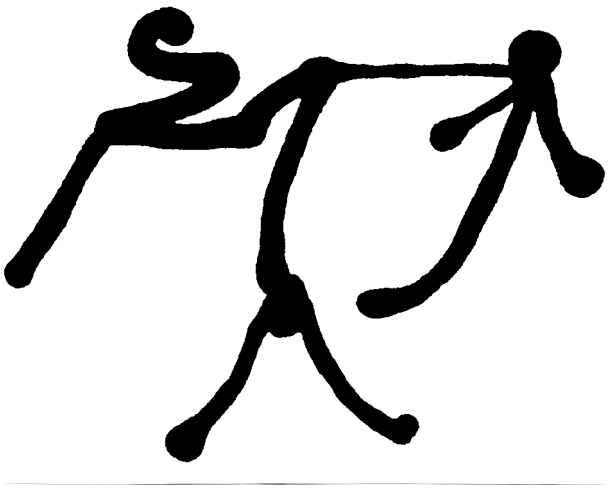
The Phencyclidine Model of Schizophrenia: Dysregulation of brain dopamine systems induced by NMDA receptor antagonists

An experimental study

Jan M. Mathé



Stockholm 1998

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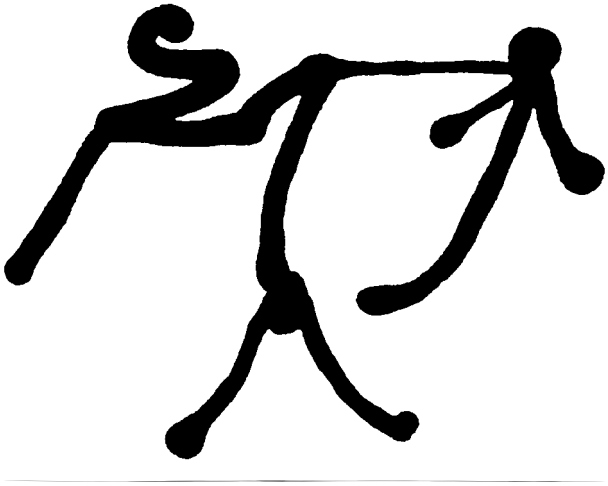

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Abstract

Non-competitive NMDA receptor antagonists, such as phencyclidine (PCP) and dizocilpine (MK-801) can even acutely induce a drug induced psychotic state which closely resembles schizophrenia. The midbrain dopamine (DA) neurons, which originate in the ventral tegmental area (VTA), are involved in motivational, attentional and cognitive processes, and have also been profoundly implicated in schizophrenia. Therefore, the present work analyzed the effects of these psychotomimetics on the mesocorticolimbic DA neurons in the rat, and associated behavioral significance, as well as the potential reversal of these effects by different types of drugs; the objectives being to reveal potential pathophysiological mechanisms underlying psychotic states and to assist the development of improved pharmacotherapy in schizophrenia.

DA neuronal activity was studied by means of single cell recordings in vivo and DA and neurotensin (NT) release in nerve terminal regions was assessed with microdialysis in freely moving animals coupled to HPLC and RIA methodology. Behavioral techniques included measurements of locomotor activity, conditioned avoidance response (CAR) and catalepsy. Systemic administration of PCP and MK-801 produced an increased firing rate and decreased variability of firing in DA neurons. Burst firing was differentially affected in different subpopulations of VTA DA cells. A high frequency, burst-like firing pattern was obtained in neurons projecting to subcortical sites, e.g. to the nucleus accumbens (NAC). In contrast, burst firing was attenuated in DA cells which largely project to the medial prefrontal cortex (MPFC). PCP increased release of DA and the colocalized peptide NT in both the ventral striatum and the MPFC. The MK-801 evoked DA output in the NAC was antagonized by inhibition of DA nerve impulse generation or by local antagonism of AMPA and kainate receptors in the VTA, a procedure which also blocked the locomotor stimulation. The MK-801 evoked DA output in the MPFC was, in contrast, not affected by inhibition of nerve impulses in the VTA. Pretreatment with the a_1-adrenoceptor antagonist prazosin also antagonized the MK-801 induced increase in DA output in the NAC, and the associated locomotor stimulation. Finally, systemic administration of the AMPA receptor antagonist LY326325 was found to specifically suppress the CAR without affecting escape behavior, and did not cause catalepsy.

PCP and MK-801 thus profoundly dysregulate mesocortical and mesolimbic DA neurons in a differential manner. The drugs cause a pronounced augmentation of mesocortical DA output that is independent of nerve impulse activity and instead is mediated at the nerve terminal level. In contrast, a nerve impulse dependent increase in mesolimbic DA output is obtained by systemic MK-801 that seems elicited in the VTA by AMPA and/or kainate receptor activation. Since both the a_1-adrenoceptor antagonist and the AMPA receptor antagonist effectively reversed several of the behavioral and biochemical correlates to the dysregulated mesocorticolimbic DA system, induced by PCP-like drugs, an antipsychotic potential of both types of drugs is indicated, which is especially supported for LY326325 by the suppression of the CAR.

Keywords: phencyclidine, MK-801, CNQX, prazosin, tetrodotoxin, dopamine, neurotensin, ventral tegmental area, nucleus accumbens, medial prefrontal cortex.

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Cover illustration: Sketch of a visual hallucination.
by anonymous, a catatonic schizophrenic.

To my family

'Twas brillig, and the slithy toves
Did gyre and gimble in the wabe;
All mimsy were the borogoves,
And the mome raths outgrabe.

Excerpt from 'Jabberwocky'
Through the Looking Glass

Lewis Carroll
nom de plume of Charles Lutwidge Dodgson (1832-1898)

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Abbreviations

5-HT	Serotonin
AMPA	a-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid
CAR	Conditioned avoidance response
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
CS	Conditioned stimulus
EAA	Excitatory amino acid
EPS	Extrapyramidal side effects
DA	Dopamine
DOPAC	Dihydroxyphenylacetic acid
HVA	Homovanillic acid
LC	Locus coeruleus
MK-801	Dizocilpine
MPFC	Medial prefrontal cortex
NAC	Nucleus accumbens
NMDA	N-methyl-D-aspartate
NT	Neurotensin
NT-LI	Neurotensin-like immunoreactivity
PBP	Parabrachial pigmented subdivision of the ventral tegmental area
PET	Positron emmision tomography
PFC	Prefrontal cortex
PCP	Phencyclidine
PN	Paranigral subdivision of the ventral tegmental area
SN-ZC	Substantia nigra, zona compacta (A9)
TTX	Tetrodotoxin
UCS	Unconditioned stimulus
VSTR	Ventral striatum
VTA	Ventral tegmental area (A10)

This thesis is based on the following articles, which are referred to in the text by their roman numerals

I: Pawlowski L, Mathé JM and Svensson TH (1990) Phencyclidine activates rat A10 dopamine neurons but reduces burst activity and causes regularization of firing. Acta Physiologica Scandinavica 139: 529-530.

II: Murase S, Mathé JM, Grenhoff J and Svensson TH (1993) Effects of dizocilpine (MK-801) on rat midbrain dopamine cell activity: differential actions on firing pattern related to anatomical localization. Journal of Neural Transmission 91: 13-25.

III: Hertel P, Mathé JM, Nomikos GG, Iurlo M, Mathé AA and Svensson TH (1996) Effects of D-amphetamine and phencyclidine on extracellular concentrations of neurotensin and dopamine in the ventral striatum and medial prefrontal cortex: A microdialysis study the freely moving rat. Behavioural Brain Research 72: 103-114.

IV: Mathé JM, Nomikos GG, Hildebrand BE, Hertel P and Svensson TH (1996) Prazosin inhibits MK-801-induced hyperlocomotion and dopamine release in the nucleus accumbens. European Journal of Pharmacology 1996; 309: 1-11.

V: Mathé JM, Nomikos GG, Schilström B and Svensson TH (1998) Non-NMDA excitatory amino acid receptors in the ventral tegmental area mediate systemic dizocilpine (MK-801) induced hyperlocomotion and dopamine release in the nucleus accumbens. Journal of Neuroscience Research 1998; 51: 583-592.

VI: Mathé JM, Nomikos GG, Hygge Blakeman K and Svensson TH (1998) Differential actions of dizocilpine (MK-801) on the mesolimbic and mesocortical dopamine systems: role of neuronal activity. Neuropharmacology, In press.

VII: Mathé JM, Fagerquist MV and Svensson TH (1998) Antipsychotic-like effects of the AMPA and kainate receptor antagonist LY326325 as indicated by suppression of conditioned avoidance response in the rat. Manuscript.

Introduction

Schizophrenia

Schizophrenia is one of the most debilitating psychiatric disorders and affects approximately 1% of the population worldwide. The onset of the disease most frequently occurs during the late teens and twenties, and episodes recur throughout life, disrupting the individuals ’most productive years. It is mostly characterized by marked distortions of the perception of reality, disturbances of intellectual functions, motivation and affect as well as motor aberrations. The etiology of schizophrenia is unknown, and there is strong indication for hereditary linkage (see Gottesman 1991). During the 1980s a neurodevelopmental perspective gained increased attention. Thus, schizophrenia might represent a long term consequence of an early, prenatal abnormality in neural development, which lies dormant until an affected region matures and is called upon to function (Weinberger 1987). Contributing factors could, for example, include a viral infection or maternal stress during pregnancy which, in turn, might interact with a genetic predisposition. Indeed, both experimental and neurohistochemical clinical studies have provided support for this hypothesis (see e.g. Jakob & Beckman 1986, Arnold et al. 1991, Akbarian et al. 1993, Lipska & Weinberger 1995, Lipska et al. 1995, Selemon et al. 1995, Weinberger 1995, Akbarian et al. 1996) Apparently, schizophrenia may be a heterogenous disorder, which is the result of several pathological processes, including structural, biochemical and functional abnormalities in brain.

The symptoms are commonly divided into three principal clusters (see Andreasen 1990). The so-called positive symptoms of schizophrenia include the more florid expression of the disorder, such as delusions, bizarre behavior and perceptual distortions, as well as auditory or visual hallucinations. In contrast, negative symptoms represent a decrease or loss of normal functions and include e.g. poverty of speech, blunting of emotions, amotivation, loss of sociability and anhedonia. In addition, schizophrenia is usually associated with several neuropsychological deficits, such as cognitive impairment, lack of insight and poor judgement. Afflicted individuals may exhibit grossly disorganized behavior and an inability to take care of their basic daily needs and, consequently, voluntary or involuntary hospitalization is not infrequently required. The combinations of symptoms may vary significantly in schizophrenia, as may the course of the disorder.

In spite of efficient antipsychotic medications coupled to psychosocial measures, a substantial number of schizophrenics do not recover completely and many schizophrenic patients lead impoverished lives. The cost of treatment of schizophrenia, as well as loss of income has been estimated to approximately $40 billion per year in the United States (Rupp & Keith 1993). Consequently, the need to improve therapy for this disorder is considerable not only from a medical perspective, but also for economical reasons. Development of more efficient pharmacological treatment requires a better understanding of the pathophysiological mechanisms involved. This task has proven difficult to achieve as reflected by the fact that to date, the only common denominator identified for the mechanisms of action of all drugs known to exert a therapeutic effect in schizophrenia is antagonism of dopamine (DA) mediated neurotransmission in brain. Such antagonism can be achieved either at the presynaptic level, as exemplified by reserpine, or at the postsynaptic level, as exemplified by all presently used antipsychotic drugs. Consequently, during the past forty years the neurotransmitter dopamine has remained at center stage in neurobiological research and drug development in this medical area.

Dopamine

Dopamine Systems

Dopamine was discovered as an independent neurotransmitter in brain during the late 1950s (Carlsson et al. 1957, 1958, Carlsson 1959). The mesotelencephalic DA systems (Fig. 1) originate in the midbrain tegmentum and the distribution of DA cell bodies in this region is restricted largely to two nuclei in the rat; the substantia nigra zona compacta (SN-ZC or A9) and the ventral tegmental area of Tsai (VTA or A10), as revealed by Dahlström and Fuxe (1964). The neurons in the SN-ZC project primarily to the caudate nucleus and putamen, i.e. striatum, and the system was thus called the nigrostriatal DA system (Andén et al. 1964). The VTA exhibits somewhat more diverse projections than the SN-ZC. Thus, the DA neurons in the VTA project to e.g. ventral striatum (VSTR) including the nucleus accumbens (NAC), amygdala, hippocampus, olfactory tubercle as well as several limbic cortical sites, such as medial prefrontal, cingulate and entorhinal cortices (Andén et al.

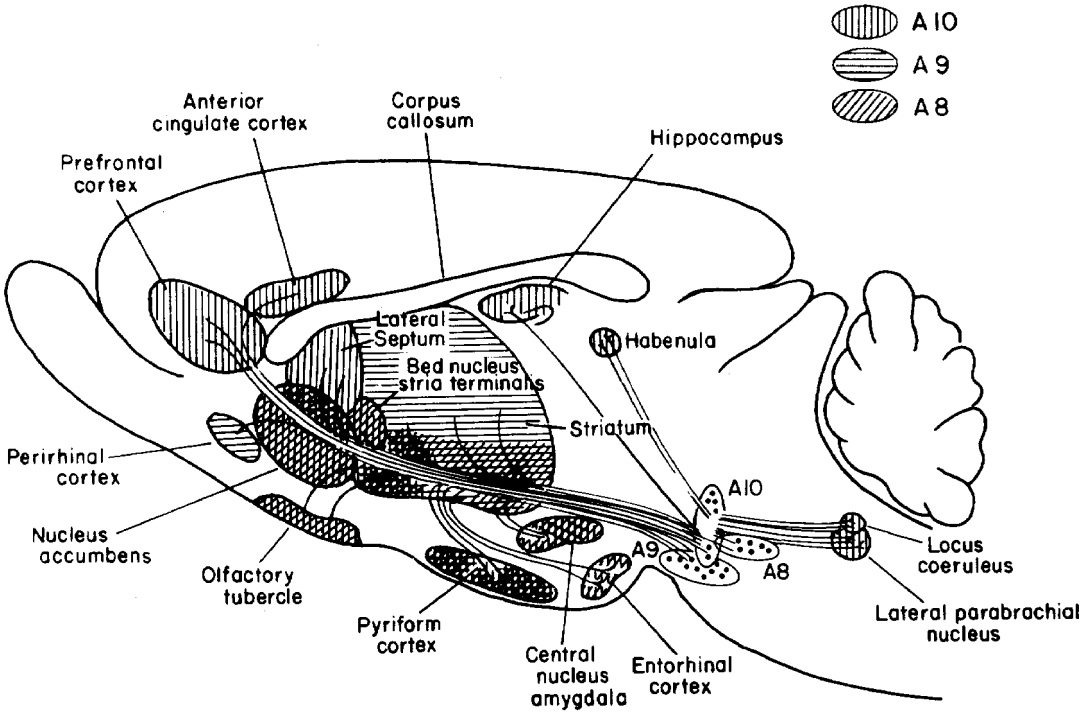

Fig. 1: Schematic drawing of the major dopamine-containing pathways in the rat brain (From Cooper et al. 1996).

1966B, Ungerstedt 1971A, Björklund and Lindvall 1984). In man, the cortical DA projection is much more widespread than in the rat, corresponding to the relatively larger size of the frontal cortex in humans. These DA pathways are often collectively referred to as the mesolimbocortical DA system. However, cortically projecting DA neurons appear not to innervate subcortical sites, and vice versa (Fallon 1981, Swanson 1982). These cortical and subcortical DA projections seem, at least partially, to arise from different anatomical subdivisions of the VTA. The ventrally located paranigral nucleus (PN) of the VTA primarily contributes the subcortical, mesolimbic DA projection, e.g. to the NAC and other striatal sites. In contrast, the parabrachial pigmented nucleus (PBP) of the VTA also contains the somata of DA neurons from which the cortical DA innervation originates (Simon et al. 1979, Deniau et al. 1980, Fallon 1981, Swanson 1982, Oades & Halliday 1987, Phillipson 1989). In addition, these mesocortical DA neurons exhibit several functional differences from the mesolimbic DA cells; mesocortical DA neurons display a more variable firing pattern, differential coexistence with neuropeptides, as well as altered autoreceptor and heteroreceptor regulation in comparison with the subcortically projecting DA neurons (see Grenhoff et

al. 1988A, Roth & Elsworth 1995).

Dopaminergic receptors are broadly divided into two families: the D_1 receptor family (i.e. D_1 and D_5) stimulates the formation of cyclic adenosine 3',5'-monophosphate (cAMP), whereas the D_2 family (D_2, D_3 and D_4) inhibits the formation of cAMP. Both D_1 and D_2 families are found postsynaptically, whereas the presynaptic receptors are regarded to belong to the D_2 family. There appears to be some differential distribution of DA receptor types in various DA terminal regions in the rat. Dense D_1 binding has been found in the dorsolateral striatum and moderate binding has been detected in the neocortex (Boyson et al. 1986). Dense D_5 binding is mainly restricted to thalamic, hypothalamic and hippocampal neurons. High D_2 binding is observed e.g. in dorsolateral striatum, NAC, VTA and SN-ZC (see Gehlert & Wamsley 1985). D_3 receptor binding is generally less abundant than D_2 receptor binding, although dense binding was found in the NAC shell subdivision, as well as in the islands of Calleja (Lévesque et al. 1992). Finally, D_4 receptors have been shown to be localized largely in limbic cortical sites in man, and have been considered as a potentially important binding site for atypical antipsychotics (see Seeman 1990, van Tol et al. 1991). D_4 receptors appear not to have been reliably detected in the rat brain (for review see Mansour & Watson 1995). However, some functional roles for this receptor have been described (Merchant, personal communication).

