = -5.277, p≤0.001; reduction similar to: Verheij and Cools, 2007).

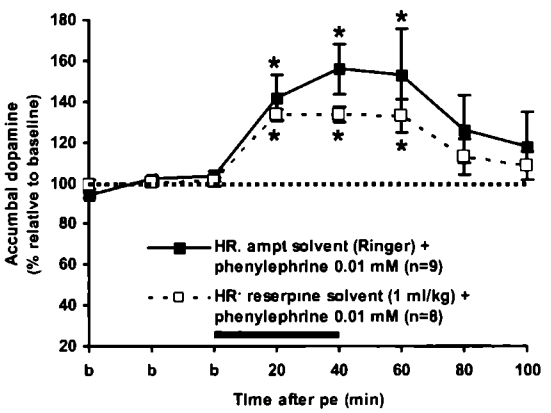


Figure 2: Effects of intra-accumbens infusion of 0.01 mM of the alpha-adrenoceptor agonist phenylephrine in rats treated with the solvent of either alpha-methyl-para-tyrosine (filled symbols) or reserpine (open symbols). Phenylephrine increased accumbal dopamine levels in these control rats The black line represents the infusion time of phenylephrine (40 min) * Significant increase relative to the baseline (one sample t-tests)

The dose of 0.01 mM of PE increased accumbal dopamine levels in rats treated with the solvent of AMPT (Fig. 2: one-way ANOVA: time effect: $F_{(7,56)}$ = 6.433, p≤0.001) and in rats treated with the solvent of RES (Fig. 2: one-way ANOVA: time effect: $F_{(7,49)}$ = 9.521, p≤0.001). The PE-induced increase of dopamine did not differ between the 2 groups of rats (Fig.2: two-way ANOVA: treatment (x time) effect: ns). PE decreased dopamine levels in rats that were treated with AMPT 40 min earlier (Fig. 3a: two-way ANOVA: treatment x time effect: $F_{(5,70)}$ = 3.688, p=0.005) and increased dopamine levels in rats that were treated with AMPT 100 min earlier (Fig. 3b: two-way ANOVA: treatment x time effect: $F_{(5,70)}$ = 10.496, p≤0.001). The PE-induced dopamine

increase in the latter group of AMPT-treated rats was equal to the PE-induced dopamine increase that was observed in the control rats that were treated with the solvent of AMPT (Fig. 3b vs Fig. 2: two-way ANOVA: treatment (x time effect): ns). PE increased dopamine levels in rats that were treated with RES 24 h earlier (Fig. 4: two-way ANOVA: treatment x time effect: $F_{(5,80)} = 8.964$, $p \leq 0.001$). The PE-induced dopamine increase in these RES-treated rats was equal to the PE-induced dopamine increase that was observed in the control rats that were treated with the solvent of RES (Fig. 4 vs Fig. 2: two-way ANOVA: treatment (x time effect) effect: ns).

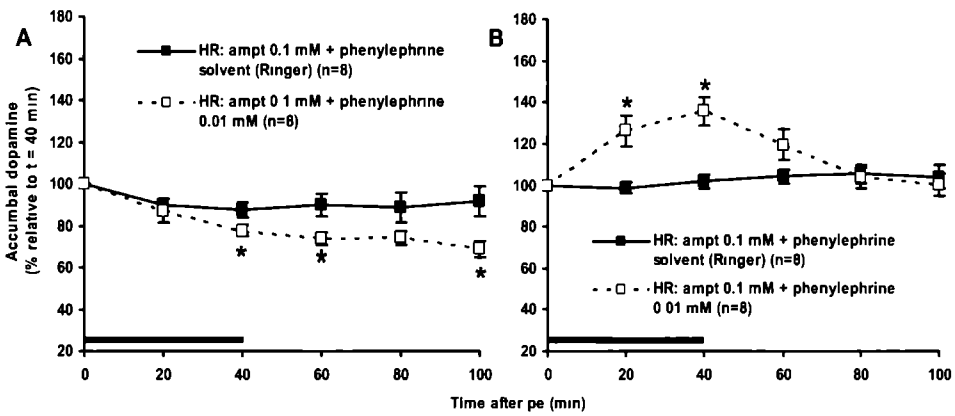


Figure 3: Effects of intra-accumbens infusion of 0.01 mM of the alpha-adrenoceptor agonist phenylephrine at 40 min (a) and 100 min (b) after 0.1 mM of alpha-methyl-para-tyrosine. Phenylephrine decreased accumbal dopamine levels 40 min after alpha-methyl-para-tyrosine The black line represents the infusion time of phenylephrine (40 min). * Significant decrease compared to control (Student's t-tests)

Discussion

The AMPT-induced changes in the baseline levels of accumbal dopamine at 40 and 100 min after its administration are very similar to our previously reported AMPT-induced changes in accumbal dopamine (Tuinstra and Cools, 2000b). The same holds true for the RES-induced changes in the baseline levels of accumbal dopamine at 24 h after its administration (Verheij and Cools, 2007). Our previous studies have shown no signs of an increased synthesis of dopamine at 40 min after AMPT or at 24 h after RES.

The alpha-1 adrenoceptor agonist PE (Ruffolo and Hieble, 1994) strongly increased accumbal dopamine levels in control rats (Fig. 2), confirming the outcome of our previous studies that PE acts at presynaptic alpha-adrenoceptors in the nucleus accumbens of otherwise untreated rats (Tuinstra and Cools, 2000a; Cools and Tuinstra, 2003; Aono et al., 2007). In contrast, PE decreased accumbal dopamine levels in rats that were treated with AMPT 40 min earlier (Fig. 3a). The possibility that PE did not increase dopamine release because the AMPT-sensitive dopamine pools of these rats were empty, can be rejected since alpha-adrenoceptors do not control the release of dopamine from this type of pool (Tuinstra and Cools, 2000b; Verheij and Cools, chapter 5; see also: Verheij and Cools, 2008). The present finding that PE reduced dopamine levels in HR treated with AMPT, therefore, demonstrates that this agonist stimulates postsynaptic alpha-adrenoceptors in these rats. This finding is in line with the fact that stimulation of accumbal postsynaptic alpha-adrenoceptors inhibits the release of dopamine from RES-sensitive pools (see introduction). Furthermore, these data confirm our hypothesis that AMPT increases the sensitivity of accumbal postsynaptic alpha-adrenoceptors (see introduction). The present study shows that noradrenaline that regulates the dopamine that is derived from RES-sensitive vesicles is derived from AMPT-sensitive pools. The finding that the PE-induced decrease of dopamine seen in rats treated 40 min earlier with AMPT (Fig. 3a) was replaced by a PE-induced increase of dopamine in rats treated 100 min earlier with AMPT (Fig. 3b) illustrates that the ability of AMPT to change the state of the alpha-adrenoceptors in the nucleus accumbens was time-dependent and reversible.

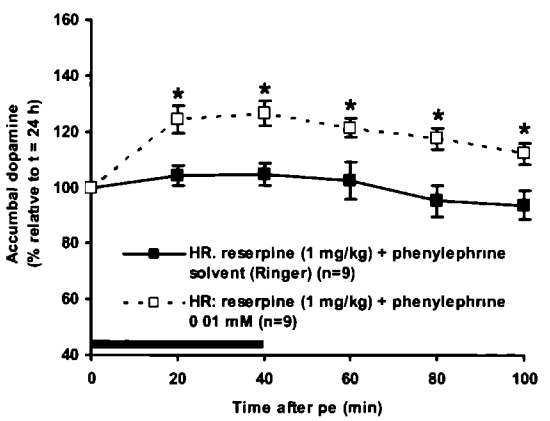


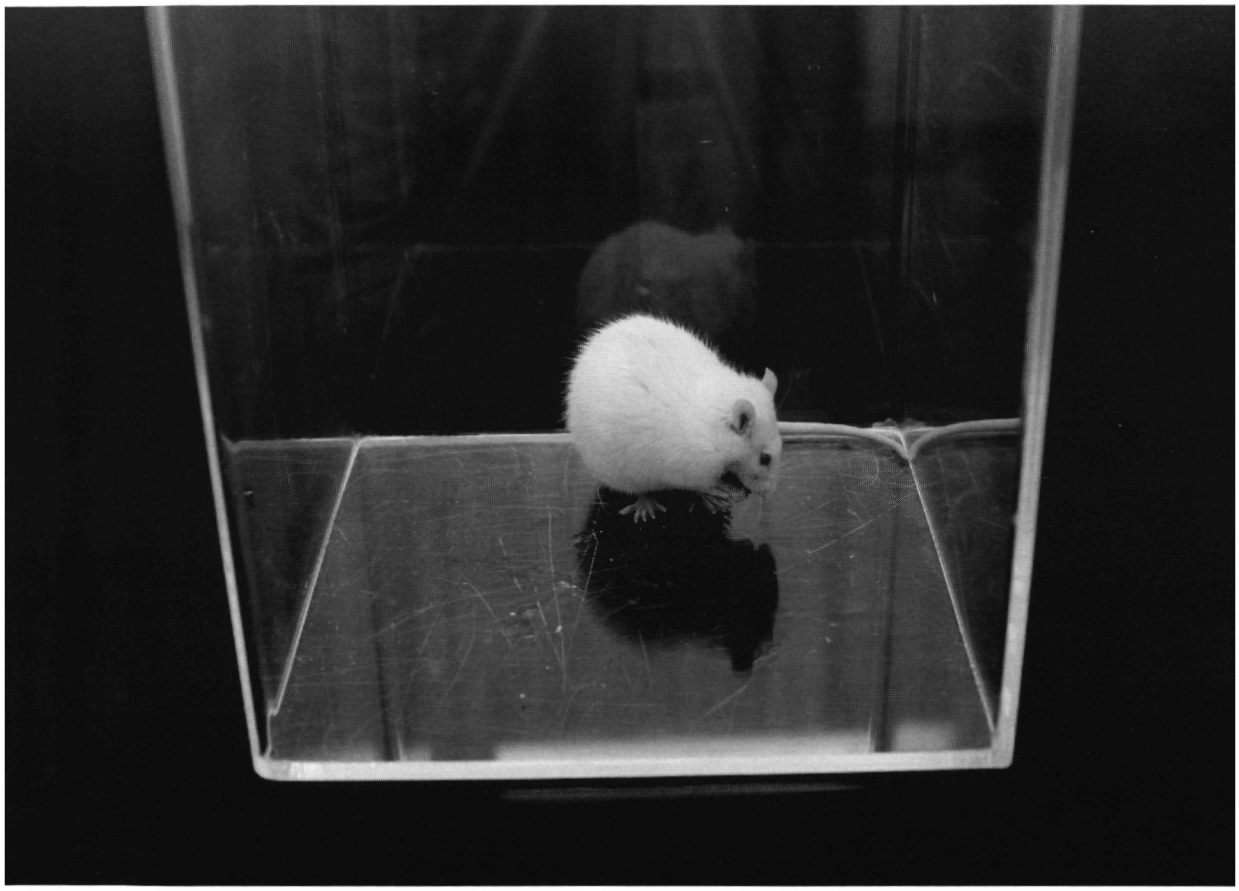
Figure 4: Effects of intra-accumbens infusion of 0.01 mM of the alpha-adrenoceptor agonist phenylephrine at 24 h after 1 mg/kg of reserpine. Phenylephrine increased accumbal dopamine levels 24 h after reserpine The black line represents the infusion time of phenylephrine (40 min). * Significant increase compared to control (Student's t-tests)

