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Novel preclinical therapeutic strategies of neurodegeneration with kynurenines: clinical perspectives

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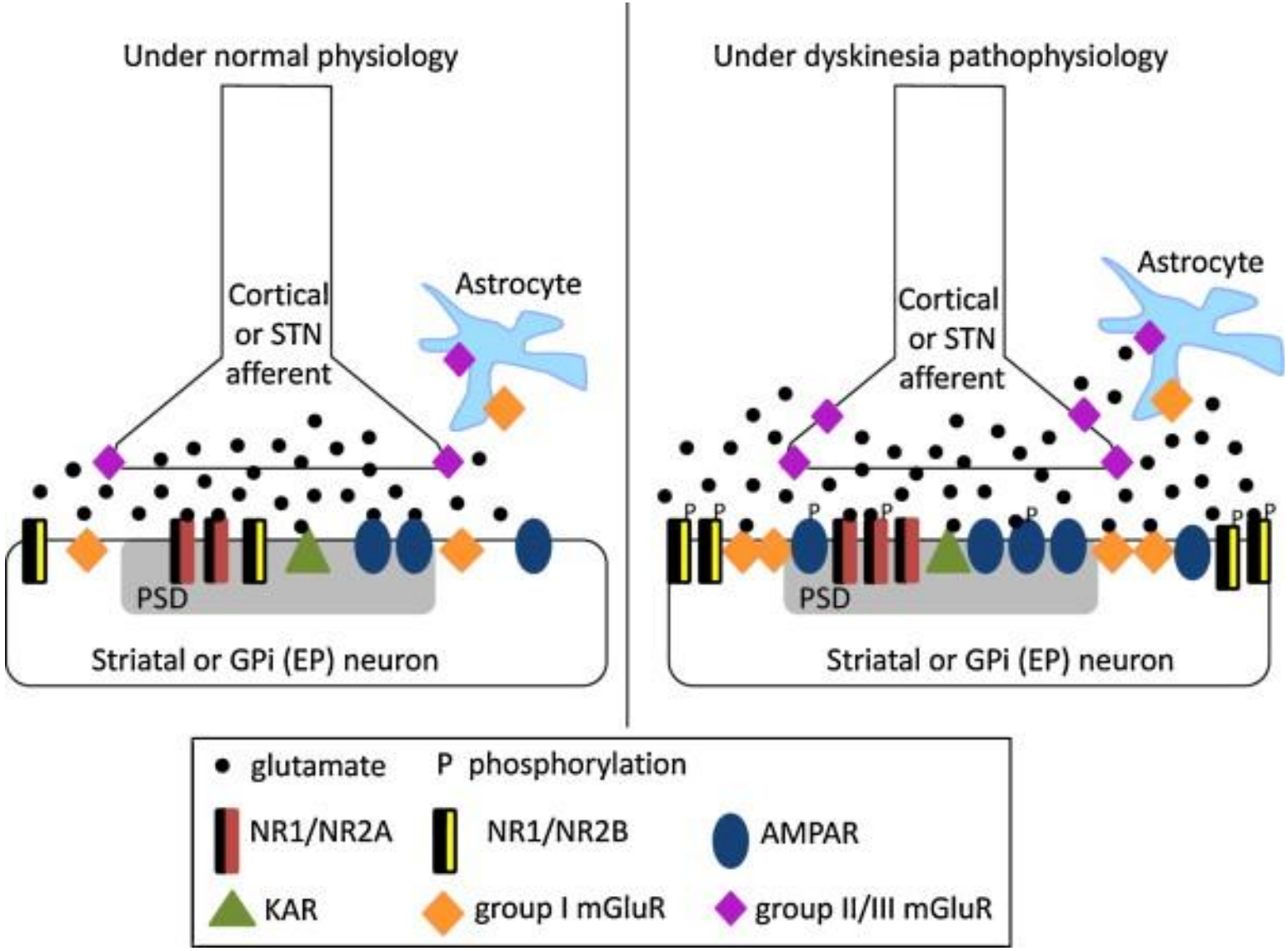
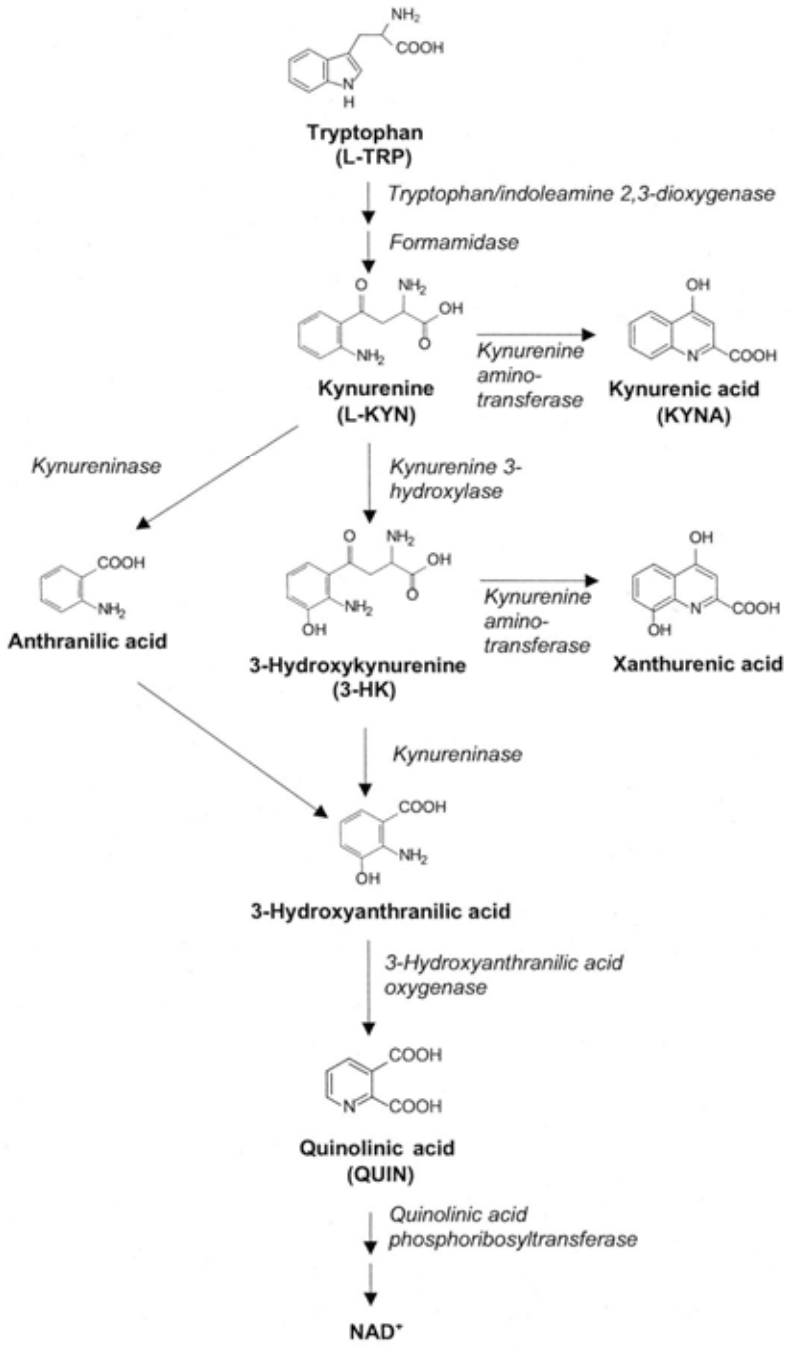
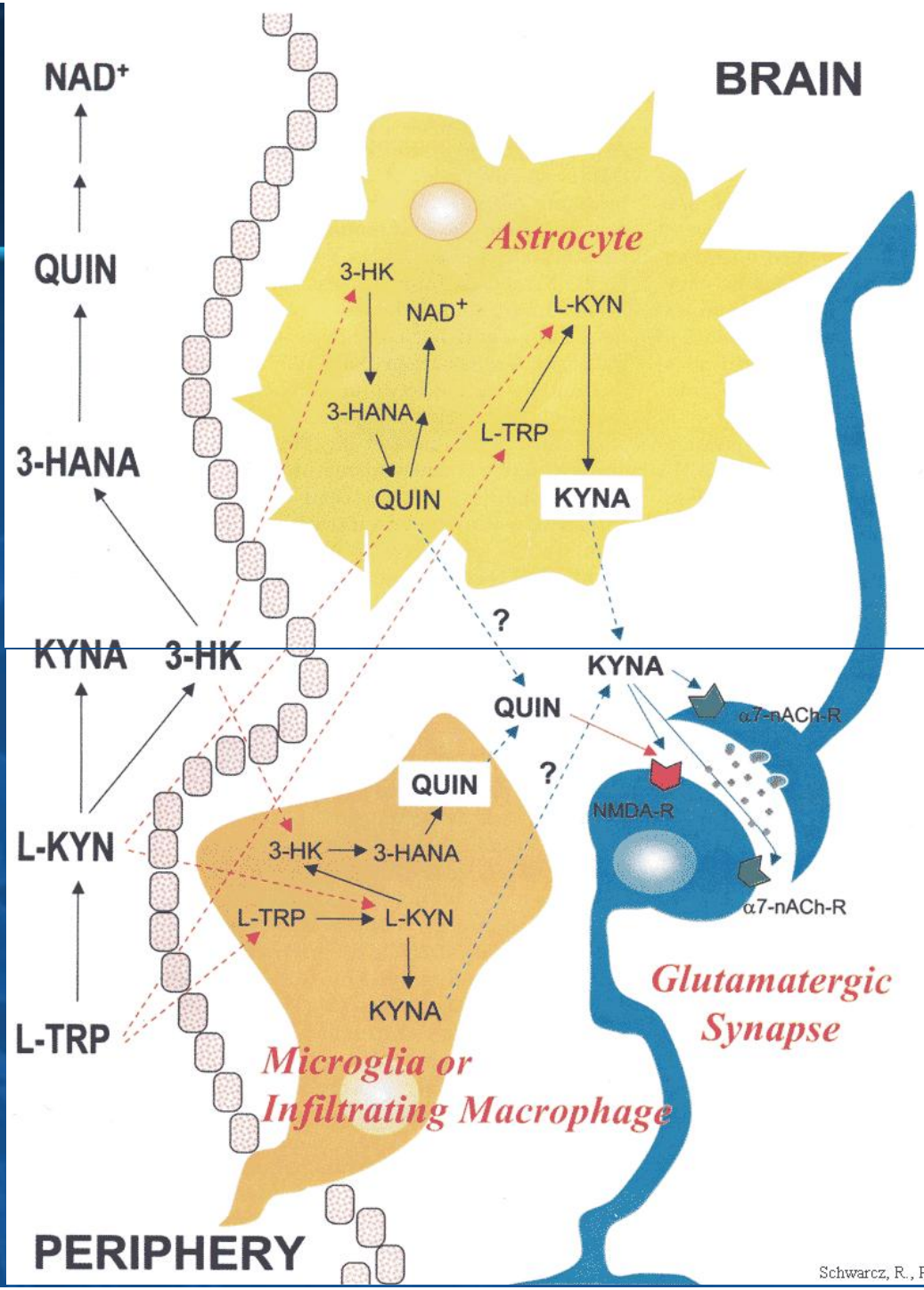


Fig. 4. Proposed glutamatergic mechanisms involved in LID or STN-HFS-induced dyskinesias. Drawings represent glutamatergic corticostriatal and subthalamo-pallidal (GPi/EP) synapses with nearby astrocytes. Ionotropic glutamate receptors (NMDA, AMPA, KA) are mainly clustered in the post-synaptic density (PSD), whereas metabotropic receptors have a peri- or extra-synaptic localization. Under physiological conditions, glutamate stimulation remains largely confined to AMPA and NMDA receptors in the post-synaptic membrane. Under conditions that elicit dyskinesia, l-DOPA administration or STN-HFS result in glutamate spillover from the synaptic cleft and/or glutamate release from astrocytes, causing a prominent activation of perisynaptic and extra-synaptic receptors. This effect concurs with changes in the subcellular localization, and phosphorylation state of iGluR subunits. Further investigations are required to clarify the causes of the large glutamate rise in the extracellular fluid occurring during the expression of dyskinesia, and the specific role of each type of glutamate receptor.



