BLOCKADE OF NMDA RECEPTORS IN POSTNATAL PERIOD
DECREASED DENSITY OF TYROSINE HYDROXYLASE
IMMUNOREACTIVE AXONAL ARBORS IN THE MEDIAL
 PREFRONTAL CORTEX OF ADULT RATS.

Institute of Pharmacology Polish Academy of Sciences, Department of Pharmacology,
Laboratory of Pharmacology and Brain Biostructure, Kraków

Malfunction of glutamatergic neurotransmission in postnatal period is considered to
be a risk factor for development of schizophrenia. Thus, the present study
investigates the impact of NMDA receptor blockade in the postnatal period on the
density of tyrosine hydroxylase immunoreactive axonal arbors in the rat medial
prefrontal cortex. Behavioral experiments revealed that adult rats (60 days old)
treated in the postnatal period with a competitive antagonist of NMDA receptors,
CGP 40116 (1.25 mg/kg on days 1, 3, 6, 9; 2.5 mg/kg on days 12, 15, 18; and finally
5 mg/kg on day 21, all injections s.c.), showed enhancement of the locomotor
activity stimulated by quinpirole (0.3 mg/kg s.c.) and amphetamine (0.5 mg/kg s.c.),
which suggests development of functional supersensitivity of dopaminergic systems.
It has been found that CGP 40116, given in postnatal period decreased the density of
tyrosine hydroxylase immunoreactive axonal arbors in the medial prefrontal cortex
of adult animals. The decrease was observed in superficial (II/III) and deep (V/VI)
layers of the medial prefrontal cortex, while the average length of tyrosine
hydroxylase immunoreactive axonal arbors was increased in both superficial and
deep cortical layers. Changes in the density of tyrosine hydroxylase immunoreactive
axonal arbors have not been followed by a significant decrease in the content of
tyrosine hydroxylase protein measured by Western blot.

Thus, NMDA receptor blockade in the early period of life evokes changes in
architecture of tyrosine hydroxylase immunoreactive axonal arbors and that
malfunction of glutamatergic neurotransmission, in early period of life may produce
anatomical changes which resemble those observed in the brains of schizophrenics.

Key words: NMDA receptors, tyrosine hydroxylase, immunocytochemistry, schizophrenia,
 locomotor activity, medial prefrontal cortex.
Schizophrenia is a devastating brain disorder of unknown etiology. It is generally believed that genetic predisposition combined with environmental factors, influencing development of certain structures of the central nervous system, lead to manifestation of the first episodes of schizophrenia in early adolescence (1).

Several experimental arguments indicate that excitatory amino acids like glutamate are engaged in multiple aspects of brain development, such as migration, differentiation and survival of neurons (2, 3). Moreover, glutamate operating via NMDA receptor ion channel complex controls the plasticity of the developing brain (4-7). Finally, it has also been shown that antagonists of NMDA receptors enhance neurodevelopmental apoptosis (8-10).

Available functional data indicate that the NMDA receptor blockade in the postnatal period may alter responsiveness of experimental animals to dopaminergic agonists (11, 12), may influence dopamine receptor density (12) and impair metabolism of dopamine (11), i.e. the neurotransmitter which is traditionally linked with pathophysiology of schizophrenia (13). Moreover, it has been found that postnatal administration of phencyclidine and MK-801 evokes alterations in sensorimotor gating and efficacy of working memory (8) as well as in spatial learning (14). Such effects and the role of glutamatergic receptors in the brain development provoke the question whether malfunction of glutamatergic neurotransmission, may be a risk factor for manifestation of neuroanatomical and neurochemical changes which are characteristic of schizophrenia.

Dysfunction of dopaminergic neurotransmission has been proposed as the fundamental neurochemical feature of schizophrenia (15, 16). It has been suggested on the basis of clinical data that overactivity of mesolimbic dopaminergic system results from attenuation of dopaminergic tone in the medial prefrontal cortex (MPC) (15, 17). Morover, hypodopaminergic state in MPC has been hypothesized as possible site of cognitive abnormalities in schizophrenia (18).