RES did not affect the ability of PE to increase the amount of accumbal dopamine (Fig 4) In fact, the PE-induced dopamine increase in the RES-treated rats was identical to the PE-induced dopamine increase that was observed in the control rats that were treated with the solvent of RES (Fig 4 vs Fig 2) These data demonstrate that PE acted at presynaptic alpha-adrenoceptors in these rats and that RES did not change the state of postsynaptic alpha-adrenoceptors Previous studies have provided evidence that alpha-adrenoceptors control the release of dopamine from RES-sensitive dopamine pools (Cools and Verheij, 2002; Verheij and Cools, chapter 6, see also Verheij and Cools, 2008) The present finding that PE still increased dopamine in RES-treated HR can be ascribed to the fact the applied dose of RES did not completely empty the dopaminergic storage vesicles in this type of rat (see: Verheij and Cools, chapter 6, Verheij et al , 2008) One could argue that the dose of 1 mg/kg of RES was too low to result in more sensitive postsynaptic adrenoceptors This explanation is quite unlikely because the applied dose of 1 mg/kg of RES strongly increased the sensitivity of the postsynaptic beta-adrenoceptors in the nucleus accumbens of HR (Verheij and Cools, chapter 6) The increase of the sensitivity of these beta-adrenoceptors can be explained by the well known action of RES to decreases accumbal noradrenaline levels (Pan et al , 1993) The notion that the noradrenaline that acts at accumbal alpha-adrenoceptors is not influenced by RES (Fig 4) whereas the noradrenaline that acts at accumbal beta-adrenoceptors is affected by this drug (Verheij and Cools, chapter 6) demonstrates that RES-induced changes in the overall amount of accumbal noradrenaline do not reflect the processes that actually take place at each distinct type of accumbal adrenoceptor. Measuring adrenoceptor-specific changes in accumbal dopamine have previously been found to be a valid and reliable method for reaching conclusions about the noradrenergic activity at the level of one single type of accumbal adrenoceptor (Tuinstra and Cools, 2000a) It is evident that for the present study, measuring adrenoceptor-specific changes in accumbal dopamine levels is preferred above measuring changes in the total amount of accumbal noradrenaline On the basis of the results obtained by this method we hypothesise that the pools that contribute to the release of noradrenaline at postsynaptic alpha-adrenoceptors of the nucleus accumbens are relatively insensitive to RES. The concept of a RES-resistant compartment of noradrenaline has also been suggested by

others (Kopin, 1964; Van Orden et al., 1970; Enna and Shore, 1974; Schwab and Thoenen, 1983; Yamazaki et al., 1997). Our data suggest that the compartment that releases noradrenaline at postsynaptic alpha-adrenoceptors in the nucleus accumbens contains AMPT-sensitive and newly-synthesised neurotransmitter that is not accumulated in RES-sensitive vesicles. It remains to be investigated whether this noradrenergic compartment consists of RES-resistant cytoplasmatic pools or RES-insensitive vesicles.

The data provide direct evidence for the previously reported hypothesis that AMPT can directly (by inhibiting dopamine synthesis) and indirectly (by inhibiting noradrenaline synthesis) change accumbal dopamine levels (Saigusa et al., 1999). The present findings reveal that compounds that directly or indirectly interact with accumbal alpha-adrenergic mechanisms should be considered as agents that can have important therapeutic effects in patients suffering from diseases in which accumbal dopamine is known to be involved. In this respect, it is important to mention that alpha-adrenergic agents have indeed been found to exert therapeutic effects in animal models of Parkinson's disease (Haapalinna et al., 2003) and addiction (Erb et al., 2000). Moreover, noradrenergic agents may also be used to treat patients with attention deficit hyperactivity disorder, schizophrenia and depression (Zhou, 2004; Buitelaar et al., 2007). This aspect is elaborated in detail elsewhere (Aono et al., 2007; Ikeda et al., 2007; for review see: Verheij and Cools, 2008).

Finally, it is important to realise that the direct and indirect therapeutic effects of noradrenergic agents strongly depend on the amount of endogenous noradrenaline in the synapse. In fact, the present study indicates that a noradrenergic agonist may have postsynaptic effects in individuals with low levels of endogenous noradrenaline whereas the same agonist may have presynaptic effects in individuals with high levels of this neurotransmitter.



9/15: Analyseren van gedrag (1/2). Rat vertoont 'grooming'.
Analysis of behaviour (1/2). Rat displays grooming.

Chapter 9

General discussion

M.M.M. Verheij and A.R. Cools (2008)

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1.1 Individual differences: accumbal noradrenaline-dopamine interactions: As already mentioned in the general introduction (section 3.2), Low Responders (LR) and High Responders (HR) are marked by a different functional activity of the noradrenergic systems of the nucleus accumbens. The results of these studies are summarised below and depicted in figure 1. The noradrenergic activity at the level of alpha adrenoceptors was found to be larger in non-challenged LR than in non-challenged HR (Tuinstra and Cools, 2000a; Cools and Tuinstra, 2003, see Fig. 1). These alpha adrenoceptors control the release of dopamine that is derived from reserpine-sensitive (Cools and Verheij, 2002; Verheij and Cools, chapter 6), but not from alpha-methyl-para-tyrosine-sensitive (Tuinstra and Cools, 2000b; Verheij and Cools, chapter 5), pools (see Fig. 1). Stimulation of alpha adrenoceptors decreases the release of this vesicular dopamine (Cools and Verheij, 2002; Verheij and Cools, chapter 6). In line with these data non-challenged LR were found to release less accumbal dopamine from storage pools than non-challenged HR (Verheij and Cools, 2007, see Fig. 1). Following exposure to novelty, the noradrenergic activity at the level of accumbal alpha adrenoceptors increases in HR and decreases in LR (Tuinstra and Cools, 2000a; Cools and Tuinstra, 2003, see Fig. 1). Accordingly, the dopamine release that is derived from storage pools decreases in novelty-challenged HR and increases in novelty-challenged LR (see Fig. 1).

The noradrenergic activity at the level of accumbal beta adrenoceptors was found to be equally low in non-challenged HR and non-challenged LR (Tuinstra and Cools, 2000a; Cools and Tuinstra, 2003, see Fig. 1). These beta adrenoceptors control the release of dopamine that is derived from alpha-methyl-para-tyrosine-sensitive (Tuinstra and Cools, 2000b; Verheij and Cools, chapter 5), but not from reserpine-sensitive (Cools and Verheij, 2002; Verheij and Cools, chapter 6), pools (see Fig. 1).

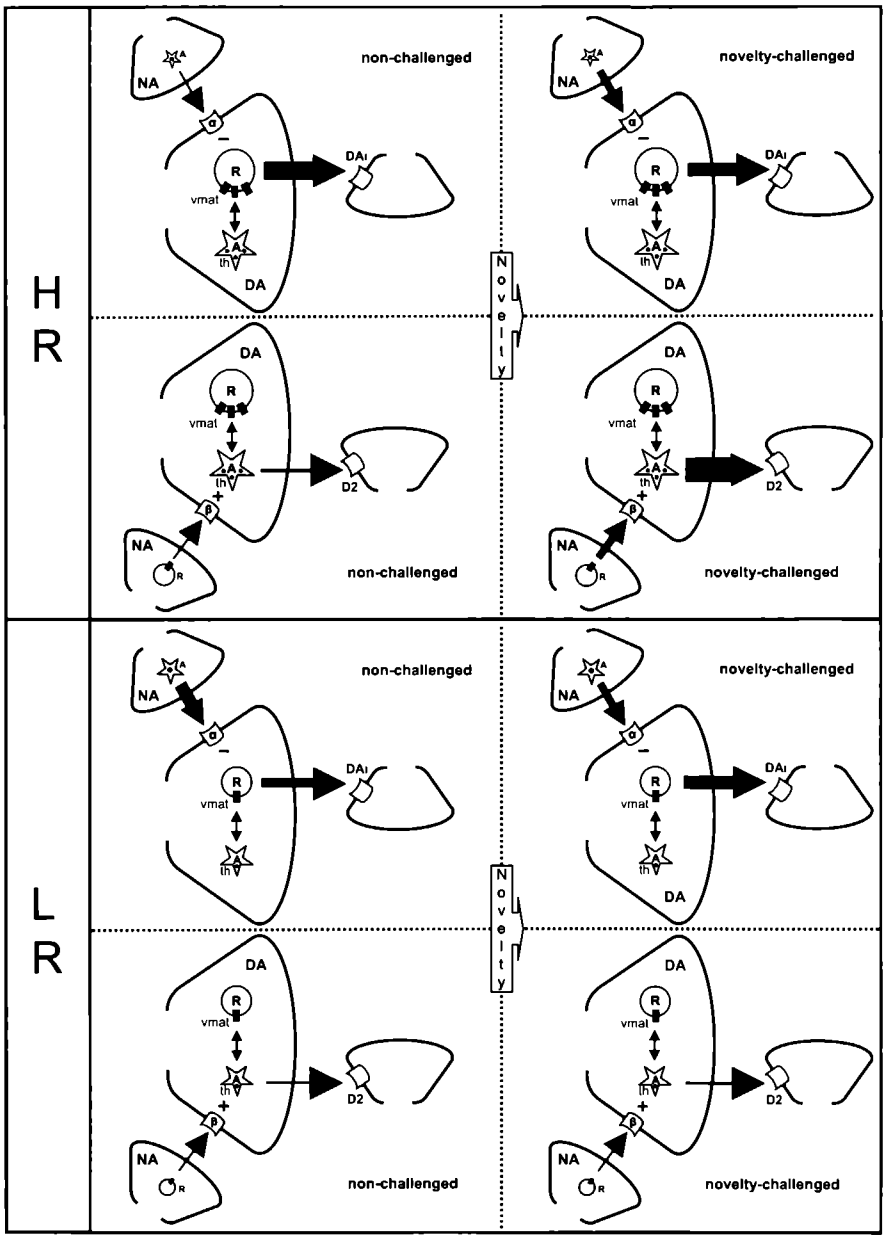


Figure 1: Summary of the noradrenaline-induced accumbal dopamine release in non-challenged (left) and novelty-challenged (right) High Responders (HR: top) and Low Responders (LR: bottom). Arrows indicate the release of noradrenaline at the level of alpha (α) or beta (β) adrenoceptors and the release of dopamine derived from reserpine-sensitive (R) or alpha-methyl-para-tyrosine-sensitive (A) pools. The dimension of the arrows represents the amount of neurotransmitter being released. NA, noradrenaline, DA, dopamine, ▮ = vmat, vesicular-mono-amine-transporters, ● = th, tyrosine hydroxylase, DA_1, inhibitory dopamine receptor; D_2, excitatory dopamine receptor.

Stimulation of beta adrenoceptors increases the release of this cytoplasmatic dopamine (Tuinstra and Cools, 2000b; Verheij and Cools, chapter 5). In line with these data, non-challenged HR were found to release as little accumbal dopamine from newly-synthesised pools as non-challenged LR (Verheij and Cools, 2007, see Fig. 1). Following exposure to novelty, the noradrenergic activity at the level of accumbal beta adrenoceptors increases in HR, but does not change in LR (Tuinstra and Cools, 2000a; Cools and Tuinstra, 2003, see Fig. 1). Accordingly, the dopamine release that is derived from newly-synthesised pools increases only in novelty-challenged HR, but not in novelty-challenged LR (see Fig. 1).

The noradrenaline that controls the alpha-adrenoceptor-mediated release of accumbal dopamine from reserpine-sensitive storage vesicles has been found to be derived from alpha-methyl-para-tyrosine-sensitive pools (Verheij and Cools, 2009, see Fig. 1). In contrast, the noradrenaline that controls the beta-adrenoceptor-mediated release of accumbal dopamine from alpha-methyl-para-tyrosine-sensitive pools has been found to be derived from reserpine-sensitive storage vesicles (Verheij and Cools, chapter 6, see Fig. 1). As elaborated elsewhere (Cools and Van Rossum, 1976; Cools and Van Rossum, 1980; Cools et al., 1991), the dopamine that is derived from reserpine-sensitive pools and under control of alpha adrenoceptors binds to the inhibitory dopamine (DAi) receptors whereas the dopamine that is derived from alpha-methyl-para-tyrosine sensitive pools and under control of beta adrenoceptors binds to the excitatory dopamine (D_2) receptors (see Fig. 1).