Kynurenine pathway in mammalian cells.

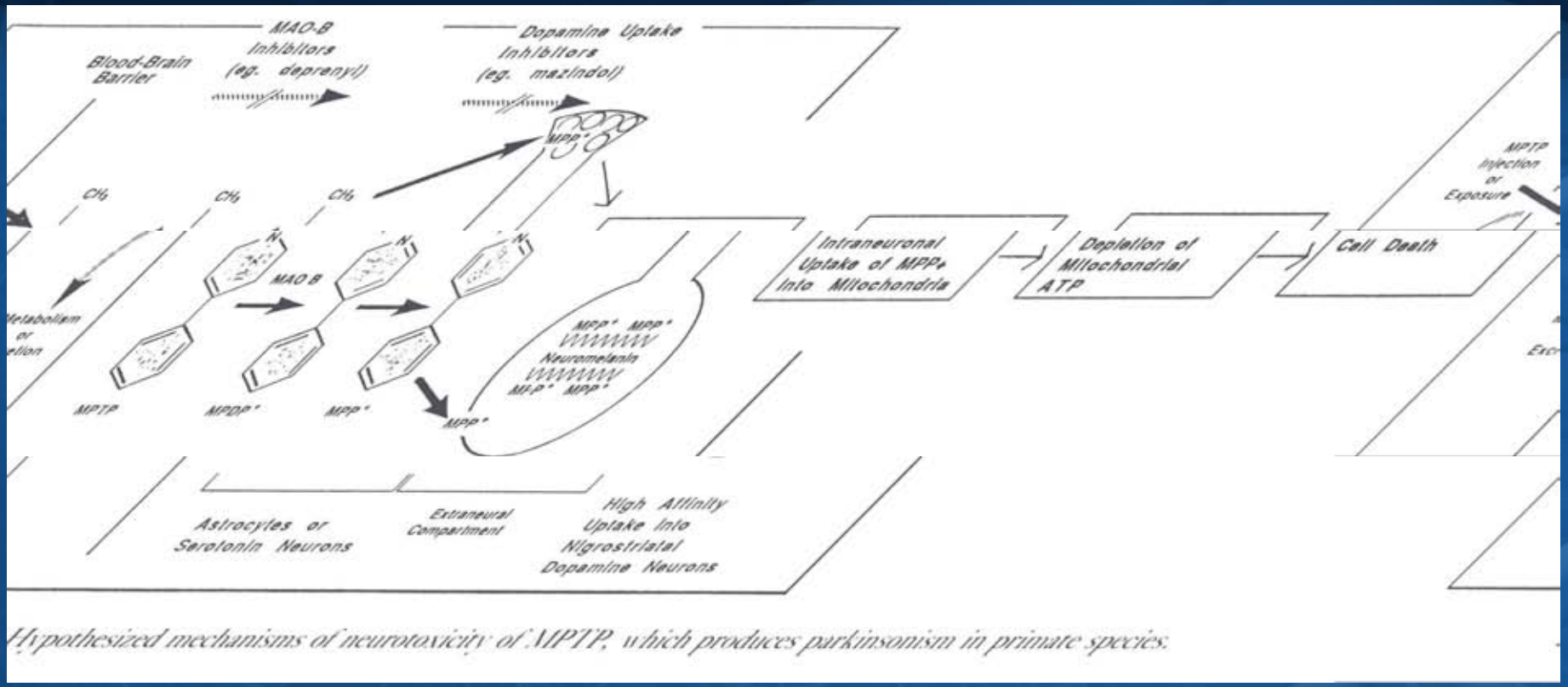


Dynamics of cerebral and extracerebral kynurenine pathway metabolism. L-TRP, L-tryptophan; L-KYN, L-kynurenine; 3-HK, 3- hydroxykynurenine; 3-HANA, 3-hydroxyanthranilic acid; QUIN, quinolinic acid; NMDA-R, NMDA receptor; α7-nACh-R, α7 nicotinic acetylcholine receptor. Broken arrows: brain entry/cellular uptake (red), release (blue). Solid arrows: enzymatic conversion (black), receptor agonist (red), receptor antagonist (blue).

Schwarcz, R., Pellicciari, R.: J.Pharmacol. Expr. Ther. 303:1-10, 2002

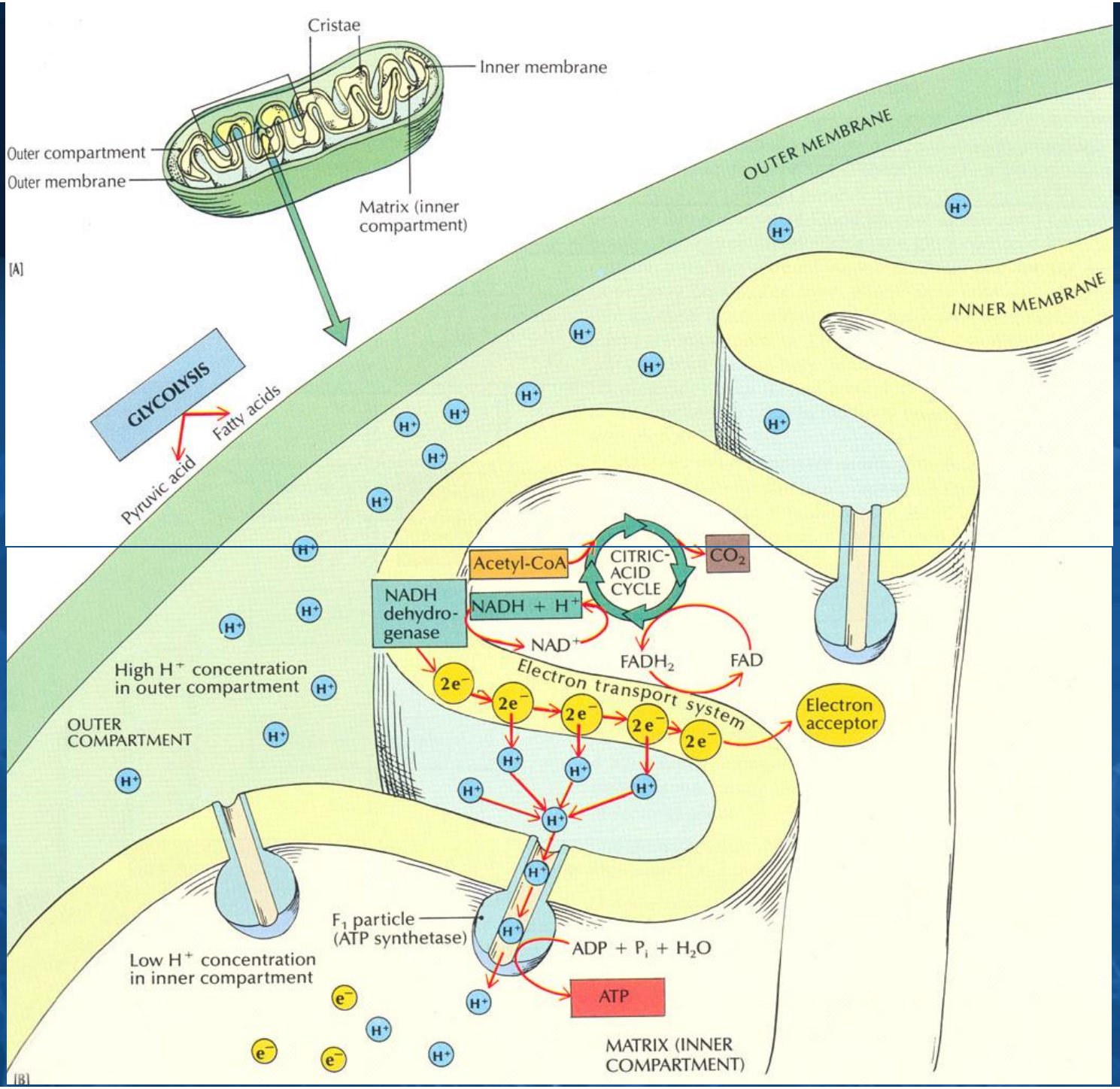
The kynurenine/tryptophan-ratios were increased both in serum and CSF of patients with Parkinson's disease as compared to controls. Serum tryptophan was lower in patients with Parkinson's disease *(Widner et al. 2002).*

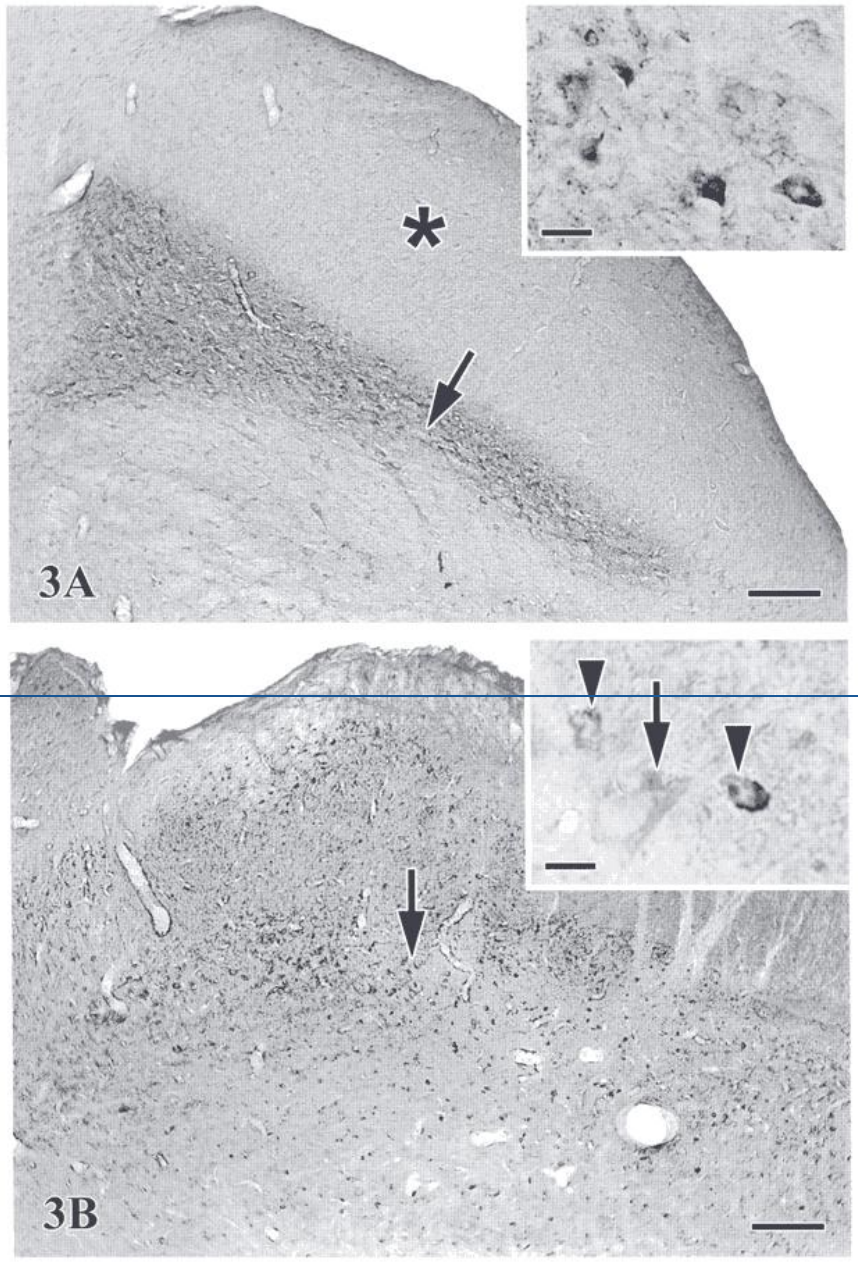
The level of 3-hydroxy-kynurenine is significantly increased in the putamen and substantia nigra in patients with Parkinson's disease, which could contribute to the neuronal loss in the disease *(Ogawa et al. 1992).*



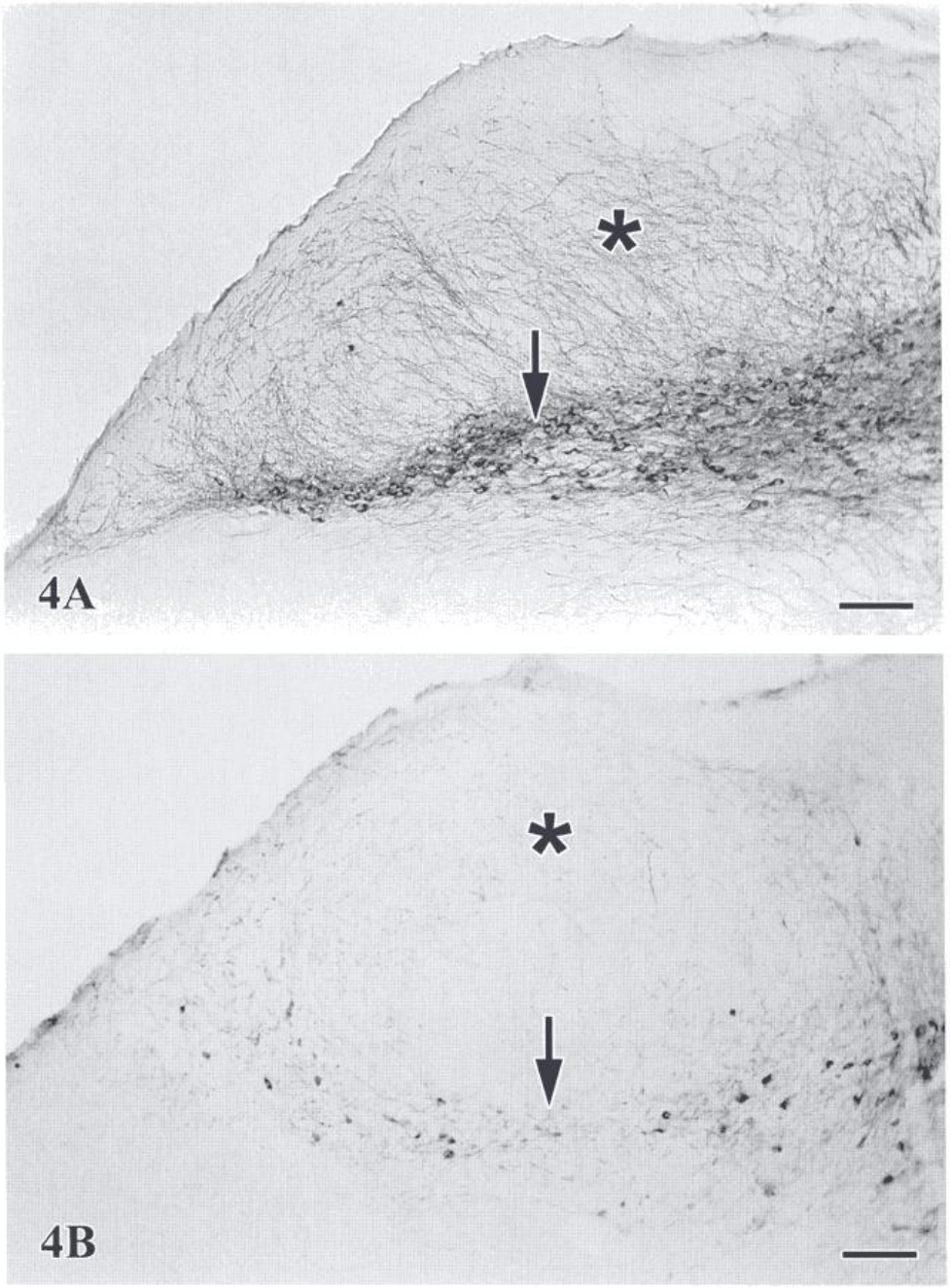
Hypothesized mechanisms of neurotoxicity of MPTP, which produces parkinsonism in primate species.

MPTP treatment and kynurenine aminotransferase-I (KAT-I) in the substantia nigra of mice

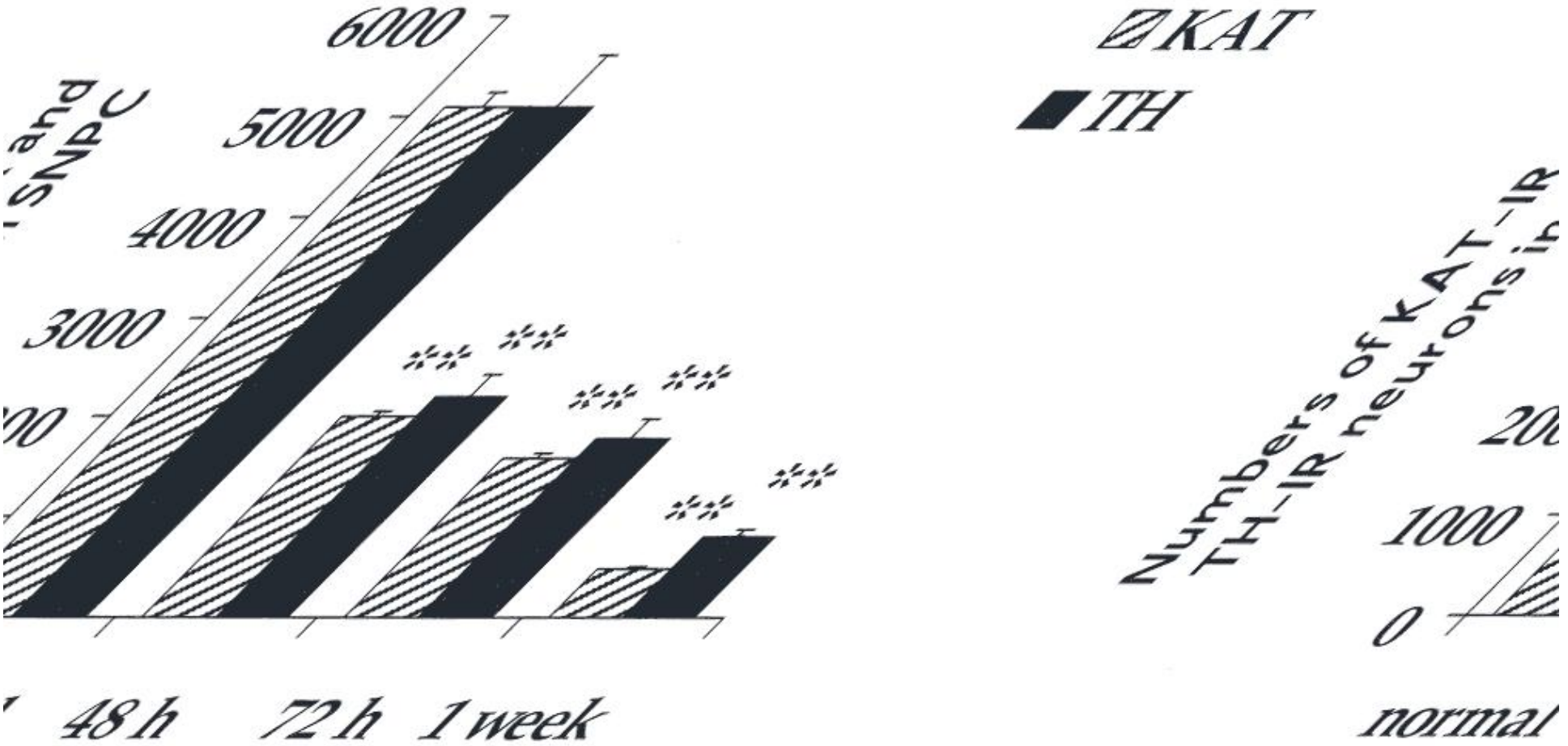

Cristae
Inner membrane
Outer compartment
Outer membrane
Matrix (inner compartment)
[A]
OUTER MEMBRANE
INNER MEMBRANE
GLYCOLYSIS
Fatty acids
Pyruvic acid
Acetyl-CoA
CITRIC-ACID CYCLE
CO_2
NADH dehydro-genase
NADH + H^+
NAD^+
$FADH_2$
FAD
Electron transport system
Electron acceptor
High H^+ concentration in outer compartment
OUTER COMPARTMENT
F_1 particle (ATP synthetase)
ADP + P_i + H_2O
ATP
Low H^+ concentration in inner compartment
MATRIX (INNER COMPARTMENT)
[B]



Effect of MPTP treatment on KAT-I IR of nerve cells in SNPC. (A) KAT-I IR in SNPC under normal conditions. Arrow points at SNPC. Asterisk denotes pars reticulata of SN. Scale bar=100 μm. Inset: KAT-I IR neurons from the SNPC. Scale bar=5 μm. (B) Strongly decreased number of KAT-I IR nerve cells 1 week after MPTP treatment. KAT-I IR mainly represents microglial cells (arrow). Scale bar=100 μm. Inset: Arrow points at pycnotic neuron. Arrowheads indicate microglial cells. Scale bar=5 μm.

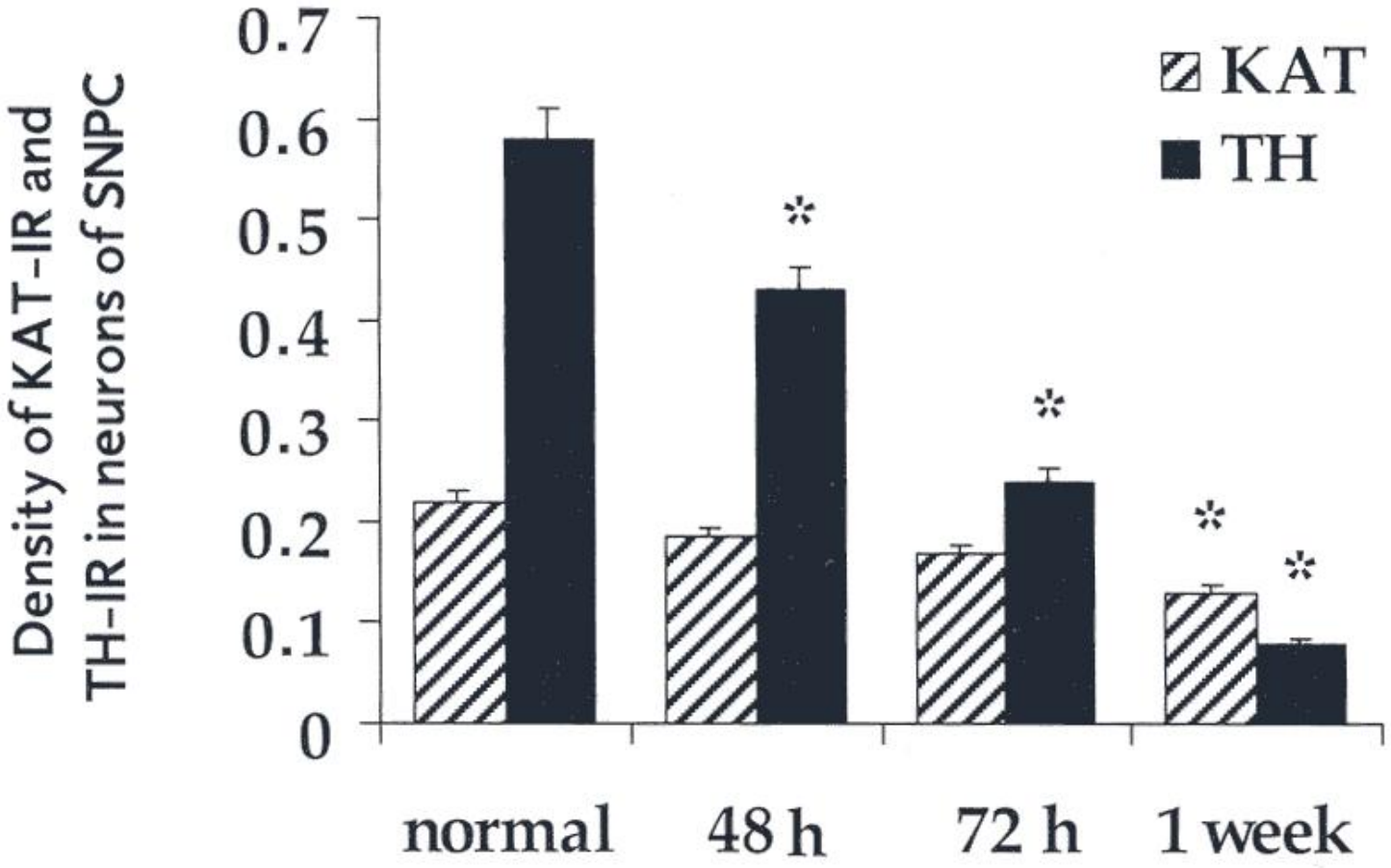


TH-IR of nerve cells in SNPC (arrow). Processes of neurons radiate in pars reticulata SN (asterisk). (A) Normal conditions. Scale bar=100 μm. (B) One week after MPTP treatment: number of nerve cells and processes is markedly decreased. Scale bar=100 μm.

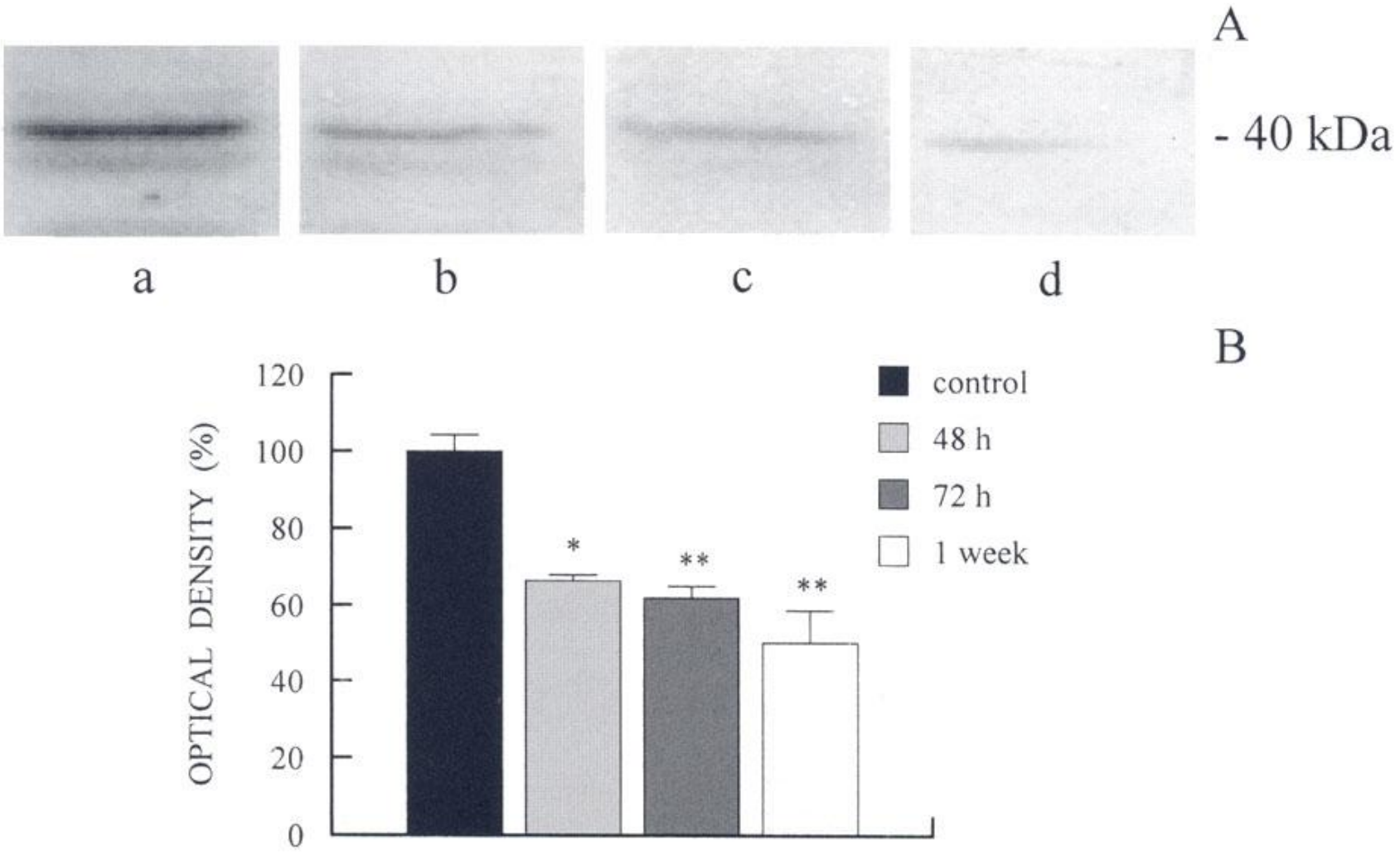


KAT-I and TH IR cells in the
by stereological methods.
-/IR cell counts are highly

Alterations in the numbers of
SNPC after MPTP treatment, determined
MPTP-induced decreases of KAT-I IR and Th
significant ($P<0.001$).



Densitometric analysis of KAT-I and TH IR of nerve cells in the SNPC after MPTP treatment. $n=15$ in each group. Asterisk indicates $P<0.01$.



Western blot analysis of KAT-I expression in SN after MPTP treatment. Data represent results from homogenates made from three mice in each group. Panel A shows typical bands. Lines relate to (a) control; (b) 48 h treated; (c) 72 h treated; (d) 1 week treated. Panel B displays the results of normalized band densities. Data are expressed as mean±S.D. and analyzed by Student's *t*-test. * $P<0.002$.

Summary
Decreased expression of kynurenine aminotrasferase-I (KAT-I) in the substantia nigra of mice after MPTP treatment

1. It has been shown that the highly selective dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-terahydropyridine (MPTP), widely used as a model of Parkinson's disease, affects not only TH dopaminergic neurons in the substantia nigra pars compacta but also their KAT-I immunoreactivity as well.

2. MPTP treatment decreased the number and optical density of KAT-I immunoreactive SNPC neurons.

3. MPTP also reduced KAT-I immunoreactivity of microglial cells, except for those involved in reactive gliosis, which were arranged in groups surrounding affected neurons of the SNPC; also the number of KAT-I immunoreactive astroglial cells was increased in SNPC.

4. It is concluded that MPTP treatment may have a dual effect: in addition to being deleterious for neurons expressing TH and KAT-I, it also affects glial cells which could exacerbate the neurodegenerative process characterizing Parkinson's disease.

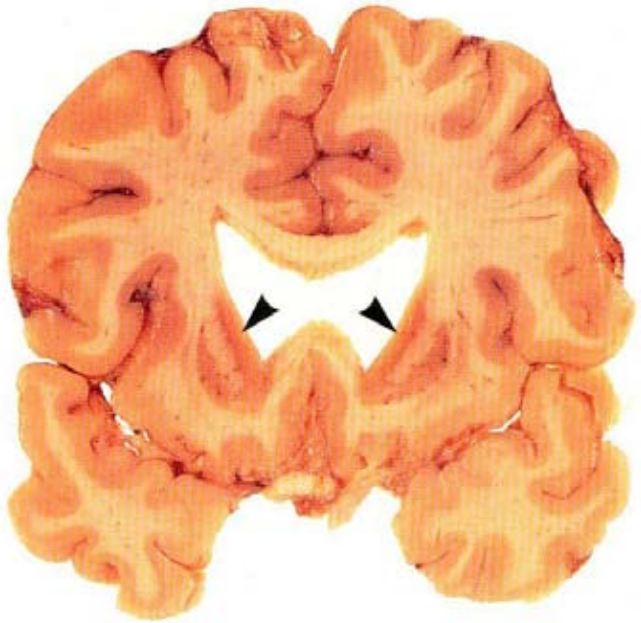


Figure 68 Coronal section of brain in Huntington's disease shows symmetrical atrophy and brown discoloration (arrowed) of the caudate and putamen together with dilatation of the lateral ventricles

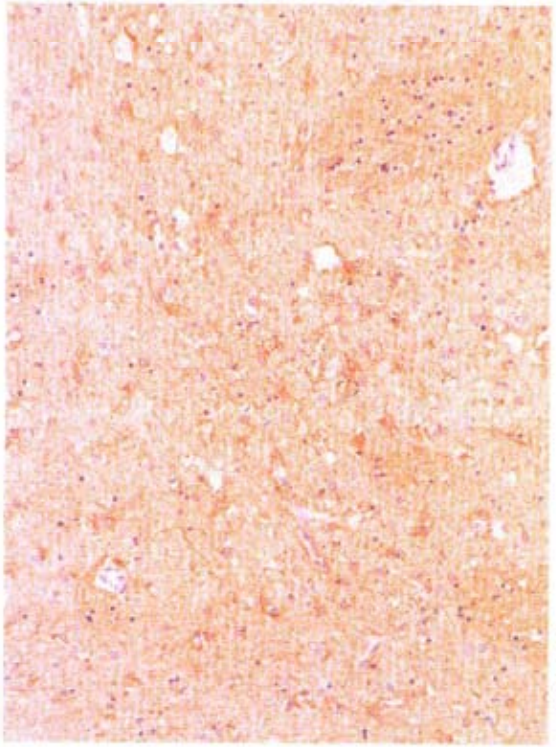
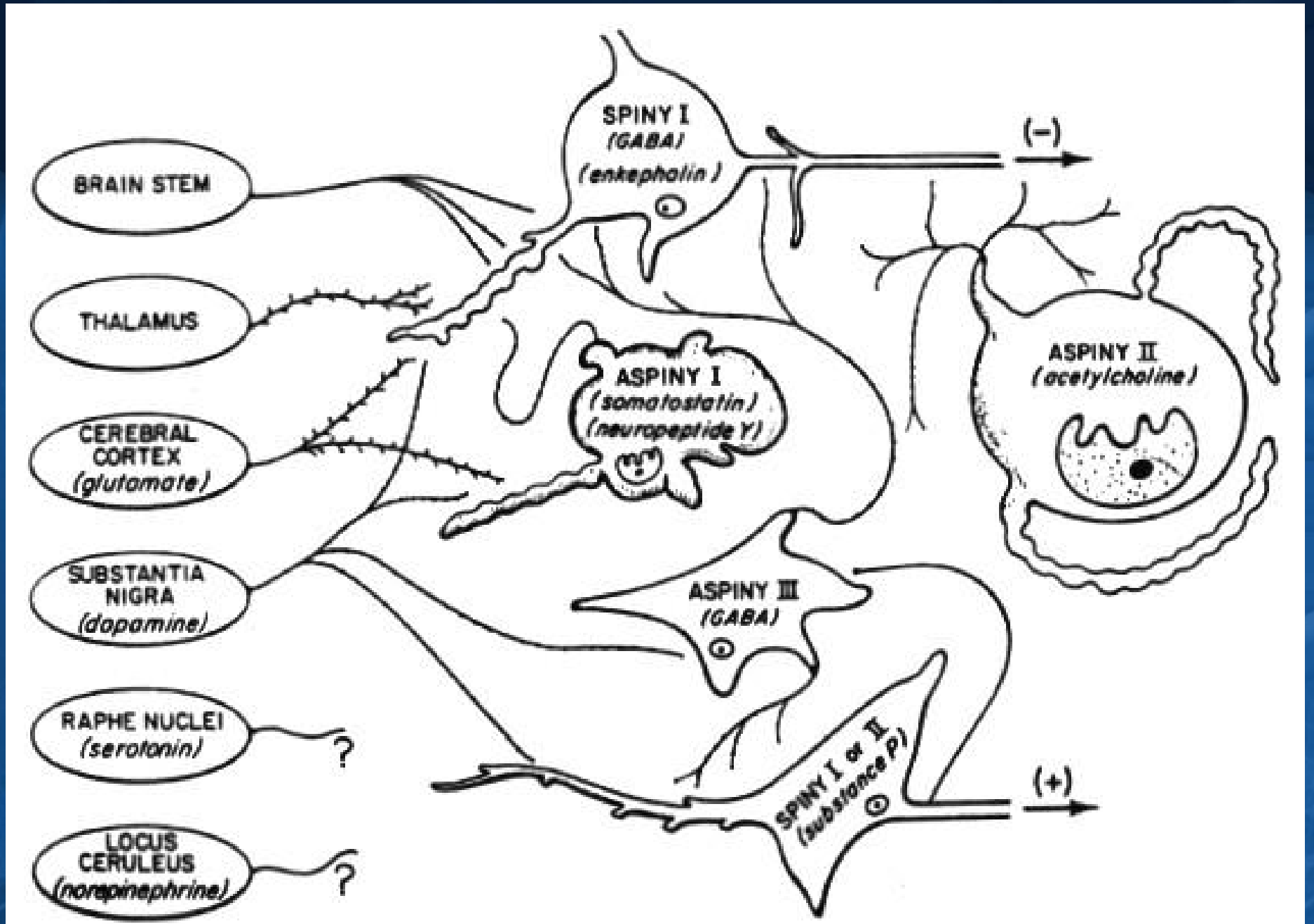
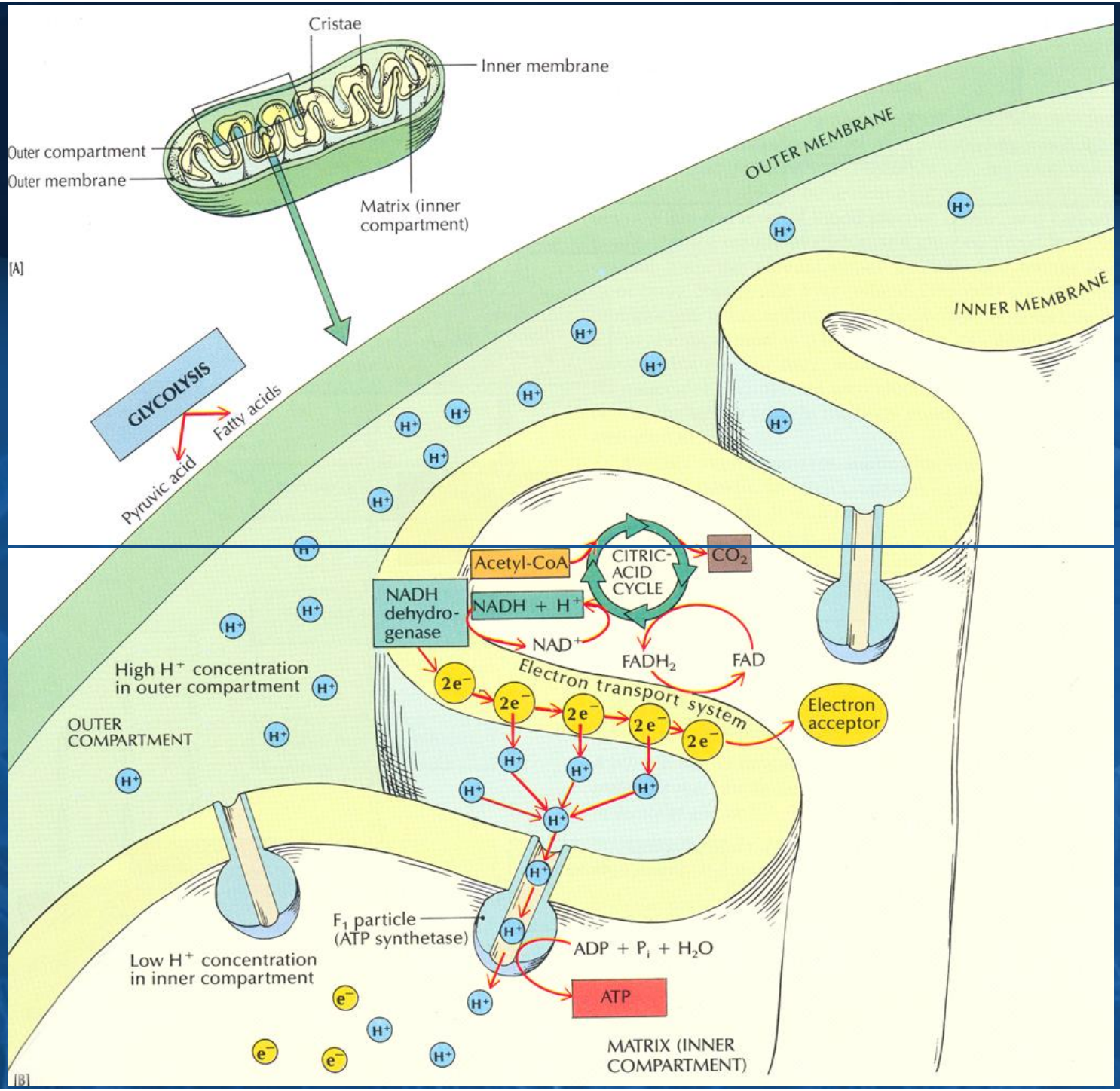


Figure 69 Histological section of brain in Huntington's disease shows atrophy with loss of neurons and astrocytic gliosis (immunocytochemistry preparation for glial fibrillary acidic protein)


BRAIN STEM
THALAMUS
CEREBRAL CORTEX (glutamate)
SUBSTANTIA NIGRA (dopamine)
RAPHE NUCLEI (serotonin)
?
LOCUS CERULEUS (norepinephrine)
?
SPINY I (GABA) (enkepholin)
(–)
ASPINY I (somatostatin) (neuropeptide Y)
ASPINY II (acetylcholine)
ASPINY III (GABA)
SPINY I or II (substance P)
(+)

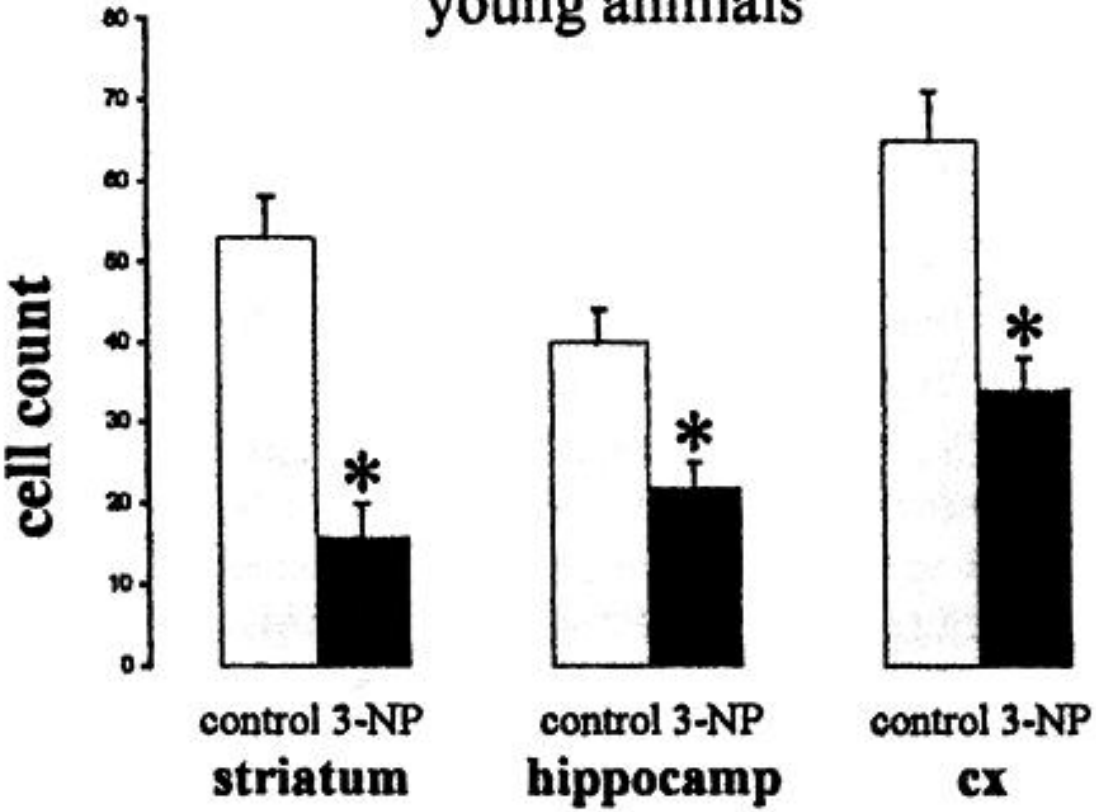

Cristae
Inner membrane
Outer compartment
Outer membrane
Matrix (inner compartment)
[A]
OUTER MEMBRANE
INNER MEMBRANE
GLYCOLYSIS
Fatty acids
Pyruvic acid
Acetyl-CoA
CITRIC-ACID CYCLE
CO_2
NADH dehydro-genase
NADH + H^+
NAD^+
$FADH_2$
FAD
Electron transport system
Electron acceptor
High H^+ concentration in outer compartment
OUTER COMPARTMENT
F_1 particle (ATP synthetase)
ADP + P_i + H_2O
ATP
Low H^+ concentration in inner compartment
MATRIX (INNER COMPARTMENT)
[B]

3-NITROPROPIONIC ACID

3-NP IS AN IRREVERISBLE INHIBITOR OF SUCCINATE DEHYDOGENASE THAT INHIBITS BOTH THE KREBS CYCLE AND COMPLEX II OF THE MITOCHONDRIAL ELECTRON TRANSPORT CHAIN.

KAT

chronic treatment
young animals



Numbers of KAT I-immunoreactive cells in the striatum, in the hippocampus (CA1) and in the temporal cortex after chronic treatment of young animals as compared to normal controls. Asterisks indicate significant alterations. Decrease of the cell count is highly significant in the striatum ($P < 0.001$), in the hippocampus ($P < 0.001$), and in the temporal cortex ($P < 0.001$).

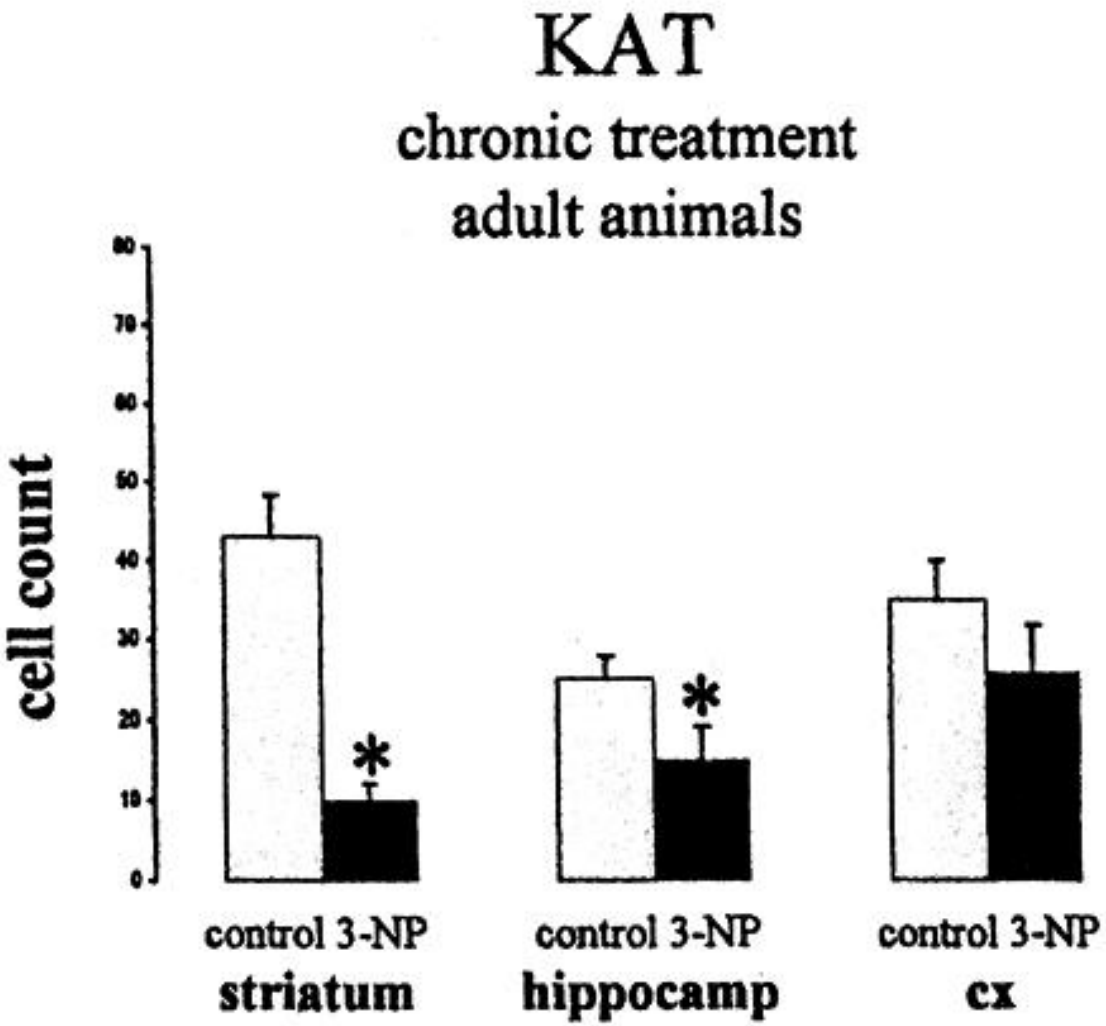


FIG. 5. Numbers of KAT I-immunoreactive cells in the striatum, in the hippocampus (CA1), and in the temporal cortex after chronic treatment of adult animals as compared to normal controls. Asterisks indicate significant alterations. Decrease of the cell count is highly significant in the striatum ($P < 0.001$) and in the hippocampus ($P < 0.001$) and significant in the temporal cortex ($P = 0.004$).

EFFECTS OF PROBENECID TREATMENTS ON KYNURENIC ACID CONCENTRATIONS IN CSF OF HUMANS

Drug free patients with either **Parkinson's disease (4)** or **Alzheimer's disease (6)** had a baseline lumbar puncture at 9 a.m. They then received probenecid (100 mg/kg) in 6 divided oral doses over 24 hours and a second lumbar puncture was performed 2 hours after the last dose of probenecid. CSF from the 5th to 8th ml was stored frozen until the time of analysis. (Dr John Growdon)

CSF LEVELS OF KYNURENIC ACID

BASELINE CONCENTRATIONS:	**3.72±0.46 nM**
PROBENECID TREATMENT:	**16.86±4.12 nM****

****=p<0.005**

the kynurenate analog SZR-72 were synthesized, which probably penetrates the blood-brain barrier according to its chemical structure

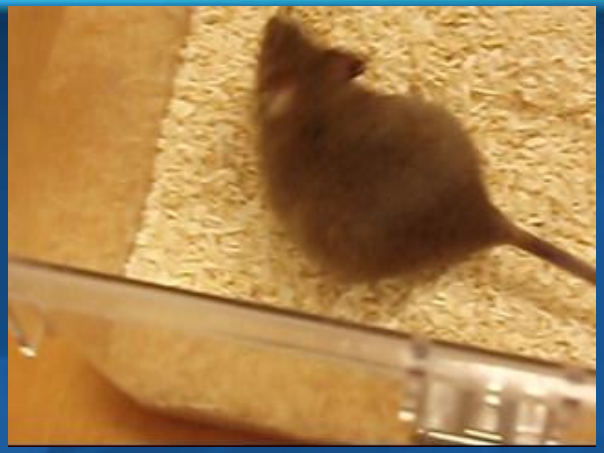
Aims

To examine:

1. whether the analog (SZR-72), or the precursor L-kynurenine could improve the survival in a transgenic mouse model of Huntington's disease
2. whether they affect the spontaneous locomotor activity of mice
3. whether the analog has any significant side effect at the applied dose
4. whether the treatments could prevent the histological alterations characteristic of the transgenic animals

Methods

- the transgenic mouse model of Huntington's disease we used: N171-82Q



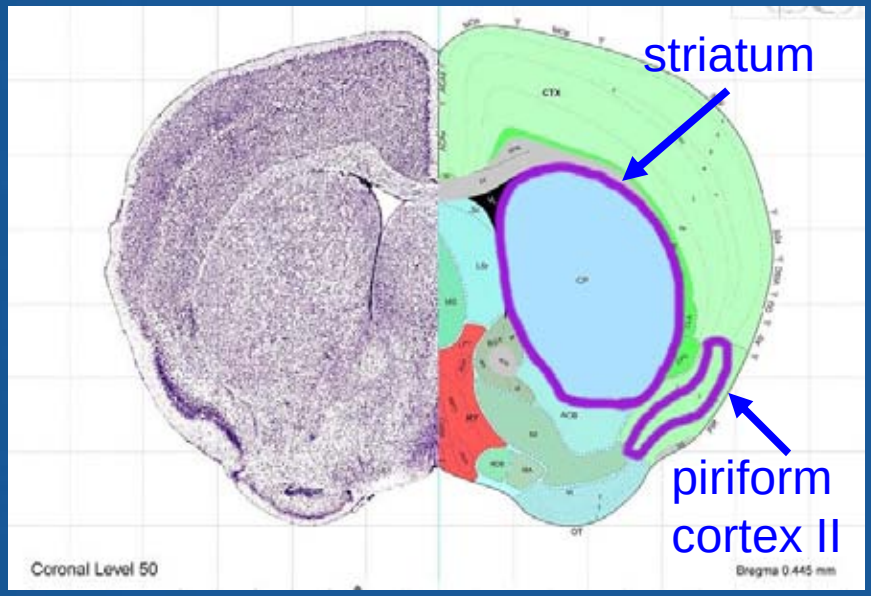
1. $n_{wt}=10$ $n_{tg}=10$ $n_{tg\text{-}kyn}=10$ $n_{tg\text{-}SZR\text{-}72}=10$
2. $n_{wt}=8$ $n_{tg}=8$ $n_{tg\text{-}kyn}=9$ $n_{tg\text{-}SZR\text{-}72}=9$
3. $n_{wt}=8$ $n_{wt\text{-}SZR\text{-}72}=8$

- intraperitoneal administration five times a week at the dose of 100 mg/kg:
  - ~ SZR-72
  - ~ L-kynurenine-sulphate
- examination of survival (1.)
- measurement of spontaneous locomotor activity with open field test (Conducta) (2. + 3.)

Methods

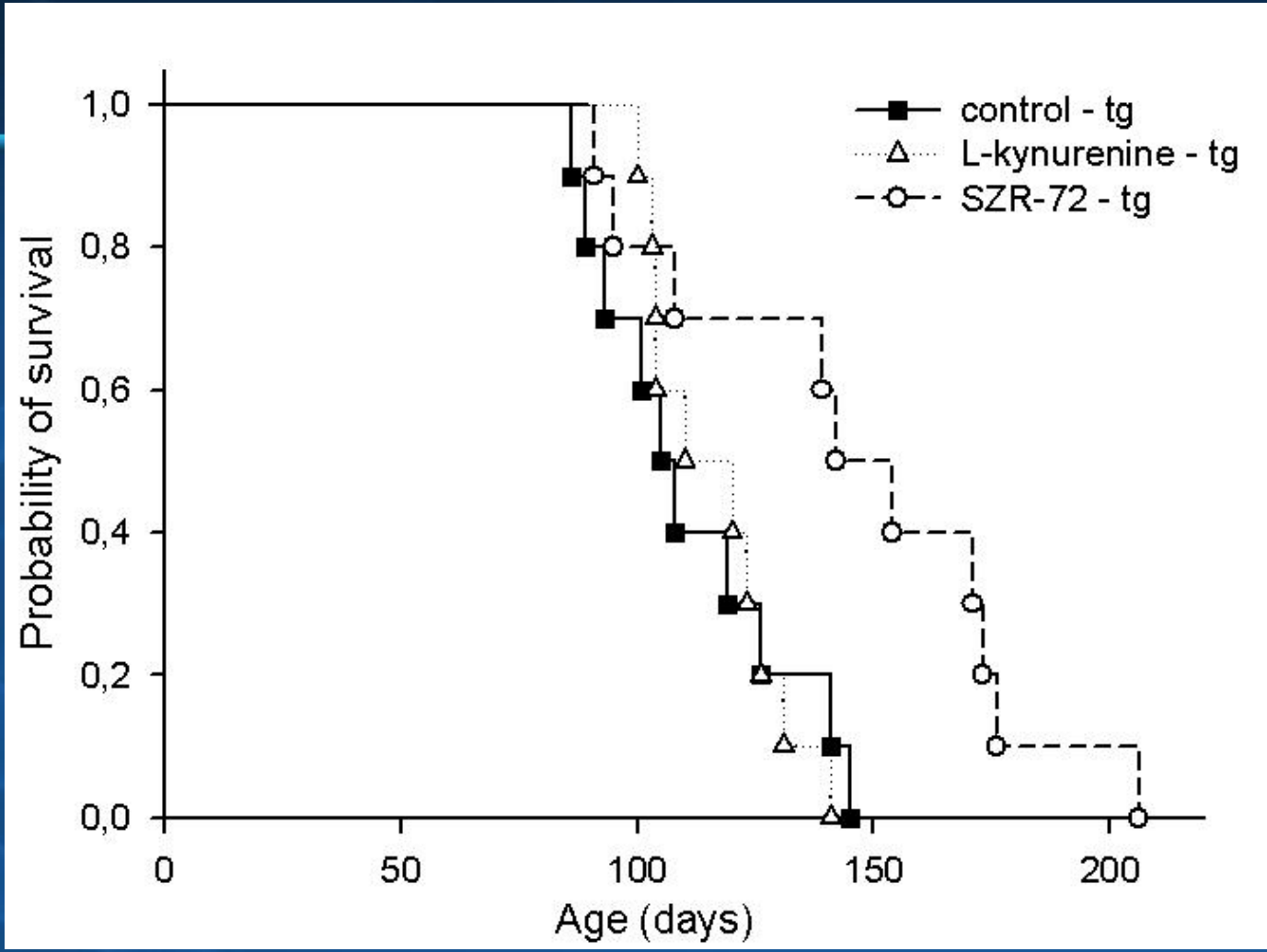
- elevated plus maze (3.)
- tail suspension test (3.)
- Porsolt's forced swimming test (3.)
- stereotypy rating scale (3.)

- histology: (2.; n=4 in all groups)
  - cresyl violet staining
    - ~ the number of neurons in the dorsomedial aspect of the striatum
    - ~ the mean area of striata in the examined section planes
  - immunohistochemistry
    - using the EM48 antibody to stain neurons containing huntingtin aggregates
    - ~ the number of EM48^{+} neurons in the second layer of the piriform cortex and in the striatum



Allen Brain Atlas: Mouse Brain

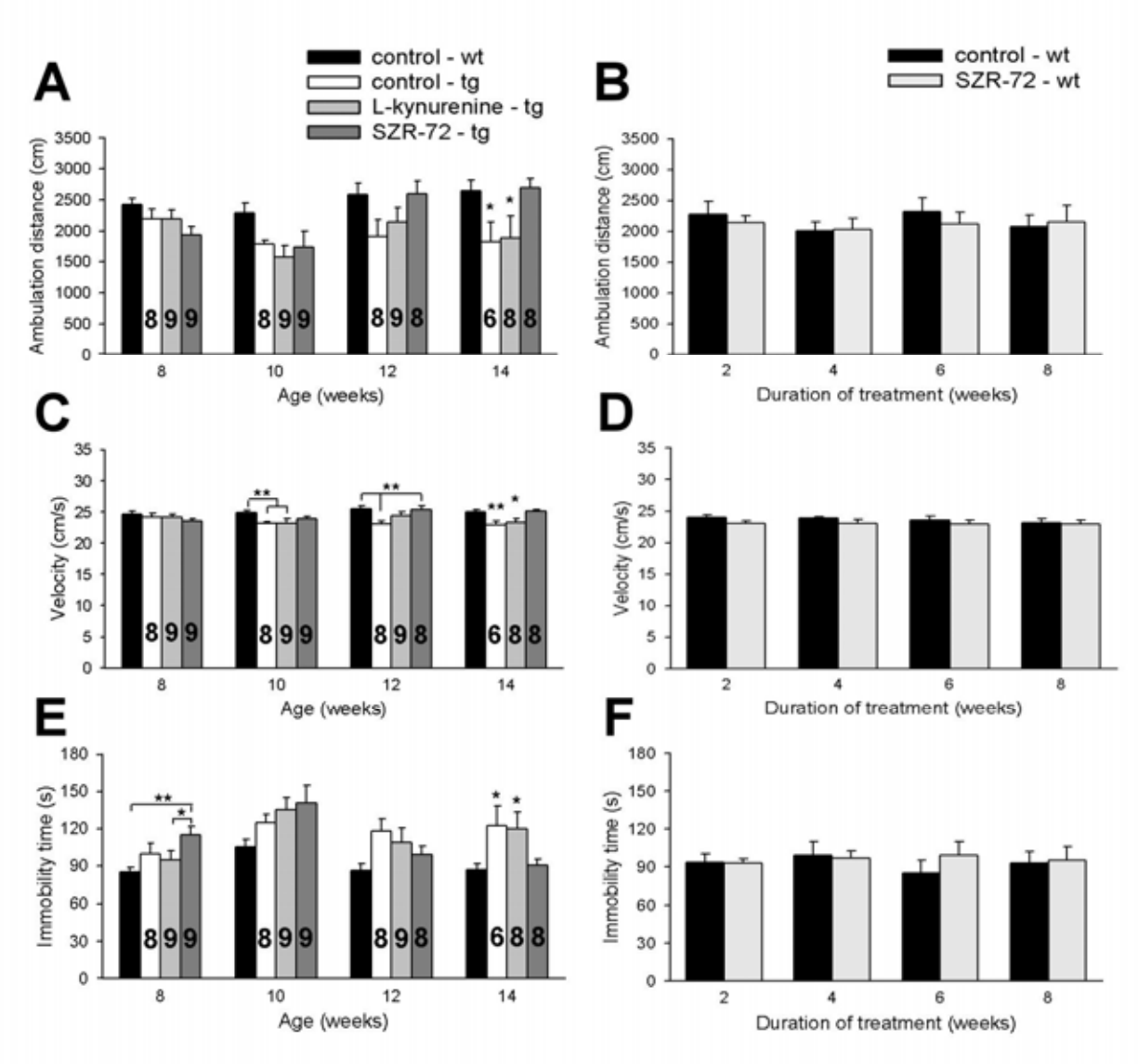
Survival



The treatment with SZR-72 significantly increased the survival of N171-82Q transgenic mice compared to the control group.

(tg: transgenic; $p < 0,05$; Mantel-Cox log rank test)

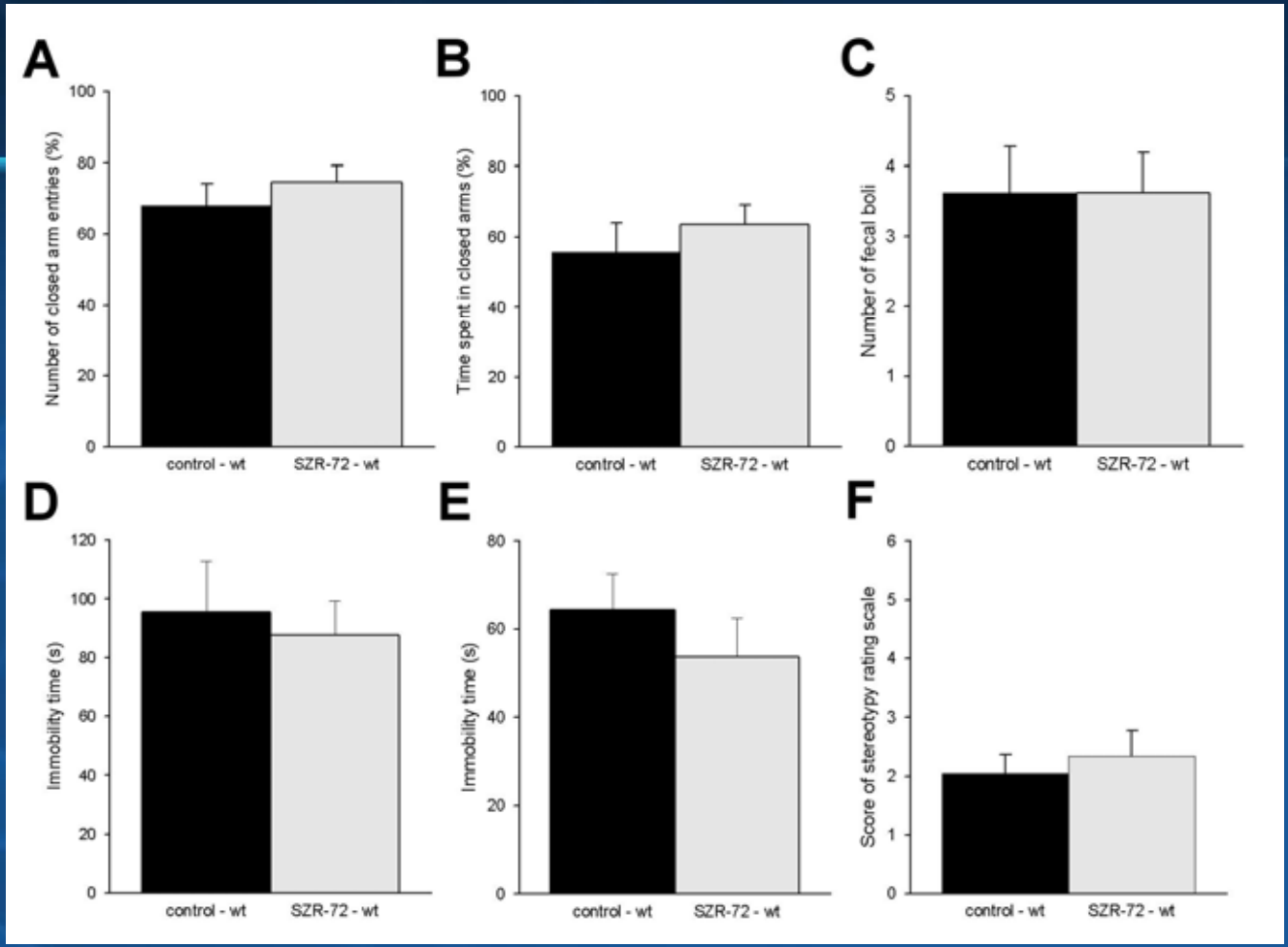
Open field test



The treatment with SZR-72 significantly ameliorated the spontaneous locomotor activity of transgenic mice, while it did not exert any effect in wild type mice according to this parameter.

(tg: transgenic; wt: wild type; data are presented as mean + S.E.M.; *p < 0,05; **p < 0,01, one-way ANOVA, Fisher's LSD post hoc test)

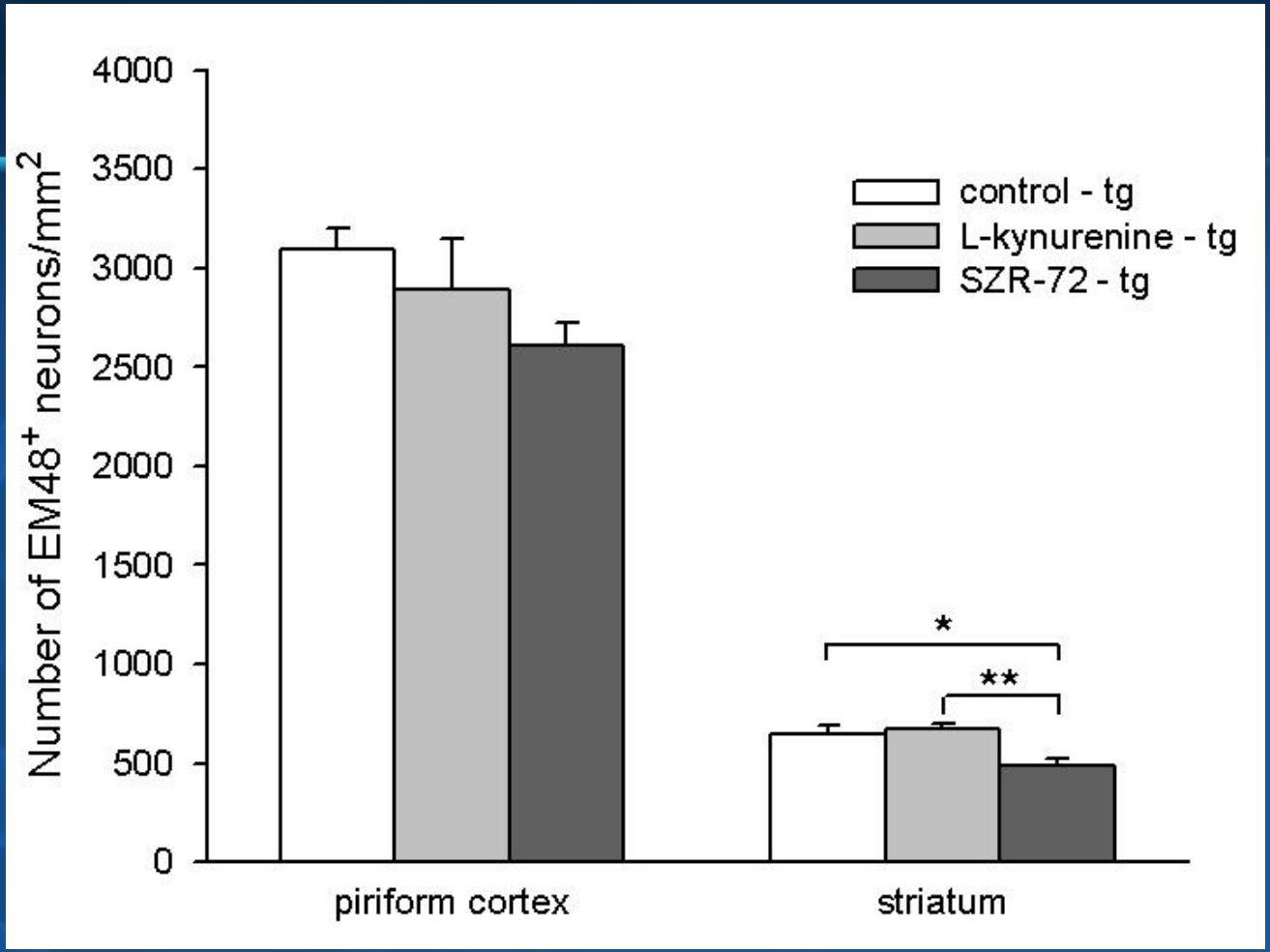
Testing side effects



Neither the elevated plus maze test (A-C), nor the tail suspension test (D), nor the Porsolt's forced swimming test (E) and nor the stereotypy rating scale (F) showed any significant difference between the two groups.

(wt: wild type; data are presented as mean + S.E.M.)

Immunohistochemistry



The treatment with SZR-72 significantly decreased the number of $EM48^+$ neurons in the striatum.

(tg: transgenic; presented parameter: mean + S.E.M.; *p < 0,05; **p < 0,01; one-way ANOVA, Fisher's LSD post hoc test)

Conclusions

- The treatment with SZR-72 significantly increased the survival of N171-82Q transgenic mice and ameliorated their spontaneous locomotor activity
- There was not any significant side effect in the used tests at the applied dose.
- The results of survival and open field test was confirmed by histology, namely the compound prevented the diminution in the number of neurons in the striatum and reduced the number of immunoreactive cells containing huntingtin aggregates.
- L-kynurenine administered at the same dose as the analog was effective in neither applied model.

Further aims

- verification of the penetration of the analog through the blood-brain barrier
- exploring the mode of action

Kynurenines in the CNS: recent advances and new questions

László Vécsei[1,2], Levente Szalárdy[1], Ferenc Fülöp[3] and József Toldi[2,4]

Abstract | Various pathologies of the central nervous system (CNS) are accompanied by alterations in tryptophan metabolism. The main metabolic route of tryptophan degradation is the kynurenine pathway; its metabolites are responsible for a broad spectrum of effects, including the endogenous regulation of neuronal excitability and the initiation of immune tolerance. This Review highlights the involvement of the kynurenine system in the pathology of neurodegenerative disorders, pain syndromes and autoimmune diseases through a detailed discussion of its potential implications in Huntington's disease, migraine and multiple sclerosis. The most effective preclinical drug candidates are discussed and attention is paid to currently under-investigated roles of the kynurenine pathway in the CNS, where modulation of kynurenine metabolism might be of therapeutic value.

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doi:10.1038/nrd3793
Published online 14 December 2012

Apart from being one of the 20 amino acids that constitute proteins, tryptophan is also a precursor for the synthesis of serotonin and L-kynurenine under physiological conditions. L-kynurenine is an intermediate metabolite of the complex metabolic pathway that ends with NAD^+, kynurenic acid and xanthurenic acid. More than 95% of tryptophan is metabolized through the kynurenine pathway[1].

Most metabolites of the kynurenine pathway are neuroactive and have essential roles in the regulation of NMDA (*N*-methyl-D-aspartate) receptor function and free radical production. NMDA receptor-mediated excitotoxicity and excessive free radical production are involved in neurodegenerative disorders such as Huntington's disease, Parkinson's disease and Alzheimer's disease. Evidence suggests that kynurenine metabolism is altered in such diseases, and the possible therapeutic potential of the pharmacological modulation of this pathway is currently being investigated in preclinical studies.

Glutamatergic neurotransmission is essential for spinal and trigeminal pain processing. Kynurenic acid, an end-product of the kynurenine pathway, acts through several mechanisms to elicit antiglutamatergic actions. As we discuss below, elevating the levels of kynurenic acid therefore offers a possible therapeutic approach in pain syndromes. A prodrug molecule has already reached the stage of clinical investigation for the treatment of neuropathic pain. However, the potential of such approaches has not yet been sufficiently investigated in migraine.

Evidence suggests that kynurenine metabolites have a role in mediating immunological tolerance. Activation of the kynurenine pathway — most probably as a compensatory response — has been detected in various autoimmune diseases, and experimental and indirect evidence indicates that the kynurenine pathway is also overactivated in multiple sclerosis. As most of the immunotolerogenic metabolites of this pathway have neurotoxic properties, it is not exactly clear how activation of the kynurenine pathway is involved in the pathogenesis of multiple sclerosis, and the potential therapeutic implications of pharmacological manipulations related to the kynurenine pathway need to be comprehensively investigated.

This Review highlights the physiological and pathological implications of kynurenine pathway activity in the central nervous system (CNS). The involvement of the kynurenine system in the pathology of neurodegenerative disorders, pain syndromes and autoimmune diseases is surveyed through a detailed discussion of its participation in Huntington's disease, migraine and multiple sclerosis. We discuss the main types of conceptual approaches and the most effective candidates in preclinical drug discovery, with an emphasis on pathological situations in which kynurenergic manipulations might be of therapeutic value.

The kynurenine pathway in the brain

Tryptophan is transported across the blood–brain barrier (BBB) with the aid of the large neutral amino acid transporter[2]. Within the brain, the metabolism of tryptophan proceeds via the serotonin pathway and the kynurenine pathway. Upon entering the kynurenine pathway, tryptophan is converted to *N*-formyl-L-kynurenine by

This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4./2-11-1-2012-0001 "National Excellence Program" and TÁMOP-4.2.2.A.11-/1/KONV-2012-0052.

Thank you for your kind attention!

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