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Chodounska et al.

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(45) **Date of Patent: Nov. 5, 2013**

(54) **STEROIDE ANIONIC COMPOUNDS, METHOD OF THEIR PRODUCTION, USAGE AND PHARMACEUTICAL PREPARATION INVOLVING THEM**

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(2), (4) Date: **Dec. 6, 2011**

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PCT Pub. Date: **Dec. 2, 2010**

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(30) **Foreign Application Priority Data**

May 28, 2009 (CZ) ..... PV 2009-348

(51) **Int. Cl.**

C07J 5/00	(2006.01)
C07J 7/00	(2006.01)
A01N 45/00	(2006.01)
A61K 31/56	(2006.01)
C07J 1/00	(2006.01)

(52) **U.S. Cl.**
USPC **552/557**; 514/169; 552/502

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**

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Assistant Examiner — Michael Schmitt
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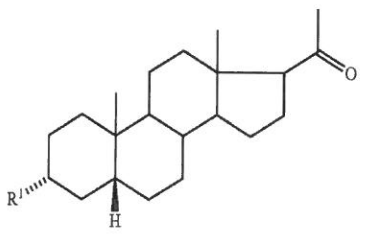
(57) **ABSTRACT**

A compound with general formula I

(I)



for treatment of various diseases of the central nervous system, in treatment of neuropsychiatric disorders related to imbalance of glutamatergic neurotransmitter system, ischemic damage of CNS, neurodegenerative changes and disorders of CNS, affective disorders, depression, PTSD and other diseases related to stress, anxiety, schizophrenia and psychotic disorders, pain, addictions, multiple sclerosis, epilepsy and gliomas.

1 Claim, 18 Drawing Sheets

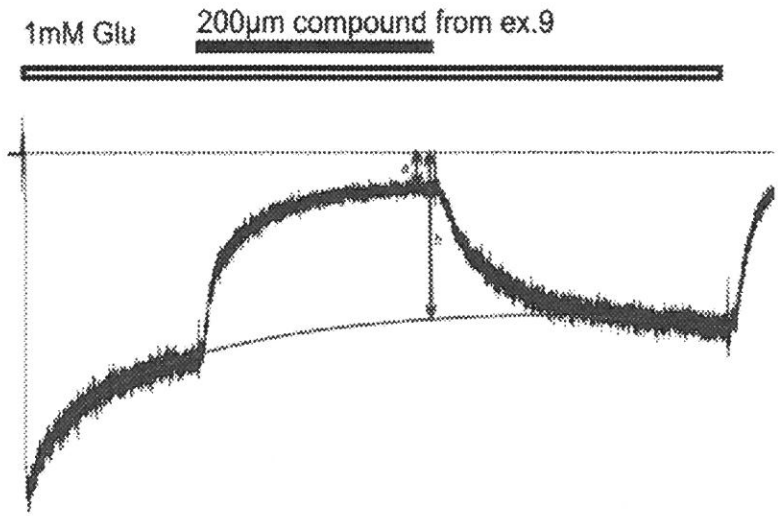


Fig. 1

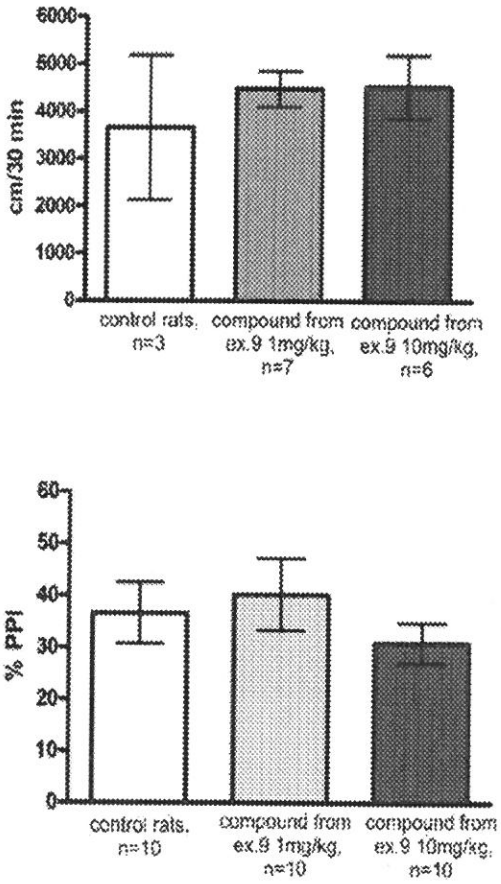

cm/30 min
6000
5000
4000
3000
2000
1000
0
control rats, n=3
compound from ex.9 1mg/kg, n=7
compound from ex.9 10mg/kg, n=6
% PPI
60
50
40
30
20
10
0
control rats, n=10
compound from ex.9 1mg/kg, n=10
compound from ex.9 10mg/kg, n=10

Fig. 2

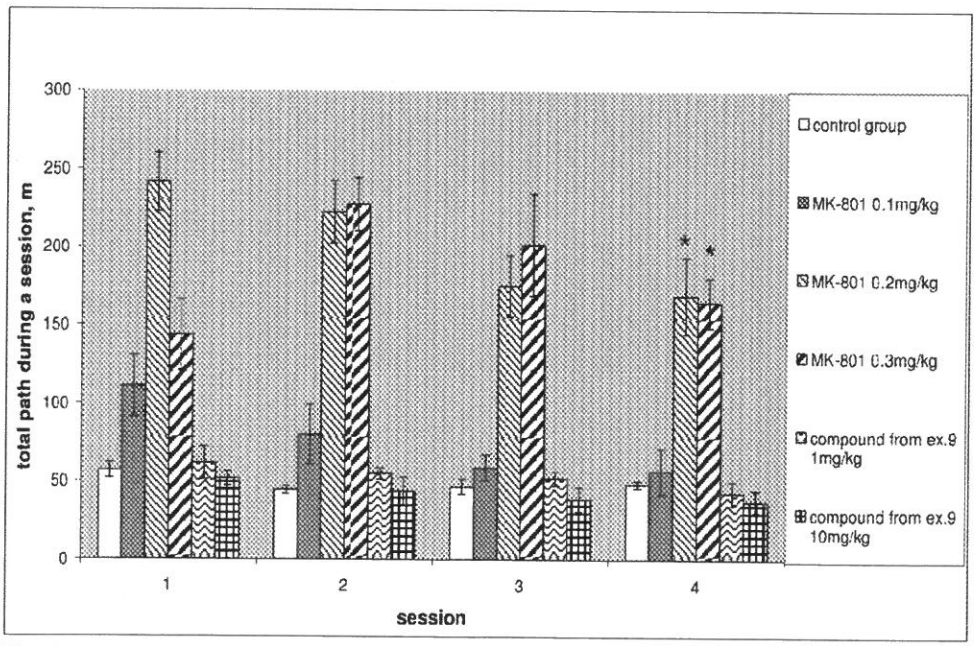

total path during a session, m
300
250
200
150
100
50
0
1
2
3
4
session
control group
MK-801 0.1mg/kg
MK-801 0.2mg/kg
MK-801 0.3mg/kg
compound from ex.9 1mg/kg
compound from ex.9 10mg/kg

Fig. 3

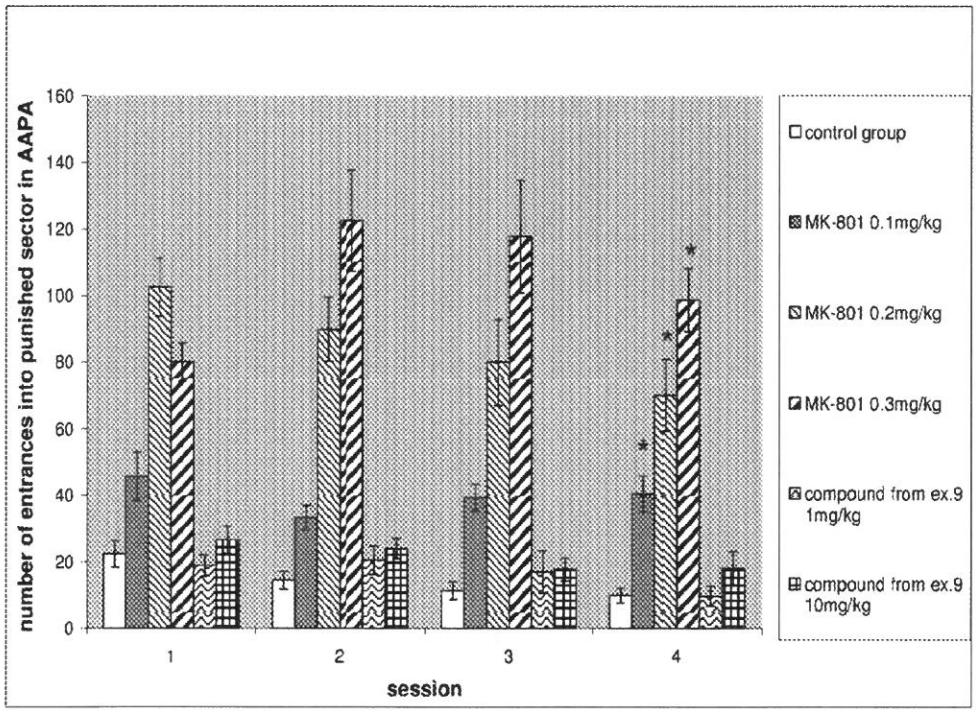

number of entrances into punished sector in AAPA
160
140
120
100
80
60
40
20
0
1
2
3
4
session
control group
MK-801 0.1mg/kg
MK-801 0.2mg/kg
MK-801 0.3mg/kg
compound from ex.9 1mg/kg
compound from ex.9 10mg/kg