1.2 Status quo of the noradrenaline-dopamine interaction in the nucleus accumbens: Our data demonstrate that the overall noradrenergic activity of the nucleus accumbens is the sum of the functional activity of two individual accumbal noradrenergic systems (see Fig. 1). According to our data, the overall noradrenergic activity (sum of noradrenergic activity at alpha and beta adrenoceptors) is larger in non-challenged LR than in non-challenged HR (see Fig. 1). In fact, this was already suggested on the basis of the outcome of a number of pharmaco-behavioural studies in the nineties (Cools et al., 1990; Roozendaal and Cools, 1994; Cools et al., 1994; Cools and Gingras, 1998). Our data also demonstrate that the overall dopaminergic activity of

the nucleus accumbens is the sum of the functional activity of two individual accumbal dopaminergic systems. According to our data, the overall dopaminergic activity (sum of dopamine derived from reserpine-sensitive and from alpha-methyl-para-tyrosine-sensitive pools) is larger in non-challenged HR than in non-challenged LR (see Fig. 1). Neurochemical studies have, indeed, revealed that non-challenged HR are marked by higher basal levels of dopamine inside the nucleus accumbens than non-challenged LR (Hooks et al., 1992a; Verheij and Cools, 2007). Figure 1 illustrates that the overall noradrenergic activity increases in novelty-challenged HR and decreases in novelty-challenged LR. Again, this is nicely in line with the outcome of the pharmaco-behavioural studies demonstrating that challenges increase the noradrenaline receptor tonus in subjects with a low baseline noradrenergic activity (HR) and decrease the noradrenaline receptor tonus in subjects with a high baseline noradrenergic activity (LR) (Cools et al., 1987; Cools, 1988; Cools et al., 1991; Roozendaal and Cools, 1994; Cools et al., 1994; Cools and Gingras, 1998). Figure 1 also illustrates that the overall dopaminergic activity increases more strongly in novelty-challenged HR than in novelty-challenged LR. In fact, this was already demonstated in the nineties (Saigusa et al., 1999) and replicated later on (Verheij and Cools, 2007). According to figure 1, the relatively small novelty-induced dopamine increase in LR is due to an increased release from storage pools whereas the relatively large novelty-induced dopamine increase in HR is due to an increased release from newly-synthesised pools. This is nicely in line with the recently reported findings that the accumbal dopamine increase to novelty can be blocked by reserpine, but not by alpha-methyl-para-tyrosine, in LR and by alpha-methyl-para-tyrosine, but not by reserpine, in HR (Saigusa et al., 1999; Verheij and Cools, 2007).

1.3 Individual differences: quantal size of the dopamine pools: HR and LR not only differ in the release of accumbal dopamine, but also in the 'hardware' that is crucial for this release. It has been demonstrated that the nucleus accumbens of HR contains more vesicular monoamine transporters (type 2) than the nucleus accumbens of LR (Verheij et al., 2008). In addition, HR are marked by more dopamine inside accumbal storage vesicles than LR (Verheij et al., 2008). Since vesicular monoamine transporters

accumulate dopamine inside storage vesicles (see general introduction, section 2.1), the individual differences in the expression of these transporters may well account for the observed individual differences in the amount of dopamine inside vesicles. Figure 1 clearly illustrates that non-challenged HR release more accumbal dopamine from storage pools than either non-challenged or novelty-challenged LR. Our data, therefore, indicate that HR are marked by a larger reserpine-sensitive storage pool than LR, allowing these rats to release a sufficient amount of dopamine when they are at rest (see Fig. 1). It was also found that the nucleus accumbens of HR contains more tyrosine hydroxylase than the nucleus accumbens of LR (see general introduction, Fig. 4). Figure 1 illustrates that novelty-challenged HR release more dopamine from newly-synthesised pools than either non-challenged or novelty-challenged LR. Therefore, our data also indicate that HR are marked by a larger alpha-methyl-para-tyrosine-sensitive newly synthesised pool than LR, allowing these rats to release a sufficient amount of dopamine when they are challenged (see Fig. 1).

2.1 Factors that may underlie the accumbal noradrenaline-dopamine interactions: Below we will discuss various factors that may contribute to the individual differences that have been found so far (see sections 1.1-1.3). These factors are incorporated into the model that is presented in figure 2 (see sections 2.2-2.5).

2.2 Putative role of hypothalamic-pituitary-adrenal-axis: The fact that exposure to a challenge, like novelty, results in a higher and more prolonged response of adrenocorticotropic hormone and corticosterone in HR and apomorphine-susceptible (APO-SUS) rats than in LR and apomorphine-unsusceptible (APO-UNSUS) rats (Piazza et al., 1991a; Rots et al., 1995; Rots et al., 1996; Kabbaj et al., 2000; Kabbaj et al., 2007), indicates that the hypothalamic-pituitary-adrenal-axis of the first mentioned rats is more reactive to mild stressors than the hypothalamic-pituitary-adrenal-axis of the second mentioned rats (Cools et al., 1993b; Lucas et al., 1998). Adrenocorticotropic hormone and corticosterone secretion have been found to change both the sensitivity of mesolimbic adrenoceptors (Mobley and Sulser, 1980; Duman et al., 1985; Volosin et al., 1992) and the release of mesolimbic dopamine (Liang et al., 1992; Piazza et al., 1996)

indicating that the observed individual differences in the novelty-induced release of accumbal noradrenaline, and the subsequent changes in the release of accumbal dopamine, may be due to individual differences in the novelty-induced secretion of these hormones (Sutanto et al , 1989, Cools, 1991)

2.3 Putative role of second messengers: The fact that binding of noradrenaline at the level of alpha adrenoceptors results in opposite effects on dopamine release compared to binding of noradrenaline at the level of beta adrenoceptors suggests that these types of receptor are coupled to different second messenger systems Stimulation of beta adrenoceptors results in an increase of the formation of the second messenger cyclic adenosine 3',5' - monophosphate (cAMP) (George et al , 1970, Kuo et al , 1972, Lee et al , 1972, Guerrero et al , 1994) whereas stimulation of alpha adrenoceptors results in an increase of the formation of the second messenger cyclic guanosine 3',5' - monophosphate (cGMP) (Haidamous et al , 1980, Perchellet and Sharma, 1980, Gross and Ferrendelli, 1982, Volle and Quenzer, 1983) The release of catecholamines is positively coupled to cAMP and negatively coupled to cGMP (Westfall et al , 1976, Pelayo et al , 1978, Weiner, 1979, Derome et al , 1981, Schoffelmeer et al , 1985, Marchi et al , 1987, Drewett et al , 1989) Accordingly, the beta adrenoceptor mediated increase and the alpha adrenoceptor mediated decrease of dopamine in novelty-challenged HR is hypothesised to be the result of an increase of respectively cAMP and cGMP (see Fig 2) In addition, the alpha adrenoceptor mediated increase of dopamine in novelty-challenged LR is hypothesised to be the result of a reduction of cGMP (see Fig 2) The finding that the beta adrenoceptor mediated dopamine levels were not altered in novelty-challenged LR indicates that cAMP levels did not change in these rats (see Fig 2)

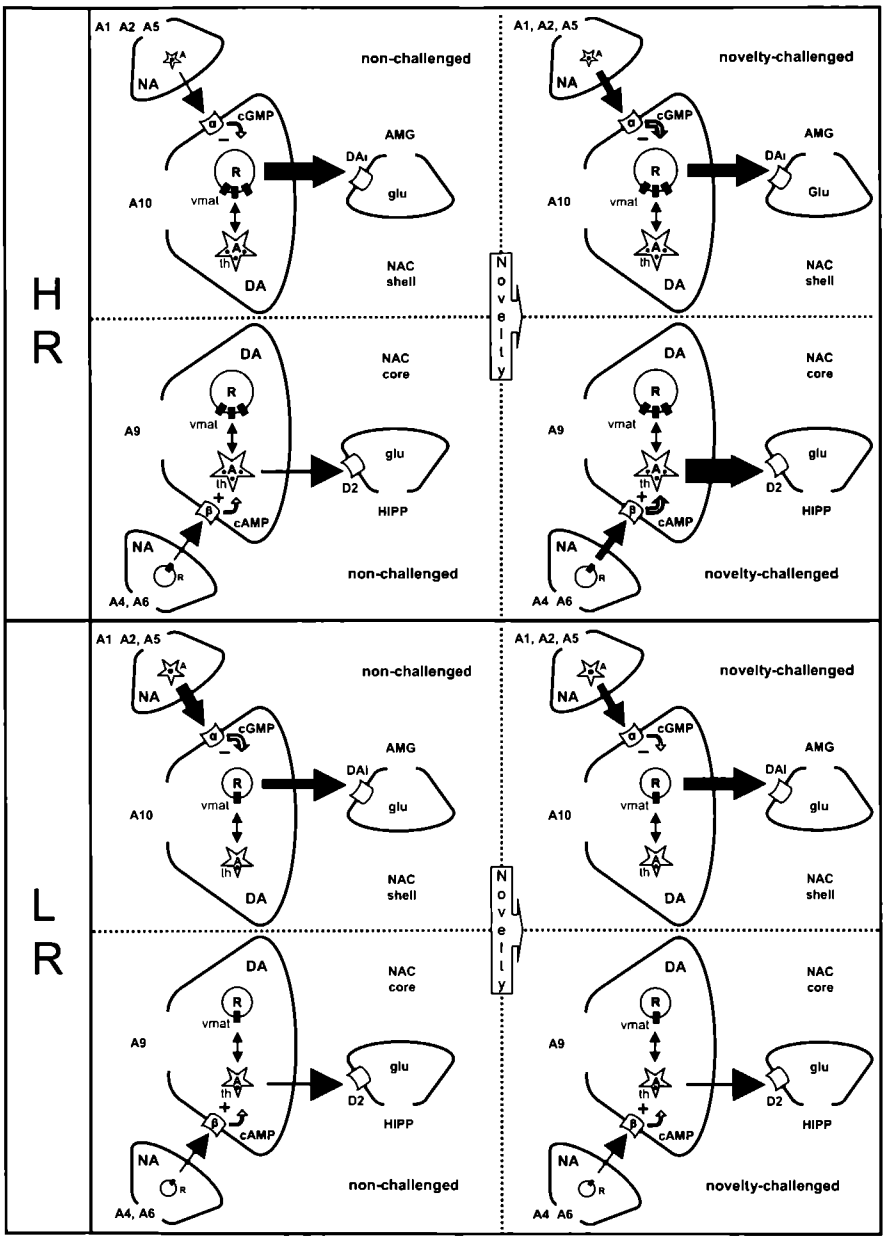


Figure 2: Model on the factors that may underlie the noradrenaline-induced accumbal dopamine release in non-challenged (left) and novelty-challenged (right) High Responders (HR: top) and Low Responders (LR: bottom). Straight arrows suggest the release of noradrenaline and dopamine in the nucleus accumbens shell and core glu glutamate, NAC, nucleus accumbens, AMG, amygdala, HIPP hippocampus, Ax, monoaminergic cell group The dimension of the curved arrows represents the amount of second messenger (cAMP or cGMP)

2.4 Putative target and origin of noradrenaline and dopamine: The fact that the dopamine release at the level of accumbal dopamine DAi receptors is different from the dopamine release at the level of accumbal dopamine D_2 receptors (Fig. 2) implies that the dopamine release in the nucleus accumbens is mediated by two distinct sets of dopaminergic neurons. Based on the localisation of the dopamine DAi and D_2 receptors in the nucleus accumbens one can predict both the target area and the origin of those dopaminergic neurons that contain either alpha or beta adrenoceptors. Pharmaco-behavioural studies, using drugs that selectively act at dopamine DAi receptors (Cools and Oosterloo, 1983; Struyker-Boudier and Cools, 1984), have demonstrated that the nucleus accumbens shell contains more dopamine DAi receptors than the nucleus accumbens core (Prinssen et al., 1994). These data nicely confirm the notion that dopamine DAi receptors are mainly found in those brain structures that are innervated by the A10 cells of the ventral tegmental area (Cools and Van Rossum, 1976; Cools, 1977; Cools and Van Rossum, 1980; Cools et al., 1989). The fact that the reserpine-sensitive dopamine release at these dopamine DAi receptors is under control of alpha adrenoceptors (see section 1.1) indicates that alpha adrenoceptors are mainly located in the shell. It is, therefore, suggested that mesolimbic alpha adrenoceptors are located on dopaminergic nerve terminals that arise in the ventral tegmental area and project to the nucleus accumbens shell (see Fig. 2). Recent binding studies, using drugs that selectively act at dopamine D_2 receptors, have demonstated that the nucleus accumbens core contains more dopamine D_2 receptors than the nucleus accumbens shell (Bardo and Hammer, 1991; Tremblay et al., 1999; Smith et al., 2005; Yaroslavsky et al., 2006). These data confirm the notion that dopamine D_2 receptors are mainly found in those brain structures that are innervated by the A9 cells of the substantia nigra (Cools and Van Rossum, 1976; Cools, 1977; Cools and Van Rossum, 1980; Cools et al., 1989). The fact that the alpha-methyl-para-tyrosine-sensitive dopamine release at these dopamine D_2 receptors is under control of beta adrenoceptors (see section 1.1) indicates that beta adrenoceptors are mainly located in the core. It is, therefore, suggested that mesolimbic beta adrenoceptors are located on dopaminergic nerve terminals that arise in the substantia nigra and project to the nucleus accumbens core (see Fig. 2).