Dopaminergic neurotransmission

Dopamine is synthesized from the amino acid tyrosine in a two-step enzymatic process. First, tyrosine is converted to L-dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine hydroxylase, which is the rate-limiting enzyme in DA synthesis. Subsequently, L-DOPA is rapidly converted to DA by aromatic L-amino acid decarboxylase. In noradrenergic neurons, DA is further converted by dopamine ß-hydroxylase to noradrenaline. DA is stored in vesicles and physiologically released by a calcium-dependent process initiated by nerve impulse activity. Released DA is to approximately 80% very effectively and rapidly transported back into the nerve terminal by a DA-specific transporter. Extravesicular DA is intracellularly metabolized by the enzyme monoamine oxidase (MAO) to dihydroxyphenylacetic acid (DOPAC). Released,

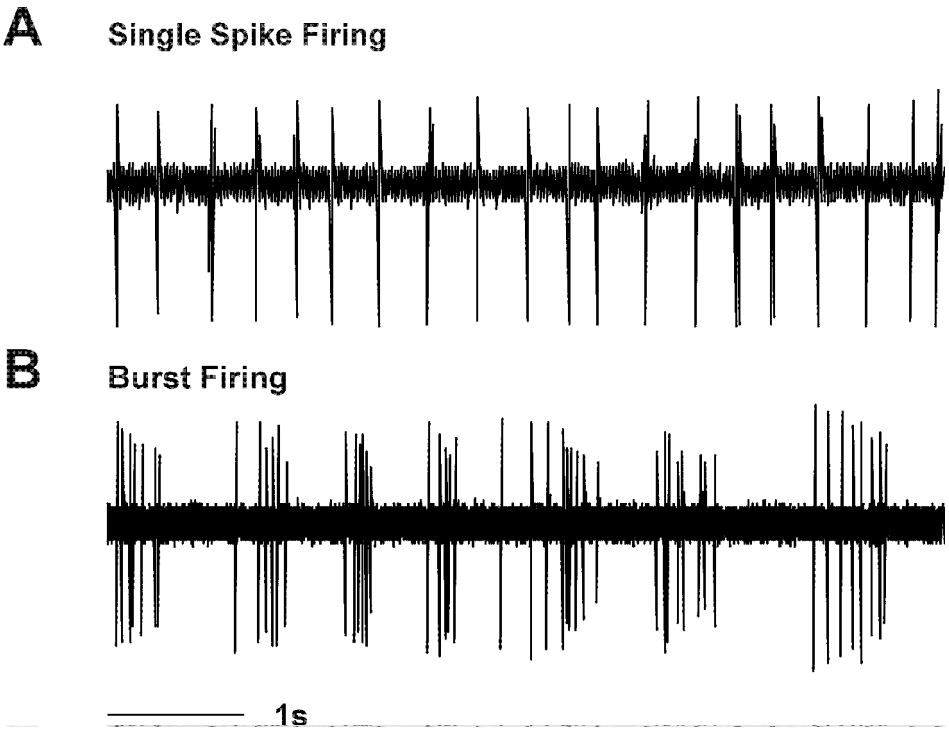

Fig. 2: Oscillgraphic traces of the two main modes of midbrain DA cell firing, i.e. single spike firing (A) and burst firing (B).

extracellular DA is sequentially degraded by the actions of catechol-O-methyl transferase (COMT) and MAO to 3-methoxytyramine (3-MT) and homovanillic acid (HVA). In the rat brain DOPAC is the major DA metabolite. Nevertheless, both DOPAC and HVA, in both sulfate-conjugated and free forms are found in high concentrations, along with small amounts of 3-MT, in rat brain. In human brain, free HVA appears instead as the main metabolite, with only small amounts of DOPAC (see Cooper et al. 1996).

Physiology and regulation of dopaminergic neurons

The impulse activity of DA neurons in the SN-ZC and VTA is characterized by two differential modes of firing; single spike firing and burst firing (Fig. 2; see Wang et al. 1991, Grace and Bunney 1983, 1984). Single spike firing is a relatively regular, low frequency firing pattern, i.e. between 1-10 Hz. In contrast, burst firing is typically recognized as the transient high-frequency discharge of multiple action potentials. The burst firing mode has been shown to elicit much more efficient release of DA in terminal areas than a regular firing pattern of the same average rate, and burst firing also

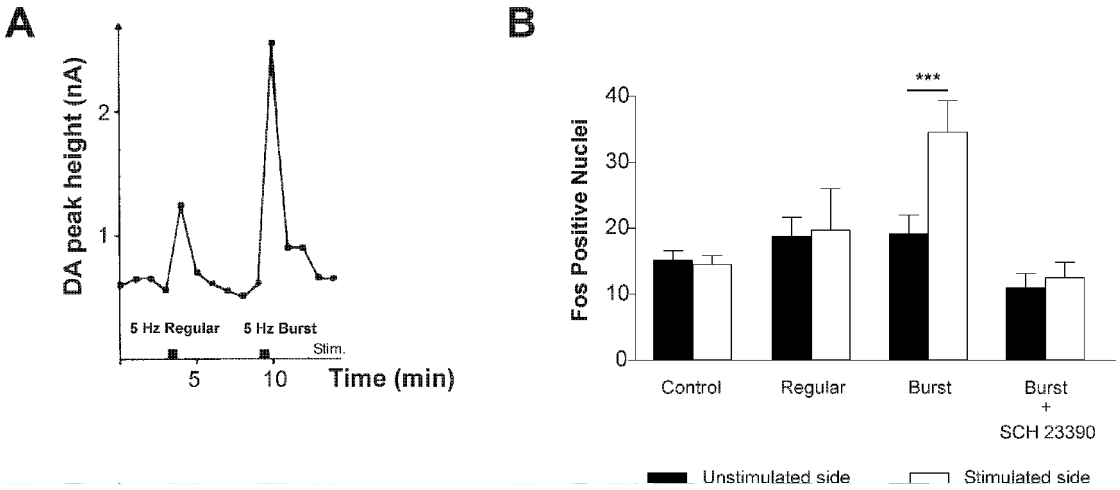

Fig. 3: Effects of regular and burst stimulation of the same average frequency on release of DA (A) and on Fos expression in postsynaptic neurons (B). Specifically, burst stimulation was up to six times as effective as regular stimulation with the same number of pulses in causing DA release, and only burst stimulation caused significant increase in the number of Fos-positive nuclei e.g. in the NAC shell subdivision, an effect blocked by administration of the D_1 receptor antagonist, SCH23390 (Adapted from Gonon 1988 (A) and Chergui et al. 1996 (B)).

causes significant activation of postsynaptic neurons (Fig. 3, Gonon 1988, Bean & Roth 1991, Suaud-Chagny et al. 1991, Chergui et al. 1996). However, this pulse of DA produced by a burst of action potentials is rapidly attenuated by efficient uptake of transmitter from the synaptic cleft (Grace & Bunney 1984). In addition, bursts seem to specifically facilitate release of colocalized neurotransmitters, such as neurotensin (NT) and cholecystokinin (Bean & Roth 1991). Such transient changes in impulse activity normally occur in relation to basic attentional and motivational processes in response to reward-predicting stimuli, and apparently serve to initiate goal-oriented behaviors (Schultz 1986, Nishino et al. 1987, Schultz et al. 1993, Schultz 1998). In both awake and anesthetized mammals in vivo, DA cells typically display a firing pattern which includes both single spike and burst firing, with frequent switches between these two modes of

firing (Grenhoff et al. 1988A). In contrast, in DA cells in a midbrain slice preparation, i.e. in cells that have been largely deprived of active neuronal inputs, burst firing is absent and little variability in the firing pattern is observed (Grace & Onn 1989). Generally, the structure of the firing patterns of midbrain DA neurons in vivo has been proposed to reflect the neurons response to coordinated synaptic inputs emerging from neuronal circuit interactions (Hoffman et al. 1995) Consequently, in view of the behavioral deficits in schizophrenia, which include both attentional dysfunction and anhedonia as well as severely impaired goal-oriented behaviors, the dynamic, physiological responsivity of mesocorticolimbic DA cells appears to be of prime interest as a potential dysfunctional mechanism in the pathophysiology of schizophrenia (Svensson & Tung 1989, see Svensson et al. 1995).

The general physiological and biochemical activity of DA neurons originating in the midbrain is profoundly regulated by DA receptors. The original experiments by Carlsson and Lindqvist (1963), which studied antipsychotic drug action utilizing biochemical methodology, proposed a feedback regulation of DA neuronal activity by postsynaptic DA receptors. This notion was subsequently confirmed by several electrophysiological studies (see Aghajanian & Bunney 1973, Bunney et al. 1973, Aghajanian & Bunney 1977, Bunney & Aghajanian 1978, Einhorn et al. 1988). Moreover, DA activity is also controlled by the so-called autoreceptors, i.e. various DA receptors located along the DA cells responding to the neuron's own neurotransmitter by an inhibition of transmitter synthesis and release, as well as of the firing rate. Conversely, a stimulation of DA activity is observed following administration of DA-D_2 receptor antagonists (Kehr et al. 1972, Andén et al. 1973, Roth 1973, Bunney & Aghajanian 1973, for review see Carlsson 1977, Roth & Elsworth 1995). Activation of somatodendritic autoreceptors by DA or DA agonists, such as apomorphine, hyperpolarize DA neurons through opening of K^+ channels via a pertussis toxin sensitive G-protein (Aghajanian and Bunney 1973, Innis & Aghajanian 1987, Lacey et al. 1987). Release of DA seems also to be modulated by presynaptic DA autoreceptors located on the nerve terminals, since local or systemic administration of DA agonists has been reported to decrease DA release in the striatum (Kehr et al. 1972, Zetterström & Ungerstedt 1984, Westerink & de Vries 1989). However, mesocortical DA neurons appear largely to lack nerve impulse and synthesis

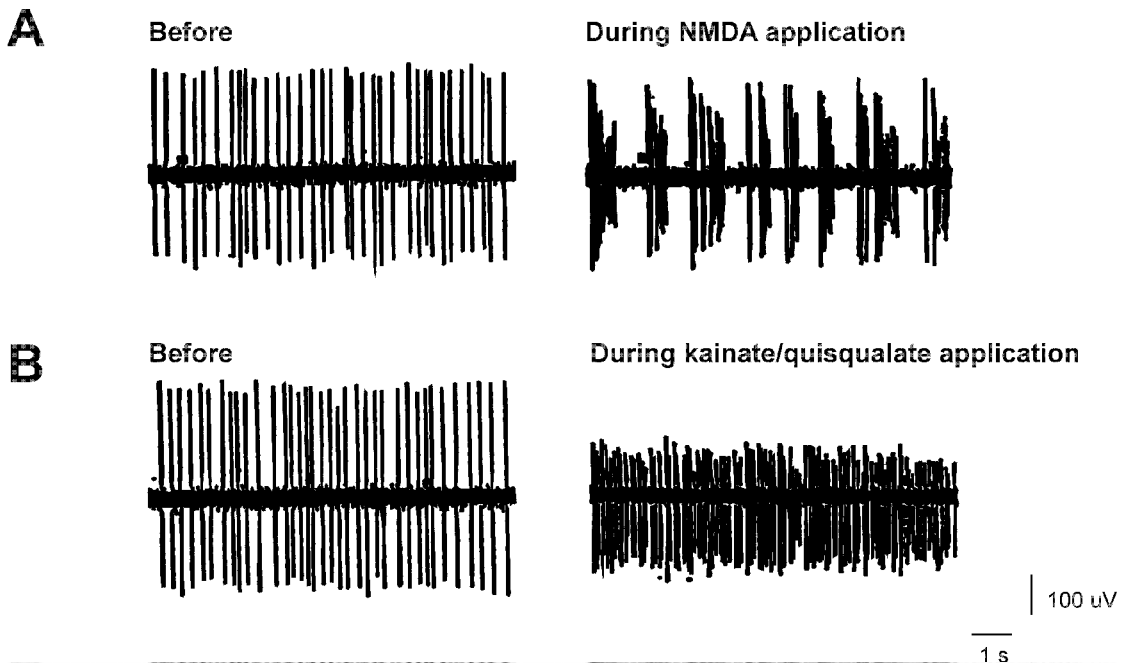

Fig. 4: Oscillographic traces of the effects of local microiontophoretic application of NMDA (A) or kainate or quisqualate (B) on the firing pattern of VTA DA neurons. Local NMDA application evoked typical bursts of action potentials. In contrast, kainate or quisqualate application caused a high-frequency, burst-like firing pattern in VTA DA cells (Adapted from Chergui et al. 1993).

modulating autoreceptors (Chiodo et al. 1984, see Roth and Elsworth 1995).

Spontaneous burst activity of DA neurons appears to be directly dependent upon activation of somatodendritic N-methyl-D-aspartate (NMDA) receptors via afferent excitatory amino acid (EAA) inputs from e.g. the prefrontal cortex (PFC) and subthalamic nucleus (Fig. 4; Christie et al. 1985, Sesack et al. 1989, Bayer & Pickel 1990): these regions seem directly involved in the control of burst firing and, secondarily, also in the release of DA in terminal regions (Gariano & Groves 1988, Grenhoff et al. 1988B, Svensson and Tung 1989, Charléty et al. 1991, Chergui et al. 1993, Murase et al. 1993, Chergui et al. 1994). In addition, the NMDA receptors are involved in the control of the regularity of firing, another functionally important determinant of DA neuronal discharge (see Grenhoff et al. 1988B, Servan-Schreiber et al. 1990). Biochemical support for differential regulation of VTA DA neuronal subpopulations by EAAs was reported in the late 1980s. Thus, although DA release in the PFC was found to be preferentially

modulated by NMDA receptors in the VTA, the DA release in the NAC seemed largely controlled by AMPA and kainate receptors in the VTA (Kalivas et al. 1989). The firing pattern of VTA DA cells was demonstrated to be influenced also by ?-amino butyric acid (GABA) receptors; $GABA_B$ receptors hyperpolarize VTA DA neurons by inhibition of voltage-dependent Ca^{2+} conductance and thus reduce the average firing rate and attenuate burst firing (Grace & Bunney 1980, Johnson & North 1992). Furthermore, burst activity of VTA DA neurons seems to be modulated by a noradrenergic input from the locus coeruleus (LC) to the VTA. Thus, administration of the a_1-adrenoceptor antagonist prazosin selectively depresses burst firing (Grenhoff et al. 1993). Electrical stimulation of the LC elicits monoamine mediated short latency bursts in VTA DA cells, an effect that in turn was specifically antagonized by prazosin administration (Grenhoff & Svensson 1993). Stimulation of a_1-adrenoceptors seems to increase the excitability of DA cells via inhibition of K^+ efflux (Grenhoff and Svensson 1993, Grenhoff et al. 1995).

Neurotensin

In rats, VTA neurons contain one or both of the neuropeptides cholecystokinin and the tridecapeptide neurotensin (NT) in neurons projecting to various structures in the limbic forebrain including the frontal cortex and ventral striatum (VSTR, see Hökfelt 1991). Some of these NT containing neurons have been shown to contain also DA (Hökfelt et al. 1984). In these mixed DA/NT neurons, which largely originate in the PBP subdivision of the VTA, a subpopulation projects to the medial prefrontal cortex (MPFC), a region where the majority, if not all, fibers contain both DA- and NT-immunoreactivity (Seroogy et al. 1987, Studler et al. 1988, Jayaraman et al. 1990). The VSTR, including the NAC, also receives both DA and NT projections from the VTA, although colocalization appears comparatively less abundant than in the MPFC. NT is thought to be stored in large dense-core vesicles, but not in small vesicles found in DA terminals. NT is released, like DA, in a Ca^{2+} and action potential dependent manner (Bean et al. 1989A, B, C). Differential storage of DA and NT is consistent with the finding that low frequency regular stimulation of DA axons selectively releases DA, whereas high frequency or burst stimulation has been shown to release both DA and NT (see Hökfelt 1991, Bean & Roth 1991). This property of the mixed DA/NT neurons may allow differential transmitter release under different functional conditions. NT binding sites have been found on

cell bodies in the VTA, as well as in many projection areas of the mesolimbic and mesocortical DA systems, where they appear to be located mostly at postsynaptic sites.

NT seems to play a modulatory role as regards VTA DA neuronal activity as well as on the effects of DA in terminal regions. Thus, NT microinjection into the VTA was found to stimulate DA cell firing rate (Seutin et al. 1989), and caused a concomitant increase in DA output in the NAC (Blaha et al. 1990). These functional effects were accompanied by an increase in locomotor activity (Kalivas et al. 1983). Conversely, NT injections into the NAC were reported to cause a decrease in NAC neuronal activity and to antagonize the locomotor stimulation caused by indirect DA agonists (McCarthy et al. 1979, Ervin et al. 1981). NT neurons, in turn, are subject to dopaminergic regulation. Early studies showed that administration of DA-D_2 receptor antagonists increases tissue levels of NT in several discrete brain nuclei, including the NAC (Govoni et al. 1980, Goedert et al. 1985).

The coexistence of DA and NT has not been observed in primates (Berger et al. 1991). Some early clinical studies indicated that certain subgroups of schizophrenic patients have decreased concentrations of NT in cerebrospinal fluid (CSF) with normalization after neuroleptic treatment (Widerlöv et al. 1982, Garver et al. 1991). In addition, a correlation between CSF NT concentrations and deficit symptoms in schizophrenia was later observed (Breslin et al 1994). In fact, NT has even been suggested to act as an endogenous neuroleptic (Lipton et al. 1979, Nemeroff 1980). Thus, NT may serve to modulate the function of mesolimbic and mesocortical DA neurons, although the role of NT in normal brain function as well as in psychiatric disorders is still far from clear.

Functional roles of the mesolimbic and mesocortical DA systems

Generally, the mesolimbic and mesocortical DA systems are involved in major behavioral functions such as motivation, emotional control and cognition (see Le Moal & Simon 1991), processes which are of prime interest in schizophrenia.

A massive set of experimental evidence supports the contention that the ventral striatum, including the NAC, plays a role in the control of motivation, emotion and ongoing behavior, including locomotion in rodents (see Mogenson 1987). Specifically, the mesolimbic DA system is regarded to be directly involved in these behavioral

functions. Indeed, stimulation of the VTA initiates locomotion (Koob & Swerdlow 1988). Furthermore, increases in locomotor activity caused by administration of several DA releasing psychostimulants, e.g. D-amphetamine and cocaine, are attenuated by lesioning of DA terminals within the NAC using the neurotoxin 6-hydroxydopamine (Wise and Bozarth 1987). Furthermore, similar lesions of DA terminals in the NAC attenuate self-administration of several drugs of abuse (Pfeffer & Samson 1988, Panocka et al. 1993), documenting a role of mesolimbic DA in the reinforcing properties of these drugs. In fact, not only DA cell firing but also the DA output in the NAC increase in response to rewarding stimuli, such as feeding or sexual behavior (Schultz et al. 1986, Nishino et al. 1987, Smith & Schnieder 1988, Fibiger et al. 1992, Westerink et al. 1997). These findings support involvement of DA both in appetitive and in consummatory aspects of motivational behaviors. In addition, the mesolimbic DA pathway to the ventral striatum appears to be of critical importance for the initiation of active avoidance behavior (Wadenberg et al. 1990, see Le Moal & Simon 1991). Interestingly, recent studies indicate that the mesolimbic DA system also may play a role in the development of long-term potentiation in corticostriatal synapses, indicating an important function of the mesolimbic DA system in learning (see Arbuthnott et al. 1998).