Therefore, in the present study, we examined whether NMDA receptor blockade in postnatal period (from birth until juvenile age) influences the dopaminergic input to the MPC. To visualize the dopaminergic fibers in the rat MPC the immunocytochemical staining of tyrosine hydroxylase (TH) has been applied. The above experiments have been inspired by the some clinical observations indicating the decreased density of tyrosine hydroxylase immunoreactivity axonal arbors THA in the prefrontal and enthorinal cortices of schizophrenic patients (19, 20) (controversies were discussed by (21)). Thus, it was of interest to investigate whether pharmacological treatment which is supposed to influence postnatal brain maturation, and which is used to model certain aspects of schizophrenia (8, 12) may lead to similar changes as those found in the brains of schizophrenics. Potential similarities may indicate that malfunction of neurotransmitter system involving NMDA receptors and respective endogenous ligands may lead to development of schizophrenia in the adult life or at least to anatomical changes which resemble
those which have been found in schizophrenia. The rat MPC (22) has been chosen for the present study, since intracerebral drug administration studies revealed that the above subregion of the cortex was involved in regulation of efficacy of the working memory (23), and correctness of sensorimotor gating (24) of cognitive functions which are impaired in the course of schizophrenia (1).

The above neuroanatomical experiments were preceded by the behavioral test investigating whether the applied regime of treatment with an NMDA antagonist CGP 40116 (25-27) evokes behavioral changes similar to those observed after other antagonists of NMDA receptors, like CGP 39555 (11, 12, 28) and phencyclidine (8), which, as mentioned above, induce functional supersensitivity of dopaminergic systems, when given in postnatal period (12).

MATERIALS AND METHODS

Animals

Pregnant dams (Wistar, Institute of Pharmacology, PAN Kraków) arrived at our experimental facilities approximately one week before parturition and were housed under standard experimental conditions (constant temperature of 22°C ± 2) with artificial light/dark cycle (12/12; the light on from 7 a.m. to 7 p.m.). All subjects used in the experiment were born in the experimental rooms of the Department of Pharmacology of the Institute of Pharmacology, PAS. Dams and pups were housed in plastic cages (54x37 cm) with wooden bedding covering the floor of the cage with free access to laboratory chow and tap water.

Administration of NMDA receptor antagonist in a postnatal period

A day of parturition was designated as a postnatal day 0. Rat pups of both sexes were injected s.c. with increasing doses of CGP 40116, a competitive NMDA receptor antagonist [Ciba-Geigy]; (25-27). The drug dose was 1.25 mg/kg on the days 1, 3, 6 and 9, then it was increased to 2.5 mg/kg on days 12, 15, 18 and finally the dose of 5 mg/kg was administered on day 21. This schedule of drug treatment was adapted from work of Facchinetti et al. (11, 28). The volume of drug solution was 0.01 ml per 1g of body weight. On day 22, the rats were separated from mothers. Control pups received only vehicle (NaCl, 0.9%), and were handled in a similar manner as CGP 40116-injected animals. The above regimen of drug administration was not lethal to any animals used in experiments. Rats were randomly assigned to groups of 6 animals per cage (54x37 cm) with a free access to standard laboratory chow and tap water till the age of 60 days. The above design allows to have experimental groups of animals having originated from different litters. All further experiments were performed on adult 60-day-old male rats weighting approximately 300 g at the time of final experiment. Rats were used only once either to behavioral test or immunocytochemistry. The experimental protocols were approved by the Committee for Laboratory Animal Welfare and Ethics of the Institute of Pharmacology, Polish Academy of Sciences in Kraków and met the criteria of the International Council for Laboratory Animals and Guide for the Care and Use of Laboratory Animals.