The fact that the noradrenaline release at the level of accumbal alpha adrenoceptors is different from the noradrenaline release at the level of accumbal beta adrenoceptors (see Fig 2) implies that the noradrenaline release in the nucleus accumbens is mediated by two distinct sets of noradrenergic neurons In fact, pharmaco-behavioural evidence (Cools et al , 1991) has indicated that the noradrenaline that acts at the level of mesolimbic alpha adrenoceptors in the shell is released by neurons that arise in the A1, A2 and A5 cells groups of the ventral bundle (see Fig 2) In contrast, electrophysiological studies (Unemoto et al , 1985a, Unemoto et al , 1985b) have demonstrated that the noradrenaline that acts at the level of mesolimbic beta adrenoceptors in the core is released by neurons that arise in the A4 and A6 cell groups of the dorsal bundle (see Fig 2) Studies to investigate the intra-accumbal distribution of these alpha and beta adrenoceptors are currently under way

2.5 Evaluation of dopamine and noradrenaline in shell and core: The concept of a heterogeneous dopamine transmission within specific subregions of the nucleus accumbens (see section 2 4) is fully in line with the outcome of a recent study demonstrating that the accumbens contains specific subpopulations of dopaminergic neurons that are each marked by their own target area and release (Wightman et al , 2007) According to figure 2, neurotransmission in the shell is different form neurotransmission in the core Several groups have reported higher basal levels of dopamine in the shell than in the core (Iyaniwura et al , 2001, Shilliam and Heidbreder, 2003, Greenslade and Mitchell, 2004) These data nicely fit in with our model indicating that more dopamine is released in the shell than in the core of both non-challenged HR and non-challenged LR (see Fig 2) It was believed for a long time that acute exposure to a challenge increases accumbal dopamine levels (Rouge-Pont et al , 1993, Rouge-Pont et al , 1998, Saigusa et al , 1999, Brake et al , 1999) This general idea, however, is based on early microdialysis studies that did not take into account that the nucleus accumbens consists of particular subregions More recent microdialysis studies in which the probe was specifically directed to the shell of the nucleus accumbens have revealed that challenges may have dual effects on dopamine release One such a challenge is the so called tail-pinch, which has been shown to result in similar individual differences in

accumbal dopamine release as novelty does (Rouge-Pont et al , 1993, Rouge-Pont et al , 1998) A tail-pinch has been found to decrease dopamine in the shell of particular individuals (Di Chiara et al , 1999) In other individuals, however, a tail-pinch has been found to increase dopamine in the shell (Giorgi et al , 2003) These data underline the fact that the dopamine changes in the nucleus accumbens are individual-specific (see Fig 2) Interestingly, the tail-pinch-induced increase of dopamine in the shell was only found in rats that are marked by a low, but not a high, response to novelty (Giorgi et al , 2003) These data fit in nicely with our model indicating that novelty results in a dopamine increase in the shell of LR, but not of HR (see Fig 2) Figure 2 also illustrates that the beta adrenoceptor mediated release of dopamine in the core of HR increases after novelty This is nicely in line with studies demonstrating that the dopamine release in the core increases after stimulation of dopaminergic nerve terminals in this region (Wieczorek and Kruk, 1995, Jones et al , 1996, Garris et al , 1999) The fact that the beta adrenoceptor mediated dopamine release in the core of novelty-challenged LR does not increase is, most likely, due to the fact that noradrenaline did not stimulate the dopaminergic nerve terminals of this region (see Fig 2)

Studies on the role of noradrenaline in the shell and the core of the nucleus accumbens are limited to just one By the use of very sensitive microdialysis equipment, Mc Kittrick and Abercrombie (2007) have recently found that the basal amount of noradrenaline is larger in the shell than in the core The outcome of this study is nicely in line with our model (see Fig 2 LR) Studies focussing on the novelty-induced or tail-pinch-induced changes of noradrenaline in the two subregions of the accumbens have, up till now, not been performed

3.1 Consequence of individual differences in the noradrenaline-dopamine system: The accumbens is known to play a key role in a variety of behavioural processes (see general introduction, sections 2 8-2 10) Below we elaborate the consequences of the individual differences in the noradrenaline-dopamine system for just three functions, namely its role in the (drug-induced) acquisition and retrieval of spatial information, the locomotor response to novelty and in the neurochemical response to psychostimulants (see general introduction, sections 2 8-2 10) The fact that the make-up and reactivity of

the nucleus accumbens differs between HR and LR (see sections 1 1-1 3) indicates that HR respond differently to these environmental and pharmacological challenges than LR (see sections 3 2-3 5)

3.2 Acquisition and retrieval of spatial information: It has previously been shown that dopamine in the nucleus accumbens controls whether information sent by the amygdala or hippocampus will arrive in the accumbens or not (Cools et al , 1993a, Roozendaal and Cools, 1994) An increase of dopamine in the nucleus accumbens inhibits the arrival of glutamatergic information from the amygdala or hippocampus whereas a decrease of dopamine in the nucleus accumbens stimulates the arrival of this information from the amygdala or hippocampus The finding that the dopamine-dependent regulation of the amygdala gate is controlled by alpha adrenoceptors in the nucleus accumbens (Roozendaal and Cools, 1994, Tuinstra et al , 2002), implies that the state of the amygdala gate is mediated by accumbal dopamine that is released from reserpine-sensitive pools and binds to dopamine DA1 receptors (see Fig 2) The finding that the dopamine-dependent regulation of the hippocampus gate is controlled by beta adrenoceptors in the nucleus accumbens (Roozendaal and Cools, 1994, Tuinstra et al , 2000), implies that the state of the hippocampus gate is mediated by accumbal dopamine that is released from alpha-methyl-para-tyrosine-sensitive pools and binds to dopamine D_2 receptors Based on these data we hypothesise that the glutamatergic fibres that transfer information from the amygdala towards the accumbens mainly terminate in the shell whereas the glutamatergic fibres that transfer information from the hippocampus towards the accumbens mainly terminate in the core (see Fig 2)

The retrieval of spatial information in the Morris water maze task is mediated, among others, by beta adrenoceptors in the nucleus accumbens (Tuinstra et al , 2000) Challenged HR show a relatively bad retrieval of recently stored spatial information compared to challenged LR (Tuinstra et al , 2000) This phenomenon becomes understandable in view of the fact that the beta adrenoceptor mediated release of dopamine from alpha-methyl-para-tyrosine-sensitive pools increases at the dopamine D_2 receptors of challenged HR, but not of challenged LR (see Fig 2) reducing thereby glutamatergic information from the hippocampus to enter the accumbens of the first

mentioned rats. The acquisition of spatial information in the radial arm maze task is mediated, among others, by alpha adrenoceptors in the nucleus accumbens (Tuinstra et al., 2002). Challenged LR show a relatively bad acquisition of this spatial task compared to challenged HR (Tuinstra et al., 2002). This phenomenon may be explained by the fact that the alpha adrenoceptor mediated release of dopamine from reserpine-sensitive pools increases at the dopamine DAi receptors of challenged LR, but not of challenged HR (see Fig. 2) reducing thereby glutamatergic information from the amygdala to enter the accumbens of the first mentioned rats.

3.3 Locomotor response to novelty: Given the direct and indirect connections between the shell and the core (see general introduction, section 2.8), neurotransmission in the shell can control neurotransmission in the core. This means that shell regulated processes are in balance with core regulated processes. The shell is strongly involved in emotional processes whereas the core is strongly involved in motor processes (see general introduction, section 2.8). Exposure to novelty corresponds to a shell-regulated emotional process resulting in a core-regulated motor response. It is suggested that the novelty-induced increase of dopamine in the shell of LR inhibits/prevents the dopamine release in the core (see Fig. 2) resulting in a relatively low locomotor response to novelty in these rats. On the other hand, the novelty-induced decrease of dopamine in the shell of HR may disinhibit/permit the dopamine release in the core (see Fig. 2) resulting in a relatively high locomotor response to novelty in these rats. The notion that a dopaminergic balance is essential for the execution of normal behaviour was already proposed in the late seventies (Cools and Van Rossum, 1976; Cools, 1977; Cools and Van Rossum, 1980).

3.4 Neurochemical response to psychostimulants: The fact that HR are marked by more dopamine inside the presynaptic pools than LR (see Fig. 2) has far-reaching consequences for understanding the individual differences in sensitivity to psychostimulants. HR have been found to be more susceptible to the behavioural and dopamine-increasing effects of cocaine than LR (Hooks et al., 1991b; Hooks et al., 1992a; Giorgi et al., 1997; Chefer et al., 2003; Verheij and Cools, chapter 4; Verheij et

al , 2008) We have recently provided evidence that HR are more sensitive to cocaine than LR for the reason that cocaine can release more dopamine from accumbal storage vesicles in HR than in LR (Verheij et al , 2008) Not only the response to cocaine, but also the response to amphetamine, depends on presynaptic pools of dopamine As discussed in the general introduction (section 2 3), the type of dopamine pool that is involved is depending on the dose of amphetamine The response to moderate doses of amphetamine primarily depends on dopamine inside newly-synthesised pools Like the behavioural response to cocaine, the behavioural response to moderate doses of amphetamine has also been found to be larger in HR than in LR (Piazza et al , 1989, Hooks et al , 1994, Gingras and Cools, 1997, Cools et al , 1997, Bevins et al , 1997) The individual differences in sensitivity to amphetamine are hypothesised to be the result of the fact that HR are marked by a larger alpha-methyl-para-tyrosine-sensitive pool than LR (see Fig 2) Studies in this respect are currently under way

3.5 Neurochemical response to psychostimulants: accumbens shell and core: A single injection of psychostimulants more strongly increases dopamine levels in the nucleus accumbens shell than in the nucleus accumbens core (see general introduction, section 2 8) The psychostimulant-induced dopamine release in the shell decreases whereas the psychostimulant-induced release of dopamine in the core increases in rats that have previously been treated with these drugs (Giorgi et al , 2005, Giorgi et al , 2007) The data of both acute and repeated administration of psychostimulants are in line with the above-mentioned notion that the neurotransmission of the shell controls the neurotransmission in the core (see section 3 3) To be more specific, a psychostimulant-induced increase of dopamine in the shell may inhibit/prevent the psychostimulant-induced release of dopamine in the core whereas a psychostimulant-induced decrease of dopamine in the shell may disinhibit/permit the psychostimulant-induced release of dopamine in the core The fact that the maximum psychostimulant-induced dopamine levels in the shell after a single injection and the maximum psychostimulant-induced dopamine levels in the core after repeated injections both reach the same value (Giorgi et al , 2005, Giorgi et al , 2007) indicates that the presynaptic dopamine pools in the shell are of the same size as the presynaptic dopamine pools in the core (see Fig 2) Which

factors contribute to the psychostimulant-induced shift from dopamine release in the shell to dopamine release in the core (see also Everitt and Robbins, 2005) remains open for speculation The fact that the same shift has been found in HR as a consequence of an increase of accumbal noradrenaline (see Fig 2), suggests that psychostimulant-induced increases of noradrenaline may play an important role in this shifting between the two brain regions