The PFC is generally involved in integrative functions, including cognitive processes, such as maintenance of focused attention, working memory, as well as planning and execution of behavior, processes that are modulated by the mesocortical DA system. Mild stressors have been shown to cause a preferential activation of the mesocortical DA neurons (Thierry et al. 1976, see Le Moal & Simon 1991), but also reward related stimuli augment mesocortical DA output (Taber & Fibiger 1997). Importantly, DA is involved in the gating of inputs to the PFC, e.g. afferents that originate in the mediodorsal nucleus of the thalamus (Ferron et al. 1984, see Thierry et al. 1998). Significantly, activation of D_1 receptors in the PFC may represent a critical step in the performance of tasks requiring working memory (Sawaguchi & Goldman-Rakic 1994). Also computer simulations of PFC circuitries suggest that DA plays a major role in the gating of excitatory and inhibitory inputs, i.e. regulation of the responsiveness or signal-to noise ratio of cortical neurons to afferent inputs (Servan-Schrieber et al. 1990, Hoffman & McGlashan 1993). Thus, from a behavioral standpoint, the mesocortical DA system appears critical for the precise control of focused attention, short term memory,

inhibitory control as well as proper planning and execution of behavioral responses to environmental stimuli.

The dopamine hypothesis of schizophrenia

The dopamine (DA) hypothesis of schizophrenia in its original form was based largely on indirect, pharmacological evidence and proposed a hyperactivity of central DA systems in schizophrenia (see Carlsson 1988). This hypothesis was based on the facts that all drugs with therapeutic effect on schizophrenia and other psychotic disorders have antidopaminergic effects, and that amphetamine and related central stimulants, which directly or indirectly activate brain DA receptors, have been found capable of eliciting or aggravating psychotic symptoms.

In early studies, Kline (1954) demonstrated for the first time in the Western world the antipsychotic effects of reserpine. Subsequent experiments by Carlsson and associates showed that the tranquilizing effect of reserpine is largely due to depletion of catecholamines such as DA in brain (Carlsson 1965, Carlsson et al. 1967, see Carlsson 1966). Subsequently, Carlsson and Lindqvist (1963) conducted the first study showing that other antipsychotic drugs, such as chlorpromazine and haloperidol, which displayed similar behavioral effects as reserpine in experimental animals, act as antagonists at postsynaptic DA receptors. Carlsson's initial results and conclusion was later confirmed by numerous experimental studies in vivo and in vitro utilizing a variety of techniques, including both functional and biochemical studies in experimental animals, as well as more recently positron emission tomography (PET) studies in humans in vivo (Andén et al. 1964, 1966A, Carlsson et al. 1966, Andén et al. 1970, Nybäck & Sedvall 1970, Aghajanian & Bunney 1973, Seeman & Lee 1975, Creese et al. 1976, Farde et al. 1988).

The classical antipsychotic drugs, such as chlorpromazine and haloperidol, are generally not equally effective against all symptoms of schizophrenia, i.e. they are most effective against the positive symptoms. In addition, classical antipsychotic drugs possess several undesirable properties, for example extrapyramidal side effects (EPS) such as Parkinsonism, akathisia, acute dystonia and tardive dyskinesia, and they also display a relatively poor effect against negative symptoms and elicit a motivational deficit syndrome (see Casey & Keepers 1988, Meltzer 1992). The EPS and the demotivational action as well as potential worsening of negative symptoms result in relatively poor

compliance with classical antipsychotic treatment. Thus, mere DA receptor antagonism has clearly not proven to be a panacea in the treatment of schizophrenia, although it represents so far the only generally recognized mechanism of action for our presently used antipsychotic drugs.

The first antipsychotic drug to be developed which in essence does not cause EPS was clozapine. In contrast to many classical antipsychotic drugs, which in clinically effective doses show approximately 75% DA-D_2 receptor occupancy, clozapine is effective already at 45-50% DA-D_2 receptor occupancy (Farde et al. 1988, Nordström et al. 1995). Moreover, it frequently exerts a therapeutic action also against negative symptoms and may be efficacious also in neuroleptic-resistant schizophrenia (Kane et al. 1988, see Meltzer 1995). Since clozapine, in addition to its DA-D_2 receptor antagonistic effects, also possesses relatively high affinity for D_1 and D_4 receptors and many neurotransmitter receptors, such as noradrenergic a_1-adrenoceptors, serotonin $(5\text{-HT})_{2A}$, 5-HT_6 and 5-HT_7 receptors, muscarinic and histaminergic receptors (see Seeman 1990, Roth et al. 1998), a crucial, as yet unresolved question is which of these other receptor affinities that, singly or in combination, contribute to its therapeutic effect.

The second major line of evidence which supports the DA hypothesis of schizophrenia is also based upon pharmacological data. Thus, high doses of amphetamine, especially when chronically administered, can induce an acute paranoid psychosis which in some ways mimics schizophrenia, particularly the positive symptoms in healthy subjects, and may also exacerbate such symptoms in schizophrenic patients (see Angrist et al. 1994). These findings are significant as amphetamine potently releases extravesicular DA (see Carlsson et al. 1967) and causes marked behavioral stimulation, i.e. hyperlocomotion and stereotyped behavior in rodents, a behavioral syndrome proposed to represent an animal model of schizophrenia (Randrup & Munkvad 1967). However, one significant shortcoming of the amphetamine model of schizophrenia is the fact that the D-amphetamine induced psychosis generally fails to reproduce negative symptoms. In fact, when D-amphetamine is given to schizophrenic patients negative symptoms may in some cases even improve (van Kammen and Boronow 1988). This finding is difficult to reconcile with the DA hypothesis of schizophrenia in its original form.

Generally, global DA hyperactivity can not readily account for all symptoms of

schizophrenia, particularly the negative symptoms. Unequivocal clinical evidence to substantiate increased activity of DA systems in the brains of schizophrenic patients has been difficult to obtain. Some clinical studies even indicated a reduction of central DA output in schizophrenia, specifically in patients with prominent negative symptoms and marked emotional withdrawal (van Kammen et al. 1986, Karoum et al. 1987). Interestingly, several early studies showed that these symptoms are associated with a decreased capacity to functionally activate the dorsolateral prefrontal cortex (Ingvar & Franzén 1974, Weinberger et al. 1986, Ingvar 1987), a brain region which contributes a major EAA input to the DA cells in the midbrain. Moreover, dopaminergic activity, in turn, appears to be one mechanism by which physiological activity of the PFC is enhanced (cf. Weinberger 1987). Therefore, it was of interest that experimentally induced, reversible "hypofrontality", i.e. impaired functional activity in the corresponding brain region in the rat, i.e the MPFC, produced by local cooling or by means of local application of lidocaine, was found to cause a nearly total extinction of phasic, burst activity in VTA DA neurons, although the average firing rate was still not significantly affected (Svensson & Tung 1989, Murase et al. 1993). In other words, a pacemaker-like firing of the VTA DA cells was obtained, reminiscent of the firing pattern of DA neurons observed in the deafferented, midbrain slice preparation. Such a dysfunction of midbrain DA cells, if also present in man, might contribute to explain the decreased capacity to process positive or negative reinforcement generally associated with impaired frontal lobe function and with schizophrenia (Svensson & Tung 1989). The specific reduction in burst firing caused by experimental hypofrontality could be significantly antagonized by pretreatment with the 5-HT_{2A-2C} receptor antagonist, ritanserin or the potent 5-HT_{2A} receptor antagonist amperozide, thus indicating that 5-HT_{2A} receptor antagonism was critically involved (Svensson et al. 1989, Grenhoff et al. 1990). Other experimental studies showed that ritanserin when given alone caused a preferential activation of VTA neuronal activity, in particular burst firing, an effect unrelated to DA-D_2 receptor activity in brain (Ugedo et al. 1989). These experimental findings appeared particularly intriguing since in early clinical studies ritanserin had been claimed to antagonize dysthymia, as well as to improve drive and motivation and, importantly, to reduce negative symptoms in schizophrenia (Reyntjens et al. 1986, Gelders et al. 1986, Duinkerke et al. 1993). Thus, the above results suggested that

negative symptoms may be a consequence of reduced, and not increased, DA activity in brain, in particular impaired phasic (dynamic) neuronal activity in VTA DA cells.

Consequently, both preclinical and clinical data are consistent with the notion that both hyperfunctioning and hypofunctioning DA systems might simultaneously occur in different brain regions in schizophrenia (cf. Weinberger 1987, Svensson et al. 1993). Such a differential dysfunction of DA systems probably reflects the differential regulation of DA neuronal subpopulations in the VTA and may be caused by an altered balance of inputs converging on the DA neurons from different sources in the brain (cf. Nauta 1976, Svensson et al. 1995). Thus, the DA hypothesis of schizophrenia in its original form, postulating a global hyperactivity of brain DA systems, appeared less attractive and, instead, the concept of dysregulation of brain DA neurons as a pathophysiological substrate for disease symptomatology has evolved. Given the fundamental role of EAA receptors in the regulation of VTA DA neurons an increased interest developed in another psychotomimetic compound, namely phencyclidine (PCP), a potent EAA receptor antagonist. PCP had already in the fifties been found to possess potent schizophrenomimetic properties (Luby et al. 1959). The newly acquired knowledge about the EAA receptor mediated regulation of the firing pattern of the mesocorticolimbic DA neurons in the VTA favored such an experimental approach.

Excitatory Amino Acids

The EAAs glutamate and aspartate are the most abundant of all amino acids in the brain, and are known to elicit fast excitatory responses in neurons in various species, from e.g. crayfish to man. The neurotransmitter nature of these amino acids has been a matter of scrutiny since the 1960s, and it is now widely recognized that glutamate is the principal neurotransmitter for fast excitatory signalling in brain, and that, in some instances also aspartate may act in a similar fashion. Although EAA receptors are now regarded to be almost ubiquitous (Orrego and Villanueva 1993), binding density varies between brain regions. Glutamate exerts its excitatory actions on neurons via activation of mainly two principal groups of EAA receptors, ionotropic and metabotropic receptors. Both groups of EAA receptors are found in the VTA. For reasons of practicality, only the ionotropic receptors will be discussed here.

Ionotropic EAA receptors are multi-subunit transmembrane proteins that

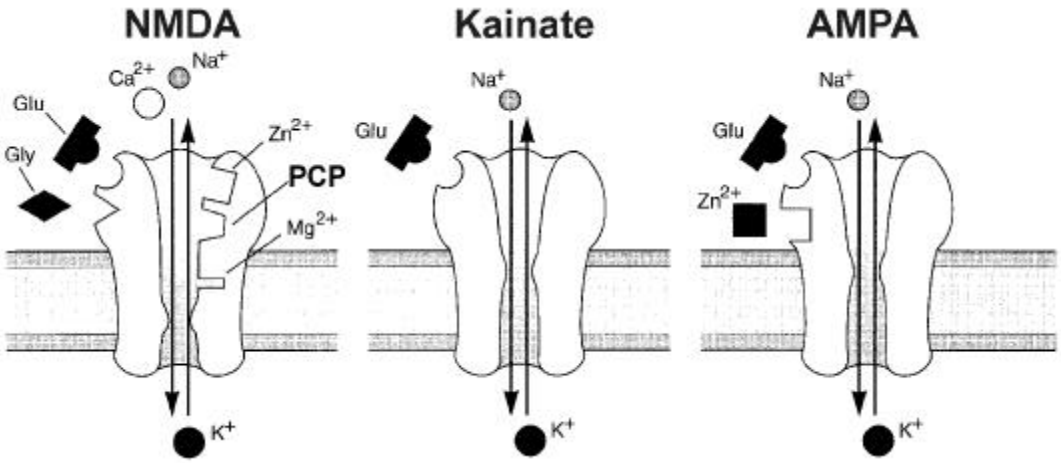

Fig. 5: Schematic drawing of the three major types of ionotropic excitatory amino acid receptors (Adapted from Kandel et al. 1991).

consist of a conducting pore embedded in the cell membrane and various binding sites on the outer, extracellular surface of the receptor (Fig. 5). The ionotropic receptors are named after the amino acid analogs to which they respond selectively. The a-amino-3-hydroxy-5-methyl-isoxazole proprionic acid (AMPA) receptor, formerly known as the quisqualate receptor, is associated with a cation channel that is nonselective with respect to Na^+ and K^+ ions, but impermeable to Ca^{2+}. AMPA receptor mediated currents exhibit very fast kinetics; with fast onset, offset and desensitization. The AMPA receptors are widely distributed in the brain, with high density in the hippocampus and olfactory tubercle (Petralia et al. 1992). This EAA receptor subtype is considered to be a major mechanism for fast excitatory signalling in the brain (Seeburg 1993). Currently there are four different subunits described for AMPA receptors termed GluR1 through GluR4, each of which occurs in two variants, "flip" and "flop" which are the result of alternative gene splicing.

Kainate receptors are similar in ion gating and kinetics to AMPA receptors and may be formed from five subunits designated GluR5, GluR6, GluR7, KA1 and KA2. (Petralia et al. 1994A). Selective antagonists AMPA or kainate receptor subtypes have been developed only relatively recently due to the structural similarities between them, which together were frequently denoted 'non-NMDA' EAA receptors.

The NMDA receptors exhibit comparatively slower kinetics than AMPA and kainate receptors and also show significant permeability to Ca^{2+} ions in addition to Na^+ and K^+. NMDA receptors may be composed of various subunits, e.g. NR1 (which exists

in at least nine isoforms) and NR2A-NR2D. The NMDA receptor has a number of regulatory sites. For example, activation of NMDA receptors by glutamate has been shown to require concomitant binding of glycine to a specific glycine binding site. In addition, NMDA receptors are blocked by relatively low concentrations of Mg^{2+} via interaction with a binding site within the ion channel complex. The NMDA receptors are widely distributed in brain, with high densities found in cortical regions and hippocampus (Petralia et al. 1994B,C).

The phencyclidine model of schizophrenia

Phencyclidine

PCP is an anesthetic agent synthesized in the early 1950s. Clinically PCP was found to cause a state of "mind-body" dissociation in which the patients were impervious to pain, yet did not lose consciousness as with the conventional general anesthetics. Due to several reports describing severe side effects in patients, including disorientation, agitation, visual and even auditory hallucinations and sometimes violent behavior, particularly during emergence from PCP anesthesia, PCP was withdrawn from clinical use in 1965 (see Domino & Luby 1981).

Subanesthetic doses of PCP reportedly cause symptoms such as apathy, but may also cause euphoria, a loss of the ability to differentiate between self and non-self, cognitive disorganization, inability to concentrate or to think abstractly, and in higher doses catatonic stupor, ataxia and rigidity can occur (see Luby et al. 1959). A number of clinical studies have reported that PCP not only can cause positive symptoms of schizophrenia, as seen also in amphetamine induced acute paranoid psychosis, but also negative symptoms, as well as formal thought disorder and cognitive deficits as otherwise encountered in schizophrenia. In contrast to almost all other drug induced psychotic syndromes, auditory hallucinations have also been reported to occur following PCP administration. Significantly, the PCP induced psychosis can persist for several weeks in some cases and has been observed even in healthy subjects after administration of a single dose of the drug (Luby et al. 1959). In addition, when given to schizophrenic patients, PCP may precipitate psychotic relapse or exacerbate pre-existing psychotic symptoms. These findings strongly suggest that PCP and schizophrenia may share common mechanisms in the generation of psychotic symptoms (for review see Domino

1964, Snyder 1980, Aniline & Pitts 1982, Javitt & Zukin 1991) and, that PCP interferes specifically with brain neurotransmitter mechanisms of significance to the generation of psychosis.