Fig. 4

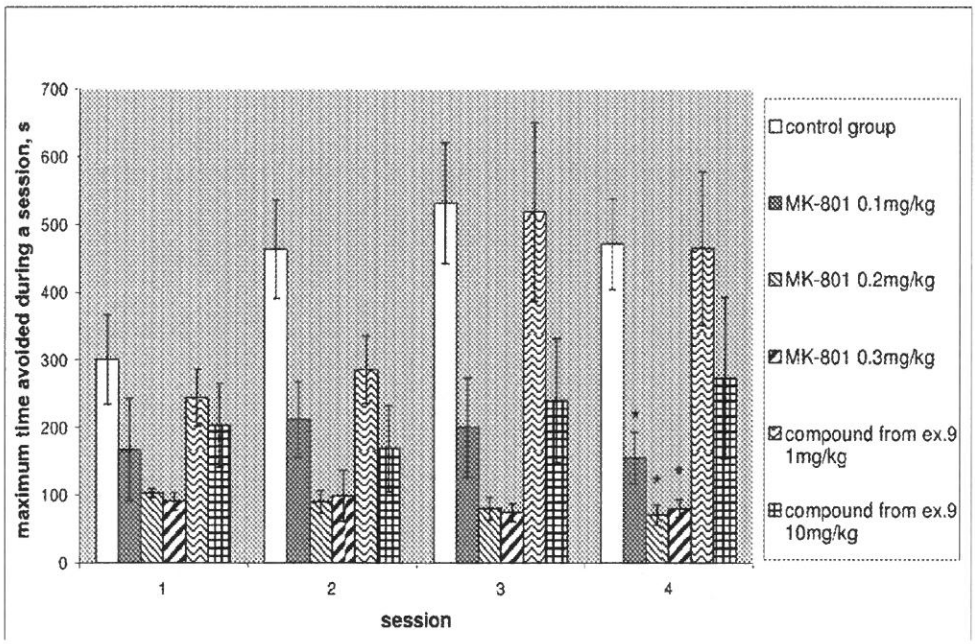

maximum time avoided during a session, s
700
600
500
400
300
200
100
0
1
2
3
4
session
control group
MK-801 0.1mg/kg
MK-801 0.2mg/kg
MK-801 0.3mg/kg
compound from ex.9 1mg/kg
compound from ex.9 10mg/kg

Fig. 5

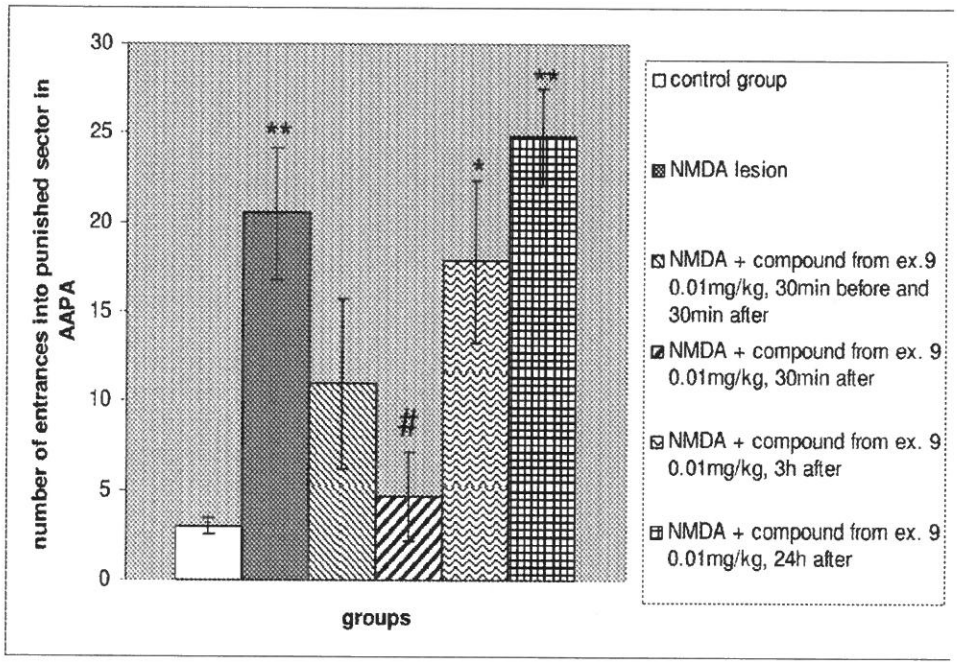

number of entrances into punished sector in AAPA
30
25
20
15
10
5
0
groups
control group
NMDA lesion
NMDA + compound from ex.9 0.01mg/kg, 30min before and 30min after
NMDA + compound from ex. 9 0.01mg/kg, 30min after
NMDA + compound from ex. 9 0.01mg/kg, 3h after
NMDA + compound from ex. 9 0.01mg/kg, 24h after

Fig. 6

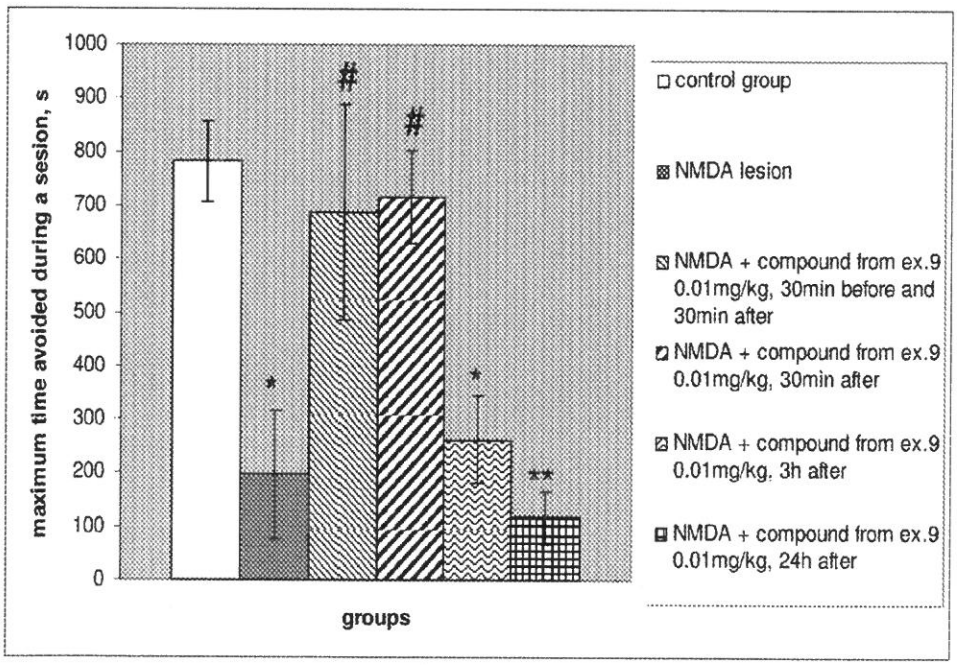

maximum time avoided during a sesion, s
1000
900
800
700
600
500
400
300
200
100
0
groups
control group
NMDA lesion
NMDA + compound from ex.9 0.01mg/kg, 30min before and 30min after
NMDA + compound from ex.9 0.01mg/kg, 30min after
NMDA + compound from ex.9 0.01mg/kg, 3h after
NMDA + compound from ex.9 0.01mg/kg, 24h after

Fig. 7

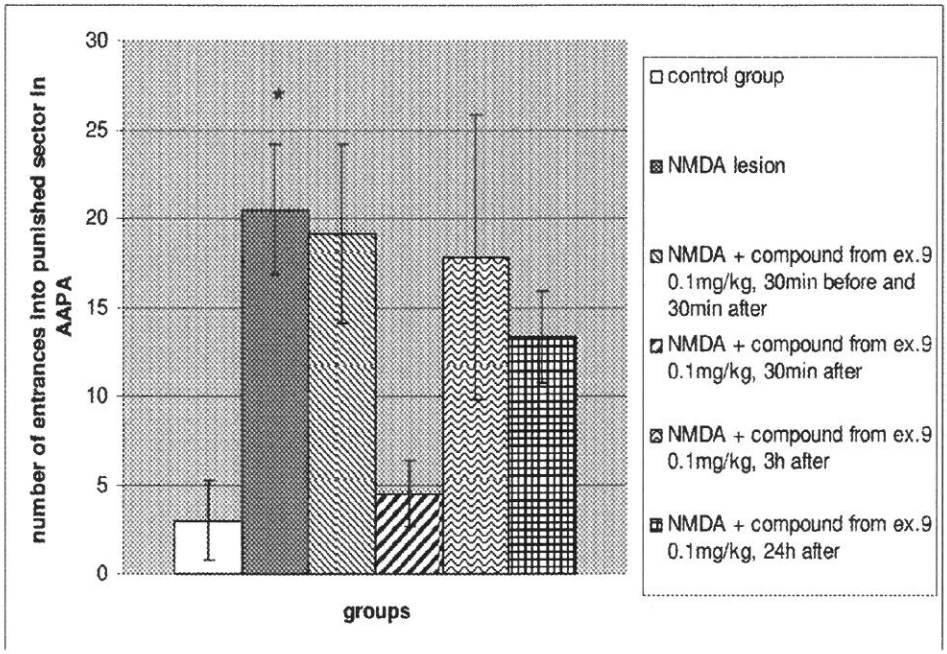

number of entrances into punished sector in AAPA
30
25
20
15
10
5
0
groups
control group
NMDA lesion
NMDA + compound from ex.9 0.1mg/kg, 30min before and 30min after
NMDA + compound from ex.9 0.1mg/kg, 30min after
NMDA + compound from ex.9 0.1mg/kg, 3h after
NMDA + compound from ex.9 0.1mg/kg, 24h after

Fig. 8

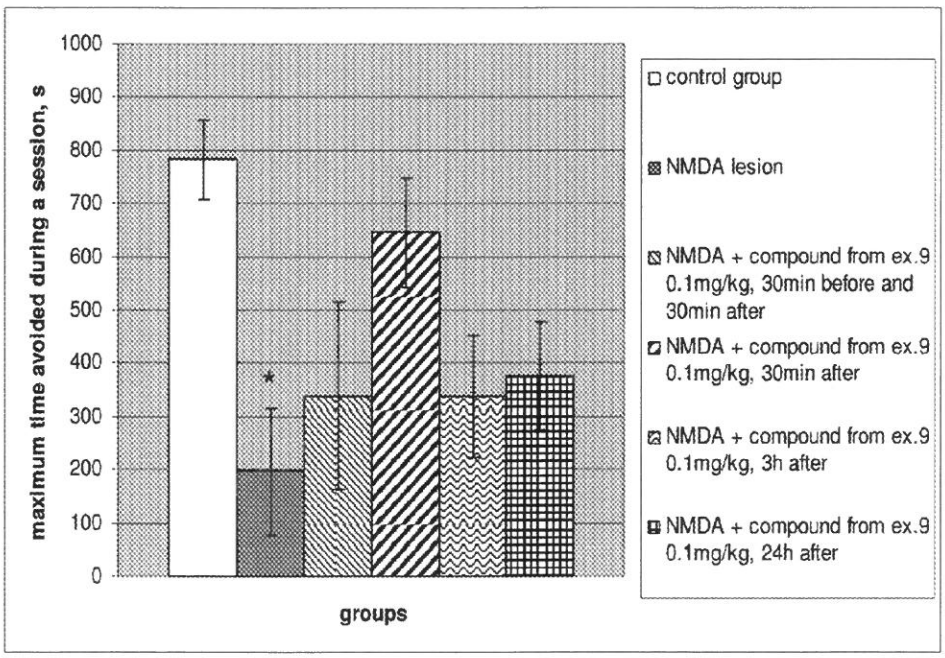

maximum time avoided during a session, s
1000
900
800
700
600
500
400
300
200
100
0
groups
control group
NMDA lesion
NMDA + compound from ex.9 0.1mg/kg, 30min before and 30min after
NMDA + compound from ex.9 0.1mg/kg, 30min after
NMDA + compound from ex.9 0.1mg/kg, 3h after
NMDA + compound from ex.9 0.1mg/kg, 24h after

Fig. 9

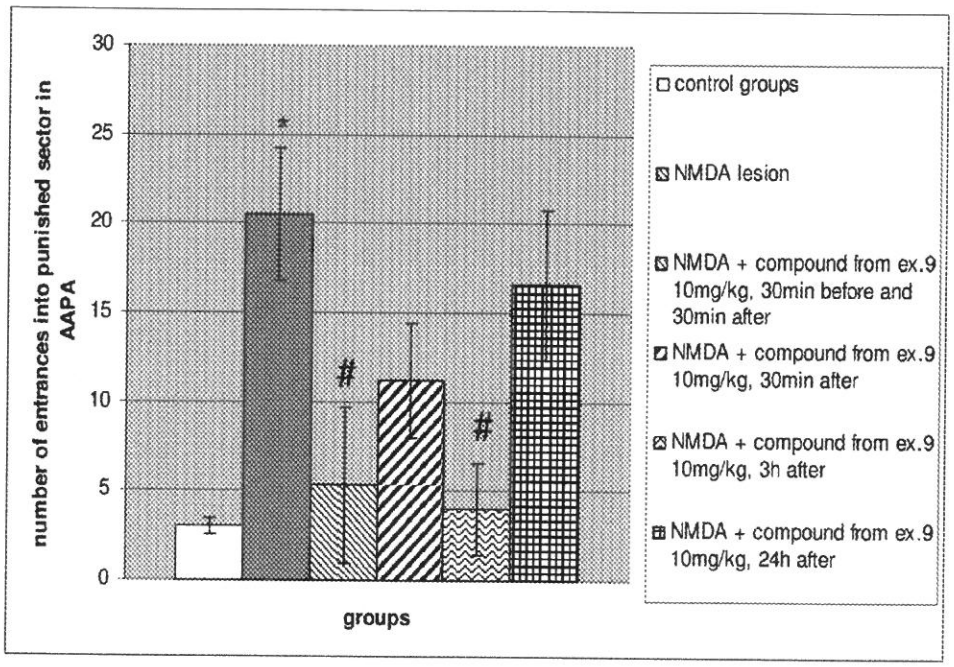

number of entrances into punished sector in AAPA
30
25
20
15
10
5
0
*
#
#
groups
control groups
NMDA lesion
NMDA + compound from ex.9 10mg/kg, 30min before and 30min after
NMDA + compound from ex.9 10mg/kg, 30min after
NMDA + compound from ex.9 10mg/kg, 3h after
NMDA + compound from ex.9 10mg/kg, 24h after

Fig. 10

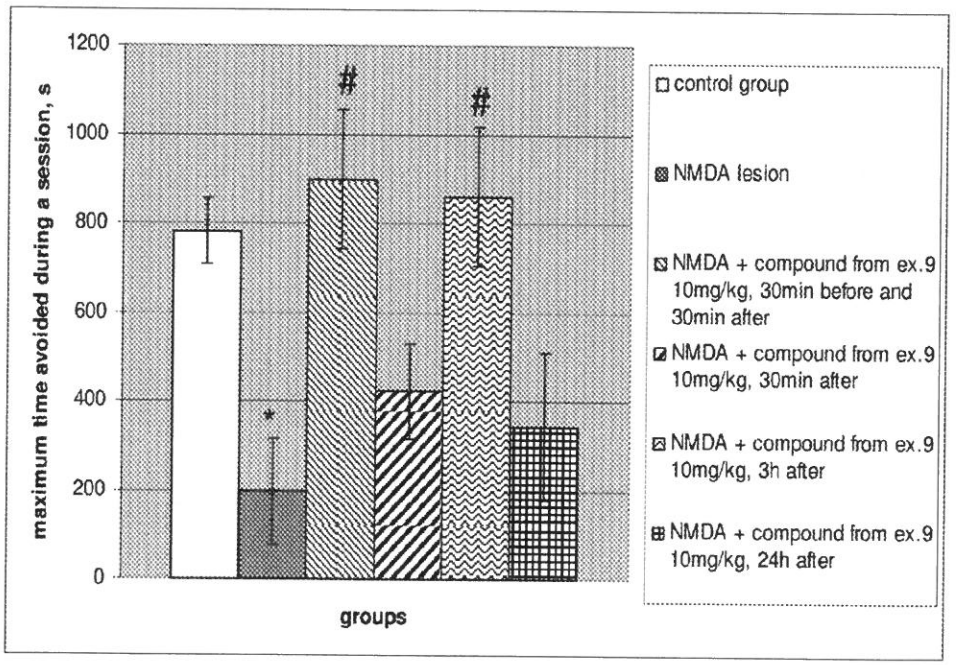

maximum time avoided during a session, s
1200
1000
800
600
400
200
0
*
#
#
groups
control group
NMDA lesion
NMDA + compound from ex.9 10mg/kg, 30min before and 30min after
NMDA + compound from ex.9 10mg/kg, 30min after
NMDA + compound from ex.9 10mg/kg, 3h after
NMDA + compound from ex.9 10mg/kg, 24h after

Fig. 11

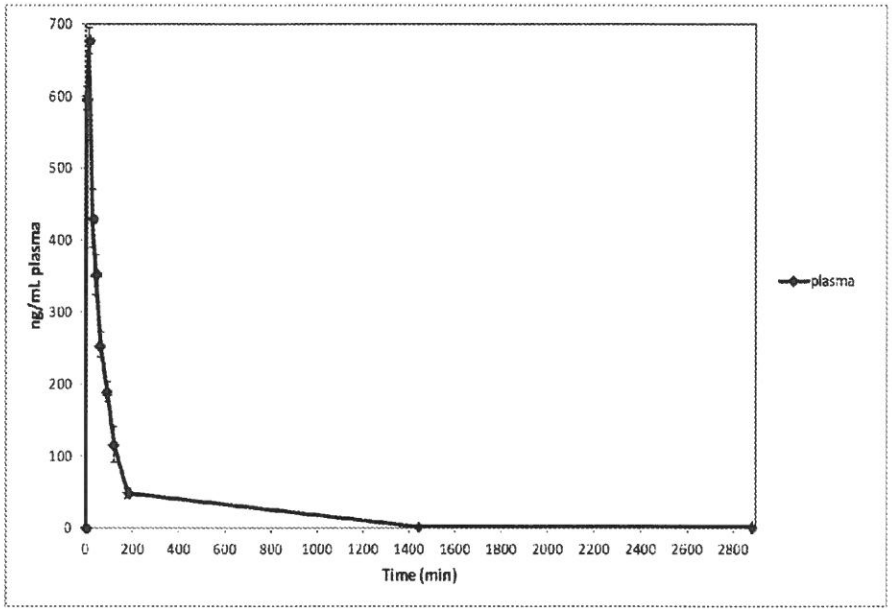

700
600
500
400
300
200
100
0
ng/mL plasma
0 200 400 600 800 1000 1200 1400 1600 1800 2000 2200 2400 2600 2800
Time (min)
plasma

Fig. 12

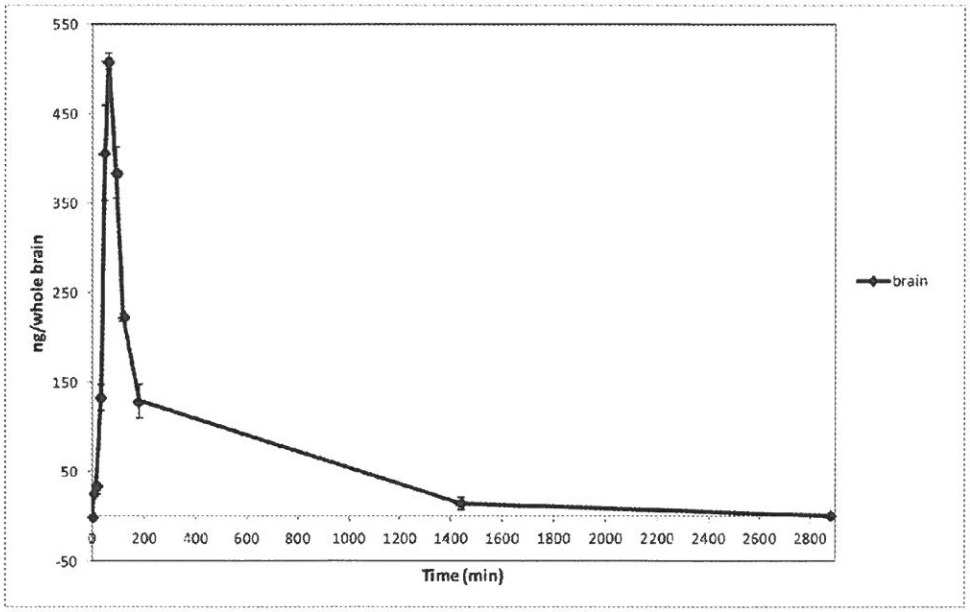

550
450
350
250
150
50
-50
ng/whole brain
0 200 400 600 800 1000 1200 1400 1600 1800 2000 2200 2400 2600 2800
Time (min)
brain

Fig. 13

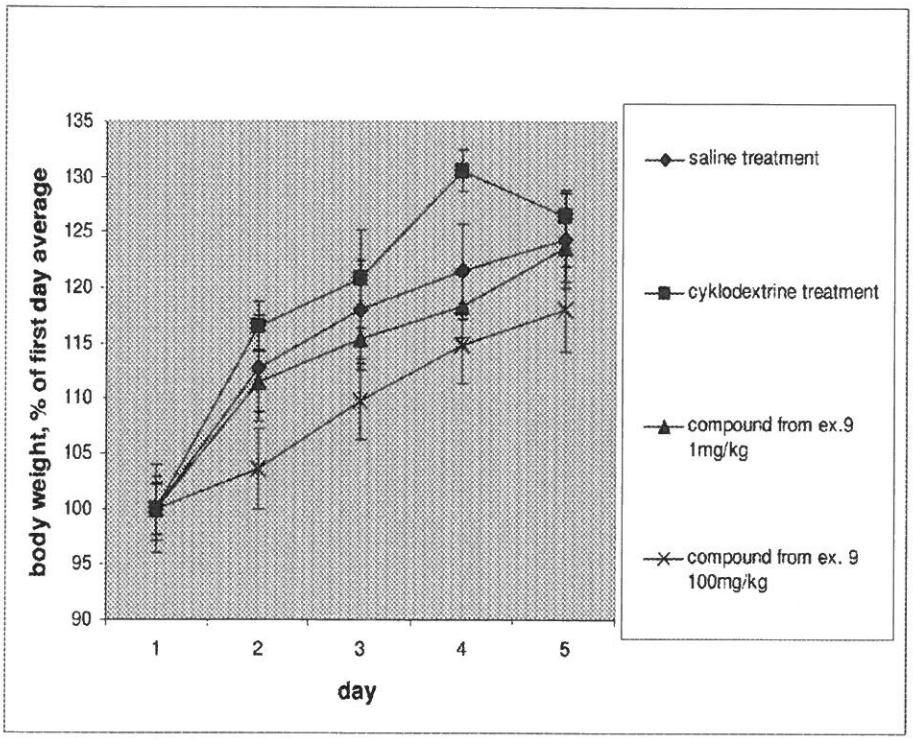

body weight, % of first day average
135
130
125
120
115
110
105
100
95
90
1
2
3
4
5
day
saline treatment
cyklodextrine treatment
compound from ex.9 1mg/kg
compound from ex. 9 100mg/kg

Fig. 14

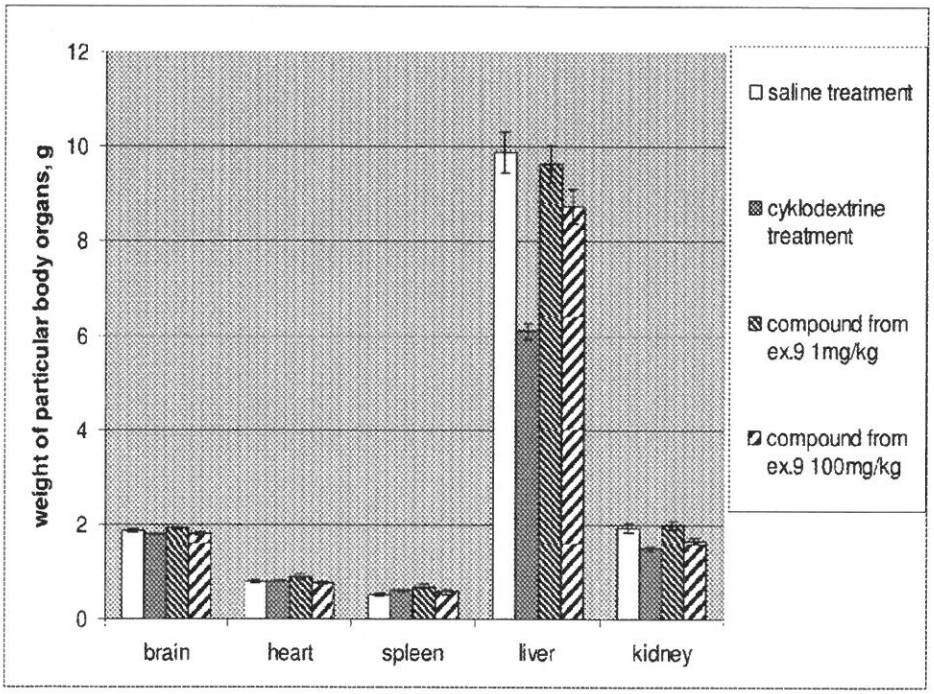

weight of particular body organs, g
12
10
8
6
4
2
0
brain
heart
spleen
liver
kidney
saline treatment
cyklodextrine treatment
compound from ex.9 1mg/kg
compound from ex.9 100mg/kg

Fig. 15

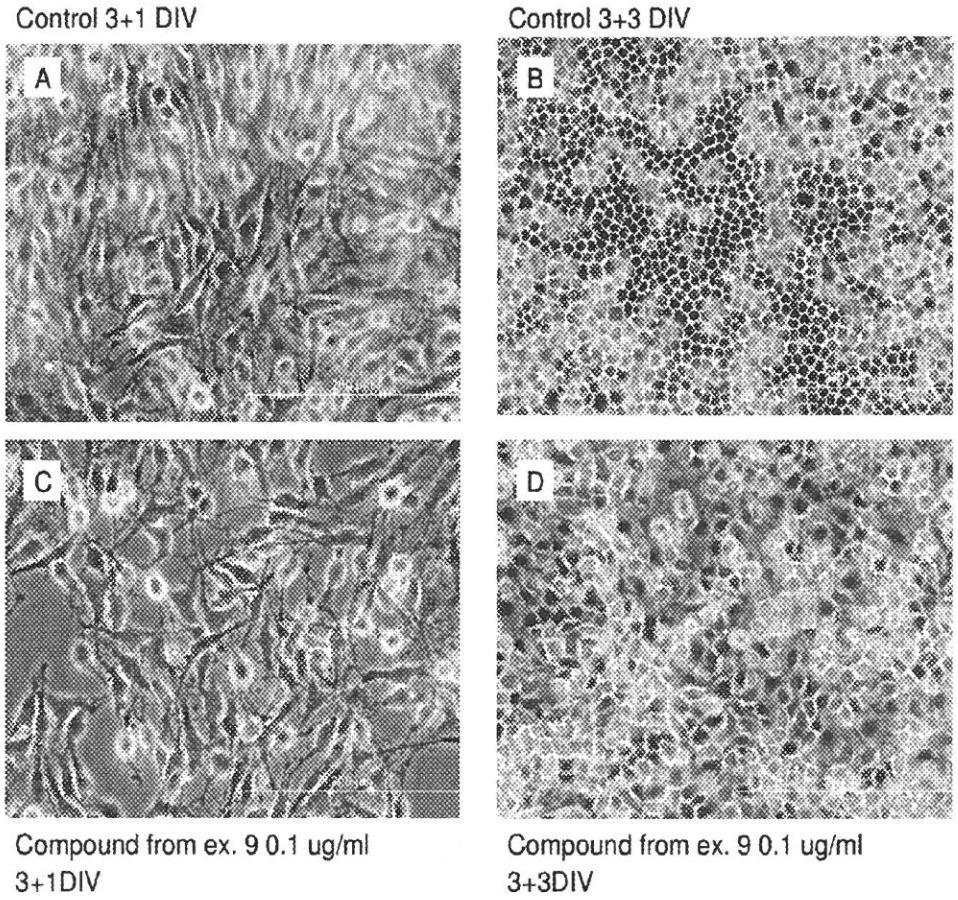

Control 3+1 DIV
Control 3+3 DIV
A
B
C
D
Compound from ex. 9 0.1 ug/ml
3+1DIV
Compound from ex. 9 0.1 ug/ml
3+3DIV

Fig. 16

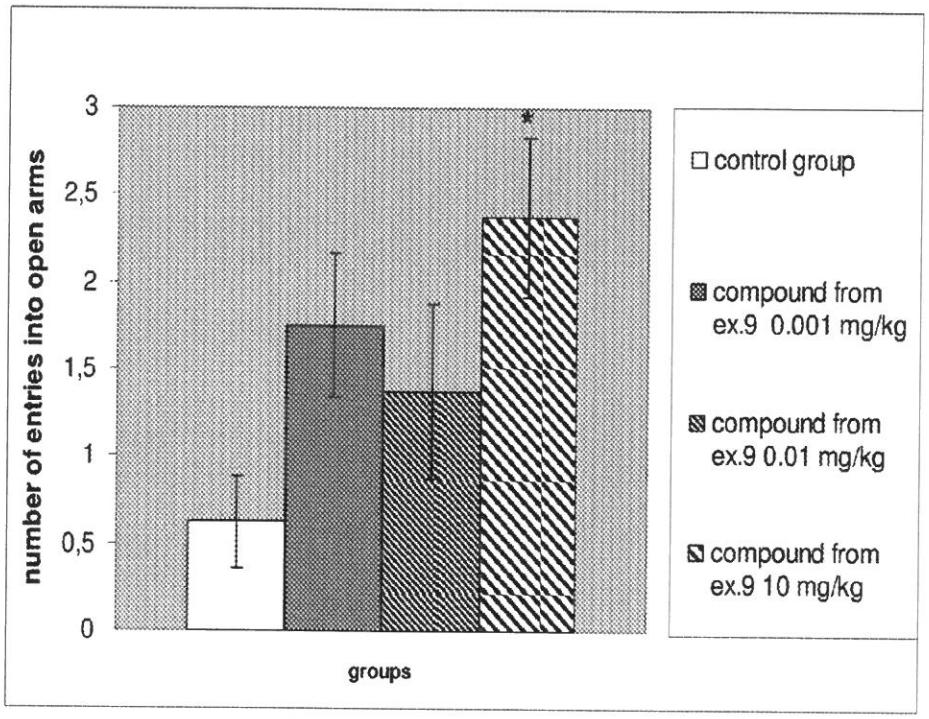

number of entries into open arms
3
2,5
2
1,5
1
0,5
0
groups
control group
compound from ex.9 0.001 mg/kg
compound from ex.9 0.01 mg/kg
compound from ex.9 10 mg/kg

Fig. 17

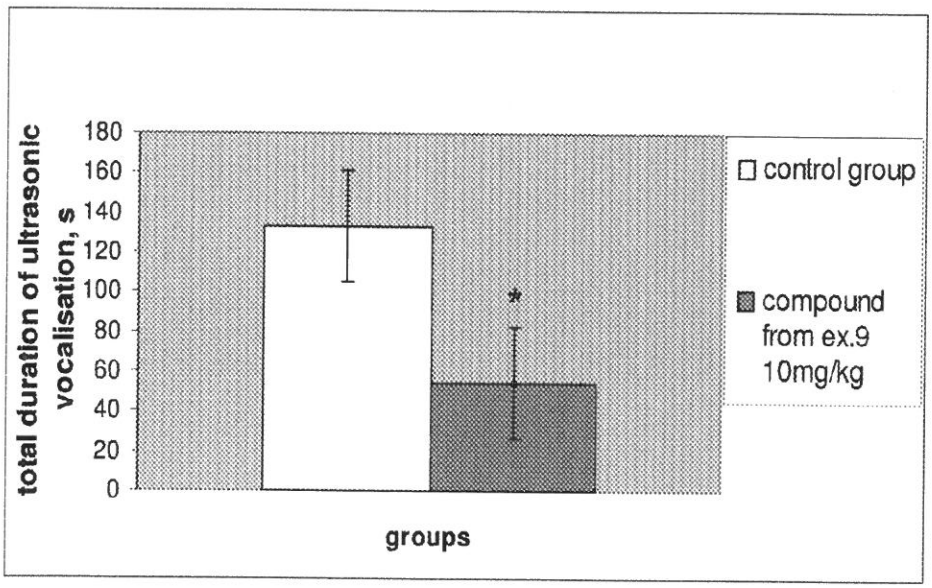

total duration of ultrasonic vocalisation, s
180
160
140
120
100
80
60
40
20
0
groups
control group
compound from ex.9 10mg/kg

Fig. 18

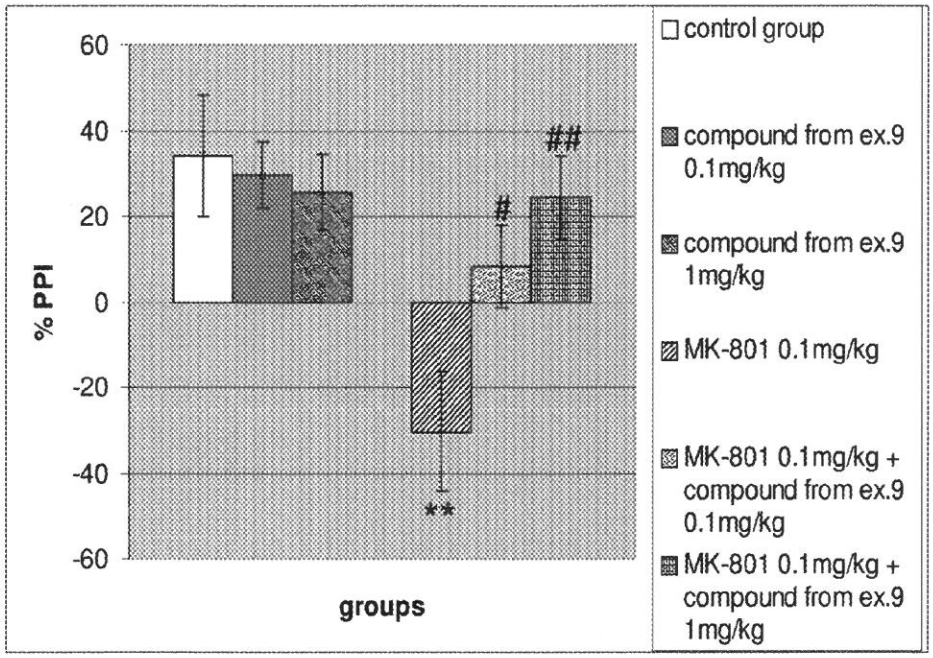

60
40
20
0
-20
-40
-60
% PPI
groups
**
#
##
control group
compound from ex.9 0.1mg/kg
compound from ex.9 1mg/kg
MK-801 0.1mg/kg
MK-801 0.1mg/kg + compound from ex.9 0.1mg/kg
MK-801 0.1mg/kg + compound from ex.9 1mg/kg

Fig. 19

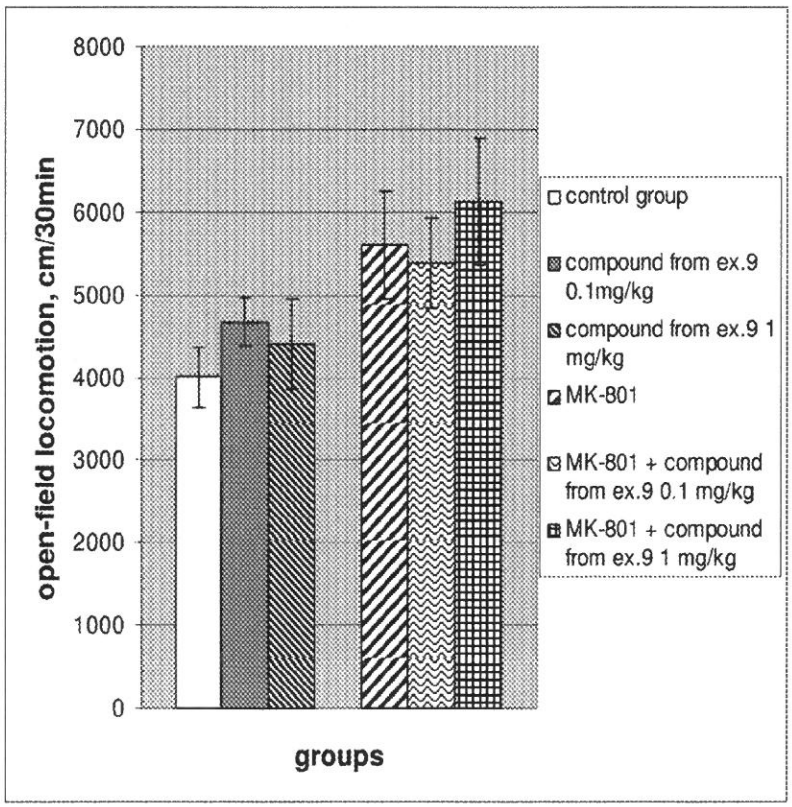

8000
7000
6000
5000
4000
3000
2000
1000
0
open-field locomotion, cm/30min
groups
control group
compound from ex.9 0.1mg/kg
compound from ex.9 1 mg/kg
MK-801
MK-801 + compound from ex.9 0.1 mg/kg
MK-801 + compound from ex.9 1 mg/kg

Fig. 20

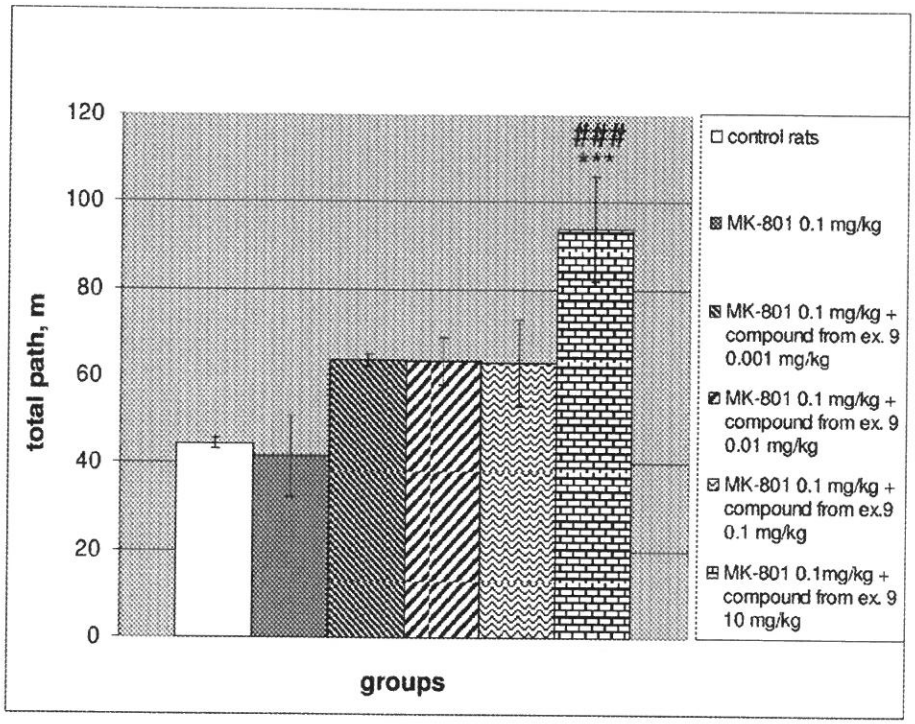

120
100
80
60
40
20
0
total path, m
###
***
groups
control rats
MK-801 0.1 mg/kg
MK-801 0.1 mg/kg + compound from ex. 9 0.001 mg/kg
MK-801 0.1 mg/kg + compound from ex. 9 0.01 mg/kg
MK-801 0.1 mg/kg + compound from ex.9 0.1 mg/kg
MK-801 0.1mg/kg + compound from ex. 9 10 mg/kg

Fig. 21

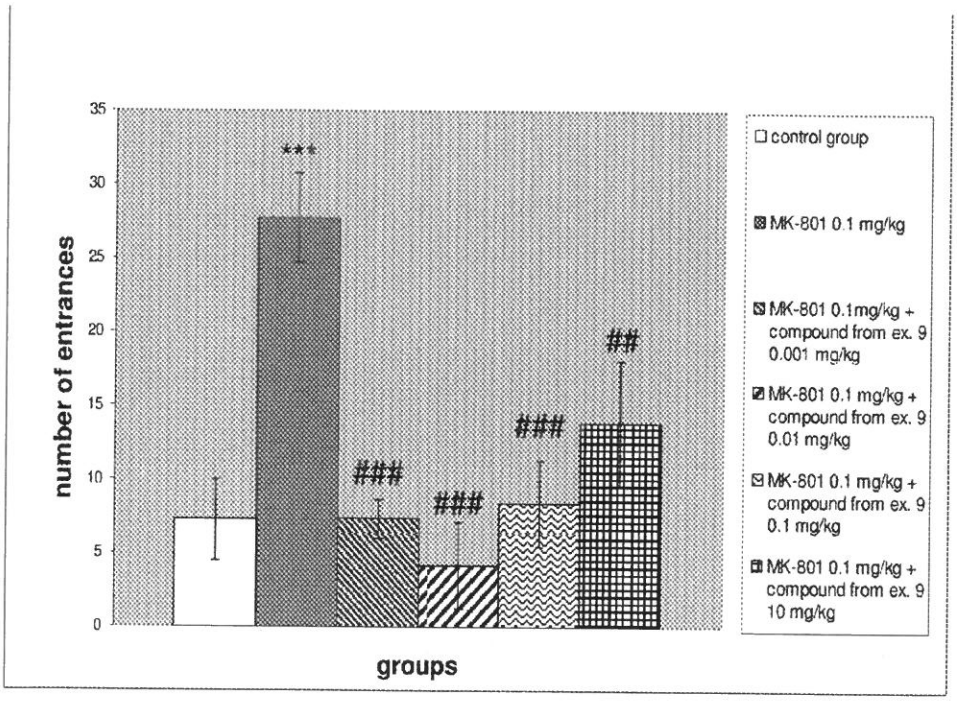

35
30
25
20
15
10
5
0
number of entrances
***
###
###
###
##
groups
control group
MK-801 0.1 mg/kg
MK-801 0.1mg/kg + compound from ex. 9 0.001 mg/kg
MK-801 0.1 mg/kg + compound from ex. 9 0.01 mg/kg
MK-801 0.1 mg/kg + compound from ex. 9 0.1 mg/kg
MK-801 0.1 mg/kg + compound from ex. 9 10 mg/kg

Fig. 22

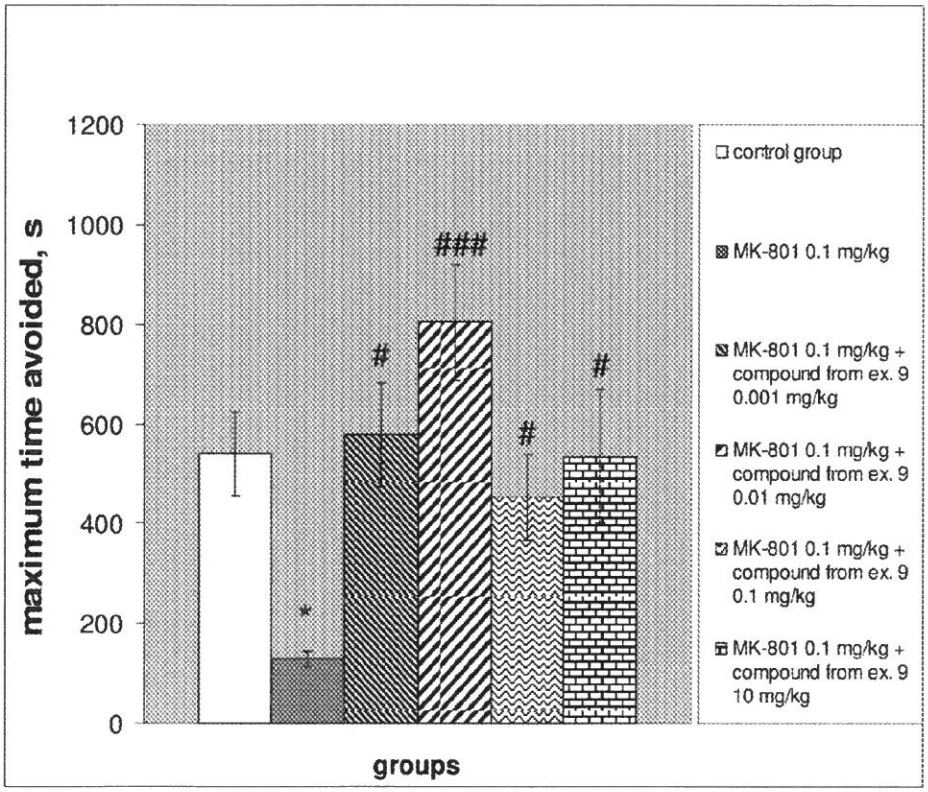

maximum time avoided, s
1200
1000
800
600
400
200
0
groups
control group
MK-801 0.1 mg/kg
MK-801 0.1 mg/kg + compound from ex. 9 0.001 mg/kg
MK-801 0.1 mg/kg + compound from ex. 9 0.01 mg/kg
MK-801 0.1 mg/kg + compound from ex. 9 0.1 mg/kg
MK-801 0.1 mg/kg + compound from ex. 9 10 mg/kg
*
#
###
#
#

Fig. 23

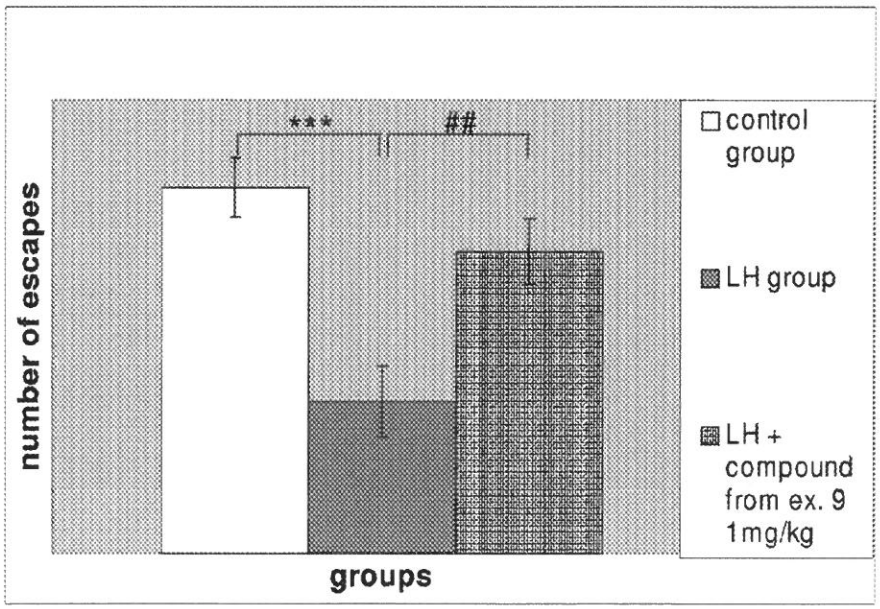

number of escapes
groups
***
##
control group
LH group
LH + compound from ex. 9 1mg/kg

Fig. 24

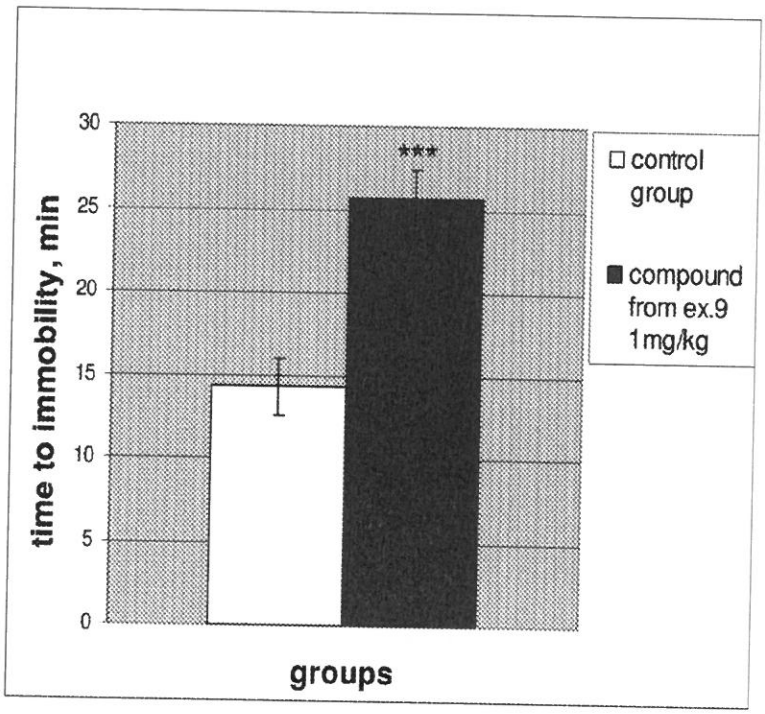

time to immobility, min
30
25
20
15
10
5
0
***
groups
control group
compound from ex.9 1mg/kg

Fig. 25

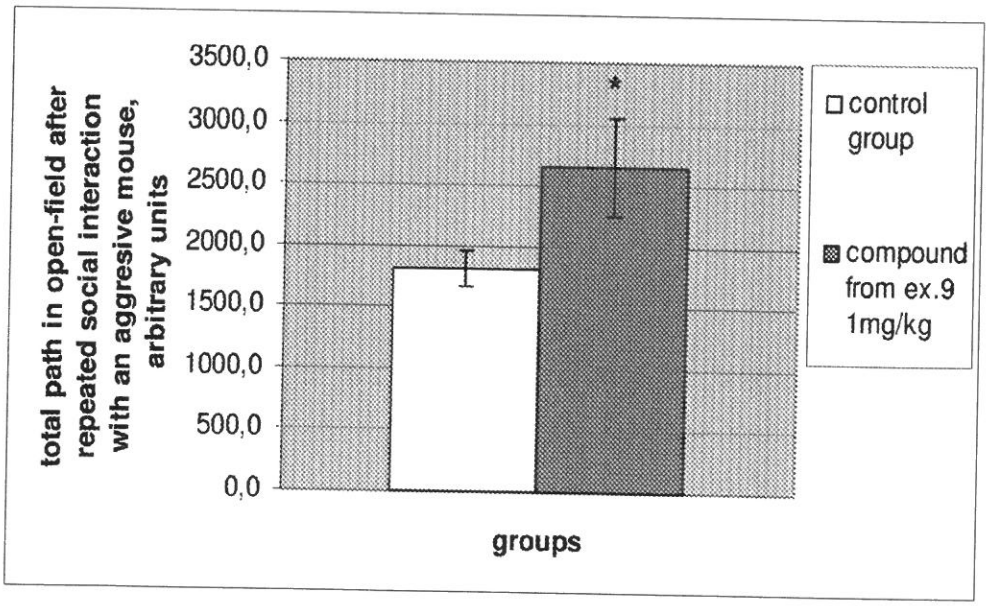

total path in open-field after repeated social interaction with an aggresive mouse, arbitrary units
3500,0
3000,0
2500,0
2000,0
1500,0
1000,0
500,0
0,0
*
groups
control group
compound from ex.9 1mg/kg

Fig. 26

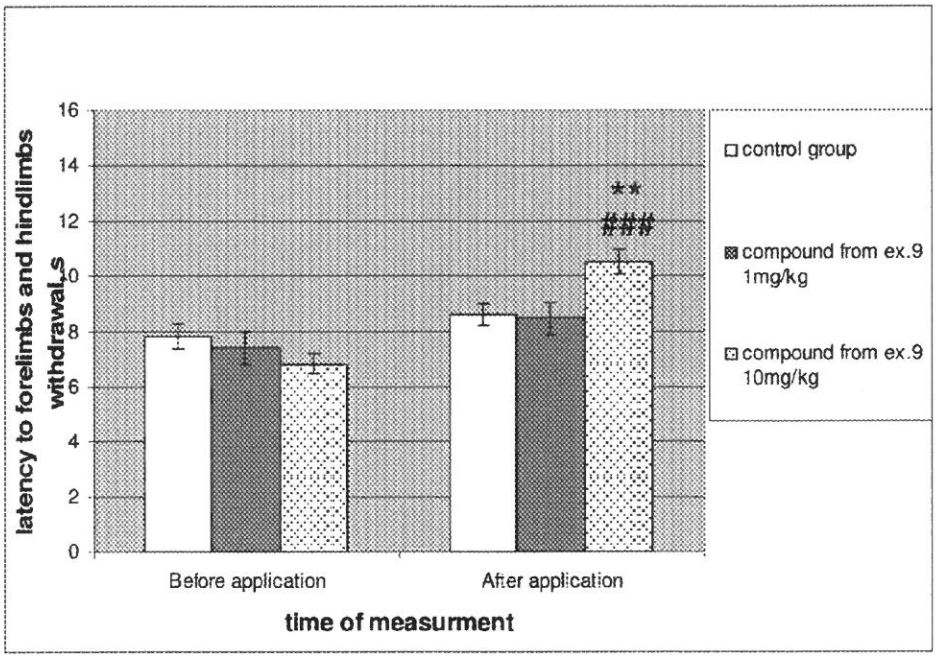

latency to forelimbs and hindlimbs withdrawal, s
16
14
12
10
8
6
4
2
0
**
###
control group
compound from ex.9 1mg/kg
compound from ex.9 10mg/kg
Before application
After application
time of measurment

Fig. 27

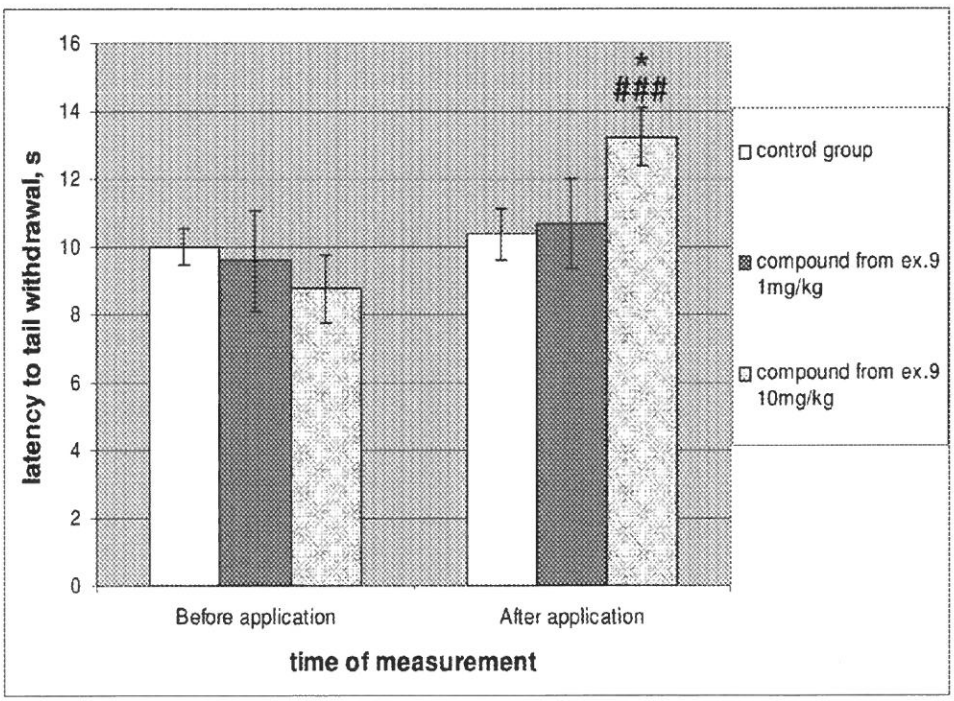
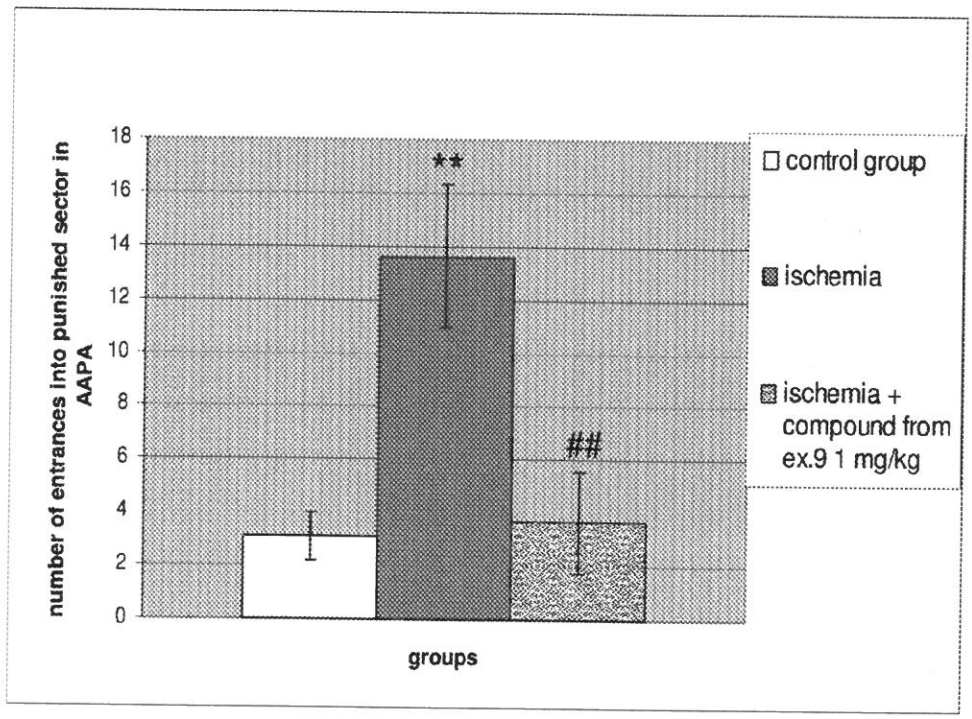

latency to tail withdrawal, s
16
14
12
10
8
6
4
2
0
*
###
control group
compound from ex.9 1mg/kg
compound from ex.9 10mg/kg
Before application
After application
time of measurement

Fig. 28


number of entrances into punished sector in AAPA
18
16
14
12
10
8
6
4
2
0
**
##
groups
control group
ischemia
ischemia + compound from ex.9 1 mg/kg

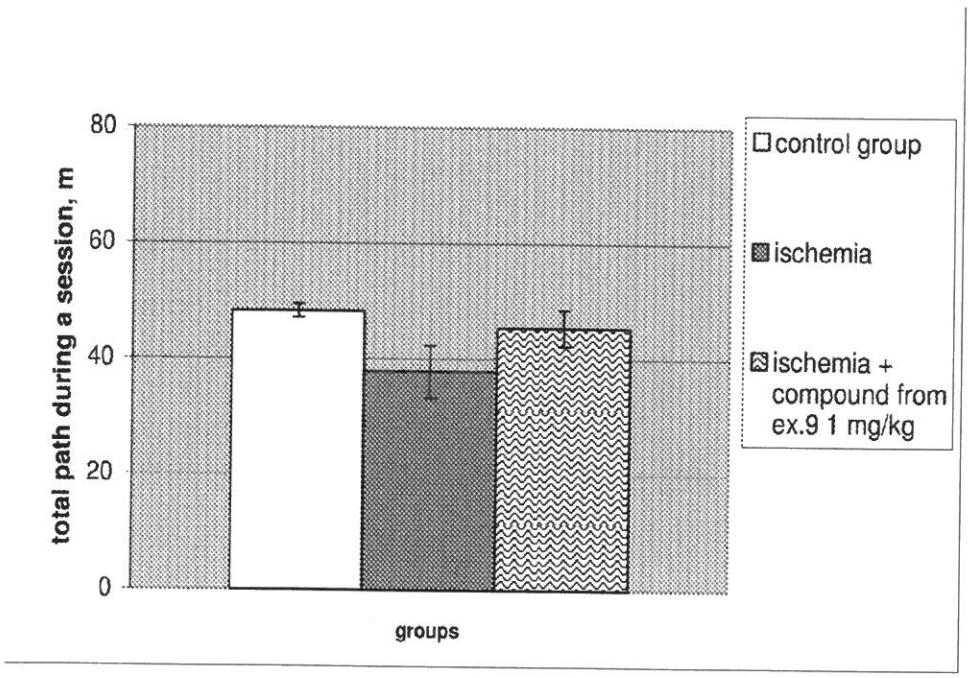

total path during a session, m
80
60
40
20
0
groups
control group
ischemia
ischemia + compound from ex.9 1 mg/kg

Fig 29

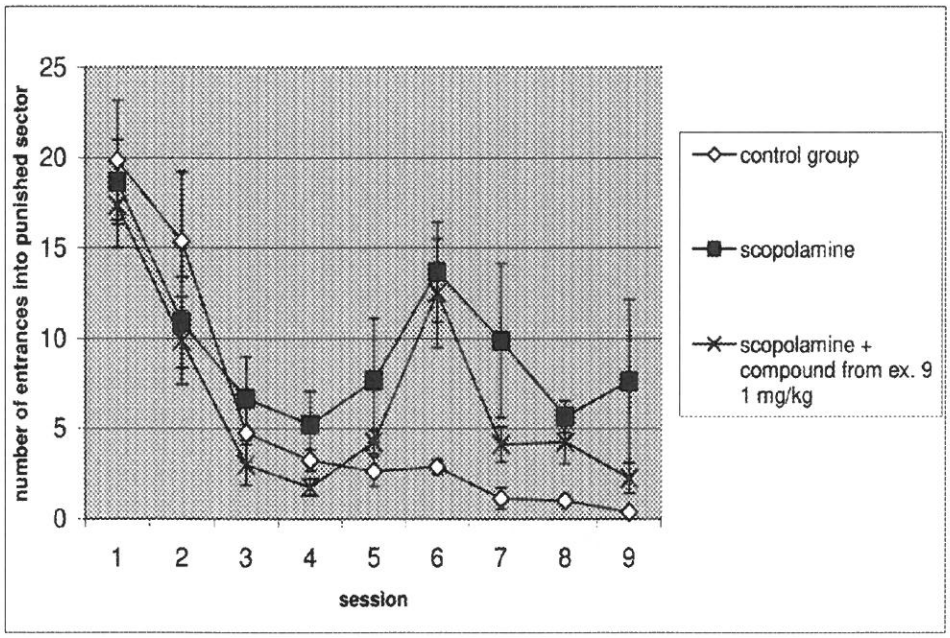

number of entrances into punished sector
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
session
control group
scopolamine
scopolamine + compound from ex. 9 1 mg/kg

Fig. 30

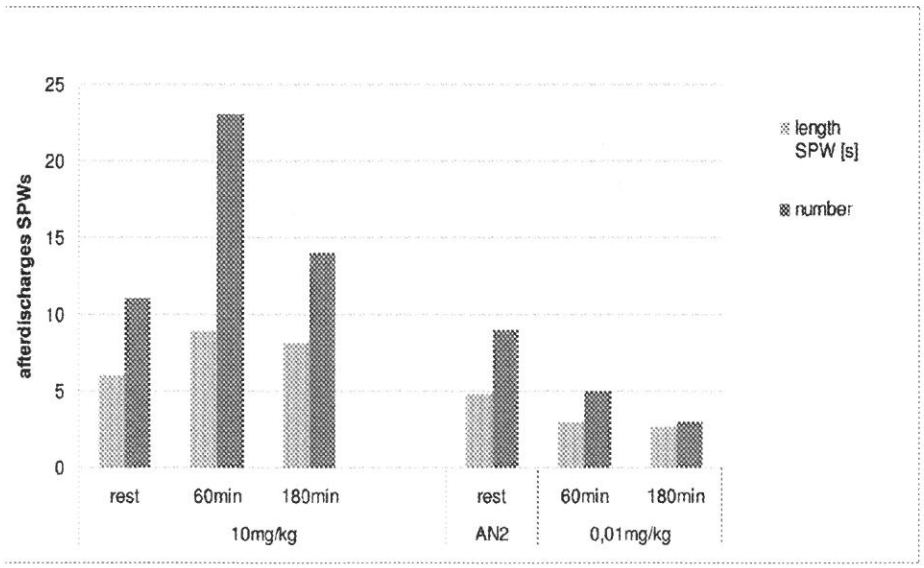

afterdischarges SPWs
25
20
15
10
5
0
rest
60min
180min
10mg/kg
rest
60min
180min
AN2
0,01mg/kg
length SPW [s]
number

Fig. 31

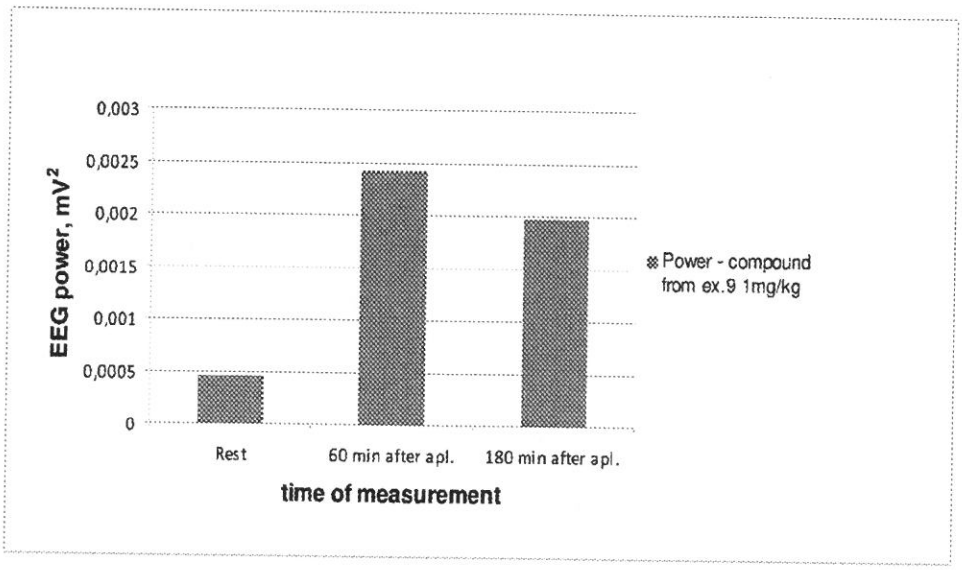

0,003
0,0025
0,002
0,0015
0,001
0,0005
0
EEG power, mV²
Rest
60 min after apl.
180 min after apl.
time of measurement
Power - compound from ex.9 1mg/kg

Fig. 32

STEROIDE ANIONIC COMPOUNDS, METHOD OF THEIR PRODUCTION, USAGE AND PHARMACEUTICAL PREPARATION INVOLVING THEM

FIELD OF THE INVENTION

This invention is represented by, anionic steroid compounds, ways of their production, their applications and pharmaceutical substances containing them. The invention particularly deals with pregnanolone derivatives substituted in 3alpha-position with the anionic group bound in this position. These derivatives may be beneficial in treatment of several central nervous system (CNS) diseases, especially ischemic CNS injury, neurodegenerative alterations and diseases, depression, post-traumatic stress disorder and other stress-related disorders, schizophrenia and various psychotic diseases, pain, addiction, multiple sclerosis and autoimmune disorders, epilepsy, and gliomas as well as other CNS tumors.

BACKGROUND ART

Glutamate is the principal excitatory neurotransmitter in the central nervous system of mammals. During synaptic transmission, the post-synaptic responses occur via ionotropic and metabotropic glutamate receptors. Metabotropic receptors operate via G-proteins and mobilize calcium ions from intracellular compartments. Activation of ionotropic receptors results in increase in permeability of postsynaptic membrane for sodium, potassium and calcium cations by opening a ion channel, which is an integral parts of the receptors.

Typical examples of ionotropic receptors are N-methyl D-aspartate (NMDA) receptors, AMPA and kainate receptors. Although current knowledge suggests specific role of various types of superfamily of glutamate receptors in the glutamate-induced excitotoxicity, ionotropic receptors are generally considered to be a key player in these processes. Activation of ionotropic receptors leads to alterations in intracellular concentrations, of various ions, mainly of Na^+ and Ca^{2+}. Current research demonstrates that beside calcium, elevated intracellular levels of sodium ions can also lead to neuronal death. In neuronal cultures and in retina the activation of glutamate receptors may lead to damage even by sodium cations in absence of extracellular calcium ions. Nonetheless, toxicity of elevated glutamate levels is usually associated with elevations in intracellular concentrations of Ca^{2+}. Currently it is well established that there is a direct relationship between excessive influx of calcium into cells and glutamate-induced damage to neurons. Glutamate-induced pathological calcium elevation is usually ascribed to prolonged activation of ionotropic receptors. Elevation in intracellular calcium then may trigger the down-stream neurotoxicity cascade, which involves uncoupling of mitochondrial electron transport from ATP production, supranormal activation of enzymes such as calpain and other proteases, induction of specific protein kinases, NO-synthase, calcineurins and endonucleases. These changes may also promote the production of toxic reactive molecules such as reactive oxygen species (ROS) and induce changes in cytoskeleton architecture and activation of signals leading to apoptosis and mitochondrial damage (Villmann and Becker, 2007).

A number of preclinical studies show a remarkable ability of NMDA receptor antagonists to prevent from the excessive exocytose of glutamate and damage to the CNS. From the clinical point of view; however, their therapeutic potential is rather limited. Regarding the fact that glutamate receptors are ones of the most abundant in the CNS, application of their antagonists leads to wide variety of side effects, ranging from motor impairment to induction of psychotic symptoms. On the contrary, a large divergence of NMDA receptors and differences in their distribution at synapses and at extrasynaptic sites offer a possibility to search for drugs which selectively influence only a limited subset of NMDA receptors and thus to avoid the induction of unexpected side effects, while retaining their therapeutic neuroprotective activity.

Previous results demonstrated that naturally occurring 3α5β-pregnanolone sulfate affects the activity of NMDA receptor by a use-dependent manner. As a consequence this molecule has a more pronounced inhibitory action on the tonically active NMDA receptors than on those phasically activated by glutamate during synaptic transmission. It was also demonstrated that activation of extrasynaptic tonically activated NMDA receptors is very important for excitotoxic action of glutamate (Petrovic et al., 2005).

Therefore, we have started the development and testing of novel NMDA receptor antagonists derived from neurosteroids. These newly synthesized drugs exhibit affinity for extrasynaptic NMDA receptors. What is more important, previous electrophysiological studies showed that these compounds bound preferentially to open NMDA receptor channels. Our compounds lack affinity for other types of receptor; it is thus presumed that they will not affect signal transmission between neurons. The suggested mechanisms of their action are the blockade of extrasynaptic tonically activated NMDA receptors and prevention of excessive action of glutamate on neurons.

In the last decade, the biomedical research focused on the study of the role of neurosteroids in the pathogenesis of number of neuropsychiatric diseases and evaluation of their therapeutic potential. Mechanisms of action of neurosteroids are conventionally associated with their activity on NMDA and GABA-A receptors. A number of experimental studies with animal models show their potential in therapy of several diseases of CNS, including neurodegenerative disorders, multiple sclerosis, affective disorders, alcoholism, pain, insomnia or schizophrenia (Morrow, 2007; Weaver, 2000).

Neurosteroids also play a crucial role in the regulation of reactivity to stress and stress-related CNS disorders. Corticosteroid levels are known to acutely increase after exposition to a stressor; this represents an adaptive mechanism. On the other hand, experimental models of chronic stress and depression in laboratory rodents show decreased levels of neurosteroids both in brain and plasma. Similar findings are often reported in patients suffering from depressions and pre-menstruation syndrome suggesting impairments in the CNS homeostatic mechanisms in stress-related neuropsychiatric disorders.

Steroid compounds affect activity and plasticity of neural and glial cells during early in life, and later in development they play an essential trophic and neuroprotective role in the adult CNS. Steroids are released by sexual and adrenal glands as well as in the CNS. Steroids secreted by peripheral glands reach brain, medulla and spinal cord via blood circulation. Nonetheless, some neural steroids (i.e., neurosteroids) are