4.1 Therapeutic effects of reserpine and alpha-methyl-para-tyrosine-like agents: The fact that the response to cocaine depends on reserpine-sensitive pools (Verheij and Cools, chapter 4, Verheij et al , 2008) opens the intriguing possibility that drugs that deplete dopaminergic storage vesicles, especially in the mesolimbic neurons, might become the drugs of choice for the treatment of cocaine abuse Interestingly, recent clinical screening trials on the effects of reserpine in cocaine-addicted subjects have already revealed promising results in this respect (Gorelick et al , 2004, Berger et al , 2005) The fact that the effects of moderate doses of amphetamine are depending on alpha-methyl-para-tyrosine-sensitive pools (see general introduction, section 2 3) opens the intriguing possibility that drugs that inhibit the synthesis of dopamine, especially in the mesolimbic neurons, might become the drugs of choice for the treatment of amphetamine abuse

4.2 Therapeutic effects of noradrenergic agents: Given that alpha adrenoceptors modulate dopamine release from reserpine-sensitive pools (see Fig 2), we hypothesise that, in addition to manipulation of reserpine-sensitive pools, manipulation with adrenergic alpha receptor agents might be a good tool to treat cocaine abuse In fact, it has recently been found that systemic alpha adrenoceptor agonists block the stress-induced reinstatement of cocaine seeking in rats (Erb et al , 2000) Given that beta adrenoceptors modulate dopamine release from alpha-methyl-para-tyrosine-sensitive pools (see Fig 2-3), we hypothesise that, in addition to manipulation of alpha-methyl-para-tyrosine-sensitive-sensitive pools, manipulation with adrenergic beta receptor agents might be a good tool to treat amphetamine abuse In fact, various studies have already found that systemic beta adrenoceptor antagonists block the effects of

amphetamine in rats (Mantegazza et al , 1968, Estler and Ammon, 1971, Berridge and Morris, 2000, Colussi-Mas et al , 2005)

Parkinsonian symptoms are seen in rats that are treated with high doses of reserpine (Gershanik et al , 1983, Colpaert, 1987, Cooper et al , 1987) These symptoms are, likely, the result of a diminished release of dopamine from storage pools Systemic administration of alpha adrenoceptor antagonists is known to reduce Parkinsonian symptoms in the 6-hydroxydopamine animal model of Parkinson's disease (Mavridis et al , 1991, Chopin et al , 1999, Haapalinna et al , 2003, Srinivasan and Schmidt, 2004, Lane et al , 2005) On the basis of our data, we suggest that alpha adrenoceptor antagonists have therapeutic effects in 6-hydroxydopamine models of Parkinson's disease because these agents can increase the release of dopamine from those reserpine-sensitive storage pools that are not affected by 6-hydroxydopamine On the basis of these findings, we suggest that adrenergic alpha receptor antagonists have also therapeutic effects in patients with Parkinson's disease Beta adrenoceptor antagonists have been found to alleviate the positive symptoms that are related to schizophrenia (Yorkston et al , 1977, Lindstrom and Persson, 1980, Hanssen et al , 1980, Sethi and Dube, 1983, Pugh et al , 1983, Eccleston et al , 1985) Positive symptoms are known to be mediated by dopamine release from amphetamine-sensitive pools (Angrist et al , 1971, Angrist et al , 1974, Angrist et al , 1980, Crow, 1980, Ellenbroek et al , 1989, Laruelle et al , 1996, Abi-Dargham et al , 1998, Angrist et al , 2001) Given that the release of dopamine from amphetamine-sensitive pools is reduced by treatment with alpha-methyl-para-tyrosine (see general introduction, section 2 3), we hypothesise that adrenergic beta receptor antagonists reduce the positive symptoms of schizophrenia because these agents reduce the dopamine release from newly-synthesised pools In addition to these drugs that act at alpha and beta adrenoceptors, drugs that inhibit the re-uptake of noradrenaline have also been found to be therapeutically effective in patients suffering from diseases in which the mesolimbic dopaminergic system is disturbed In fact, noradrenaline transporter inhibitors may be used to treat patients with attention deficit hyperactivity disorder and depression (Zhou, 2004, Buitelaar et al , 2007) Additional studies, not only focussing on the therapeutic effects of noradrenergic agents, but also on the possible side-effects of these agents, are required

10/15: Analyseren van gedrag (2/2). Rat vertoont 'rearing'.
Analysis of behaviour (2/2). Rat displays rearing.

Chapter 10

Summary

1. Overall aim

Individual differences in both the sensitivity to stress and the response to drugs of abuse have extensively been reported Individual differences in the reactivity of the dopaminergic system of the nucleus accumbens have frequently been used to explain these individual differences (see introductory **chapter 1**) The factors underlying the individual differences in the reactivity of accumbal dopamine are poorly understood In this thesis experiments are described to investigate the factors that contribute to the individual differences in the reactivity of accumbal dopamine in rats Subjects of all experiments were Low Responders (LR) and High Responders (HR) to novelty These animals were selected from an outbred population of Wistar rats using a novel open-field LR are animals that travel a small distance on the open-field and habituate to this novel environment relatively fast whereas HR are animals that travel a large distance on the open-field and habituate to this novel environment relatively slow It has previously been reported that both exposure to novelty (mild stressor) and exposure to cocaine increase accumbal dopamine levels more strongly in HR than in LR

Dopamine can be released from two distinct pools, which are presynaptically located in the nerve terminal One pool contains newly-synthesised dopamine whereas the other pool contains dopamine that is stored The overall aim of the studies described in this thesis was to investigate whether the individual differences in the reactivity of accumbal dopamine are due to individual differences in the release of this newly-synthesised and/or stored dopamine

The release of accumbal dopamine can be inhibited by different drugs Alpha-methyl-para-tyrosine was used to inhibit the release of newly-synthesised dopamine whereas reserpine was used to inhibit the release of previously stored dopamine The results presented in this thesis demonstrate that LR and HR are differentially sensitive to the dopamine decreasing effects of both alpha-methyl-para-tyrosine and reserpine Interestingly, the effects of these drugs were found to depend on the condition the rats

are exposed to The collected data were incorporated in a model that explains the putative 'hardware and software' underlying the individual differences in accumbal dopamine release in LR and HR (see overall conclusion below)

It has been shown that LR and HR not only differ in their sensitivity to cocaine, but also in their sensitivity to amphetamine and alcohol In addition, previous studies have revealed that HR can be used as an animal model for schizophrenia whereas LR can be used as an animal model for Parkinson's disease Dopamine is known to play a key role in the pathogenesis of these diseases Unravelling the mechanisms underlying individual differences in the dopaminergic system may help to understand why particular individuals suffer from 'dopaminergic diseases' whereas others do not, and why particular dopaminergic agents have therapeutic or side effects in one group of patients, but not in another

2. Summary per chapter

The aim of the experiments described in **chapter 2** was to investigate whether the above-mentioned individual differences in the reactivity of the mesolimbic dopamine system are due to individual differences in the reactivity of the two types of dopamine pool Accordingly, LR and HR were treated with reserpine or alpha-methyl-para-tyrosine and accumbal dopamine release was measured The experiments were performed in rats that were either novelty-challenged or fully habituated The experiments demonstrated that habituated HR are marked by higher basal levels of dopamine in the nucleus accumbens than habituated LR These results were explained by the finding that habituated HR release more dopamine from storage vesicles than habituated LR As expected, HR were marked by a larger novelty-induced increase of dopamine in the nucleus accumbens than LR Reserpine prevented the novelty-induced increase of accumbal dopamine in LR, demonstrating that novelty-challenged LR release accumbal dopamine from storage vesicles Because alpha-methyl-para-tyrosine did not affect the novelty-induced increase of accumbal dopamine in these rats, we concluded that novelty-challenged LR do not release accumbal dopamine from pools of newly-synthesised dopamine Given that reserpine did not affect the novelty-induced increase

of accumbal dopamine in HR, we concluded that novelty-challenged HR do not release accumbal dopamine from storage vesicles. The finding that alpha-methyl-para-tyrosine prevented the novelty-induced increase of accumbal dopamine in these rats demonstated that novelty-challenged HR release accumbal dopamine from pools of newly-synthesised dopamine.

From the studies described in the general introduction (see chapter 1) it can be concluded that the effects of drugs of abuse are depending on presynaptic pools of neurotransmitter. It has previously been shown that the cocaine-induced dopamine increase in the neostriatum is depending on reserpine-sensitive storage pools. In addition to the neostriatum, the nucleus accumbens plays an important role in the effects of drugs of abuse as well. The aim of the experiments described in **chapter 3** was to investigate whether accumbal storage pools play a role in the dopamine increasing effects of cocaine. Accordingly, cocaine-treated LR and HR were pre-treated with reserpine and accumbal dopamine release was measured. The dose of 1 mg/kg of reserpine prevented the cocaine-induced accumbal dopamine increase in LR, but not in HR. In fact, the higher dose of 2 mg/kg of reserpine was required to inhibit the cocaine-induced accumbal dopamine increase in HR. These data indicate that drugs that are known to empty dopaminergic storage vesicles may have therapeutic effects in the treatment of cocaine abuse. LR were also found to display smaller amounts of dopamine inside the reserpine-sensitive storage vesicles of the nucleus accumbens than HR. These individual differences in the dopaminergic vesicular storage capacity could be ascribed to the fact that the nucleus accumbens of LR displays lower levels of both tyrosine hydroxylase (see general introduction, Fig. 4) and vesicular-monoamine-transporters (see chapter 3) than the nucleus accumbens of HR. Finally, we confirmed the original finding of Hooks et al. (1991b) that cocaine increases accumbal dopamine levels more strongly in HR than in LR. The individual differences in accumbal dopamine levels after cocaine could not be explained by individual differences in the re-uptake of dopamine. Our data indicate that HR are more sensitive to the dopamine increasing effects of cocaine than LR, because cocaine can release more accumbal dopamine from storage vesicles in HR than in LR.

The aim of the experiments described next, was to investigate whether the individual differences in sensitivity to reserpine not only result in individual differences in the neurochemical response to cocaine, but also in the behavioural response to this drug **Chapter 4** describes the behavioural effects of reserpine in cocaine-treated LR and HR Cocaine increased walking and normal rearing more strongly in HR than in LR The dose of 1 mg/kg of reserpine reduced these cocaine-induced behavioural items in LR, but not in HR The higher dose of 2 mg/kg of reserpine was required to inhibit cocaine-induced walking and normal rearing in HR These behavioural data nicely fit in with the neurochemical data that higher doses of reserpine are required to change the accumbal dopamine response to cocaine in HR than in LR (see chapter 3) It was hypothesised that HR are more sensitive to the locomotor effects of cocaine than LR, because cocaine can release more accumbal dopamine from storage vesicles in HR than in LR

The above-mentioned studies focused on the role of dopamine in the nucleus accumbens The fact that noradrenaline regulates the release of dopamine (see general introduction chapter 1), indicates that noradrenergic drugs may have therapeutic effects in patients with a disturbed dopaminergic system At this moment, little is known about the interactions between noradrenaline and dopamine in the brain The remaining part of this thesis, therefore, focused on the noradrenaline-dopamine interactions in the nucleus accumbens The studies below are based on our previously reported finding that stimulation of accumbal alpha-adrenoceptors inhibits accumbal dopamine release, in contrast to stimulation of accumbal beta-adrenoceptors that stimulates the release of accumbal dopamine The presynaptic pools that are involved in this noradrenaline-induced release of dopamine were still unknown The aim of the experiments described in **chapter 5** was to investigate whether alpha-methyl-para-tyrosine-sensitive pools are involved in the noradrenaline-induced dopamine release Alpha-methyl-para-tyrosine prevented the accumbal dopamine release evoked by the beta-adrenoceptor agonist isoproterenol, but not by the alpha-adrenoceptor antagonist phentolamine These data demonstrate that mesolimbic beta-, but not alpha-adrenoceptors control accumbal dopamine release from pools of newly-synthesised dopamine The experiments of chapter 5 also indicated that presynaptic alpha-adrenoceptors are located on

noradrenergic nerve-terminals that impinge on those dopaminergic neurons that contain postsynaptic alpha-, but not beta-adrenoceptors