Mechanism of action of phencyclidine

PCP interacts with a number of binding sites in brain, e.g. NMDA receptors, monoamine reuptake carriers, sigma binding sites and a number of other receptors and ion channels, in order of decreasing affinity. However, when administered in psychotomimetic doses, PCP acts predominantly as a non-competitive antagonist at the NMDA subtype of EAA receptors. Other non-competitive NMDA receptor antagonists, e.g. ketamine and memantine, have also been reported to cause psychotic symptoms to an extent that is well correlated with their affinity for NMDA receptors (Fig. 6, Krystal et al. 1994, Lahti et al. 1995, see Kornhuber & Weller 1995). In addition, several competitive NMDA receptor antagonists have subsequently been associated with psychotomimetic effects when administered to human subjects (Kristensen et al. 1992, Clark & Coull 1994, Grotta et al. 1995). Systemic administration of MK-801, the most specific non-competitive NMDA receptor antagonist to date, was also found to elicit the typical psychotomimetic effects of PCP in human volunteers, an observation that

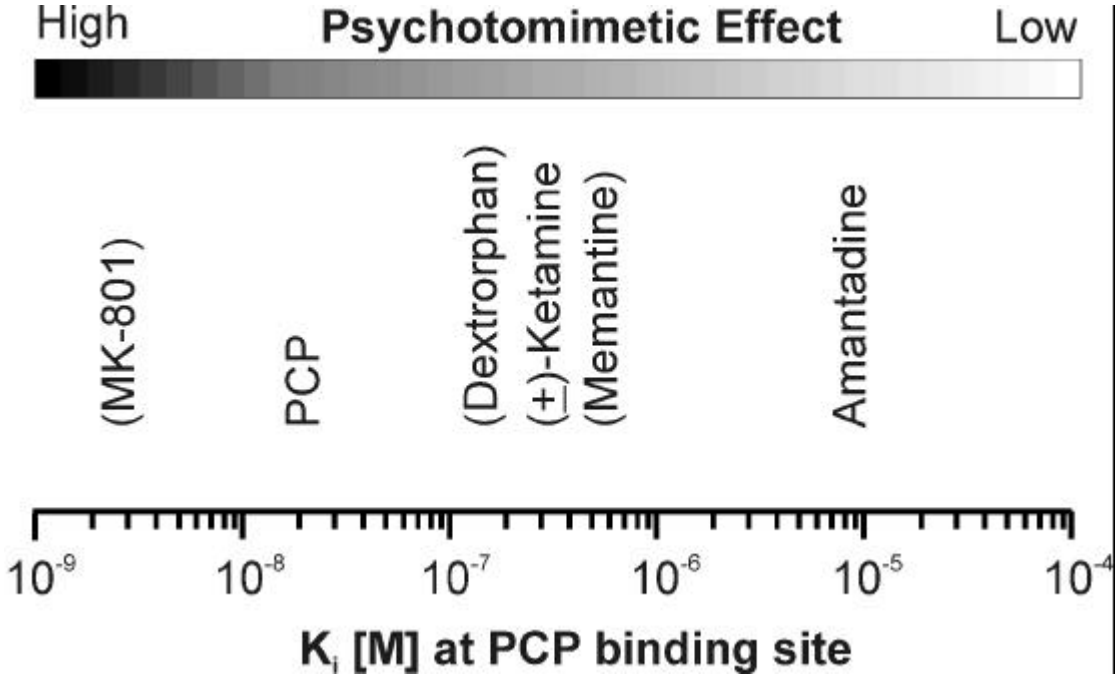

Fig. 6: Correlation between psychotomimetic effect of non-competitive NMDA receptor antagonists and their affinity for the PCP binding site within the NMDA receptor ion channel. Compounds in parentheses indicate that their psychotomimetic effects have only incompletely been characterized (Adapted from Kornhuber & Weller 1995).

precluded further clinical development of this compound (L. Iversen, personal communication). In addition, PCP is a relatively potent monoamine reuptake blocker, a property it shares with cocaine and D-amphetamine, two other psychotomimetic compounds. However, the doses of PCP required to cause significant monoamine reuptake blockade are sublethal or lethal in humans, a finding which implies that this mechanism probably only to a minor extent contributes to the induction of PCP psychosis. Consequently, the psychotomimetic effects of PCP-like drugs generally appear to stem from drug induced impairment of NMDA receptor function in brain and the subsequent consequences thereof (Javitt & Zukin 1991, Kornhuber & Weller 1995).

A hypothesis of decreased glutamatergic function in brain in schizophrenia has also been proposed based on findings indicating that schizophrenic patients may have decreased concentrations of glutamate in cerebrospinal fluid (Kim et al. 1980). In addition, in post-mortem brains both glutamate uptake and kainate receptor binding may be increased in the PFC in schizophrenics, a finding which was interpreted to

indicate an impaired glutamatergic neurotransmission (Nishikawa et al. 1983, Deakin et al. 1989). Such results, in addition to the findings with the PCP model of schizophrenia, contributed to support the glutamate hypothesis of schizophrenia (see Kim et al. 1980). In essence, this hypothesis postulates that reduced activation of the NMDA subtype of glutamate receptor or a hypoglutamatergic state in brain might play a pivotal role in the generation of psychotic symptoms (for review see Bunney et al. 1995).

Pharmacological properties of non-competitive NMDA receptor antagonists

PCP binds to a specific binding site within the NMDA receptor ion channel complex, similarly to the selective ligand dizocilpine (MK-801) which, however, is approximately 10-fold more potent than PCP (Fig. 6). Thus, PCP displaces [^{3}H]-MK-801 binding in brain slices with a potency well correlated to its antagonistic action at NMDA receptors (Anis et al. 1983, Wong et al. 1986). This antagonistic effect appears to be both use- and state-dependent; it requires the NMDA receptor ion channel to be open and the voltage-dependent Mg^{2+} blockade of channel opening to be overcome by membrane depolarization to allow the antagonist to reach its binding site. Similar ligands such as PCP, ketamine and MK-801 bind rapidly but dissociate slowly from the ion channel complex (Wong et al. 1986, Lodge et al. 1987, Lodge & Johnson 1990, Wong & Kemp 1991). When administered systemically to rodents, MK-801 rapidly enters the brain, attaining maximal concentrations within ten minutes of injection, and its plasma half-life is approximately two hours (Hucker et al. 1983). Furthermore, MK-801 generalizes to PCP in discriminative stimulus tests and vice versa (Tricklebank et al. 1987, 1989).

Systemic administration of non-competitive NMDA receptor antagonists, including PCP and MK-801, causes a behavioral syndrome in rodents characterized by e.g. increased horizontal locomotor activity with frequent turning. Also, a stereotyped behavioral repertoire is observed, especially with high doses, including pronounced head weaving (repetitive side-to-side, left-to-right swaying movements of the head and upper torso) and sniffing. When higher doses are administered (i.e.>0.3 mg/kg to rats), increasing ataxia is obtained, an effect characterized by tottering of the hindquarters, abduction and dragging of hindlimbs, flat body posture, and loss of balance (Clineschmidt et al. 1982, Lehmann-Masten & Geyer 1991, Bubser et al. 1992, Löscher & Hönack 1992). Recent studies have shown that non-competitive NMDA receptor

antagonists also cause deficits in prepulse inhibition as assessed by acoustic startle response (see Zhang et al. 1997). PCP-like drugs have also been shown to impair social interaction in rodents (Steinpreis et al. 1994). Retention of new information and learning are impaired under the influence of PCP-like drugs (Wozniak et al. 1990). In view of these effects, it seems reasonable to assume that the PCP model of schizophrenia currently represents the most accurate, pharmacologically induced behavioral model of the disease. A major question remained, namely to what extent this model is compatible with, or relate to, the DA hypothesis of schizophrenia, an issue which will be briefly discussed below.

Involvement of DA in behaviors induced by NMDA receptor antagonists

Several early studies reported an increase in VTA DA cell firing rate following systemic administration of low doses of PCP (Freeman & Bunney 1984, French 1986). However, administration of high doses, i.e >2 mg/kg intravenously, was associated with a decrease in firing rate, an effect which was attributed to the DA reuptake blocking effect of the drug. Subsequent studies of other non-competitive NMDA receptor antagonists revealed a stimulating effect on firing rates of midbrain DA neurons similar to that of PCP (Freeman & Ceci 1990). However, the questions about the effects of PCP-like drugs on the physiological regulation of the firing patterns and dynamic response range of the mesocorticolimbic DA neurons remained to be elucidated. A PCP induced increase in DA metabolism was also observed and found to be associated with locomotor hyperactivity, an effect that could be blocked by 6-hydroxydopamine lesions of the VTA (French et al. 1985). The hyperlocomotion induced in rodents by systemically administered NMDA receptor antagonists in relatively low doses could also be abolished by depletion of DA from neuronal stores by pretreatment with reserpine and/or a-methyl-*p*-tyrosine (Fessler et al. 1980, Clineschmidt et al. 1982), or attenuated by systemic administration of DA receptor antagonists (Murray & Horita 1979, Clineschmidt et al. 1982). These observations indicate that brain DA systems are critically involved in the mediation of behavioral effects induced by PCP and MK-801 in low, non-ataxic doses. Later studies reported that high doses of MK-801, i.e. >1 mg/kg, could also induce locomotor hyperactivity in monamine-depleted mice (Carlsson & Carlsson 1989). However, such high doses were reported to be associated with profound

ataxia in rats (Criswell et al. 1993). These findings indicate a contribution also of DA-independent mechanisms to the locomotor hyperactivity induced by very high doses of MK-801. However, questions as regards the putative effects of PCP-like drugs on DA release in different nerve terminal regions, such as the cortical and subcortical projections of the VTA, remained elusive. In addition, the relationship between drug induced changes in electrophysiological activity of the DA neurons and transmitter release remained to be investigated. Since the psychotomimetic actions of PCP-like drugs basically seem to be related to NMDA receptor antagonism this analysis was especially focussed on the central actions of systemically administered MK-801, the most specific of the non-competitive NMDA receptor antagonists.

Consequently, the precise way in which PCP-like drugs affect the function of mesolimbic and mesocortical DA neurons appeared to be of major scientific interest, since insights gained within this framework might aid in the conceptualization of the emergence of psychotic pathophysiology. A second rationale for our work was that such insights might also be used to facilitate development of novel pharmacological strategies to treat psychoses. The present work was directed towards these two ends.

Specific aims

- To study the effects of acute administration of non-competitive NMDA receptor antagonists on

  - (I) The firing patterns of mesolimbic and mesocortical DA neurons.
  - (II) Regional release of DA and NT in mesolimbic (VSTR) and mesocortical (MPFC) DA terminal regions.

- To study the precise mechanisms underlying the effects of systemically administered non-competitive NMDA receptor antagonists on the mesocorticolimbic DA systems, i.e.

  - (III) The significance of nerve impulse activity in the DA neurons for basal and evoked DA release in the NAC and MPFC.
  - (IV) The significance of AMPA and/or kainate receptors in the VTA for basal and MK-801 evoked DA release in the NAC and locomotor stimulation.

- (V) To study the tentative antagonistic action of a_1-adrenoceptor blockade on MK-801 evoked DA release in the NAC and locomotor stimulation.

- (VI) To study the effects of AMPA receptor antagonists on the conditioned avoidance response and catalepsy score.

Materials and Methods

Animals and general experimental protocols

Male albino rats (Bantin and Kingman Universal AB, Sollentuna, Sweden) weighing between 250-350 g were used. BKl:WR (i.e. Wistar) rats were used in microdialysis and locomotor activity studies, and BKl:SD (i.e. Sprague-Dawley) rats were used in electrophysiological, conditioned avoidance and catalepsy experiments. Animals arrived at least one week before use and were housed five per cage under standard laboratory conditions, maintained on a 12 h light:dark cycle with lights on at 06:00, except for conditioned avoidance and catalepsy experiments where a reversed light:dark cycle was used, i.e. lights on at 18:00. Animals had access to R34 rat chow and water ad libitum. Only experimentally naive rats were used. All experiments were conducted with the permission and in accordance with the guidelines of the local ethical committees (Stockholms Norra och Södra Försöksdjursetiska Kommittéer).

Drugs

Phencyclidine hydrochloride (PCP; a generous gift from Astra Arcus AB), dizocilpine maleate (MK-801; (+)-5-methyl-10,11-dihydroxy-5H-dibenzo-(a,d)cyclohepten-5,10-imine maleate; Research Biochemicals Inc.), and D-amphetamine (Sigma) were dissolved in 0.9% saline solution for systemic injection. Prazosin (1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furanylcarbonyl)piperazine; Pfizer AB), an a_1-adrenoceptor antagonist, was dissolved in a drop of glacial acetic acid and titrated to volume using 5.5% glucose solution for systemic administration. LY326325 (LY293558 monohydrate; [3S(3a,4aa,6ß,8aa)]decahydro-6-[2-(1H-tetrazol-5-yl)ethyl]3-isoquinolinecarboxylic acid monohydrate; a generous gift from Eli Lilly and Company), an AMPA receptor antagonist, was dissolved in distilled water. CNQX (6-cyano-7-nitroquinoxaline-2,3-dione; Research Biochemicals Inc.), an AMPA and kainate receptor antagonist, was dissolved to a concentration of 100 mM in pure dimethylsulfoxide (DMSO). Immediately before use, this CNQX stock was diluted with perfusion solution (147 mM sodium chloride, 3.0 mM potassium chloride, 1.3 mM calcium chloride, 1.0 mM magnesium chloride, and 1.0 mM sodium phosphate, pH 7.4) to 0.3 and 1 mM for local perfusion via a microdialysis probe. Tetrodotoxin (TTX; Research Biochemicals Inc.), a sodium channel blocker which inhibits action potential generation, was dissolved in perfusion solution to a concentration of 1 µM for local perfusion via a microdialysis probe. Sodium pentobarbital was purchased as a solution, ready for systemic injection (Apoteksbolaget, Umeå). Chloral hydrate (Merck) was dissolved in 0.9% saline solution for systemic injection.

Extracellular single cell recording

Surgery and experimental procedures

The electrophysiological experiments followed the general procedures routinely used in this laboratory since more than a decade (see Grenhoff et al. 1986, Murase et al. 1993). Rats in studies I and II were anesthetized with chloral hydrate (400 mg/kg, i.p.) and maintained under surgical anesthesia throughout the experiments. A tracheal cannula and a jugular vein catheter for i.v. drug administration were inserted. The animal was subsequently mounted in a stereotaxic apparatus (David Kopf). Body temperature was kept at 37EC by means of a thermostatically controlled heating pad. A hole was drilled overlying the VTA, i.e. 3.0 mm anterior and 0.7 mm lateral to lambda (Paxinos and Watson 1986), and the dura mater was removed. At the end of each experiment a negative current of 5 µA was passed through the electrode for 10 min to mark the recording site (Lodge et al. 1974). Subsequently, rats were killed by overdose of anesthesia, brains were removed and stored in 25% sucrose in 10% formaldehyde. The brains were finally sliced in 50 µm thick sections and stained with neutral red. All recording sites included in these studies were confirmed under microscope to be correctly located within the VTA or the SN-ZC. In addition, anatomical localization of recording sites within subnuclei of the VTA were made in accordance with the atlas of Paxinos and Watson (1986) by three independent observers who were blind to the obtained effect on the recorded cell.

Extracellular recordings

Recording electrodes were pulled from Omegadot glass capillaries in a vertical electrode puller (Narshige) and filled with 2M sodium acetate saturated with Pontamine Sky Blue. The tips were broken back under microscope to an impedance of 2.0-3.0 MO measured at 135 Hz. Electrodes were lowered into the brain by means of a hydraulic microdrive (David Kopf). A reference electrode was also inserted into the subcutaneous tissue. The signal was amplified and visualized on a digital oscilloscope (Tektronix TDS 310) connected to an IBM compatible computer which allowed screen captures from the oscilloscope. Spikes were discriminated from background by means of a window discriminator. Subsequently, discriminated spikes were fed via a CED 1401 interface (Cambridge Electronic Design Ltd.) to a second computer running Spike2 software.

Identification of dopamine neurons and data analysis

Presumed DA neurons were found 7.5 - 8.5 mm from the brain surface and were recognized by their characteristic triphasic action potential waveforms of more than 2.0 ms duration, basal firing rates of 1 - 10 Hz and frequent occurrence of burst firing (Wang et al. 1981). The firing pattern was analyzed for average firing rate,

percentage of action potentials fired in bursts and variation coefficient, parameters which were calculated over a period of 500 consecutive inter-spike time intervals, utilizing an analysis script developed in our laboratory by the author. Average firing rate was calculated as the ratio between the number of spikes and the time elapsed, expressed in spikes per second (Hz). The onset of a burst was defined as an interval of less than 80 ms, burst termination as the next interspike time interval exceeding 160 ms (Grace & Bunney 1984), bursts exceeding 18 spikes were ignored. Burst firing was quantified as the percentage ratio of spikes in bursts and the total number of spikes. Variation coefficient was defined as the percentage ratio between the standard deviation and the mean of the inter-spike time intervals (Werner and Mountcastle 1963). Intravenous drug injections were administered 3 - 5 min apart. Only one DA cell was studied in each animal.

Statistical analysis

Electrophysiological data were presented as means ± standard error of the mean (S.E.M.) with the exception of burst firing values which are presented as means only, since they deviate from a normal distribution. Five hundred intervals preceding the first drug injection and five hundred intervals after each injection were used for calculation of control values and drug-induced changes, respectively. For statistical evaluation, firing rate and variation coefficient values were analyzed by Student's paired T-test, whereas burst firing values were analyzed with Wilcoxon's matched-pairs signed ranks test. Comparisons of burst firing values between groups were made with the Mann-Whitney U-test. Comparison of anatomical localization within the VTA was made with Fisher's exact test. P values <0.05 were considered significant.

Microdialysis in freely moving rats

Surgery and microdialysis

Rats in study III-VI were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and mounted in a stereotaxic frame (David Kopf). Body temperature was maintained at 37°C with a thermostatically controlled heating pad. Vertical probes of concentric type were stereotaxically implanted into the VSTR, i.e. AP +1.6 mm, ML -1.3 mm, DV -8.5 mm, the NAC, i.e. AP + 1.6 mm, ML - 1.4 mm, DV -8.2 mm, or the MPFC, i.e. AP +3.2 mm, ML -0.6 mm, DV -5.2 mm, relative to bregma. In some experiments, a second microdialysis probe was implanted into the ipsilateral VTA, i.e AP +3.8 mm, ML -0.5, DV -8.6 mm relative to lambda. Dialysis occurred through a semipermeable membrane (copolymer of acrylonitrile and sodium methallyl sulfonate, i.d. = 0.24 mm, 40,000 Da molecular weight cutoff, AN69 Hospal) with an active surface length of 1.0, 2.25, 3 and 4 mm for VTA, NAC, VSTR and MPFC, respectively.

After surgery, the animals were housed individually in plexiglass cages (32 x 35 x 50 cm) and given ad libitum access to food and water. All experiments were conducted approximately 48 h after surgery in awake, freely moving animals during the light cycle. All drug administrations were carried out after stable baseline conditions were achieved (<10% variation).

Microdialysis was performed using automated on-line sampling (Nomikos et al. 1989; Nomikos et al. 1994). The dialysis probe was perfused with a physiological perfusion solution (147 mM sodium chloride, 3.0 mM potassium chloride, 1.3 mM calcium chloride, 1.0 mM magnesium chloride, and 1.0 mM sodium phosphate, pH 7.4) at a rate of either 2.5 or 5 µl/min set by a microinfusion pump (Harvard Apparatus). The perfusate was loaded directly into the sample loop of the injector (Valco). An IBM compatible computer controlled the loading and injection modes of the injector. Samples were automatically injected into the analytical system every 20 min for NAC samples in studies 4-6, every 30 min for VSTR in study 3 and MPFC in studies 3 and 6 for analysis of DA and its metabolites. Samples designated for NT assay were collected every 60 min and frozen at -20EC and analyzed within one month. Upon completion of the experiments, rats were killed by an overdose of sodium pentobarbital and the brains were removed and stored in 25% sucrose in 10% formaldehyde. Each brain was sectioned on a microtome (50 µm), stained with neutral red, and examined for probe placement. Only rats with probes verified to be located within the correct region(s), according to the anatomical atlas of Paxinos and Watson (1986), were included in these studies.