Measurement of the locomotor activity

The locomotor activity of rats was recorded individually for each animal in Opto-Varimex cages (Columbus Instruments, OH), linked on-line to an IBM-PC compatible computer. Each cage (43/44
cm) was equipped with 15 infrared emitters, located on the x and y axes, and with an equivalent amount of receivers on the opposite walls of the cage. The locomotor activity of rats was analyzed using Auto-Track software (Columbus Instruments, OH). The locomotor activity was defined as a trespass of three consecutive photo-beams, while other movements (e.g. repeated interruption of the same photo-beams) were regarded as stereotypy-like movements (data not given). The above procedure differentiated between the locomotor activity associated with horizontal locomotion, and stereotypy-like or not locomotor movements of the body (29). The locomotor activity is expressed as a distance traveled by animals in respective time intervals (10 or 120 minutes). The impact of CGP 40116 given in a postnatal period on locomotor hyperactivity induced by amphetamine and quinpirole was analyzed in four groups of animals (9 animals per group), two treated with CGP 40116 in a postnatal period and two treated with saline in the respective period, in the session lasting 270 min. Before recording, the animals were habituated to the Opto-Varimex cages for 30 min (data not shown) and after that period, they were injected with vehicle (0.9 % NaCl), and their spontaneous locomotor activity was recorded for 120 min at 10 min intervals. At the end of that session, the rats were injected again with amphetamine (0.5 mg/kg, s.c. RBI, Natick MA) or quinpirole (0.3 mg/kg, s.c. RBI, Natick MA) and again their locomotor activity was registered for 120 min at 10 min intervals. Rats were used only once for locomotor activity test. All the data are given as the average distance traveled within 10 min intervals (time course) or in a cumulative manner (120 min) ± SEM.

Euthanasia and perfusion

Eight rats treated with CGP 40116 in a postnatal period and eight respective controls were deeply anesthetized with pentobarbital (100 mg/kg) and were intracardially perfused first with 50 ml of 0.9% NaCl and then with 200 ml of a fixative solutions, i.e. 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS) pH 7.2. Their brains were then removed and postfixed for additional 24 hours (in the above fixative).

Immunocytochemistry

The brains were pre-cut at the level 7 mm from rostral beginning of the brain using brain matrix (ASI Instruments) and secured to the stage of a microtome (Leica VT1000) which automatically displayed level of sectioning, and serially cut into sections 50 µm thick in the coronal plane at the level of MPC (30). Application of the brain matrix allows to have similar cutting planes in all brains used in the above experiments. Every third section obtained at the level between 2.2 and 3 mm from rostral beginning (measured on the vibratome display) of brains was selected for further analysis. Selected sections corresponded approximately to the plane 3.2 - 2.2 according to Paxinos and Watson stereotaxic atlas (30). In order to visualize THA, free floating sections were processed according to standard procedures and instructions of the ABC kits from Vector Laboratories used in our laboratory (31, 32). In brief, the sections were rinsed with 0.01 M PBS. Then, they were placed in a blocking solution (0.01 M PBS containing, 5% normal horse serum (Vector Laboratories) and 0.2 % Triton X-100 for 1 hr, and incubated in primary monoclonal antibody recognizing the epitope of TH (Boehringer) diluted in 0.01 M PBS supplemented with 3% horse serum and 0.2 % Triton X-100, 4°C. Anti-TH antibody was used at working dilutions of 1: 5000; time of incubation was 48 hours. After exposure to primary antibody, the tissue sections were rinsed again with 0.01 M PBS and incubated in biotinylated secondary antibody (anti-mouse, rat adsorbed, Vector Laboratories, diluted at 1:200 as suggested by the manufacturer) for 1 hr at room temperature, rinsed again with 0.01 PBS. In order to reduce non-specific labeling, the sections were rinsed with 0.1 M PBS and then incubated in 1% H2O2 for 30 min and after extensive washes, they were incubated in avidin-
biotin-complexed horseradish peroxidase (ABC kit, Vector Laboratories; 1 hr, room temperature, diluted at 1:100 as suggested by the manufacturer). Finally, the sections were rinsed thoroughly with PBS and immunoreactivity was visualized using 0.07% 3,3'-diaminobenzidine (DAB) as chromogen, and 1% H$_2$O$_2$. In order to minimize variation of staining, slices form CGP 40116-treated animals were run in parallel with section obtained form control animals, and duration of finale reaction with DAB (lasting 5 min) was controlled with a stopwatch (to minimize variation between individual sets of experiments).