The aim of the experiments described in **chapter 6** was to investigate whether reserpine-sensitive pools are, like alpha-methyl-para-tyrosine-sensitive pools, involved in the noradrenaline-induced dopamine release as well Reserpine reduced the accumbal dopamine release evoked by the alpha-adrenoceptor antagonist phentolamine, but not by the beta-adrenoceptor agonist isoproterenol These data demonstrate that mesolimbic alpha-, but not beta-adrenoceptors control accumbal dopamine release from storage pools The dose of 1 mg/kg of reserpine prevented the phentolamine-induced increase of accumbal dopamine completely in LR, but only partially in HR These results were explained by the above-mentioned finding that 1 mg/kg of reserpine empties the accumbal storage vesicles of LR, but not of HR (see chapter 3)

Based on the results of chapter 6 it was hypothesised that the behavioural effects of phentolamine are depending on previously stored dopamine In **chapter 7** experiments were described demonstrating that reserpine counteracts the behavioural response to phentolamine, but not to isoproterenol The behavioural data were ascribed to the fact that mesolimbic alpha-, but not beta-adrenoceptors mediate accumbal dopamine release from storage vesicles

The experiments of chapter 6 indicated that the accumbal noradrenaline that controls the beta-adrenoceptor-mediated release of newly-synthesised dopamine is derived from reserpine-sensitive storage vesicles In **chapter 8** we showed that alpha-methyl-para-tyrosine, but not reserpine, made accumbal postsynaptic alpha-adrenoceptors more sensitive to the alpha-adrenoceptor agonist phenylephrine This study confirmed our previously reported hypothesis that the accumbal noradrenaline that controls the alpha-adrenoceptor-mediated release of previously stored dopamine is derived from alpha-methyl-para-tyrosine-sensitive pools

In summary, the data described in the chapters 5-8 illustrate that the interaction between noradrenaline and dopamine in the nucleus accumbens is not only mediated by different adrenoceptors (alpha-receptors and beta-receptors), but also by different pools of neurotransmitter (AMPT-sensitive and RES-sensitive) in both dopaminergic and noradrenergic nerve terminals

3. Overall conclusion

It is concluded that the individual differences in accumbal dopamine release are due to individual differences in 1) the functional reactivity of the noradrenergic system, 2) the accumbal concentration of vesicular-monoamine-transporters and tyrosine hydroxylase and 3) the size of the presynaptic dopamine pools As elaborated elsewhere, individual differences in the reactivity and make-up of the monoaminergic systems of the nucleus accumbens are due to a combination of both (early) environmental and genetic factors (Ellenbroek et al , 2000, Ellenbroek and Cools, 2002)

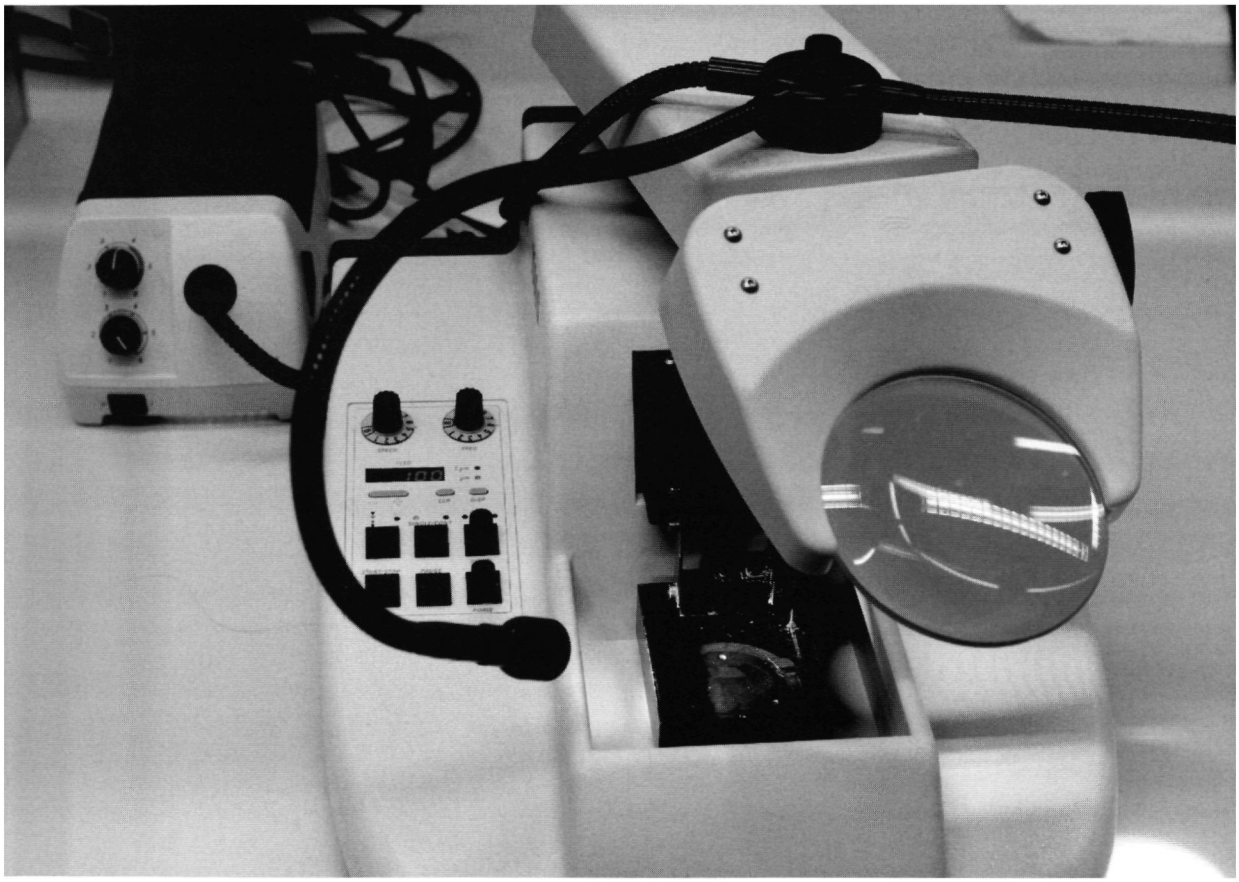
The collected data were summarised in figure 1 of the general discussion (see page 173) This figure shows that HR are marked by a larger amount of dopamine inside the presynaptic pools of the nucleus accumbens than LR (see chapter 3) The functional activity of these presynaptic pools of accumbal dopamine is controlled by noradrenaline The release of dopamine from reserpine-sensitive storage pools is controlled by alpha-adrenoceptors (see chapter 6), whereas beta-adrenoceptors control the release of dopamine from alpha-methyl-para-tyrosine-sensitive pools of newly-synthesised dopamine (see chapter 5) HR are marked by a low noradrenergic activity that increases during exposure to novelty resulting in a relatively large dopamine increase derived from alpha-methyl-para-tyrosine-sensitive pools (see chapter 2) In contrast, LR are marked by a high noradrenergic activity that decreases during exposure to novelty resulting in a relatively small dopamine increase derived from reserpine-sensitive pools (see chapter 2) These data were used to develop the model that is depicted in figure 2 of the general discussion (see page 178) The model combines the results of our studies with the available knowledge about the neuroanatomy, neurochemistry and neuropharmacology of the nucleus accumbens It was hypothesised that accumbal alpha-adrenoceptors are

postsynaptic receptors of neurons that arise in the adrenergic A1, A2 and A5 regions and that accumbal beta-adrenoceptors are postsynaptic receptors of neurons that arise in the adrenergic A4 and A6 regions. Moreover, alpha-adrenoceptors were suggested to be located on dopaminergic nerve terminals of neurons that arise from the A10 region and innervate the shell whereas beta-adrenoceptors were suggested to be located on dopaminergic nerve terminals of neurons that arise from the A9 region and innervate the core. The region-specific distribution of adrenoceptors may be responsible for the fact the dopamine release in the shell differs from the dopamine release in the core (see **chapter 9**).

4. Impact

The HR/LR dichotomy is not only limited to rats, but is present across a great variety of species (Ellenbroek and Cools, 2002). HR rats have been described as a valid animal model of the human sensation seeker, whereas LR rats have been described as a valid animal model of the human non-sensation seeker (Dellu et al., 1996b; Cools and Ellenbroek, 2002; Kabbaj, 2006). Human sensation seekers are more sensitive to the effects of cocaine than human non-sensation seekers (Ball et al., 1994b). Our studies suggest that individual differences in the make-up and reactivity of the nucleus accumbens may play an important role in the individual-specific responses to cocaine in man. Individual differences in the aminergic systems of the nucleus accumbens may explain why some individuals perish under conditions that other individuals flourish (Cools et al., 1993b). Studies using HR and LR may also help to understand why particular individuals are more vulnerable to develop 'dopaminergic diseases' than others and why some patients are more sensitive to dopaminergic drugs or develop more side-effects to these drugs than others.

Based on our studies it is suggested that noradrenergic agents as well as agents that interact with vesicular-monoamine-transporters (reserpine) or tyrosine hydroxylase (alpha-methyl-para-tyrosine) may have therapeutic effects in subjects that are suffering from addiction, schizophrenia or Parkinson's disease. The fact that the make-up and reactivity of both the noradrenergic and the dopaminergic system is individual-specific indicates that these disorders must be treated by individual instead of group therapy. Future studies should focus not only on the therapeutic effects, but also on the side effects of noradrenergic agents.



11/15: Coupes snijden met behulp van een vibratoom.
Cutting brain sections using a vibratome.

Chapter 11

Samenvatting

1. Algemeen doel

Individuele verschillen in de respons op stress alsmede individuele verschillen in de gevoeligheid voor verslavende middelen zijn veelvuldig beschreven Vele studies hebben aangetoond dat deze individuele verschillen het gevolg zijn van verschillen in de afgifte van de neurotransmitter dopamine in een hersenstructuur met de naam nucleus accumbens (zie **hoofdstuk 1**) De factoren die ten grondslag liggen aan deze individuele verschillen in de dopamine afgifte in de nucleus accumbens waren onbekend Dit proefschrift beschrijft een groot aantal experimenten om de factoren die bijdragen aan de individuele verschillen in de afgifte van dopamine in de nucleus accumbens van ratten te achterhalen Er is gebruik gemaakt van zogenaamde Low Responders (LR) en High Responders (HR) to novelty Deze ratten worden geselecteerd op basis van de exploratie respons in een nieuwe omgeving (= novelty) LR leggen een kleine aftand af in een nieuwe omgeving en gaan al vroeg stil zitten (= korte habituatie tijd), terwijl HR een grote afstand afleggen in een nieuwe omgeving en pas laat stil gaan zitten (= lange habituatie tijd) Zowel de introductie van een nieuwe omgeving als een injectie met cocaine resulteren in een grotere toename van dopamine in de nucleus accumbens van HR dan in de nucleus accumbens van LR Dopamine wordt afgegeven uit twee verschillende compartimenten Deze pools zijn gelegen in het zenuwuiteinde (= presynaptisch) en bestaan uit recent gesynthetiseerde of reeds opgeslagen dopamine Het algemene doel van dit proefschrift was om te onderzoeken of de individuele verschillen in de toename van dopamine in de nucleus accumbens het gevolg zijn van individuele verschillen in de afgifte van dopamine uit de twee presynaptische pools

Er zijn verschillende stoffen die de dopamine afgifte beinvloeden Alpha-methyl-para-tyrosine remt de afgifte van recent gesynthetiseerde dopamine, terwijl reserpine de afgifte van voorheen opgeslagen dopamine remt De experimenten beschreven in dit proefschrift tonen aan dat HR en LR verschillen in de gevoeligheid

voor zowel alpha-methyl-para-tyrosine als reserpine. De effecten van deze farmaca op de dopamine afgifte bleken afhankelijk van de conditie waaraan de rat was blootgesteld. De verkregen gegevens vormen de basis voor een model dat de factoren beschrijft die ten grondslag liggen aan de individuele verschillen in de dopamine afgifte in de nucleus accumbens van HR en LR (zie algemene conclusie hieronder).