Biochemical assays

Concentrations of DA, DOPAC and HVA were determined by high performance liquid chromatography with electrochemical detection (HPLC-ED) as previously described by Nomikos et al. (1989). Separation of DA and its metabolites was achieved by reverse phase chromatography (column: 150 x 4.6 mm, Nucleosil 5 µm, C18) with a mobile phase consisting of 0.055 M sodium acetate with 0.1 mM octanesulfonic acid, 0.01 mM Na_2EDTA, and 5% methanol, pH 3.8, adjusted with glacial acetic acid. The mobile phase was delivered by an HPLC pump (LKB; 2150) at 0.8 ml/min. Before entering the detector, the analysate was passed through a guard cell with an oxidizing potential of 50 mV. Electrochemical detection was accomplished using a coulometric detector (Coulochem II model 5200; ESA) with a high sensitivity analytical cell (5011) in which amine detection was achieved by the sequential oxidation and reduction of the eluent (coulometric electrode=+0.4 V; amperometric electrode=-0.2 V). Chromatograms were generated by a two-pen chart recorder (Kipp & Zonen) and simultaneously recorded and analyzed by an IBM compatible computer (Turbochrom

software; Perkin Elmer). The limit of detection was 1 fmol for DA and DOPAC and 3 fmol for HVA.

Neurotensin-like immunoreactivity (NT-LI) in the dialysates were quantified in study III by radioimmunoassay (RIA). Standards (140 µl, 1.95 - 125 pmol/l, Peninsula Laboratories, prepared in the same buffer as perfusion solution) and samples were incubated at 4°C for 48 hours with 25 µl of NT-antiserum (Cambridge Research Biochemicals) diluted to give approximately 30% total binding. Following this incubation, 25 µl of ^{125}I-NT (approximately 3500 cpm; Amersham) was added. After an additional 24 h at 4°C the bound and free antigen were separated by addition of 50 µl donkey anti-rabbit antibody-coated cellulose suspension (Sac-Cel; Wellcome Diagnostics) followed by centrifugation. The antibody is directed against the -COOH terminal of NT and its specificity is: NT_{1-13} 1.00, NT_{7-13} 0.83, NT_{1-8} and NT_{1-9} <0.001. Using high performance liquid chromatography, it has previously been shown that antibodies with immunoreactive characteristics similar to these we used recognize authentic NT from rat brain dialysates (Bean et al. 1989A). The intra- and interassay coefficient of variance for 3.7 pmol/l were 8 and 11%, respectively. The limit of RIA detection was 0.05 fmol.

Statistical analysis

For graphical representation of microdialysis data, the average of four baseline samples immediately preceding drug injection was defined as 100%. All subsequent measurements were transformed to a mean percentage of baseline values for each subsequent sampling period. Statistical evaluation was performed using the Statistica software suite (StatSoft Inc.). For evaluation of changes in dialysate levels of the various compounds, a one- and two-way (treatment x time) analysis of variance (ANOVA) with repeated measures was used, followed by the post-hoc Newman-Keuls test for multiple comparisons. In all statistical tests, a P value <0.05 was considered significant.

Behavioral studies

Locomotor activity

Locomotor activity in studies III and IV was assessed by means of computer-monitored photocell-equipped boxes (see Ericson et al. 1991). Each animal was placed in a square plexiglass open-field arena (68 x 68 x 45 cm) within a sound-attenuated and fan ventilated box, which was kept dark during the duration of the testing session. Two rows of photocells lined the exterior of the arena. All photobeam interruptions were recorded and stored on disk by an IBM-compatible computer. Computer recordings of horizontal activity (lower row interruptions) and rearing (upper row interruptions) were taken. In study III, peripheral activity (photobeam interruption adjacent to any wall of

the arena) and forward locomotion (successive interruptions of photobeams when the animal is moving in the same direction) were also assessed.

On the day of the experiment, rats were brought to the behavioral testing room in their home cages and allowed to become accustomed to the new environment for at least 60 min. Rats received drugs systemically and were immediately placed in the locomotor activity boxes. The interior of the locomotor activity boxes was wiped clean after each session. All behavioral monitoring was conducted during the light cycle between 08:30 and 17:00.

Visual assessment of MK-801 evoked behaviors

In study V, behavioral assessment was carried out simultaneously with ongoing microdialysis experiments (cf. above); thus, the behavior of the above animals was continuously observed to ascertain any overt changes. Characteristic MK-801 induced behaviors (Clineschmidt et al. 1982, Löscher & Hönack 1992) were rated by two independent observers, and were measured by the time during which the rats exhibited the following behaviors: Locomotor activity, ipsi- and contralateral turning, sniffing, head weaving and ataxia. Head weaving was determined as repetitive side-to-side, left-to-right swaying movements of the head and upper torso. Ataxia was defined as tottering of the hindquarters, abduction and dragging of hindlimbs, flat body posture, and loss of balance (Löscher & Hönack 1992). All behavioral monitoring was conducted during the light phase of the daily cycle between 12:00 and 17:00.

Conditioned avoidance response

In study VII, a shuttle-box (530 x 250 x 225 mm) divided into two compartments by a partition was used (see Wadenberg et al. 1990). Upon presentation of a conditioned stimulus (CS; 80 dB white noise) the animals had 10 s to move into the adjacent compartment of the shuttle-box. If the rat remained in the same compartment for longer than 10 s, the unconditioned stimulus (UCS) was presented, i.e. an intermittent electric shock in the floor grid (4 shocks per 10 s, duration 0.5 s, approximately 0.2 mA), until an escape response was performed, i.e. moving into the other compartment. Avoidance was recorded as a response to the CS within 10 s, escape as response to CS and UCS, i.e. > 10 s, and intertrial crosses, i.e. movement between compartments between trials. The animals were trained for three consecutive days and were initially habituated to the shuttle-box for 5 min, and subsequently trained. Each training session consisted of 20 trials randomly distributed over 15 min.

Experimental trials were preceded by a pre-test to reaffirm the rats' maintenance of CS responding ($80% avoidance). All pre-tests and experimental trials consisted of 10 trials randomly distributed over 7.5 min. Test sessions were conducted 20, 90 and 240

minutes after systemic administration of drug or vehicle. Animals were subjected to repeated observations using a cross-over design (Li, 1964) with a one week inter-trial delay period.

Catalepsy

Animals were placed on an 60E inclined grid in study VII and, excluding the first 30 s, the time the rat remained in the same position was measured, for a maximum of 2.5 min. The catalepsy was scored from 0-5 according to the (square root transformation) immobility time (min): 0 = 0-0.08, 1 = 0.09-0.35, 2 = 0.36-0.80, 3 = 0.81-1.42, 4 = 1.43-2.24, 5 $ 2.25.

Statistical analysis

Photocell measurements of behavior are presented as means ± S.E.M. of raw values of behavioral parameters over time. Data were analyzed by a two-way ANOVA, followed by LSD test in paper III or the Neuman-Keuls test for multiple comparisons in paper IV.

Visual assessments of behaviors are presented as mean ± S.E.M. times the animals displayed the various behaviors. Data were analyzed using one-way ANOVA and the post-hoc Neuman-Keuls test.

In the CAR experiments, avoidance data are presented as medians ± semi-interquartile range. CAR data and intertrial crosses were analyzed using the Friedman's analysis of variance (ANOVA) followed by the Wilcoxon's signed ranks test. Catalepsy scores were statistically analyzed using the Kruskal-Wallis ANOVA followed by the Mann-Whitney U-test (Ahlenius and Hillegaart 1986). P values < 0.05 were considered significant.

Results and Discussion

I: *Effects of PCP and dizocilpine (MK-801) on the firing pattern of midbrain dopamine neurons* (Papers I & II)

In an initial study we characterized the effects of a single, relatively low systemic dose of PCP (1 mg/kg, i.v.) on the firing pattern of VTA DA neurons, utilizing extracellular single cell recording techniques. While this dose of PCP elevated the average firing rate of VTA DA cells, in agreement with the previous results (Freeman & Bunney 1984, French 1986), it also caused differential effects on the firing pattern of VTA DA neurons. Thus, DA cells which initially displayed a bursty firing pattern responded to PCP administration by a regularization of the firing pattern and a reduction in burst firing. In contrast, DA neurons displaying relatively little burst firing before PCP administration responded with an increase in the variation coefficient (deregularization) and an increase in the percentage of spikes in bursts, as defined by the computer program used at this time(Grenhoff et al. 1988A). Thus, the overall VTA DA cell discharge was increased, yet abnormal as regards its temporal distribution. Other previous experiments had indicated that the VTA DA cell firing pattern is a major determinant of normal reward motivated behavior (Schultz et al. 1986, Nishino et al. 1987) as well as DA release from DA neurons (Gonon 1988). These conclusions have been supported by many subsequent studies (see Bunney 1992, Murase et al. 1993, Suaud-Chagny et al. 1992, Chergui et al. 1993, Schultz et al. 1993, Chergui et al. 1996, Schultz 1998). Consequently, we hypothesized that the abnormal functioning of subgroups of VTA DA neurons induced by PCP might contribute to the generation of abnormal behaviors, e.g. psychotic symptomatology.

PCP had been reported to reduce VTA DA cell firing rate in rats when doses >2.0 mg/kg i.v. were used, a finding that might reflect the dopamine reuptake blocking effect of PCP (Freeman & Bunney 1984, French 1986, Zhang et al. 1992). We therefore chose to subsequently investigate the actions on midbrain DA cell firing patterns of the more selective non-competitive NMDA receptor antagonist MK-801, which displays approximately 10 times more potent binding to the PCP site within the NMDA receptor ion channel than PCP, but lacks reuptake inhibitory properties.

Systemic administration of MK-801 (0.01-1 mg/kg, i.v.) caused a significant and

dose dependent increase in the average firing rate of DA neurons in both the VTA and the SN-ZC, in similarity to previous findings with PCP and MK-801 (Freeman & Bunney 1984, French 1986, French & Ceci 1990). In addition, we demonstrated that systemic MK-801 also significantly decreased the variability of DA cell impulse activity in a dose-dependent manner, as assessed by the variation coefficient, in both VTA DA and SN-ZC cells. Previous studies indicate that the dynamic response range, or variability, of neuronal activity is a necessary and adaptive feature of neuronal systems, in particularly within the context of learning or adaptation to novel situations (Servan-Schreiber et al. 1990). Thus, the reduced variability of firing of midbrain DA neurons caused by systemically administered psychotomimetic NMDA receptor antagonists may per se imply a generally reduced adaptive capacity of midbrain DA neurons to respond adequately to environmental demands. In SN-ZC cells, a modest yet significant increase in burst firing was obtained. Moreover, different VTA DA cell groups responded to MK-801 in a differential manner with respect to burst firing, in similarity to our previous findings with PCP. These effects were significant and also dose-dependent, indicating that they were, indeed, drug induced. Thus, in one group of VTA DA neurons, a marked decrease in burst firing was obtained (Fig. 7), in similarity to the previously observed effect of the broad-spectrum EAA receptor antagonist, kynurenate (Grehoff et al. 1988B). In contrast, in a second group of VTA DA neurons, several cells were activated by MK-801 to such an extent that they were interpreted by the computer as being essentially continuously bursting (Fig. 8). Thus, the average inter-spike interval often was shorter than 160 ms,theminimum interval required to signal burst termination in DA cells, due to the large increase in firing rate following MK-801 administration. Since this firing pattern was essentially devoid of post-burst pauses in DA cell activity and displayed a low variation coefficient, this firing pattern will henceforth be referred to as 'burst-like', even though it formally meets the previously established criteria for burst firing proposed by Grace & Bunney (1984).

Post mortem histological inspection revealed the following picture: Cells which displayed a high frequency, burst-like firing pattern after MK-801 administration were preferentially located within the paranigral (PN) subdivision of the VTA, that projects to

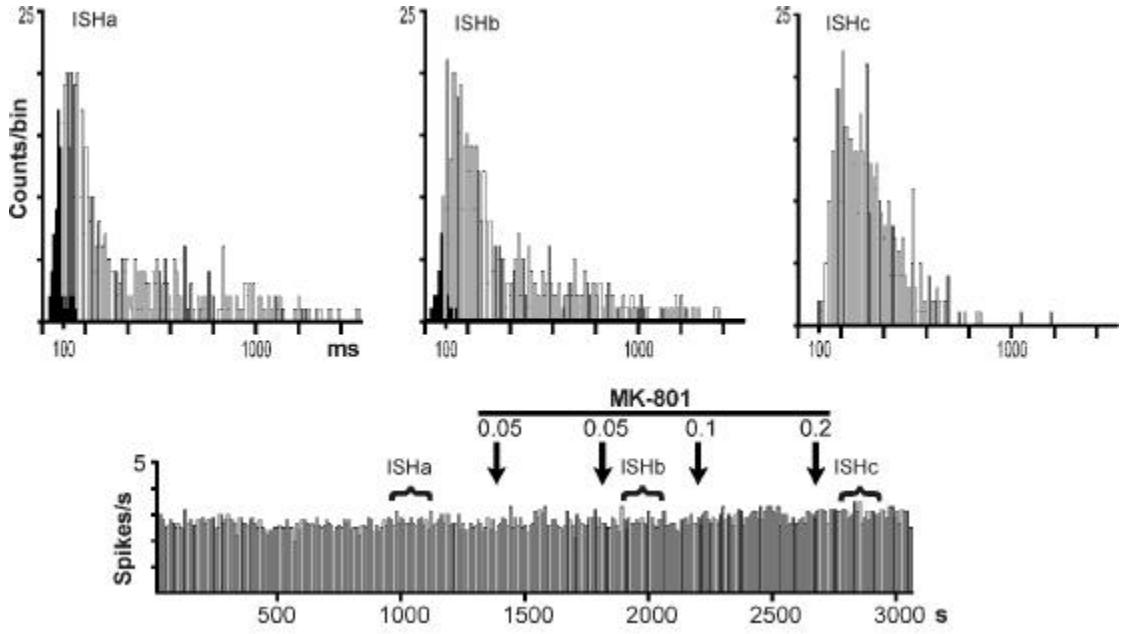

Fig. 7: Effects of systemic administration of MK-801 on the firing pattern of a VTA DA cell in the PBP, as shown by sequential interspike time interval histograms (ISH:es), burst firing illustrated by black bars (upper panel), and ratemeter recording (lower panel). Drug injections indicated at arrows. MK-801 administration caused only a minor increase in the average firing rate, whereas the firing pattern was clearly regularized, as shown by a gathering of the ISH around the mean interspike time interval (ISHc). Burst firing decreased in a dose-dependent manner, and was abolished following administration of the final dose of MK-801 (ISHc, 0.4 mg/kg, i.v.).

subcortical areas, which are involved in motor function and reward related behaviors, i.e. caudate-putamen, NAC and septum (cf. introduction). In contrast, VTA DA neurons that responded to MK-801 with a decreased burst firing were instead mainly localized within the parabrachial pigmented (PBP) subdivision of the VTA, an area which essentially provides the DA innervation of the PFC. Statistical evaluation revealed that in response to MK-801 administration cells localized in the PN, analyzed as a group, displayed significantly augmented burst-like firing, whereas PBP neurons displayed a significant decrease in the percentage of bursts. We subsequently reinvestigated the histological localization of the recording sites from our previous study on PCP and the above described pattern of distribution was also found in cells responding differentially to PCP (unpublished observations). Consequently, relatively low doses of both MK-801 and PCP potently activate burst-like firing in subcortically projecting, mesolimbic DA

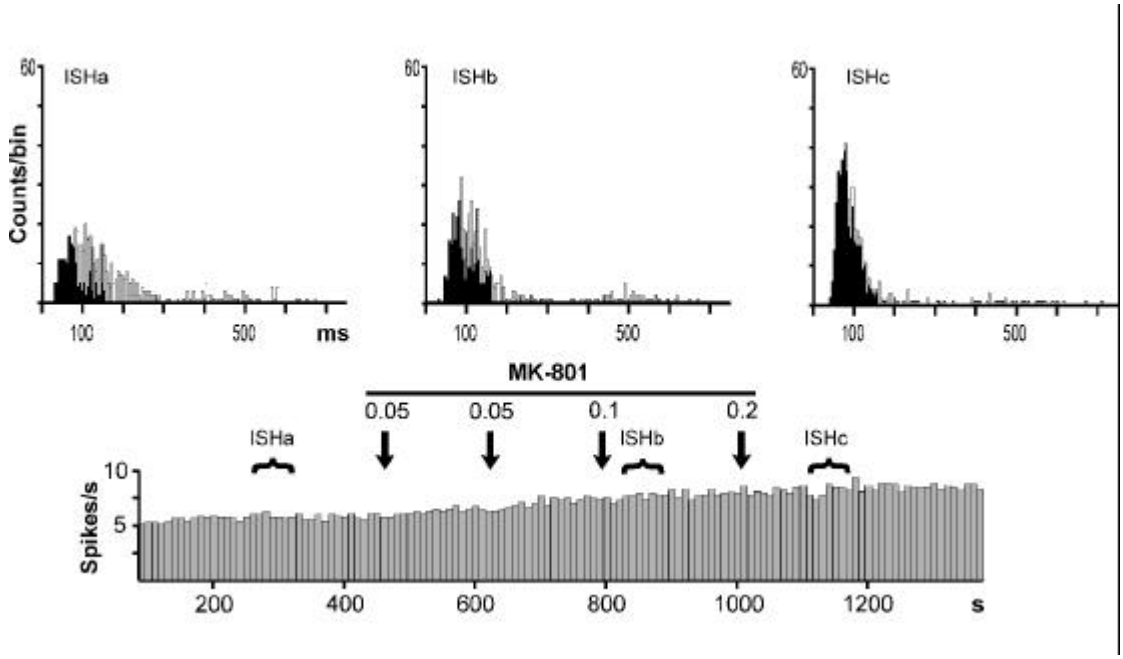

Fig. 8: Effects of systemic administration of MK-801 on the firing pattern of a VTA DA cell in the PN, as shown by sequential interspike time interval hisograms (ISH:es), burst firing illustrated by black bars (upper panel), and ratemeter recording (lower panel). Drug injections indicated at arrows. MK-801 administration caused an increase in average firing rate and a regularization of firing, as shown by a gathering of the ISH around the mean interspike time interval (ISHc). MK-801 administration evoked a high frequency burst-like firing pattern (ISHc).

neurons, while both drugs concomitantly attenuate burst firing in DA cells, which largely, but not exclusively project to the prefrontal cortical region.