**Silver-gold intensification**

The DAB reaction product was intensified according to methods of Melchitzky and Lewis (33). DAB-reacted slide-mounted sections were incubated in 1% silver nitrate (pH=7.45, 55°C), briefly rinsed with distilled H$_2$O, and then incubated in 0.1% gold chloride (10 min, room temperature). Sections were rinsed again with distilled H$_2$O and fixed in 5% sodium thiosulfate (10 min, room temperature), and placed under cover slips. Again, in order to minimize variation of staining, slices form CGP 40116-treated animals were run in parallel with section obtained form control animals.

**Quantitative evaluation**

Quantitative analyses of fiber density was performed on Camera Lucida drawings made of immunoreactive fibers visualized under bright-field illumination (Leica DMLB microscope) using a high aperture Plan Apo 63x oil immersion objective and drawing tube. Densities of THA were examined in layers II/III and V/VI of MPC. Six sections taken at the level of MPC from each animal (see Fig. 2), and three to four not overlapping drawings from each cortical layer were analyzed and averaged. Camera lucida drawings were digitalized as quality black and white photographic images (Mustek scanner), and exported into Image-Pro Plus (Media Cybernetics) image analysis software. Fiber densities were evaluated automatically in circles (7.1 mm$^2$) placed approximately at the level of layers II/III and V/VI. Images were first skeletonized to replace lines of varying width with ones of uniform thickness of one pixel and mean pixel density of the selected area was measured. In order to verify correctness of transformation to one-pixel lines, the transformed images were pseudo-colorized and overlay on original untransformed images. For further details see (34, 35).

In order to measure the length of individual THA, live images from deep and superficial cortical layers of MPC were captured, spatially calibrated and each center of THA was manually traced with trace-option subroutines of Image-Pro Plus software. The above procedure allows for reconstruction of the length of THA segments present within a section 50 µm thick, and for adjustment of the focal plane of THA during tracing. Traces were converted into the black and white digital images and their length was measured with semiautomatic trace option subroutines of the Image-Pro Plus program. Since similar effects were observed in the above-mentioned regions, the results for both regions were averaged. The lengths of individual THA were measured on sections which were not processed for silver-gold intensification.

**Tyrosine hydroxylase electrophoresis and Western blot**

Brains from eight rats from two independent groups treated with CGP 40116 in a postnatal period and eight respective controls were removed after decapitation, cooled on ice and sliced into 1 mm coronal sections using rodent brain matrix as described previously (29). Prefrontal cortex was punched out from coronal sections by use of a 15-gauge syringe needle (29). The isolated brain region was homogenized (80 mg/ml) in 2% SDS, and protein levels were determined using BCA Protein Assay Kit (modification of the Lowry assay, Sigma). Then equal samples in respect of protein contents were adjusted to contain final concentration with 50 mM Tris, pH 6.8, containing
2% SDS, 8% glycerol, 2% 2-mercaptoethanol with bromophenol blue as a marker and boiled for 5 min. Aliquots (containing 100 µg of protein) were separated by one-dimensional SDS-polyacrylamide gel electrophoresis on 0.75 mm thick 7.5% polyacrylamide resolving gels using a Bio-Rad Mini-Protean II. Proteins were electroblotted to nitrocellulose with a Bio-Rad Mini Trans-blot electrophoretic transfer cell. Blots were routinely stained with 0.5 % Ponceau S (Sigma), and correctness of protein loading per each lane, and effectiveness of transfers were inspected. Then the Ponceau S was washed out and the blots were subjected to reaction with 0.2 µg/ml of anti-TH monoclonal antibody (Boehringer) and the reaction was visualized by chemiluminescence using BM Chemiluminescence Western Blotting Kit (Boehringer). Each gel/nitrocellulose membrane contained equal number of samples form CGP 40116-treated animals and respective controls, and a protein molecular weight standard (BioRad). Levels of TH immunoreactivity were determined by computerized video-densitometry (Fuji Las 1000 imager and Image-Pro Plus software). All values are expressed as a percentage of vehicle-treated controls.

**Data presentation and statistics**

All data are given as the means ± SEM. In behavioral experiments, two-way ANOVA with time as a repeated measure and treatment (saline or CGP) as an independent variable was used to test significance of saline and amphetamine/quinpirole treatment. In immunohistochemical experiments, two to three reconstructions from superficial and two to three reconstructions from deep cortical layers per animal were averaged and used as independent variable in two-way ANOVA where cortical layers (layer II/III or V/VI) and treatment (saline or CGP 40116) were used as independent variable. One-way ANOVA was used for Western blot experiments. The Duncan test was used for post hoc comparisons.

**RESULTS**

**Locomotor activity - effects of amphetamine and quinpirole**

The spontaneous locomotor activity of rats treated in a postnatal period with CGP 40116 was not different from the activity of control rats, which in a postnatal period received injections of saline: treatment (saline vs. CGP 40116 in postnatal period as independent variables and time factor as a repeated measurement) F(11,176)=0.19, ns and = 0.65, ns respectively for groups which subsequently were treated with amphetamine and quinpirole, (for cumulative data presentation F(1,14)=1.8 and 3.5 ns, respectively) (Fig.1). Amphetamine at a dose of 0.5 mg/kg evoked enhancement of the locomotor activity lasting 120 min and reaching its peak 40 min. after the administration, F(11, 176)= 142.2 p<0.001 (Fig.1). The effect of amphetamine on adult rats, treated in a postnatal period with CGP 40116 had similar time course as the time course of respective controls (Fig.1) but it was stronger; main effect: (amphetamine in saline- vs. amphetamine in CGP-treated animals as independent variables and time factor as a repeated measurement) F(11,176)=2.62 p< 0.05, reaching respectively ca. 220 % of controls when analyzed in a cumulative manner F(1,32)=5.04, p<0.05 (Fig.1, left inset). Quinpirole at a dose of 0.3 mg/kg i.p. only slightly enhanced locomotor activity in control rats, habituated to actometer cages which did not reach criteria
of statistical significance $F(11, 176) = 1.12 \, \text{ns}$, while the same dose of quinpirole given to adult rats treated in the postnatal period with CGP 40116 evoked robust enhancement of locomotor activity; main effect: (quinpirole in saline- vs. quinpirole in CGP-treated animals as independent variables and time factor as a repeated measurement $F(1,176) = 2.54 \, p<0.05$) (Fig. 1). When cumulative data were considered, the locomotor activity of CGP 40116-treated animals in a postnatal period after quinpirole reached 550% of respective controls $F(1,32) = 4.7, \, p<0.05$ (Fig. 1).

**Impact of CGP 40116 given in a postnatal period on the density of tyrosine hydroxylase immunoreactive axonal arbors in the rat medial prefrontal cortex**

Monoclonal antibody used in the present study displayed a high degree of specific labeling of THA in the rat MPC (Fig. 2, for discussion of selectivity see (35)), with a typical pattern of distribution of THA described previously in detail (36). Clear and increasing density gradient was seen in a lateral direction (statistically significant, $F(1, 28) = 10.21, \, p<0.01$) (Fig. 2). It was found that CGP 40116 given in a postnatal period decreased the density of THA both in a superficial (layers II/III) and deep cortical layers (layers V/VI). The above decrease reached ca. 24 and 31 % in comparison to the respective controls.
Statistical analysis showed that administration of CGP 40116 in a postnatal period had a profound impact on density of THA, $F(1,28)=5.71$, $p<0.05$, and that there was no interaction between treatment vs. cortical layers indicating that the above treatment in a postnatal period to the same extent influenced density of axonal arbors in both regions under analysis $F(1,28)=0.52$, ns. In the fragments from brain sections which were not amplified by silver method, analysis of the lengths of fragment of axonal arbors revealed that administration of CGP 40116 produced an increase in the average length of THA in the prefrontal cortex, $F(1,14)=21.56$, $p<0.001$.

(Fig. 3). Statistical analysis showed that administration of CGP 40116 in a postnatal period had a profound impact on density of THA, $F(1,28)=5.71$, $p<0.05$, and that there was no interaction between treatment vs. cortical layers indicating that the above treatment in a postnatal period to the same extent influenced density of axonal arbors in both regions under analysis $F(1,28)=0.52$, ns. In the fragments from brain sections which were not amplified by silver method, analysis of the lengths of fragment of axonal arbors revealed that administration of CGP 40116 produced an increase in the average length of THA in the prefrontal cortex, $F(1,14)=21.56$, $p<0.001$ (Fig. 4).
Fig. 3. The impact of NMDA receptor blockade in postnatal period on the density of tyrosine hydroxylase immunoreactive axonal arbors (THA) in the medial prefrontal cortex of rat. Left panel illustrates reconstruction of THA (camera lucida drawings) observed in control animals (A,B) and animals treated in the postnatal period with CGP 40116 (C,D). Panels A,C and B,D are from layers II/III and layers V/VI, respectively. Right panel shows quantitative analysis of density of THA. Bars illustrate average density of TH positive terminals ± S.E.M of 8 rats per group. Asterisk indicates statistically significant effects of administration of CGP 40116 in postnatal period in comparison with the respective controls. Two-way ANOVA (treatment vs. layer), followed by Duncan test.

Fig. 4. The impact of NMDA receptor blockade in postnatal period on the lengths of hydroxylase immunoreactive axonal arbors (THA) in the medial prefrontal cortex (MPC) of adult rats. Bars illustrate average length of THA positive terminals ± S.E.M of 8 rats per group. Asterisk indicates statistically significant effects of administration of CGP 40116 in postnatal period in comparison with the respective controls. One-way ANOVA, followed by Duncan test. Panels A and B show examples of THA positive terminals in the rat MPC of control (A) and CGP 40116-treated animals (objective 63 X, Nomarski optic, scale bar 10 µm).
Impact of CGP 40116 given in a postnatal period on tyrosine hydroxylase protein levels - Western blot analysis

The Western blot analysis revealed that monoclonal anti-TH antibody, recognizing specific epitope of TH, labeled only one band of tissue homogenates of the rat prefrontal cortex (Fig.5). Moreover, the molecular weight of the band corresponded to the molecular weight of TH calculated from its amino acid sequence and available in protein data banks. It was found that the administration of CGP 40116 in a postnatal period slightly decreased the density of respective bands indicating a slight decrease in the TH content in the examined brain region by ca. 17% in comparison with the control values, however, the above value did not reach criteria of statistical significance [F(1,14)=0.27, ns (Fig.5)].

DISCUSSION

Our initial behavioral experiments revealed that blockade of NMDA receptors in a postnatal period led to a persistent functional supersensitivity of dopaminergic
systems, contributing to alterations of the locomotor activity induced by direct (quinpirole) and indirect (amphetamine) agonists of dopamine receptors. This supersensitivity is possibly associated with the functional up-regulation of dopamine D2/D3 receptors since it was observed not only after treatment with amphetamine which releases dopamine but also after a direct agonist of D2/D3 dopamine receptors (37). Moreover, the above behavioral effects indicate that the applied regimen of administration of the NMDA receptor antagonist in a postnatal period produces qualitatively similar responses to those recently described in literature after postnatal administration of CGP 39555 (11, 12, 28) and phencyclidine (8) and used to model schizophrenic symptoms in adult rats.

Results of immunocytochemical analysis indicate that NMDA receptor blockade in the postnatal period leads to persistent decrease in the density of THA in the MPC of adult rats. This decrease was observed in the adult animals (60 days old) in superficial (II/III) and deep (V/VI) cortical layers. Since in the MPC, THA represent 99 % of dopaminergic terminals (38), the observed decreases reaching 24 and 31 % in superficial and deep cortical layers, respectively, indicate that generally dopaminergic terminals are affected in these structures, however, it cannot be ruled out that NMDA receptor blockade in the postnatal period also influences density of noradrenergic terminals, which are also positive for TH (36).

The above conclusions are based on the assumption that the observed decrease in the immunoreactivity is due to the decreased number of THA (20, 39). It might be, however, argued that, alternatively, the number of axons is not altered but the attenuation of rate of TH synthesis or impairments of TH transport to the dopaminergic terminals leads to weaker staining of axons whose anatomical structure per se is not modified. Further experiments will be required to answer that question, however, the observation that average length of THA after such treatment is increased speaks against such explanation and suggests again that the above changes are due to the alteration of the neuroanatomy of the axonal arbors. Interestingly, the observed decrease in density of dopaminergic innervations with subsequent increase in the length of individual axons is in line with the findings that in the cultures of hippocampal neurons, blockade of NMDA receptors decreased the neuronal branching with the subsequent increase in the length of axonal arbors (40, 41).

Our anatomical observations are not fully supported by the results of the Western blot experiment since we observed a slight decrease in the amount of TH protein (by ca. 17 % in comparison to the respective control), however, the above effects did not reach the criteria of statistical significance. It is possible that a decrease in the density of cortical TH positive innervation under discussion is too subtle to be visualized with Western blot technique, measuring the TH protein content in the entire MPC.

At present, the mechanisms which may lead to a decrease in density of THA in the MPC may be only a matter of speculation. Firstly, NMDA receptor blockade in the postnatal period enhances neurodevelopmental apoptosis in the
MPC (9). Thus, it is conceivable that a drop in the number of cortical neurons which are the targets for the dopaminergic terminals will reduce trophic chemotaxis and subsequently decrease the in-growth of dopaminergic terminals into the MPC. Moreover, it cannot be ruled out that blockade of NMDA receptors may also reduce the number of THA positive neurons via the same mechanism, since postnatal apoptosis has also been observed in dopaminergic cells (42) that possess NMDA receptors (43, 44), which could make them vulnerable to the proapoptotic action of the NMDA receptor antagonists administered during postnatal period of life. Thus, it cannot be excluded that blockade of NMDA receptors in the postnatal period decreases the number of dopaminergic neurons forming mesocortical dopaminergic system and, subsequently, lowers the density of cortical innervation, or number of axonal arborizations. Finally, it has to be taken into account that antagonists of NMDA receptors attenuate the number of social contacts, at least in the adult animals (45). Hence, it is plausible that NMDA receptor blockade in a postnatal period reduces the mother-pup interactions which in the pharmacological way may lead to the maternal deprivation. Interestingly, it has been observed in Octodon degus that maternal deprivation decreases the density of cortical THA with concomitant increase in density of serotonergic axons (46). Notably, maternal separation has been used in a certain strain of rats to produce deficits in sensorimotor gating in order to model some cognitive deficits typical of schizophrenia (47, 48), thus the same deficits as those observed after administration of NMDA antagonists in a postnatal period according to our preliminary studies (49) and literature data (8). Available data indicate that alteration of dopaminergic cortical neurotransmission may cause impairments of cognitive functions like disruption of sensorimotor gating and impairments of the efficacy of working memory, two important elements of cognitive processes which are disturbed in the course of schizophrenia (24, 50). It has also been demonstrated that not only blockade of dopaminergic receptors belonging to D1 and D2 family, which are localized in MPC (24), but also lesion of catecholaminergic terminals in this region disrupted sensorimotor gating (50). Efficacy of working memory is altered both when dopamine level or activation of dopaminergic D1 receptors is below or above the optimum level (23).

Decreased dopaminergic neurotransmission in the prefrontal cortex increases the dopaminergic tone in the subcortical areas (51), while its enhancement attenuates behavioral effects mediated by the subcortical dopaminergic structures, like for example nucleus accumbens (52), which has previously been shown in behavioral (53) and neurochemical experiments (51, 52). In the context of our and literature data, the decreased dopaminergic tone in MPC, suggested by our present findings, may at least partially explain functional supersensitivity to direct and indirect dopaminergic agonist which we observed in our study.

As mentioned above, the blockade of NMDA receptors in the postnatal period has been analyzed in order to investigate whether such treatment leads to qualitatively similar effects, as those found in the brains of the schizophrenic.
patients. In the course of schizophrenia, dopaminergic neurotransmission is increased in the subcortical structures (striatum and accumbens) and decreased in the cortical regions (for a review article see (1)). The latter effect is linked with the metabolic hypofrontality, which is typical of schizophrenia (1) and, in the functional terms, is related to cognitive deficits and negative symptoms of schizophrenia (1). The results showing a substantial decrease in the density of THA in the prefrontal and enthorinal cortex of schizophrenics are in line with the above hypothesis, since from functional point of view, this may suggest a decrease in the cortical dopaminergic neurotransmission (20, 54). Apparent alterations were not followed by any significant changes in the average length of THA (20, 55). In comparison with those findings, our results are slightly different since we observed consistent decrease in THA in both deep and superficial layers of the MPC with a subsequent increase in the average length of THA. Thus, in terms of modeling anatomical changes characteristic of schizophrenia, only the effect which we observed in the deep cortical layer i.e. decreased density of THA fits in the clinical picture of schizophrenic brain.

Apart from the fact that available data suggest that decreased dopaminergic neurotransmission in the prefrontal cortex may be related in a cause-effect manner to development of schizophrenic symptoms (56, 57), it will be of interest to investigate in the future whether postnatal administration of CGP 40116 or other treatment/factors which decrease density of THA in MPC also evoke behavioral or neurochemical "equivalents" of cognitive impairments which are typical of schizophrenia (56, 57). For instance, it has been found that maternal separation (46) and certain type of gonadolectomy (34, 35, 58, 59) decreased the density of THA in the cortex. Apart from methodological differences, maternal separation may cause such deficits in certain population of rats (47, 60). To our knowledge, it is not known whether postnatal gonadolectomy evoked such changes. Development of cognitive deficits after the above treatments/factors which reduced density of THA in MPC may further support the role of dopamine in the prefrontal cortex as a factor responsible for appearance of schizophrenic symptoms or provide arguments that alterations of TH density alone are not sufficient for induction of schizophrenia or its cortical symptoms, but possibly should be regarded as an element of entire set of changes which are responsible for schizophrenia. The latter conclusion seems to be supported by observations that chronic administration of haloperidol does not influence the density of cortical dopaminergic terminals (20), which might indicate that restoration of the normal pattern of cortical THA is not required for therapeutic effects of haloperidol.

In conclusion, our present study indicates that malfunction of the glutamatergic neurotransmission in the postnatal period may alter the anatomy of the cortical dopaminergic THA and secondly that impairments of glutamatergic transmission associated with NMDA receptors in postnatal period may induce anatomical changes which resemble those observed in the brains of schizophrenic patients. These similarities rise again the question whether malfunction of
glutamatergic neurotransmission associated with NMDA receptors in a postnatal period may constitute a risk factor for development of schizophrenic symptoms in the adult life.

**Acknowledgments:** We would like to thank Dr. DS Melchitzky and Dr. DA Lewis for experimental protocols of silver-gold intensification of TH immunoreactivity. This study was supported by grants from Polpharma Foundation, grant number 008/2002 to KW.

**REFERENCES**


18. Goldman-Rakic PS, Castner SA, Svensson TH, Siever LJ, Williams GV. Targeting the dopamine D1 receptor in schizophrenia: insights for cognitive dysfunction. Psychopharmacology (Berl) 2004; 174: 3-16.
29. Wedzony K, Czyrak A, Mackowiak M, Fijal K. The impact of a competitive and a non-competitive NMDA receptor antagonist on dopaminergic neurotransmission in the rat ventral tegmental area and substantia nigra. Naunyn Schmiedebergs Arch Pharmacol 1996; 353: 517-527.


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


Received: February 15, 2005
Accepted: May 16, 2005

Author's address: K. Wędzony, Institute of Pharmacology, Polish Academy of Sciences, 31-343 Kraków, 12 Smętna Street, Poland, phone (4812) 6623253; fax: (4812) 6374500. E-mail: nfwedzony@cyf-kr.edu.pl