LR en HR verschillen niet alleen in gevoeligheid voor cocaïne, maar ook in de gevoeligheid voor andere verslavende middelen zoals amphetamine en alcohol. Het is in het verleden gebleken dat HR een goed diermodel zijn om schizofrenie te onderzoeken, terwijl LR eerder verschijnselen van de ziekte van Parkinson ontwikkelen. Bij al deze ziekten speelt dopamine een sleutelrol. Het ontrafelen van de mechanismen die ten grondslag liggen aan individuele verschillen in het dopamine systeem is van groot belang om te achterhalen waarom slechts bepaalde mensen bovengenoemde ziekten krijgen of waarom in bepaalde mensen een behandeling aanslaat (of juist leidt tot bijwerkingen) en in andere mensen niet.

2. Samenvatting per hoofdstuk

Het doel van de studie beschreven in **hoofdstuk 2** was om te onderzoeken of de hierboven genoemde individuele verschillen in de reactiviteit van het mesolimbische dopamine systeem het gevolg zijn van verschillen in de reactiviteit van de twee dopamine pools. We hebben gekeken naar de effecten van reserpine en alpha-methyl-para-tyrosine op de dopamine afgifte in de nucleus accumens van LR en HR. De effecten van deze stoffen zijn onderzocht in gehabitueerde ratten en in ratten in een nieuwe omgeving. Uit de experimenten is gebleken dat gehabitueerde HR gekenmerkt worden door een grotere dopamine concentratie in de nucleus accumbens dan gehabitueerde LR. Deze bevinding kon worden toegeschreven aan het feit dat de afgifte van dopamine uit opslag pools groter is in gehabitueerde HR dan in gehabitueerde LR. Er is eveneens aangetoond dat de introductie in een nieuwe kooi (= novelty) resulteert in een grotere dopamine afgifte in de nucleus accumbens van HR dan in de nucleus accumbens van LR. Uit de bevinding dat reserpine de novelty-geïnduceerde dopamine afgifte in LR kon remmen, blijkt dat de dopamine ten gevolge van een nieuwe omgeving in LR

voornamelijk wordt vrijgemaakt uit opslag pools Omdat de novelty-geinduceerde dopamine afgifte in deze ratten niet kon worden geremd door alpha-methyl-para-tyrosine, hebben we geconcludeerd dat de dopamine ten gevolge van een nieuwe omgeving in LR niet wordt vrijgemaakt uit pools met nieuw gesynthetiseerde neurotransmitters Uit de bevinding dat alpha-methyl-para-tyrosine de novelty-geinduceerde dopamine afgifte in HR kon remmen, blijkt dat dopamine ten gevolge van een nieuwe omgeving in HR voornamelijk wordt vrijgemaakt uit pools met nieuw gesynthetiseerde neurotransmitters Omdat de novelty-geinduceerde dopamine afgifte in deze ratten niet kon worden geremd door reserpine, hebben we geconcludeerd dat de dopamine ten gevolge van een nieuwe omgeving in HR niet wordt vrijgemaakt uit opslag pools Deze studie toont aan dat de bron waaruit dopamine wordt vrijgemaakt 1) individuspecifiek is en 2) afhankelijk is van de conditie waaraan het individu is blootgesteld

Uit de studies die staan beschreven in hoofdstuk 1 blijkt dat verslavende middelen een beroep doen op presynaptische pools met neurotransmitters Het is bijvoorbeeld gebleken dat de cocaine-geinduceerde dopamine toename in de hersenstructuur met de naam neostriatum afhankelijk is van reserpine-gevoelige pools Naast het neostriatum speelt de nucleus accumbens ook een grote rol bij de effecten van verslavende middelen Het was onbekend of de reserpine-gevoelige pools in de nucleus accumbens ook een rol spelen in de dopamine afgifte ten gevolge van cocaine Het doel van de studie beschreven in **hoofdstuk 3** was om de effecten van reserpine op de cocaine-geinduceerde dopamine toename in de nucleus accumbens van LR en HR te onderzoeken De dosis van 1 mg/kg reserpine voorkwam de cocaine-geinduceerde dopamine toename in LR, maar had geen effect in HR In HR was een dosis van 2 mg/kg reserpine nodig om de dopamine toename ten gevolge van cocaine te remmen Deze resultaten geven aan dat farmaca die resulteren in lege dopaminerge opslag pools mogelijkerwijs een therapeutisch effect hebben in de behandeling van cocaine verslaving Ook is aangetoond dat LR kleinere hoeveelheden dopamine opslaan in de reserpine-gevoelige opslag pools van de nucleus accumbens dan HR Deze individuele verschillen in opslagcapaciteit kunnen worden toegeschreven aan het feit dat de nucleus

accumbens van HR meer tyrosine hydroxylase (zie introductie, Fig 4) en meer vesiculaire-monoamine transporters bevat dan de nucleus accumbens van LR Tot slot hebben de experimenten van hoofdstuk 3 laten zien dat cocaine leidt tot een sterkere verhoging van de dopamine in de nucleus accumbens van HR dan in de nucleus accumbens van LR (zie algemeen doel hierboven) Deze individuele verschillen in de gevoeligheid voor cocaine konden niet worden verklaard met individuele verschillen in de heropname van dopamine Onze gegevens suggereren dat cocaine-behandelde HR een grotere dopamine toename in de nucleus accumbens laten zien dan cocaine-behandelde LR omdat cocaine meer dopamine kan mobiliseren uit de grote opslag pools in HR dan uit de kleine opslag pools in LR

Het doel van de volgende studie was om te onderzoeken of de individuele verschillen in gevoeligheid voor reserpine niet alleen leiden tot individuele verschillen in de neurochemische effecten, maar ook tot individuele verschillen in de gedragseffecten van cocaine In **hoofdstuk 4** zijn de effecten van reserpine op de cocaine-geinduceerde gedragsrespons van LR en HR beschreven Er is gebleken dat cocaine leidt tot een sterkere toename van horizontale en verticale activiteit in HR dan in LR Deze toename in gedrag werd geremd door de dosis van 1 mg/kg reserpine in LR, maar niet in HR In HR was een dosis van 2 mg/kg reserpine nodig om de horizontale en verticale activiteit te inhiberen Deze gedragsmatige gegevens ondersteunen de hierboven beschreven neurochemische gegevens van hoofdstuk 3 Onze gegevens duiden erop dat cocaine-behandelde HR meer activiteit vertonen dan cocaine-behandelde LR omdat cocaine meer dopamine kan mobiliseren uit de grote opslag pools in de nucleus accumbens van HR dan uit de kleine opslag pools in de nucleus accumbens van LR

De hierboven beschreven studies zijn gericht op de rol van dopamine in de nucleus accumbens Het feit dat noradrenaline de afgifte van dopamine reguleert (zie hoofdstuk 1), betekent dat noradrenerge farmaca therapeutische effecten kunnen hebben bij patienten waarbij het dopamine systeem is aangedaan Momenteel is er nog maar weinig bekend over de noradrenaline-dopamine interacties in het brein Het resterende deel van dit proefschrift richt zich op de interactie tussen noradrenaline en dopamine in

de nucleus accumbens. De studies zijn gebaseerd op onze bevinding dat stimulatie van noradrenerge alpha-receptoren de dopamine afgifte in de nucleus accumbens remt, terwijl stimulatie van noradrenerge beta-receptoren de afgifte van dopamine in de nucleus accumbens bevordert. De presynaptische pools die betrokken zijn bij de noradrenaline-geïnduceerde dopamine afgifte waren onbekend. Het doel van de studie beschreven in **hoofdstuk 5** was om te onderzoeken of alpha-methyl-para-tyrosine-gevoelige pools een rol spelen bij de noradrenaline-geïnduceerde dopamine afgifte. Alpha-methyl-para-tyrosine remde de dopamine afgifte ten gevolge van de beta-receptor-agonist isoproterenol, maar niet de dopamine afgifte ten gevolge van de alpha-receptor-antagonist phentolamine. Deze experimenten tonen aan dat de beta-, maar niet de alpha-, receptoren in de nucleus accumbens de afgifte van nieuw gesynthetiseerde dopamine reguleren. De experimenten van hoofdstuk 5 hebben ook geleid tot de suggestie dat presynaptische alpha-receptoren van de nucleus accumbens zijn gelegen op de noradrenerge zenuwuiteinden die eindigen op de dopaminerge neuronen die postsynaptische alpha-, maar geen beta-, receptoren bevatten.

Het doel van de studie beschreven in **hoofdstuk 6** was om te onderzoeken of de reserpine-gevoelige pool ook een rol speelt bij de noradrenaline-geïnduceerde dopamine afgifte. Reserpine remde de dopamine afgifte ten gevolge van de alpha-receptor-antagonist phentolamine, maar niet de dopamine afgifte ten gevolge van de beta-receptor-agonist isoproterenol. Deze experimenten tonen aan dat de alpha-, maar niet de beta-, receptoren van de nucleus accumbens de afgifte van voorheen opgeslagen dopamine reguleren. De dopamine afgifte ten gevolge van phentolamine kon volledig worden geremd door de dosis van 1 mg/kg reserpine in LR, maar niet in HR. Deze resultaten kunnen worden verklaard door de bevinding dat 1 mg/kg reserpine resulteert in lege opslag pools in de nucleus accumbens van LR, maar niet in lege opslag pools in de nucleus accumbens van HR (zie hoofdstuk 3).

Het doel van de volgende studie was om te onderzoeken of niet alleen de neurochemische effecten, maar ook de gedragseffecten van phentolamine afhankelijk zijn van de afgifte van voorheen opgeslagen neurotransmitters. Uit **hoofdstuk 7** bleek

dat reserpine de gedragsrespons ten gevolge van phentolamine, maar niet de gedragsrespons ten gevolge van isoproterenol, kon remmen. De gedragsmatige bevindingen zijn toegeschreven aan het feit dat de alpha-, maar niet de beta-, receptoren in de nucleus accumbens de dopamine afgifte uit reserpine-gevoelige opslag pools reguleren (zie ook hoofdstuk 6).

De experimenten van hoofdstuk 6 duiden er op dat de noradrenaline die aangrijpt op de postsynaptische beta-receptoren van de nucleus accumbens afkomstig is uit reserpine-gevoelige pools. In **hoofdstuk 8** hebben we aangetoond dat alpha-methyl-para-tyrosine, maar niet reserpine, de postsynaptische alpha-receptoren van de nucleus accumbens gevoeliger maakt voor de alpha-receptor-agonist phenylephrine. Deze studie bevestigt onze voorheen beschreven hypothese dat de noradrenaline die aangrijpt op de alpha-receptoren van de nucleus accumbens afkomstig is uit alpha-methyl-para-tyrosine-gevoelige pools. De gegevens beschreven in hoofdstuk 5-8 illustreren dat de interactie tussen noradrenaline en dopamine in de nucleus accumbens niet alleen het gevolg is van inhibitie of stimulatie van verschillende noradrenerge receptoren (alpha- of beta-receptoren), maar ook van afgifte uit verschillende pools (gevoelig voor alpha-methyl-para-tyrosine of reserpine) die gelegen zijn in zowel dopaminerge als noradrenerge neuronen.