The increased firing rate in DA cells observed following administration of low doses of PCP must thus be related related, albeit indirectly, to the non-competitive NMDA receptor antagonistic properties of this drug since MK-801 produced the same effect. The reduced burst firing observed in VTA DA cells in the PBP is very likely a direct consequence of NMDA receptor antagonism of DA cells, since local application of NMDA receptor antagonists to the VTA DA neurons has been shown to specifically attenuate the burst firing mode (Chergui et al. 1993, Tong et al. 1996). However, the pronounced increase in firing rate and burst-like firing pattern found in VTA DA cells in the PN following systemic administration of PCP or MK-801 could not readily be explained by such a mechanism. Tentative mechanisms might instead involve a decreased GABAergic inhibition of the DA cells (Zhang et al. 1993), or an enhanced activation of the DA neurons by other, non-NMDA EAA receptors (cf. introduction),

or both, a question that we subsequently addressed in other studies (see section IV). Interestingly, PBP and PN neurons have been reported to differ with respect to their expression of co-existing peptides. In particular, NT seems to be localized to DA neurons in the PBP rather than those in the PN (Jayaraman et al. 1990, Bayer et al. 1991). Thus, administration of PCP might cause differences in regional DA and NT release in cortical versus subcortical DA projection areas, a question we also addressed in subsequent experiments (see section II). In conclusion, the marked burst-like activation of DA neurons projecting subcortically to brain regions involved in motivational control and the concomitantly decreased burst firing in DA neurons which project to the PFC, a region which is involved in cognition and planning of behavior, might infer a possible basis for the dissociation of cognitive and motivational functions frequently described following systemic administration of PCP-like drugs (see Luby et al. 1959, Javitt & Zukin 1991).

II: *Effects of PCP on regional release of dopamine and neurotensin in the ventral striatum and the medial prefrontal cortex and on locomotor activity: comparison with D-amphetamine* (Paper III)

Several studies had emphasized the importance of the pattern of monoaminergic neuronal activity, specifically burst firing, rather than the average rate of discharge for the release of DA and NT from nerve terminals (see introduction; Gonon 1989, Bean and Roth 1991). Thus, the above electrophysiological data suggested that non-competitive NMDA receptor antagonists might differentially affect DA and/or NT-LI output in cortical and limbic DA terminal regions (see section I). Therefore, we subsequently studied the effects of systemic PCP and D-amphetamine on extracellular DA levels in the VSTR and MPFC, using microdialysis in freely moving rats coupled on line to a HPLC-ED system. In separate animals we also analyzed the PCP and D-amphetamine evoked changes in dialysate concentrations of NT-LI in the same regions with RIA.

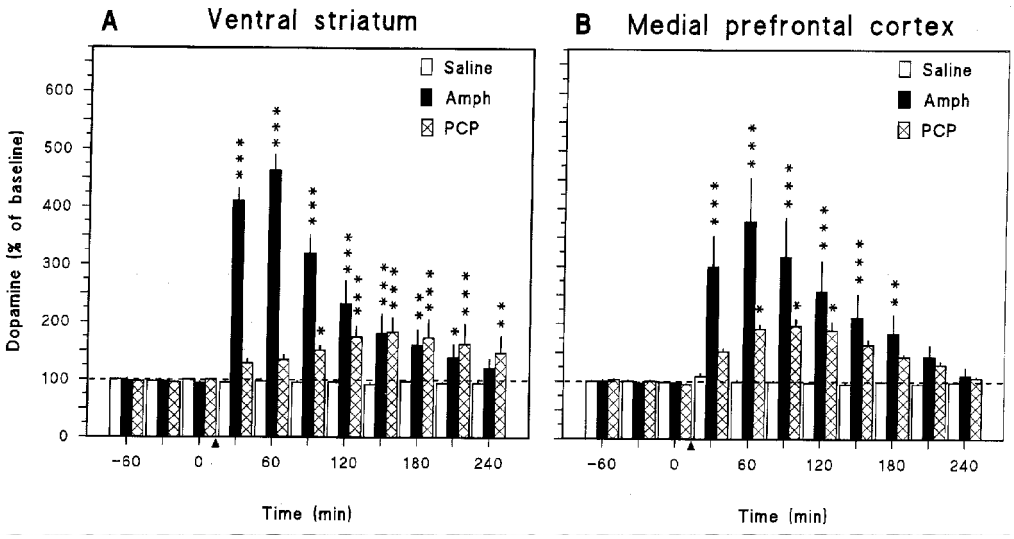

Fig. 9: Effects of systemic PCP and D-amphetamine administration on dialysate concentrations of DA in the VSTR (A) and the MPFC (B). Arrowheads indicate drug injection.

Systemic D-amphetamine administration (1.5 mg/kg, s.c.) caused a pronounced increase in DA concentrations from the VSTR and the MPFC (Fig. 9). The maximal increase occurred within 60 min of injection, and was of comparatively larger magnitude than that observed for PCP, in both regions. The increases in DA concentrations evoked by D-amphetamine were long lasting; DA levels remained significantly elevated for 210 and 180 min in VSTR and MPFC, respectively. In addition, D-amphetamine administration significantly increased NT-LI levels in MPFC dialysates during the first one hour sample, but did not affect NT-LI in VSTR dialysates (Fig. 10).

Similarly, PCP administration (2.5 mg/kg, s.c.) also significantly elevated DA levels in both VSTR and MPFC dialysates (Fig. 9). Thus, in the VSTR, the maximal increase was achieved at 150 min after PCP injection. In the MPFC, DA levels were also significantly increased by systemic PCP, reaching a maximal effect sooner than the increase observed in the VSTR, i.e. 90 min after administration. In parallel to the effects on DA, NT-LI levels in the first one hour sampling period in both MPFC and VSTR dialysates were also significantly increased by PCP (Fig. 10).

Animals treated with D-amphetamine showed significant and long-lasting increases in all behavioral parameters measured, including total horizontal activity, forward locomotion, peripheral activity and rearing. D-amphetamine decreased the

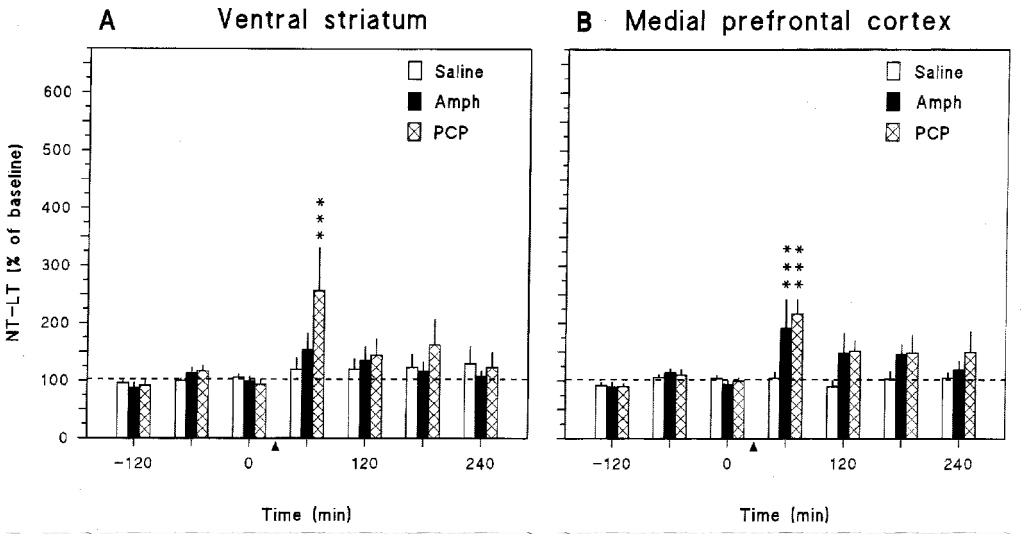

Fig. 10: Effects of systemic PCP and D-amphetamine administration on dialysate concentrations of NT-LI in the VSTR (A) and the MPFC (B). Arrowheads indicate drug injection.

percentage of peripheral activity as a ratio of total horizontal activity, which indicates a decrease in thigmotactic scanning, i.e. a common mode of exploratory locomotion in many species of mammals, including rats. In addition, D-amphetamine caused an increase in forward locomotion when expressed as a percentage of total horizontal activity, i.e. an increase in the perseverance of linear movement.

Similarly to D-amphetamine, PCP administration increased several parameters of locomotor activity, i.e. total horizontal activity and peripheral activity. However, the PCP induced behavioral activation was of smaller magnitude. In fact, during the first 30 min measurement period, PCP administration even decreased rearing. PCP also decreased the percentage ratio of peripheral activity to total horizontal activity and increased the ratio of forward locomotion to total horizontal activity.

In summary, these experiments show that D-amphetamine and PCP enhance DA output in the VSTR and MPFC in a similar manner, evoke marked behavioral stimulation and also alter the pattern of locomotor activity. The D-amphetamine and PCP evoked changes in the pattern of locomotor activity were temporally associated with the changes in DA output. However, although the effects of these two compounds on NT-LI levels in the MPFC were similar, only PCP elevated NT-LI levels in the VSTR. In view of the inhibitory effect of NT on DA receptor evoked responses in the

VSTR the temporal association of increases in NT-LI in the VSTR and the generally poor locomotor stimulation and reduced rearing following PCP administration, a causal relationship between these effects of PCP is suggested. Since several studies indicate that release of coexisting peptides as well as DA might be particularly effective during burst activation of monoamine containing neurons, the relatively large increase in release of both DA and NT in the MPFC following PCP administration may seem surprising since PCP administration decreased burst firing in cortically projecting VTA DA cells (see section I). These results might indicate that the PCP induced release of both DA and NT in the MPFC to a significant extent is independent of changes in nerve impulses, a hypothesis that we subsequently decided to investigate (see below, section III).

III: *Significance of nerve impulse activity in the ventral tegmental area for basal and MK-801 evoked dopamine release in the nucleus accumbens and medial prefrontal cortex* (Paper VI)

We had previously shown that systemic PCP and MK-801 cause reduced burst firing in VTA DA neurons in the PBP, which provides the DA projection to the MPFC. Yet, several subsequent microdialysis studies reported a marked increase in DA output in this region following systemic administration of these drugs (see section II, W“dzony et al. 1993, Wolf et al. 1993). These findings raised the possibility that PCP-like drugs evoke DA release in the MPFC largely via a mechanism independent of DA neuronal activity, i.e. it could be locally elicited in the DA nerve terminal region within the MPFC (see section II). Therefore, we studied the importance of nerve impulse activity in the VTA DA neurons for basal and MK-801 evoked DA output in the MPFC. Dual-probe microdialysis was used and the results were compared to those of corresponding experiments on the DA release in the NAC.

TTX perfusion of the VTA (1 µM) significantly attenuated basal DA output in both the MPFC and the NAC (Fig. 11). Thus, basal DA output in both these regions appears entirely dependent upon action potential generation in the VTA.

Two hour TTX perfusion of the VTA abolished the effect of MK-801 on DA

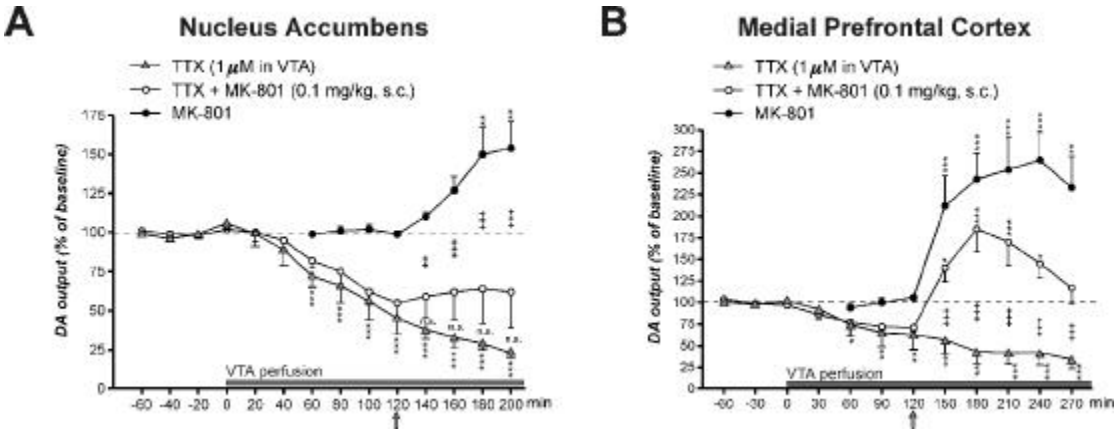

Fig. 11: Effects of TTX perfusion of the VTA on DA output in the NAC (A) and the MPFC (B), as assessed by dual-probe microdialysis in freely moving rats. Gray bars indicate TTX perfusion of the VTA. Arrows indicate the MK-801 injection.

release in the NAC (Fig. 11A) but not in the MPFC (Fig. 11B) where a significant increase was still observed.

In summary, the present data demonstrate that the increase in DA release in the NAC evoked by systemic MK-801 is critically dependent on nerve impulses generated in the VTA, i.e. it is largely caused by the increased neuronal activity in the VTA DA cells. In contrast, the pronounced increase in DA levels in the MPFC evoked by MK-801 is largely independent of action potentials generated in the VTA, as we had hypothesized. In other words, this increase is in all probability due to facilitation of DA output at the DA nerve terminal level in the MPFC. This facilitatory effect of systemic MK-801 on DA output in the MPFC might be caused, at least partially, by decreased GABAergic inhibition of DA terminals in the MPFC (Yonezawa et al. 1998). In addition, an EAA mediated mechanism in the MPFC has been proposed (Moghaddam et al. 1997), since local perfusion of the MPFC with the AMPA and kainate receptor antagonist CNQX partially attenuated systemic ketamine induced DA release in this brain region (cf. below). Our results indicate that a major dysfunction of brain DA systems induced by non-competitive NMDA receptor antagonists is the uncoupling of the mesocortical DA projection from its basal, physiological regulation by nerve impulse generation at the level of the VTA, in particular as regards its functionally most important mode, i.e. burst firing. However, the mechanism for the increased, burst-like firing of subcortically projecting VTA DA neurons in the PN induced by these drugs remained to be clarified.

IV: *Significance of AMPA and kainate receptors in the ventral tegmental area for basal and MK-801 evoked dopamine release in the nucleus accumbens and locomotor stimulation*

(Paper V)

During the experiments described in section I, we observed an unexpected increased neuronal activity and, particularly, burst-like firing in VTA DA neurons in the PN following administration of PCP or MK-801. This was associated with a markedly increased DA release in the major projection area of the VTA PN neurons, the NAC (see section II & III). Since burst firing had been shown to depend on tonic activation of NMDA receptors in the VTA, the mechanism of the high frequency burst-like firing remained obscure. Although NMDA receptor in different anatomical localizations might be involved, significant NMDA-, AMPA-, and kainate receptor binding had been demonstrated in the VTA (Albin et al. 1992). We observed a marked similarity between the MK-801 induced firing pattern in VTA DA neurons in the PN and that obtained following local microiontophoretic application of the AMPA and kainate receptor agonists quisqualate and kainate (Chergui et al. 1993). Moreover, previous results indicated that mesolimbic and mesocortical DA neurons are differentially modulated by EAA receptor subtypes in the VTA: although the mesocortical DA projection appeared largely modulated by NMDA receptors, subcortically projecting VTA DA cells seemed primarily regulated by the AMPA and kainate subtypes of EAA receptors (Kalivas et al. 1993). Thus, we hypothesized that the high-frequency burst-like firing in the PN neurons of the VTA following systemic PCP or MK-801 might be caused indirectly by means of activation of AMPA and/or kainate receptors in the VTA, an effect that also could be responsible for the increased DA release found in the NAC. Therefore, in this series of experiments we assessed to what extent local application into the VTA of an AMPA and kainate receptor antagonist, CNQX, could antagonize the evoked DA release in the NAC and the associated behavioral stimulation caused by systemically administered MK-801. MK-801 evoked behaviors were assessed by direct observation in conjunction with the ongoing microdialysis experiments, i.e. locomotor activity, turning, head weaving, sniffing and ataxia.

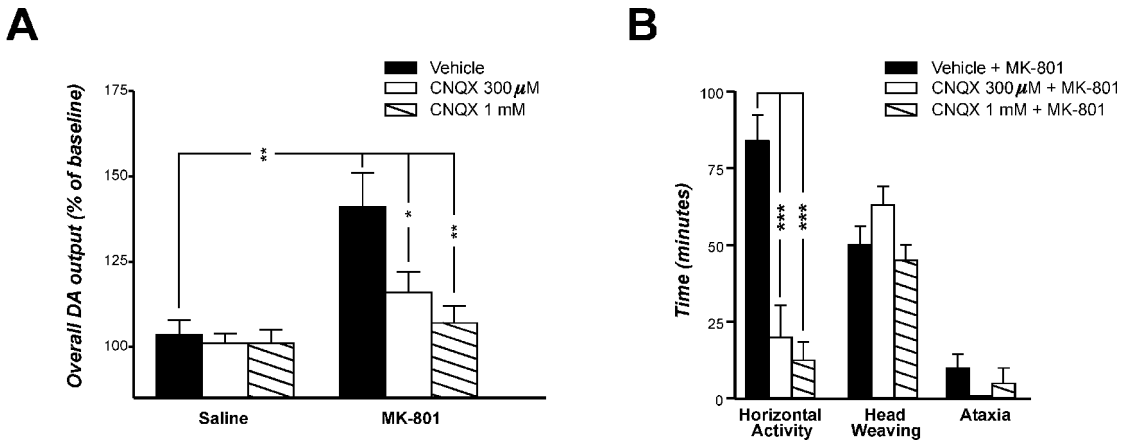

Fig. 12: Effects of local perfusion of the VTA with the AMPA and kainate receptor antagonist, CNQX, on systemic MK-801 evoked DA release in the NAC as assessed by microdialysis (A) and typical MK-801 evoked behaviors (B).

Perfusion of the VTA with CNQX for forty minutes effectively antagonized the increase in DA output in the NAC evoked by subsequent systemic MK-801 administration in a concentration-dependent manner. When the highest concentration of CNQX was used, i.e. 1 mM, systemic administration of MK-801 completely failed to affect DA output in the NAC (Fig. 12A). In addition, we observed that animals perfused with CNQX in the VTA exhibited significantly shorter periods of locomotor activity following MK-801 injection. None of the other measured MK-801 evoked behaviors was affected by CNQX perfusion of the VTA (Fig. 12B). Perfusion of the VTA with the AMPA and kainate receptor antagonist CNQX (300 µM and 1 mM) alone did not significantly affect DA output in the NAC, nor did it cause any change in the rated behaviors.

Consequently, MK-801 evoked locomotor activity and DA release in the NAC appear critically dependent upon AMPA and/or kainate receptor activation in the VTA. Indirectly, this finding suggests that MK-801 causes an augmented EAA input to the VTA neurons, a notion that has gained some support from preliminary results, demonstrating an apparently increased extracellular concentration of glutamate and aspartate in the VTA following s.c. administration of MK-801, 0.1 mg/kg (Svensson et al. 1998A). In fact, the non-competitive NMDA receptor antagonist ketamine has recently been shown to elevate extracellular concentrations of glutamate in the MPFC (Moghaddam et al. 1997). Our behavioral results are indirectly supported by Narayanan

et al. (1996), data demonstrating that local inhibition of VTA neuronal activity with the $GABA_B$ agonist baclofen also antagonizes the increase in locomotion caused by systemic MK-801. In addition, in the same study local bilateral microinjection of MK-801 into the VTA was found to mimic the behavioral effects of systemic MK-801 injection. Thus, both the locomotor stimulatory and the DA releasing effects in the NAC of systemic MK-801 seem to be elicited within the VTA. By inference, our present and previous findings suggest that the hyperlocomotion caused by systemic MK-801 is causally related to the increased DA output in the NAC which, ultimately, is due to the AMPA and/or kainate receptor mediated increase in DA neuronal activity.

In summary, the data presented in this section show that the dose-dependent high frequency activation of mesolimbic DA neurons, induced by low doses of systemically administered MK-801 (see section I) represents the major underlying mechanism both for the associated increased DA release in the NAC and the ensuing hyperlocomotion. Moreover, the burst-like activation of the subcortically projecting DA neurons of the PN subdivision of the VTA is indirectly mediated via activation of AMPA and/or kainate receptors in the VTA. Most significantly, although CNQX, locally applied to the VTA effectively suppressed both the DA releasing effect and the hyperlocomotion induced by administration of MK-801, it did not affect basal DA release or spontaneous locomotor activity. Since antagonism of hyperlocomotion induced by PCP-like drugs has been shown to be of some predictive value in assessment of putative antipsychotic drugs (see Ögren et al. 1996), we hypothesized that AMPA and/or kainate receptor antagonists might possess antipsychotic activity. This idea was also proposed by other researchers, and was in those cases based upon behavioral evidence alone (see section VI). The present study supports a causal relationship between the MK-801 induced DA release and the associated behavioral stimulation and, hence, provides evidence for a presynaptic DA inhibitory effect of the AMPA and kainate receptor antagonists, an action that might act synnergistically with DA receptor blockade to suppress psychotic symptomatology.

V: *Tentative antagonistic action of a_1- adrenoceptor blockade on MK-801 evoked dopamine release in the nucleus accumbens and behavioral stimulation* (Paper IV)

Previous results infer blockade of other excitatory inputs to the VTA might suppress the behavioral stimulation as well as the evoked accumbal DA release caused by the psychotomimetic NMDA receptor antagonists. Since several studies from this laboratory provided evidence for a noradrenergic excitatory regulation of VTA DA neuronal activity, the putative utility of blocking this a_1-adrenoceptor mechanism was explored.

Nearly all clinically utilized antipsychotic drugs display a_1-adrenoceptor antagonistic properties, in addition to their DA-D_2 receptor blocking action (see Carlsson & Lindqvist 1963, Peroutka & Snyder 1980, Cohen & Lipinski 1986). Although brain noradrenergic hyperactivity has been proposed in schizophrenia, especially preceding and during acute psychotic relapse, the putative contribution of a_1-adrenoceptor antagonism to the antipsychotic efficacy of neuroleptics remains to be established (van Kammen & Kelley 1991, Maas et al. 1993, van Kammen et al. 1994).

In initial electrophysiological experiments we observed that the increased burst-like firing in subcortically projecting VTA DA neurons induced by systemic MK-801 could be effectively suppressed by subsequent administration of prazosin (Svensson et al. 1995). In the present experiments we therefore studied the modulation by prazosin of MK-801 effects on output of DA and its metabolites in the NAC, as assessed by microdialysis, and on locomotor activity. The two doses of MK-801 chosen were 0.1 mg/kg s.c., a dose which has been shown to evoke hyperlocomotion which is highly sensitive to catecholamine depletion, and 0.3 mg/kg, s.c., reported to represent the highest dose which may be administered to rats without concomitant induction of severe ataxia (cf. Criswell et al. 1993).

Systemic administration of MK-801 caused significant 60% overall increase in output of DA and its metabolites in the NAC that reached a plateau approximately three hours after injection (Fig. 13A). The magnitude and time course of effect of MK-801 was essentially identical for the two doses, suggesting that already at 0.1 mg/kg a maximal DA release in the NAC was achieved (data not shown). MK-801 administration

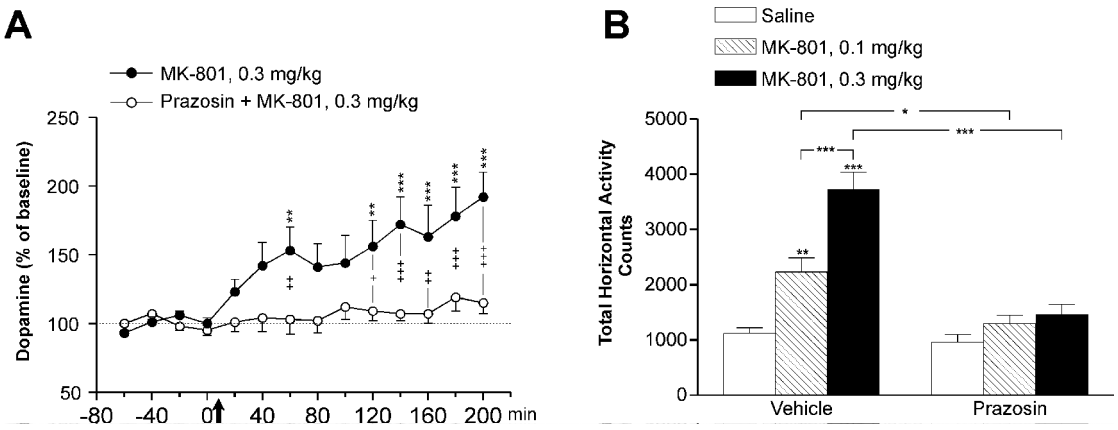

Fig. 13: Effects of systemic pretreatment with the a_1-adrenoceptor antagonist, prazosin, on MK-801 evoked DA release in the NAC (A) and on locomotor activity (B). Arrow indicates MK-801 injection.

also caused dose-dependent increases in dialysate concentrations of DOPAC and HVA, with a time course that parallelled that of DA, as well as a significant and dose-dependent increase in horizontal locomotor activity (Fig. 13B). However, similarly to PCP, MK-801 also reduced rearing in dose-dependent manner. An increased contribution of non-DA dependent mechanisms to the generation of hyperlocomotion when higher doses (i.e. >0.25 mg/kg s.c. to rats) of MK-801 are used (see Carlsson & Carlsson 1989) is supported by the finding that the higher dose of MK-801 increased locomotor activity further while DA output in the NAC was not additionally augmented compared to the effects of the low dose.

Prazosin administration alone did not affect basal DA output in the NAC or horizontal locomotor activity. However, it significantly reduced rearing. In contrast, pretreatment with prazosin for twenty minutes significantly attenuated the elevation in DA output in the NAC induced by both doses of MK-801 and significantly antagonized MK-801 evoked increases in locomotor activity.

In summary, the present data demonstrate that systemic administration of MK-801 significantly augments DA output in the NAC, in accordance with previous microdialysis data (see section III, Wolf et al. 1993). Moreover, systemic pretreatment with the a_1-adrenoceptor antagonist prazosin, did not affect basal locomotor activity or DA output in the NAC, but blocked both the evoked DA release in the NAC and the behavioral stimulation caused by MK-801, effects which thus seem to be causally related.

Since prazosin also blocked the MK-801 induced intense burst-like firing in the VTA DA neurons projecting e.g. to the NAC, these results are in consonance with the view that both the observed biochemical and behavioral effects are causally related to the evoked neuronal activity in the subcortically projecting VTA DA cells. One mechanism that may be involved in the suppression of MK-801 evoked DA output is that prazosin blocks the excitatory a_1-adrenoceptors within the VTA. However, a_1-adrenoceptors in other parts of the brain might also be involved. Thus, a_1-adrenoceptor antagonism appears to specifically inhibit evoked, but not basal, DA output, an effect which may be of clinical interest, in view of the recent evidence for an enhanced evoked DA release in vivo in striatal tissue of schizophrenic patients (Laruelle et al. 1996, Breier et al. 1997). Therefore, the a_1-adrenoceptor blocking action of most clinically available antipsychotic drugs may well contribute to their overall DA antagonistic actions at a presynaptic level. Since brain noradrenergic neurons, e.g. of the LC, are known to be activated by stress (Foote et al. 1983), and environmental stress has been claimed to contribute to precipitation or aggravation of psychotic behavior or relapse (van Kammen & Kelley 1991, Maas et al. 1993, van Kammen et al. 1994), the a_1-adrenoceptor blocking action of most antipsychotics, which with some drugs such as clozapine is very prominent, may be particularly valuable during conditions of environmental stress or at the beginning of a psychotic episode.

VI: *Effects of AMPA receptor antagonism on the conditioned avoidance response and on catalepsy score* (Paper VII)

In the previous experiments, we observed that AMPA and/or kainate receptor antagonists block both behavioral stimulant and central, dopaminergic effects of psychotomimetic NMDA receptor antagonists (see section IV, Willins et al. 1993, Bubser et al. 1995, Vanover 1998). These studies suggest, although their predictive value of this type of behavioral studies with regard to antipsychotic efficacy seems not to be conclusively established, that AMPA and/or kainate receptor antagonists may possess antipsychotic efficacy. Since suppression of the conditioned avoidance response (CAR) is a behavioral effect that is shared by all effective antipsychotics, and potencies in the

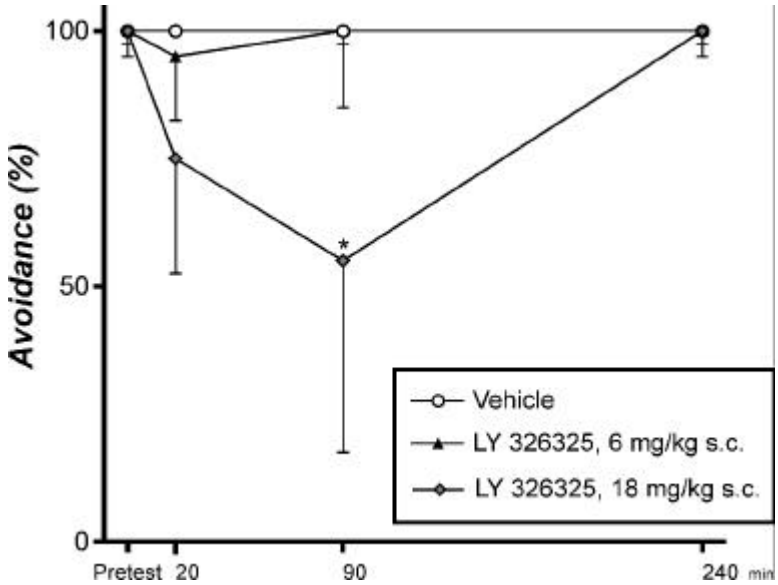

Fig. 14: Effects of the AMPA receptor antagonist, LY326325, on the conditioned avoidance response.

CAR test are highly correlated with and specifically predict therapeutic efficacy in schizophrenia (Janssen et al. 1965, Arnt 1982), we decided to study the effects of systemically administered AMPA receptor antagonists within this experimental paradigm. Interestingly, systemic administration of GYKI52466, a non-competitive AMPA receptor antagonist, was found to cause a significant suppression of CAR in the rat, without any effect on escape behavior (Svensson et al. 1998B). Therefore, in the present study we analyzed the putative effect of LY326325, a novel and specific AMPA receptor antagonist, on CAR performance. In parallel experiments we studied its tentative cataleptogenic properties, since the induction of catalepsy is generally recognized and utilized as a predictive test for EPS liability of antipsychotics or potentially antipsychotic drugs.

Our experiments revealed a reduction in avoidance behavior induced by systemic administration of LY326325, 18 mg/kg, s.c., which was significant at 90 min following injection (Fig. 14), without any impairment of escape behavior and, in fact, no response failures were observed. LY326325 did not affect CAR when given at 6 mg/kg s.c. Moreover, LY326325 did not cause significant catalepsy. When given in high doses LY326325 (18 mg/kg, s.c.) caused some reduction of spontaneous locomotion and mild

ataxia, although no significant change in inter-trial crosses was obtained. However, a selective suppression of CAR is a specific effect of antipsychotic drugs which cannot be attributed to a general sedative action of the drugs (Courvoisier 1957, Verhave et al. 1958).

In summary, LY326325 exhibits a pharmacological profile consonant with antipsychotic efficacy, as assessed by the CAR paradigm, an effect which is similar to our recent results obtained with the non-competitive AMPA antagonist, GYKI52466. Since two chemically different AMPA receptor antagonists were both found to cause selective suppression of CAR, our findings provide strong support for the notion that AMPA receptor blockade is the mechanism involved. These results were obtained without any significant general attenuation of locomotor activity, thus indicating that the suppression of CAR was not related to impairment of locomotion per se. Moreover, since LY326325 did not cause significant catalepsy, it may not cause extrapyramidal side effects, in contrast to classical antipsychotic drugs. Previous studies have emphasized that clozapine more effectively ameliorates the behavioral effects of PCP-like drugs than classical antipsychotic drugs, including superior efficacy against hyperlocomotion and stereotypies, as well as amelioration of deficits in delayed alternation performance (Tiedtke et al. 1990, Hoffman 1992, Hauber 1993). Since LY293558, another structurally similar AMPA receptor antagonist, has been shown to attenuate cognitive deficits induced by ketamine, as assessed by delayed alternation (Moghaddam et al. 1997), the antipsychotic efficacy of AMPA receptor antagonists may also include cognitive symptoms. Thus, LY326325 appears to possess a pharmacological profile that is more similar to the atypical antipsychotic profile of clozapine than to that of classical neuroleptics.

General Discussion

In 1959, Luby et al. described the astonishing finding that an intravenous injection of PCP to healthy volunteers even within an hour could elicit a mental state that was virtually indistinguishable from the primary symptomatology of acute schizophrenia, including e.g. auditory hallucinations and formal thought disorder, but also blunted affect (see Javitt & Zukin 1991). Moreover, administration of subanesthetic doses of PCP to a small group of chronic schizophrenic patients was reported to reinstate the symptomatology of the acute, agitated form of the illness. Such psychotomimetic properties have subsequently been found to be shared by all clinically evaluated NMDA receptor antagonists (cf. introduction, Kornhuber & Weller 1995). Consequently, it can be assumed that the behavioral state modelled by acute administration of PCP-like drugs may reflect pathophysiological mechanisms in brain that are involved at the onset of schizophrenia. Since early effective treatment might possibly contribute to reduce continued deterioration frequently associated with chronic schizophrenia (Remington et al. 1998, Wyatt et al. 1998), such initial pathophysiological mechanisms may be of particular importance in the search for novel pharmacological strategies to treat schizophrenia at an early stage of illness. Generally, our findings show that following acute systemic administration of low, non-ataxic doses of psychotomimetic NMDA receptor antagonists, such as PCP or MK-801, a profound amd differential dysregulation of the mesolimbic and mesocortical DA systems occur. Since several sets of experimental data show that major behavioral effects of low doses of these drugs in the rat, tentatively relevant to the human experience, are mediated largely via brain catecholamines, in particular DA, which exerts a pivotal role in the control of psychotic symptomatology, the precise nature of these dysregulations has a considerable interest.

Our findings demonstrate a general deficit in the NMDA receptor mediated phasic neuronal activation of mesocorticolimbic DA neurons and markedly reduced variability of firing, superimposed upon an increased basal activity, i.e. a reduction in the signal-to-noise ratio in DA signalling. Also previously, dopaminergic dysfunction in schizophrenia has been proposed to involve a generally inappropriate modulation of DA responsiveness to environmental stimuli, although in this case all stimuli were hypothesized to elicit a maximal phasic DA release, an effect elicited essentially at the

DA nerve terminal level in the VSTR (Grace 1993). However, more recent results have emphasized the importance of corticofugal EAA inputs to the VTA in the reward and stress related augmentation of DA output in the NAC, i.e. phasic nerve impulse mediated responses mediated by EAA receptors in the VTA (Murase et al. 1993, Taber et al. 1995, Murphy et al.1996, Westerink et al. 1997, Enrico et al. 1998). In fact, a previous study from our laboratory directly demonstrated a decreased sensory responsiveness of other catecholamine neurons in the brain, i.e. in the LC, which receives an EAA input from the MPFC (Jodo & Aston-Jones 1997, Jodo et al. 1998), following systemic administration of PCP or MK-801. These neurons also displayed a reduced varability of firing, but an increased basal firing rate (Murase et al. 1992). These findings support the notion of a general reduction of dynamic neuronal responsivity to environmental stimuli of central catecholamine neurons. Our present results support the contention that the augmentation of subcortical DA output induced by MK-801 largely is elicited in the VTA, and that it is nerve impulse dependent. Thus, the dysregulations of DA neuronal activity induced by PCP-like drugs indicate that an augmented tonic, but attenuated phasic DA neuronal signalling in the mesolimbic DA projection may be associated with the onset of psychotic symptoms. Such a dysregulation might well have bearing on symptoms such as emotional blunting and motivational dysfunction. Since a dysregulation of DA signalling also was observed in the nigrostriatal DA projection, a neuronal pathway inter alia regulated by proprioceptive inputs and fundamentally involved with the specific control of movements and posture (Romo & Schultz 1989), and an even more pronounced dysregulation was observed in the prefrontal DA projection, a brain region heavily involved with cognitive and integrative functioning, an imbalance emerges within the striato-pallido-thalamo-cortical circuitry that may tentatively have bearing on primary symptoms such as disturbances of body image and depersonalization.

Our findings demonstrate that the locomotor hyperactivity induced by acute systemic administration of low doses of MK-801 is associated with, and largely dependent upon, augmentation of DA output in the NAC, in similarity to the psychostimulant effect of D-amphetamine. Previously it has been postulated that hyperlocomotion induced by psychotomimetic drugs may primarily reflect mechanisms involved in the generation of positive symptoms of schizophrenia (see Martin 1998).

Dopaminergic mechanisms have also been implicated in various other PCP induced behaviors in rodents, e.g. hyperlocomotion, some stereotypies, impaired cognitive function and deficits in social behavior vide supra (Murray & Horita 1979, Fessler et al. 1980, Clineschmidt et al. 1982, Gratton et al. 1987, Ögren & Goldstein 1994, Steinpreis et al. 1994, Jentsch et al. 1997). Needless to say, not all MK-801 evoked behaviors were attenuated by reduction of concomitant DA output in the NAC since, for example, head weaving and sniffing were not affected. Consequently, some of these effects, e.g. stereotyped behaviors, may instead be related to augmentation of DA output in nigrostriatal DA terminal regions, e.g. dorsolateral striatum (Miller & Abercrombie 1996), or to non-dopaminergic mechanisms. Thus, PCP and MK-801 evoked head weaving has been shown to be related to enhanced 5-HT_{1A} receptor activity (Yamaguchi et al. 1987, Löscher & Hönack 1992), and our experiments showed that reduction of rearing, as induced by systemic administration of PCP, appears to be temporally related to the increase in NT output in the VSTR. In addition, higher, ataxic doses of MK-801 have been reported to evoke pronounced locomotor activity also in monoamine-depleted mice (Carlsson & Carlsson 1989), although the threshold dose was subsequently shown to be approximately tenfold higher than that in monoamine-intact animals (Martin et al. 1994). These observations are not, in principle, contradictory to our findings in rats. Rather, following the probably more extensive NMDA receptor blockade induced by high ataxic doses of MK-801, local EAA mechanisms within the NAC may contribute to further exaggerate behavioral output in a manner which is functionally disconnected from the DA release process (see Carlsson 1993). Several lines of evidence indicate that in schizophrenia impairments of the connectivity and synchronization of discrete brain regions may facilitate the generation of abnormal output from these structures, e.g. contribute to the emergence of local "ectopic foci", which autonomously generate abnormal behaviors (Weinberger 1987, Hoffman & McGlashan 1994). Tentatively, the presently observed differential dysregulations of mesolimbic and mesocortical DA neurons may be seen as an early sign of such regional desynchronization. Physiologically both DA projections are largely regulated at the VTA level. However, administration of PCP-like drugs largely shifts this control of the mesocortical, but not the mesolimbic, DA projection to a local control mechanism within the PFC region. Thus, the PFC becomes functionally more autonomous in

comparison to subcortical structures such as the ventral striatum. Consequently, the emergence of DA independent behaviors with very high doses of NMDA receptor antagonists might constitute a pharmacological model of more chronic schizophrenia, whereas the largely DA dependent behavioral abnormalities, acutely obtained with low, non-ataxic doses of these drugs, rather might reflect pathophysiological mechanisms that may be involved in the onset of acute psychosis. The clinical observation that acute psychotic states generally are more responsive to treatment with DA-D_2 receptor antagonists than chronic schizophrenia is clearly compatible with this notion. A logical consequence of this notion is that pharmacological treatment at the early onset of schizophrenia might require somewhat different drugs than those currently used to combat the chronic disorder (see below). Potentially, the acute PCP model of schizophrenia could therefore help to identify such drugs.

Repeated PCP administration, similarly to acute administration, is associated with profound cognitive deficits, although the effects of chronic administration appear more long-lasting and severe than those observed following a single injection. Also non-human primates exposed to chronic PCP exhibit marked cognitive impairment, a phenomenon associated with reduced DA turnover in the MPFC (see Jentsch et al. 1998). Consequently, these data indicate that a dysregulation of the mesocortical DA system may play a role in the behavioral actions of PCP also in primates, and vide infra also in schizophrenia, as previously suggested (Weinberger 1987, Svensson et al. 1993). Cognitive impairments have also been observed in rats following acute administration of PCP-like drugs, but were associated with an increased DA output in the MPFC (present study, Verma & Moghaddam 1996, Moghaddam et al. 1997). However, several lines of evidence indicate that PFC functioning may be highly sensitive to changes in DA receptor activation, whether increased or decreased (Brozoski et al. 1979, Sawaguchi & Goldman-Rakic 1994, Murphy et al. 1996, Verma & Moghaddam 1996). Moreover, optimal PFC function in relation to cognitive activity, e.g. executive function and planning of behavior in a contextually relevant manner, seems not only to require sufficient basal release of DA, but also adequate and transient variations of DA output in response to reward-predicting stimuli or failure of reward prediction. In other words, impulse dependent phasic alterations in VTA DA neuronal activity appear necessary for adequate cognitive functions involving working memory as well as the general adaptation

to novel situations (Sawaguchi & Goldman-Rakic 1994, Schultz et al. 1993, Schultz 1998). Therefore, the fact that in the MK-801 treated animal the physiological, impulse dependent DA release in the MPFC has been largely replaced or superseded by locally generated DA release within this brain region implies a dramatic change in the physiological control of prefrontal dopamine release. Such a dysregulation of mesocortical DA function should have particular behavioral impact in stressful situations requiring rapid alterations in the signal processing characteristics of the postsynaptic cortical neurons (Servan-Schreiber et al. 1990).

Pioneering studies in a neurodevelopmental model of schizophrenia, created by neonatal lesions of the ventral hippocampus, have revealed in post-pubertal rats the emergence of behavioral hyperresponsiveness to stress or DA agonists (see Lipska & Weinberger 1993) and, in the adult lesioned rats, an increase in DA turnover in the VSTR, concomitant with a decrease in the MPFC were observed (Lipska et al. 1995, see Weinberger & Lipska 1995). Thus, this neurodevelopmental model of schizophrenia, perhaps particularly in the stressed state, offers an intriguing functional similarity with the PCP-induced dichotomy in the dynamic function and dysregulation of the mesocortical and the mesolimbic DA neurons.

In our previous experiments, the reduction of burst activity in the mesocortical DA cells following MK-801 administration could be specifically reversed by subsequent injection of the potent 5-HT_{2A-2C} antagonist, ritanserin, which still did not affect the tonic activity of the cells, i.e. their average firing rate (Svensson et al. 1995). In addition, atypical antipsychotic drugs with potent 5-HT_{2A} antagonistic properties, e.g. clozapine and amperozide, preferentially augment DA output in the limbic cortical related sites including the MPFC in rats (Moghaddam & Bunney 1990, Nomikos et al. 1994, Marcus et al. 1996). These and other findings from this laboratory (e.g. Grenhoff et al. 1990) suggest that 5-HT_{2A} receptor antagonistic effect of atypical antipsychotics may specifically help to improve the nerve impulse dependent, phasic signalling of cortically projecting DA cells. Such an improvement of the timing of DA release in the PFC may lead to an improved PFC functioning, which according to recent data appears to require a precise timing and magnitude of DA release for effective cognitive behavior (Williams & Goldman-Rakic 1997). A secondary consequence of an augmentation of phasic DA output in the PFC is probably an associated reduction of DA output in striatal sites

(Kolachana et al. 1995). Consequently, a facilitation of cortical DA functioning caused by potent 5-HT_{2A} antagonists, may indirectly suppress a hyperactive or hyperreactive subcortical DA projection at the presynaptic level and, hence, act synergistically with postsynaptic DA-D_2 receptor antagonism.

Our results, which may reveal fundamental pathophysiological mechanisms with tentative bearing on the emergence of schizophrenia, might thus afford insights that could prove useful to reveal novel strategies for pharmacological treatment of this disorder. Several potential strategies could be utilized to reduce a hyperactive or hyperreactive mesolimbic DA system and the concomitant behavioral activation without directly antagonizing DA receptors. Our data suggest that one means of achieving such an effect is antagonism of a_1-adrenoceptors. The mechanism(s) may involve reduction of VTA DA cell excitability by blocking an excitatory, noradrenergic input to the DA cells and thus provide a presynaptic mechanism to antagonize the augmented subcortical DA release. In fact, presynaptic attenuation of DA output has previously successfully been utilized in the treatment of schizophrenia in clinical trials (Carlsson et al. 1973, Walinder & Carlsson 1973) An obvious advantage of this strategy might be a reduced risk of EPS, which is substantial with classical antipsychotics and directly reflects the degree of postsynaptic DA-D_2 receptor blockade (Farde & Nordström 1993). Another putative advantage of this strategy is that a_1-adrenoceptor blockade seems to preferentially suppress evoked mesolimbic DA release and the associated behavioral stimulation, leaving basal DA release and behavioral functions relatively undisturbed, as demonstrated by our results. Prazosin has recently been shown also to prevent PCP induced deficits in prepulse inhibition, a behavioral test considered to possess predictive value in the assessment of putative antipsychotic efficacy (Bakshi & Geyer 1997). Consequently, these findings suggest that a_1-adrenoceptor antagonism may contribute to antipsychotic activity. This is consistent with the a_1-adrenoceptor antagonistic action of a large number of antipsychotic drugs in general, and clozapine in particular. Although a clinical trial reported that addition of prazosin to neuroleptic treatment of chronic schizophrenic patients failed to augment the therapeutic effect of the ongoing treatment (Hommer et al. 1984), this finding may relate to a relatively limited capacity of prazosin to penetrate the blood-brain barrier in man (Pfizer AB, personal communication, Cubeddu 1988, van Kammen & Kelley 1991), although it effectively does so in rodents.

In addition, the fact that an antipsychotic drug was used in the study by Hommer et al. (1994), that by itself possesses some a_1-adrenoceptor antagonistic action might have confounded the outcome of this study. Thus, a_1-adrenoceptor antagonists with improved capacity to enter the CNS might represent an intriguing pharmacological principle to explore for its clinical usefulness, perhaps particularly at the onset of schizophrenia. At any rate, our results suggest that even if such a_1-adrenoceptor antagonists were to be found ineffective in the management of schizophrenia when given alone, the combination of effective central a_1-adrenoceptor blockade with DA-D_2 antagonists might imply a clinical advantage over DA-D_2 antagonists given alone (see Svensson et al. 1998B).

A second, novel pharmacological principle to treat psychotic disorders, that is supported by our experimental results is the use of AMPA receptor antagonists. Thus, following administration of MK-801, an augmentation of the EAA input to the VTA seems to occur, which via stimulation of AMPA receptors in the VTA causes an unphysiological activation of the mesolimbic DA neurons, as well as an increased DA output in the NAC and concomitant behavioral stimulation. Our results, as well as those of others, accordingly showed that local intra-VTA- as well as systemic antagonism of AMPA receptors in turn antagonizes the PCP or MK-801 induced hyperlocomotion (Willins et al. 1993, Bubser et al. 1995, Vanover 1998). On the basis of our findings that the AMPA receptor antagonists attenuated the behavioral stimulation and also suppressed the evoked DA release in the NAC, without effect on basal DA release or behavior, we hypothesized that the AMPA receptor antagonists might possess an antipsychotic potential. This notion gained substantial novel support from our subsequent studies showing suppression of CAR by two, chemically different, AMPA antagonists GYKI52466 (Svensson et al. 1998B) and LY326325, since CAR represents a classical preclinical test of high predictability as regards the clinical antipsychotic effect of experimental drugs. However, our observations indicate that side effects such as ataxia might limit the usefulness of this type of compounds if high doses were to be needed (Maj et al. 1995, Schoepp et al. 1995, Vanover 1998). Clearly, other preclinical observations obtained with selective AMPA receptor antagonists, such as suppression of the morphine withdrawal reaction at the level of the LC (Rasmussen et al. 1996), also underline the potential usefulness of this type of drugs in psychiatry.

A major consequence of the glutamate/dopamine dysregulation hypothesis of schizophrenia may be that the objective for pharmacological treatment perhaps should no longer be to generally block dopaminergic neurotransmission in brain, not even selectively within the mesocorticolimbic DA system, a previous strategy used to circumvent the EPS liability of antipsychotic drugs drugs. Rather, early pharmacological intervention might aim at correcting a differential dysregulation of different DA projections in brain. Our results lead to the heuristic hypothesis that the PCP induced, psychotic symptomatology may not only be a direct consequence of reduced NMDA receptor activation in brain, but also indirectly be a consequence of enhanced AMPA receptor activation. Thus, a novel pharmacological treatment strategy in psychosis might involve both facilitation of NMDA receptor function, e.g. as achieved by administration of glycine (Heresco-Levy et al. 1996), and AMPA receptor antagonism. Clearly, this pharmacological approach is different from that suggested by unifying theories for antipsychotic drug action, such as the depolarization-block theory (see Grace 1993), which apart from several points of technical criticism raised by other researchers (Moghaddam & Bunney 1993, Mereu et al. 1995, Klitenick et al. 1996, Melis et al. 1998) also fails to encompass the probable regional differences between the dysregulations of mesocortical and mesolimbic DA systems as suggested by both the PCP model and the neurodevelopmental model of schizophrenia. The finding that clozapine differentially affects these two dopaminergic projections vide supra provides indirect support for this notion (Moghaddam & Bunney 1990, Nomikos et al. 1994, Marcus et al. 1996). Perhaps, in this way, clozapine may not only clinically, but also theoretically be regarded as a truly pioneering antipsychotic drug.

Summary

- Systemic administration to the rat of psychotomimetic NMDA receptor antagonists, such as PCP and MK-801, similarly dysregulates the firing pattern of VTA DA neurons, an effect which indicates that these effects of PCP are largely due to NMDA receptor antagonism.

- VTA DA and SN-ZC neurons respond to systemic administration of PCP or MK-801 with an increased average firing rate and increased regularity of firing. Burst firing in VTA DA neurons is differentially affected dependent upon the anatomical localization of the DA neurons; essentially subcortically projecting VTA DA neurons in the PN display a high frequency burst-like firing pattern, whereas VTA DA cells in the PBP, which largely provide the DA innervation of the PFC, respond with a decreased phasic burst activity. Thus, the temporal distribution of DA impulse activity in the mesolimbic and mesocortical DA projections is dysregulated, which may impair the adequate responsivity of the DA neurons to salient and reward predicting stimuli.

- Systemic administration of PCP and D-amphetamine significantly augments DA output in the VSTR and MPFC, although D-amphetamine is more potent that PCP in this regard. The spatial disorganization of behavior caused by systemic D-amphetamine and PCP appears largely related to the DA releasing effects of these compounds.

- Systemic administration of PCP and D-amphetamine also increases NT-LI levels in the MPFC, although only PCP elevates NT-LI levels in the VSTR. In view of the antagonistic action of NT on DA mediated behaviors in the VSTR, the capacity of PCP to reduce rearing may, at least partially, be related to the increase in NT output in the VSTR caused by PCP.

- The systemic MK-801 evoked stimulation of DA release in the NAC, a major terminal area of the mesolimbic DA system, as well as concomitant locomotor stimulation, are largely dependent upon the augmented nerve impulse activity in the DA neurons. These effects seem dependent on increased AMPA and/or kainate receptor mediated activation of DA neurons in the VTA.

- The increased DA output in the MPFC evoked by systemic MK-801 is, instead, largely independent of DA neuronal activity. Instead, it is probably caused locally within the DA nerve terminal region. Thus, the mesocortical DA neurons become uncoupled from their afferent regulation at the cell body level, an effect which may contribute to impair the neuronally mediated, phasic responsivity of DA neurons to salient and reward predicting environmental stimuli.

- Systemic pretreatment with the a_1-adrenoceptor antagonist prazosin significantly attenuates both MK-801 induced DA release in the NAC and concomitant hyperlocomotion. Thus, a_1-adrenoceptor antagonism should reduce the impact of noradrenergic activation of VTA DA neurons, an effect that may be particularly important in association with pronounced environmental stress. Consequently, a_1-adrenoceptor antagonistic properties of antipsychotic drugs may act, presynaptically, in concert with concomitant postsynaptic DA-D_2 antagonism to specifically reduce evoked subcortical DA hyperactivity.

- Administration of the AMPA receptor antagonist LY326325 suppresses the conditioned avoidance response without effect on escape behavior, effects similar to those of essentially all antipsychotic drugs. Simultaneously, LY326325 does not attenuate general locomotor activity, nor does it cause significant catalepsy. The effects of LY326325 are in these respects similar to those of clozapine rather than classical antipsychotics and support the hypothesis that AMPA receptor antagonists may possess an atypical antipsychotic profile.

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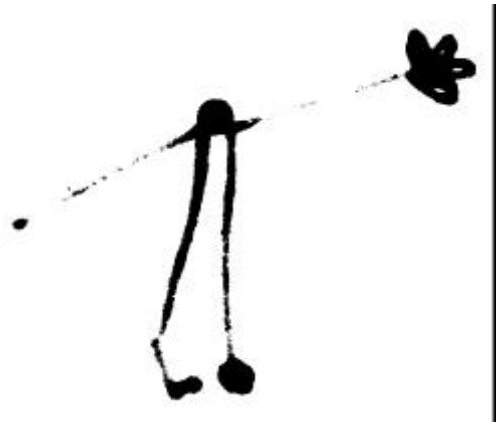

'Curiouser and curiouser!' cried Alice

(she was so much surprised that for the moment she quite forgot how to speak good English).