3. Algemene conclusie

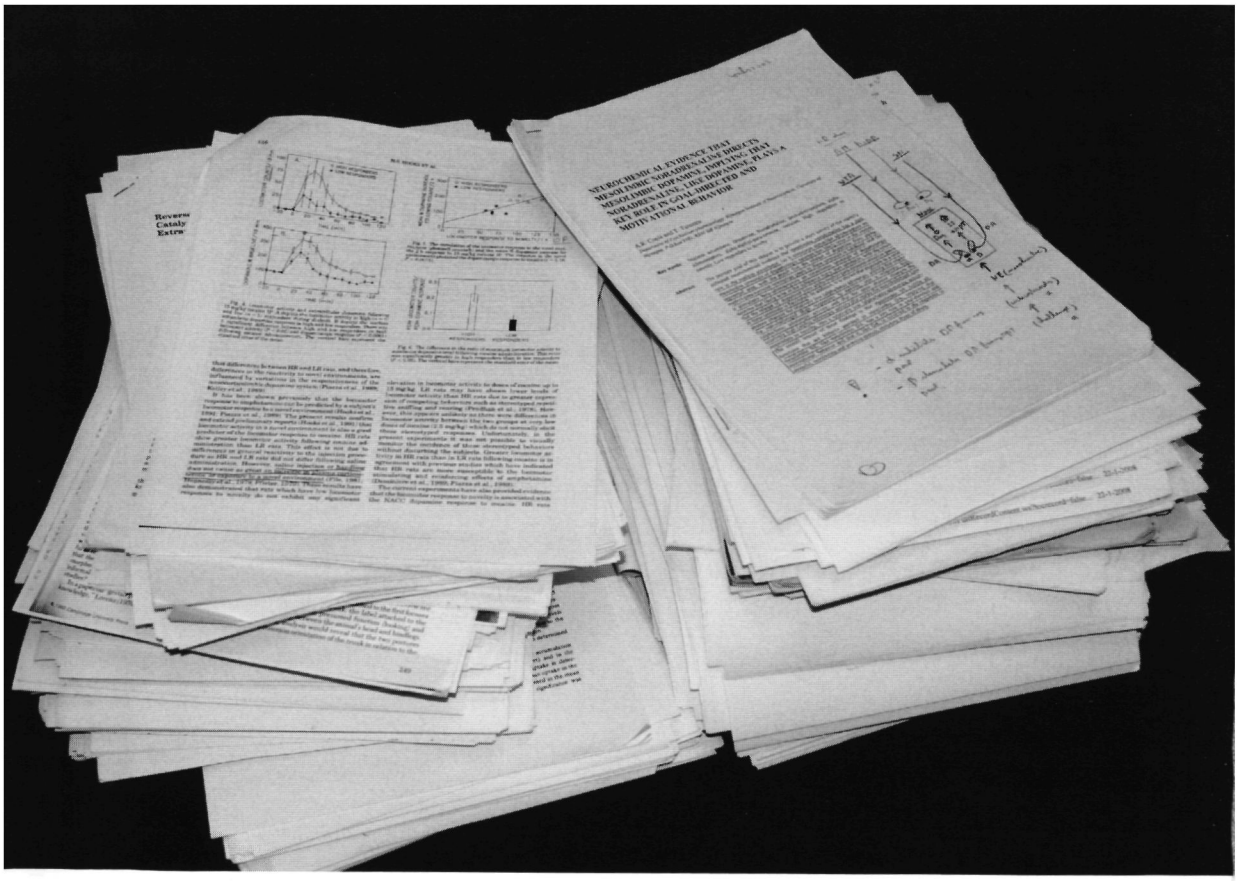
Op basis van bovenstaande informatie kunnen we concluderen dat de individuele verschillen in de afgifte van dopamine in de nucleus accumbens onder andere het gevolg zijn van 1) de functionele reactiviteit van het noradrenerge systeem, 2) de hoeveelheid tyrosine hydroxylase en de hoeveelheid vesiculaire monoamine transporters en 3) de grootte van de presynaptische dopamine pools. Deze individuele verschillen in de reactiviteit en opmaak van het monoaminerge systeem van de nucleus accumbens zijn waarschijnlijk het gevolg van zowel (postnatale) omgevingsfactoren als genetische factoren.

Zoals beschreven in hoofdstuk 3 en samengevat in figuur 1 van de algemene discussie (pagina 173), zijn HR gekenmerkt door een grotere hoeveelheid dopamine in de presynaptische pools van de zenuwuiteinden van de nucleus accumbens dan LR De functionele activiteit van deze presynaptische dopamine pools staat onder controle van noradrenaline De afgifte van dopamine uit reserpine-gevoelige opslag pools staat onder controle van alpha-receptoren (zie hoofdstuk 6), terwijl de afgifte van dopamine uit nieuw gesynthetiseerde pools van neurotransmitters onder controle staat van beta-receptoren (zie hoofdstuk 5) HR worden gekenmerkt door een lage noradrenerge tonus welke toeneemt in een nieuwe omgeving Dit heeft een grote afgifte van dopamine uit pools die gevoelig zijn voor alpha-methyl-para-tyrosine tot gevolg (zie ook hoofdstuk 2) LR worden gekenmerkt door een hoge noradrenerge tonus welke afneemt in een nieuwe omgeving Dit heeft een kleine afgifte van dopamine uit pools die gevoelig zijn voor reserpine tot gevolg (zie ook hoofdstuk 2) Deze gegevens vormen de basis voor het model dat is weergegeven in figuur 2 van de algemene discussie (pagina 178) Het model combineert de resultaten die zijn beschreven in dit proefschrift met de huidige stand van zaken over de neuroanatomie, neurochemie en neurofarmacologie van de nucleus accumbens Het model suggereert dat de noradrenerge neuronen die eindigen op de zenuwen die postsynaptische alpha-receptoren bevatten, ontspringen in de A1, A2 en A5 regio's van het brein en dat de noradrenerge neuronen die eindigen op de zenuwen die postsynaptische beta-receptoren bevatten, ontspringen in de A4 en A6 regio's van het brein De alpha-receptoren worden verondersteld zich te bevinden op de zenuwuiteinden van de dopaminerge neuronen, die ontspringen in de A10 regio van het brein en de shell van de accumbens innerveren De beta-receptoren worden verondersteld zich te bevinden op de zenuwuiteinden van de dopaminerge neuronen, die in de A9 regio van het brein ontspringen en de core van de accumbens innerveren Deze regiospecifieke distributie van noradrenerge receptoren speelt mogelijk een rol bij de verschillen in dopamine afgifte in de shell versus de core (zie **hoofdstuk 9**)

4. Impact

De HR/LR verdeling is niet alleen aanwezig in ratten, maar is ook gevonden in mensen. Zo zijn HR ratten beschreven als een diermodel voor mensen die sensatie zoeken, terwijl LR ratten zijn beschreven als een diermodel voor mensen die sensatie mijden. De effecten van cocaïne zijn groter in sensatie zoekers dan in niet-sensatie zoekers. Onze resultaten suggereren dat individuele verschillen in de opmaak en reactiviteit van de accumbens een grote rol spelen bij de individu-specifieke respons op cocaïne bij mensen. Individuele verschillen in de reactivitcit van de accumbens kunnen verklaren waarom sommige individuen opbloeien onder bepaalde omstandigheden, terwijl andere individuen onder deze omstandigheden falen. Studies met HR en LR kunnen bovendien inzicht verschaffen in de vraag waarom slechts een bepaalde groep mensen 'dopaminerge aandoeningen' krijgt of waarom in bepaalde mensen een dopaminerge behandeling aanslaat (of juist leidt tot bijwerkingen) en in andere mensen niet.

Wij voorspellen dat noradrenerge farmaca alsmede farmaca die interfereren met tyrosine hydroxylase (alpha-methyl-para-tyrosine) en vesiculaire monoamine transporters (reserpine) een therapeutisch effect hebben in de behandeling van verslaving, schizofrenie en de ziekte van Parkinson. De aanwezigheid van individuele verschillen in de opmaak en reactiviteit van zowel het noradrenerge als het dopaminenerge systeem suggereert dat individuele therapie van deze ziekten valt te prefereren boven groepstherapie. Het is aan te bevelen de therapeutische effecten van noradrenerge farmaca alsmede de eventuele bijwerkingen van deze stoffen in de toekomst verder te onderzoeken.



12/15: Herevaluatie van de literatuur.
Re-evaluation of the literature.

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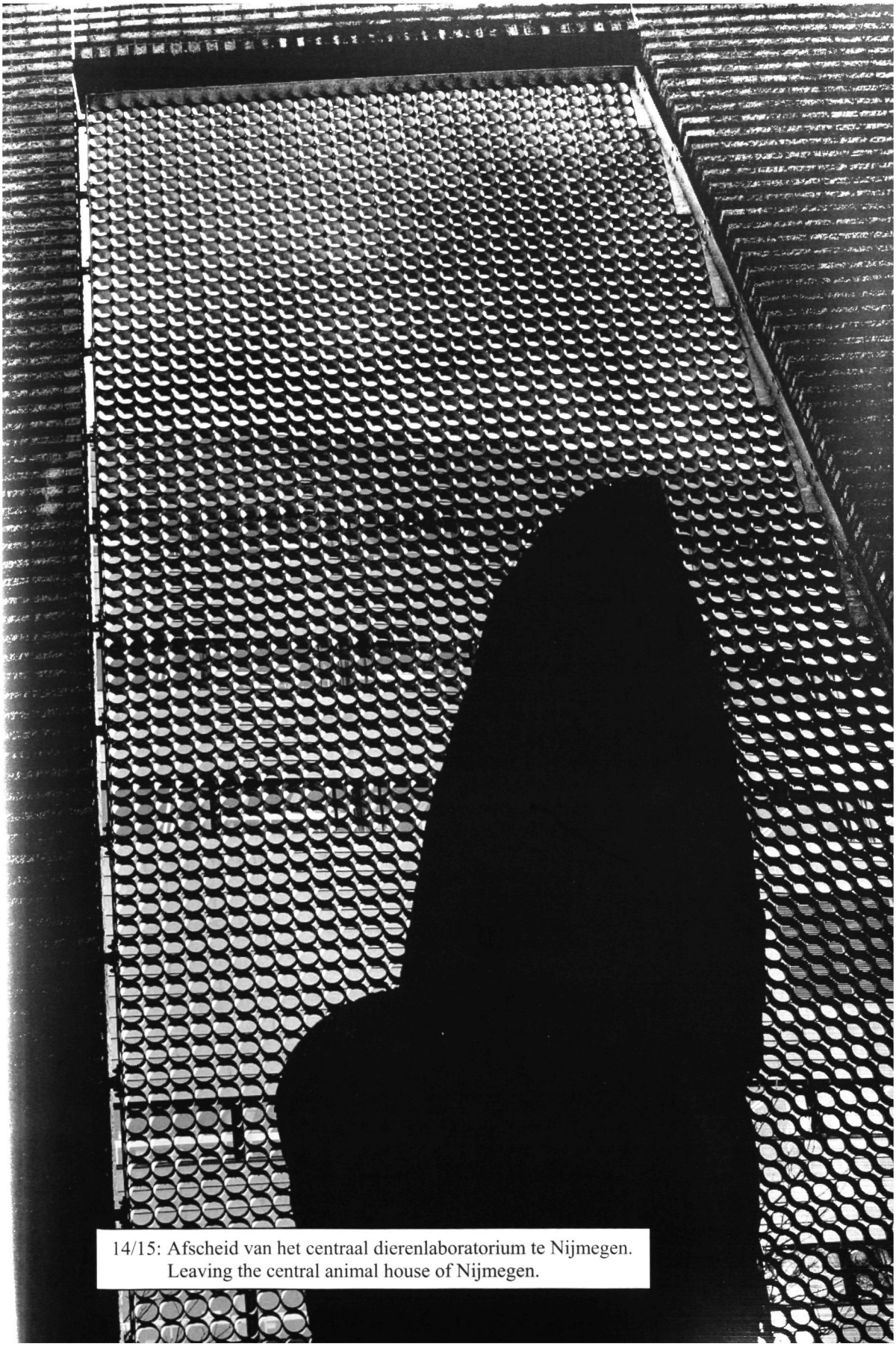
Curriculum Vitae

Michel Verheij was born on the first of December 1974 in Silvolde, the Netherlands. In 1994, he graduated from the Isala College (VWO) in Silvolde. In that same year, he continued his education at the Hoger Laboratorium Onderwijs (HLO) in Utrecht to become a research technician. His internship was performed at the department of Psychoneuropharmacology of the Radboud University of Nijmegen. The research project concerned the neurobiology of individual differences in the serotonergic system of rats. In 1998 he obtained his BS in Zoology. From 1998 until 2003 he was appointed as a research technician at the department of Psychoneuropharmacology. In 2003, he became a PhD student at the same department. The research conducted during this period is summarised in this thesis. In addition, he was involved in an animal study to investigate the working mechanism of a potential new anti-Parkinson agent. Since August 2008, he is employed as a postdoctoral researcher at the department of Molecular Animal Physiology of the Radboud University of Nijmegen.



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15/15: Verdedigen van dit proefschrift.
Defence of this thesis.

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Notes: