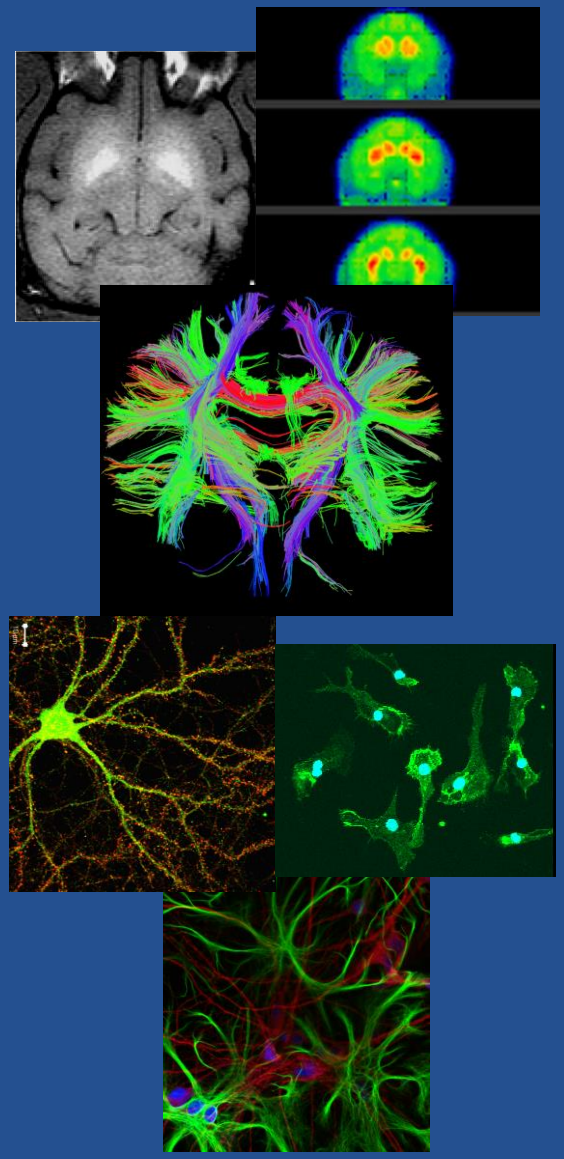

Approaches to Studying Neurotoxic Effects of Environmental Pollutants: Manganese – A Case Study

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Manganese Neurotoxicity

Manganese (Mn) is an essential nutrient but excess levels in the brain produces a complex neurological syndrome with expression of psychiatric symptoms, cognitive impairment and parkinsonism. [Perl and Olanow, 2007; Guilarte, 2010]

Occupational and environmental exposures to Mn cause problems of attention and cognitive impairment as well as increased expression of neuropsychological symptoms. [Josephs et al., 2006; Bouchard et al., 2006; Bowler et al., 2006; Kloes et al., 2006]

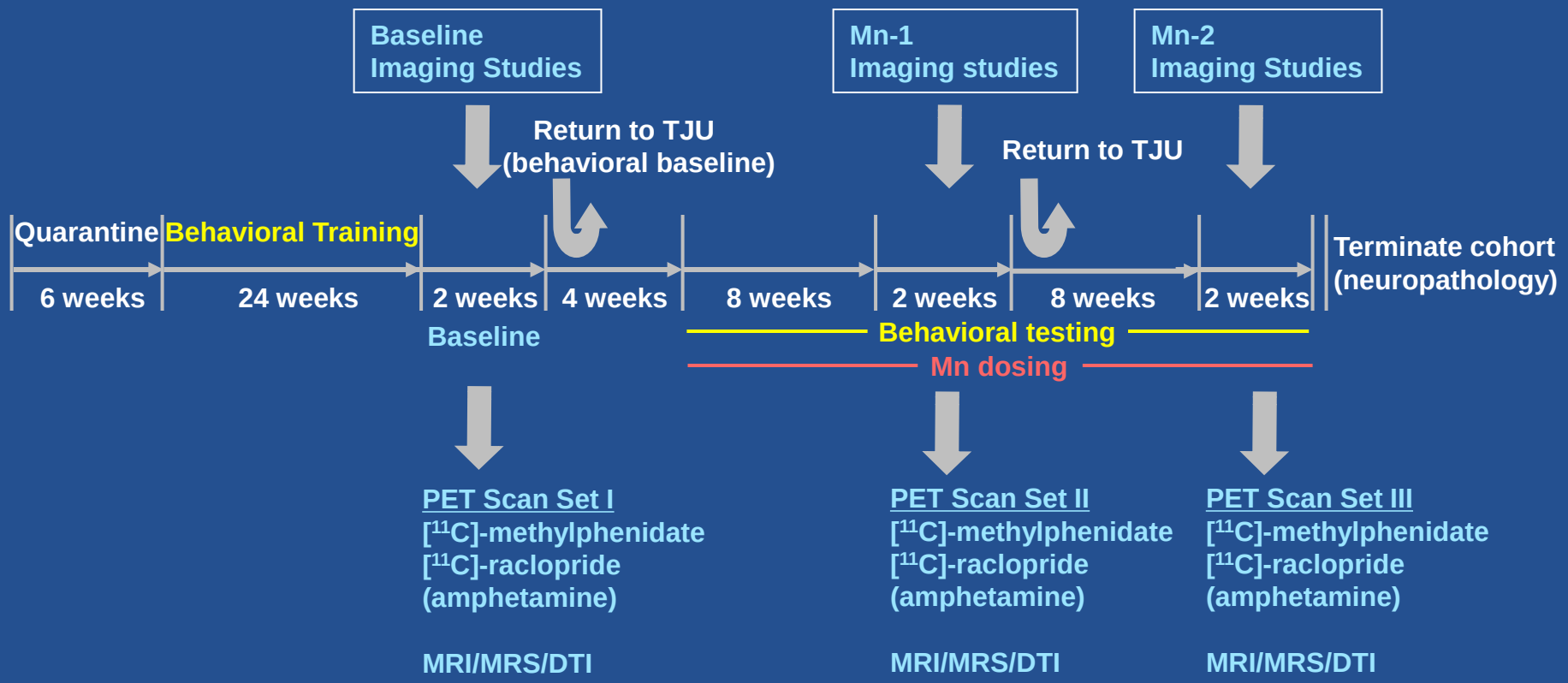
An association between environmental exposure to Mn and deficits in measures of intellectual function has been described in children. [Takser et al., 2003; Wasserman et al., 2006]

Recent studies also show that chronic Mn exposure in non-human primates may be associated with early signs of Alzheimer's disease-like pathology and neurodegeneration in the cerebral cortex [Guilarte et al., 2008]

Sources of Human Mn Exposure

- Environmental: water (>300 µg/L): automobile combustion of gasoline containing the additive MMT (methylcyclopentadienyl manganese tricarbonyl).
- Occupational: smelting industry, mining, welding (inhalation)
- Other human conditions in which Mn concentrations increase in the brain: liver disease, parenteral nutrition
- Drug users: Ephedrine-based illicit psychostimulant drugs [Russian Cocktail]. Blood [Mn] ranging from 2100-3200 µg/L. Normal: 5-15 µg/L.
- Inherited Genetic mutation- *SLC30A10* loss of function; Mn transporter

Experimental paradigm for manganese studies in non-human primates: Integration of behavioral, neuroimaging and neuropathological studies



Research naïve, male, 5-6 years of age *Cynomolgus macaques*

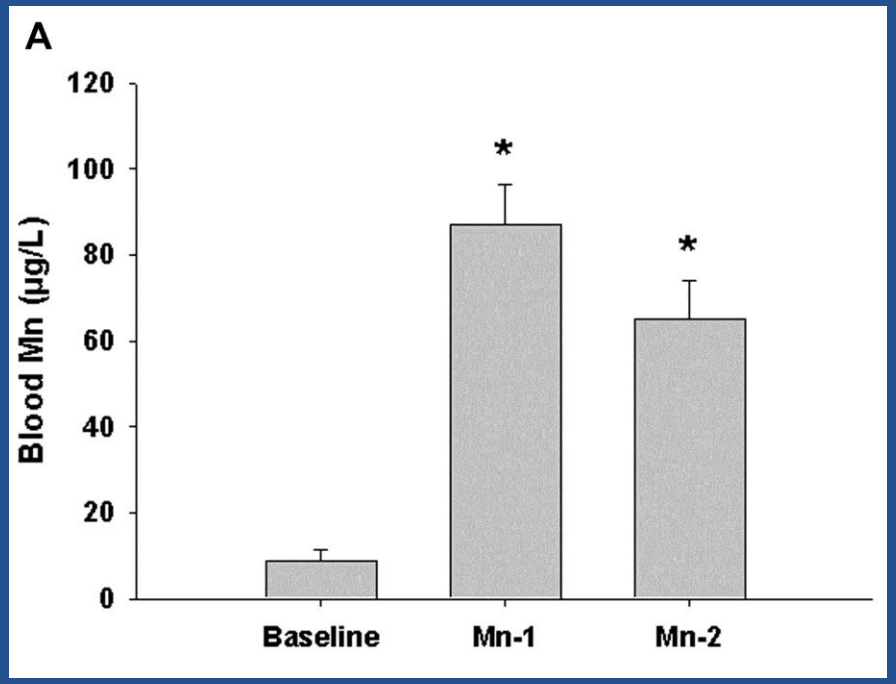
Exposure Paradigm and Animals

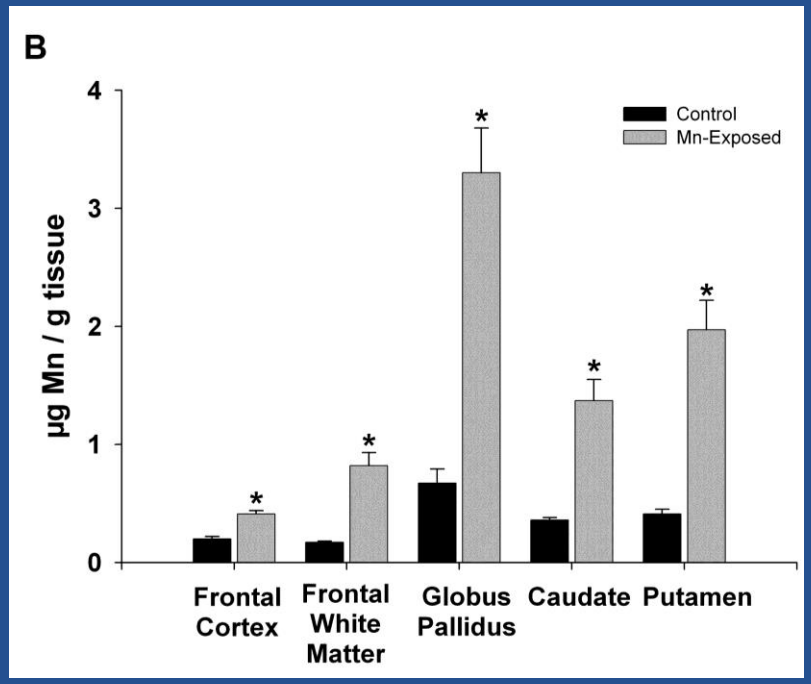
Animal ID	Dose Level ($MnSO_4$)	Dose Level (Mn)	Dosing Interval	Exposure Duration (weeks)	Cumulative $MnSO_4$	Cumulative Mn
75W	10-15 mg/kg	3.3-5.0 mg/kg	1/wk	44	455mg/kg	151.7mg/kg
144T	10-15 mg/kg	3.3-5.0 mg/kg	1/wk	50	515mg/kg	171.7mg/kg
107-705	10-15 mg/kg	3.3-5.0 mg/kg	1/wk	42	500mg/kg	166.7mg/kg
3154	10-15 mg/kg	3.3-5.0 mg/kg	1/wk	45	515mg/kg	173.8mg/kg
3114	15-20 mg/kg	5.0-6.7 mg/kg	1/wk	46	635mg/kg	206.4mg/kg
7782	15-20 mg/kg	5.0-6.7 mg/kg	2/wk*	27	435mg/kg	141.4mg/kg
9093	15-20 mg/kg	5.0-6.7 mg/kg	2/wk*	59	770mg/kg	250.8mg/kg
7469	15-20 mg/kg	5.0-6.7 mg/kg	2/wk*	32	525mg/kg	170.7mg/kg
00-8001	15-20 mg/kg	5.0-6.7 mg/kg	2/wk*	34	535mg/kg	173.9mg/kg
001-1099	15-20 mg/kg	5.0-6.7 mg/kg	2/wk*	32	535mg/kg	173.9mg/kg
7839	25-30mg/kg	8.3-10.0 mg/kg	2/wk*	38	640mg/kg	218.3mg/kg
6697	25-30mg/kg	8.3-10.0 mg/kg	Bolus, 2/wk*	7	206mg/kg	68.3mg/kg
7426	25-30mg/kg	8.3-10.0 mg/kg	Bolus, 2/wk*	15	340mg/kg	113.3mg/kg

Importantly, we included two different control groups:

1) Naïve controls: No Mn exposure or behavioral/imaging studies
2) Imaged controls: No Mn exposure but experienced behavioral/imaging studies

Blood and Brain Manganese Concentrations





Blood Mn in non-occupationally exposed populations

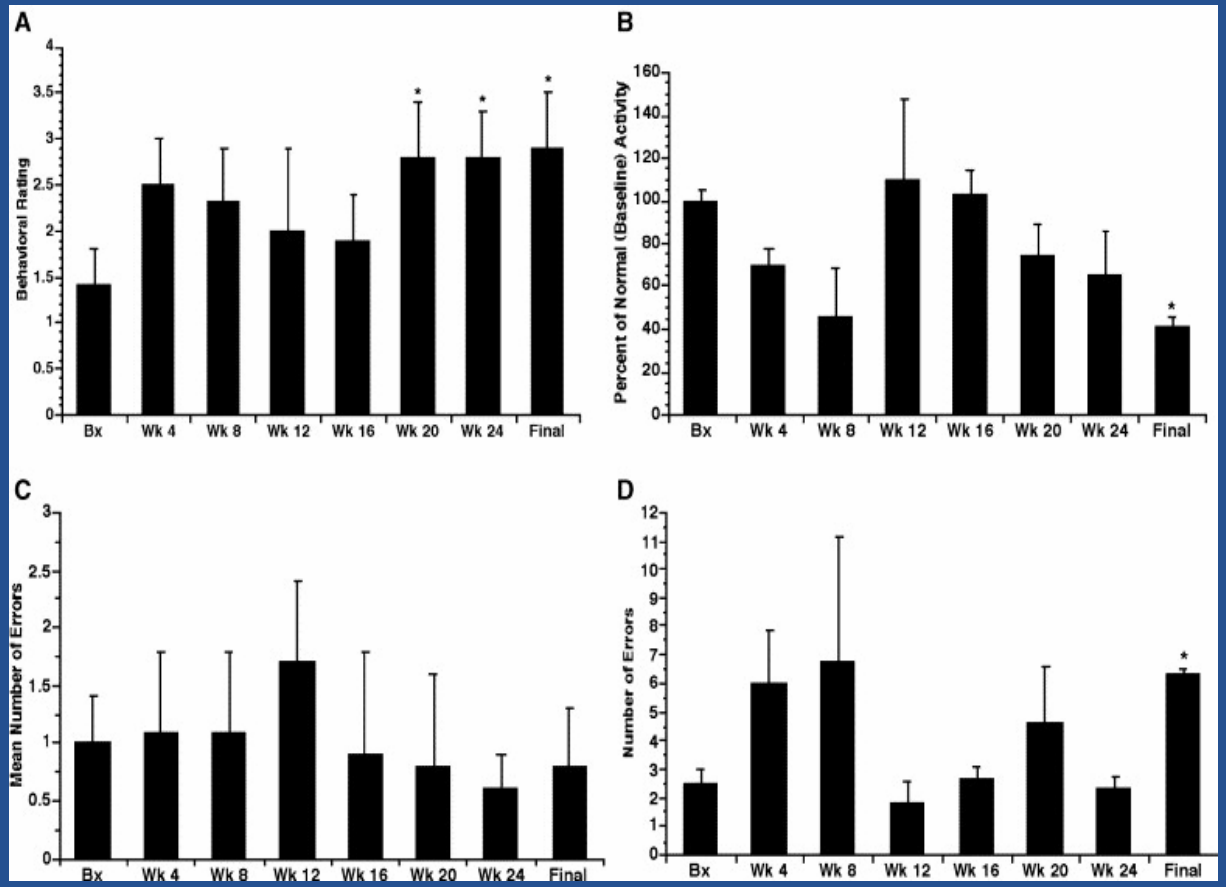
	Blood Mn Levels (µg/L)				
Age (yrs)	**Mean**	**Minimum Value**	**Maximum Value**	***N***	**Reference**
0.29-2.4	12.3	1.8	45.0	254	children in Australia *Gulson et al. (2006)*
7.1	9.8	3.6	26.5	384	school children *Rollin et al. (2005)*
6.7	6.74	1.6	32.8	430	school children *Rollin et al. (2005)*
29 ± 4 (mothers)	20.4	6.3	151.2	222	general population *Takser et al. (2003)*
Newborns	38.5	14.9	92.9	222	cord blood in newborns *Takser et al. (2003)*
15-93	18.3	10	88	46	general population *Santos-Burgoa et al. (2001)*
14-75	16.8	7.5	45	27	general population *Santos-Burgoa et al. (2001)*
3.4-20.8	31.7	18	51	7	children on parenteral nutrition *Iinuma et al. (2003)*

"Behavioral Studies"

Motor Function Tests

Working Memory

Summary of motor function findings



A: parkinsonian rating scale

B: overall activity levels

C: test of fine motor skill-"easy"

D: test of fine motor skill-"difficult"

Schneider et al., Brain Res, 2006

BRAIN RESEARCH 1258 (2009) 86–95

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BRAIN RESEARCH

Research Report

Effects of chronic manganese exposure on working memory in non-human primates

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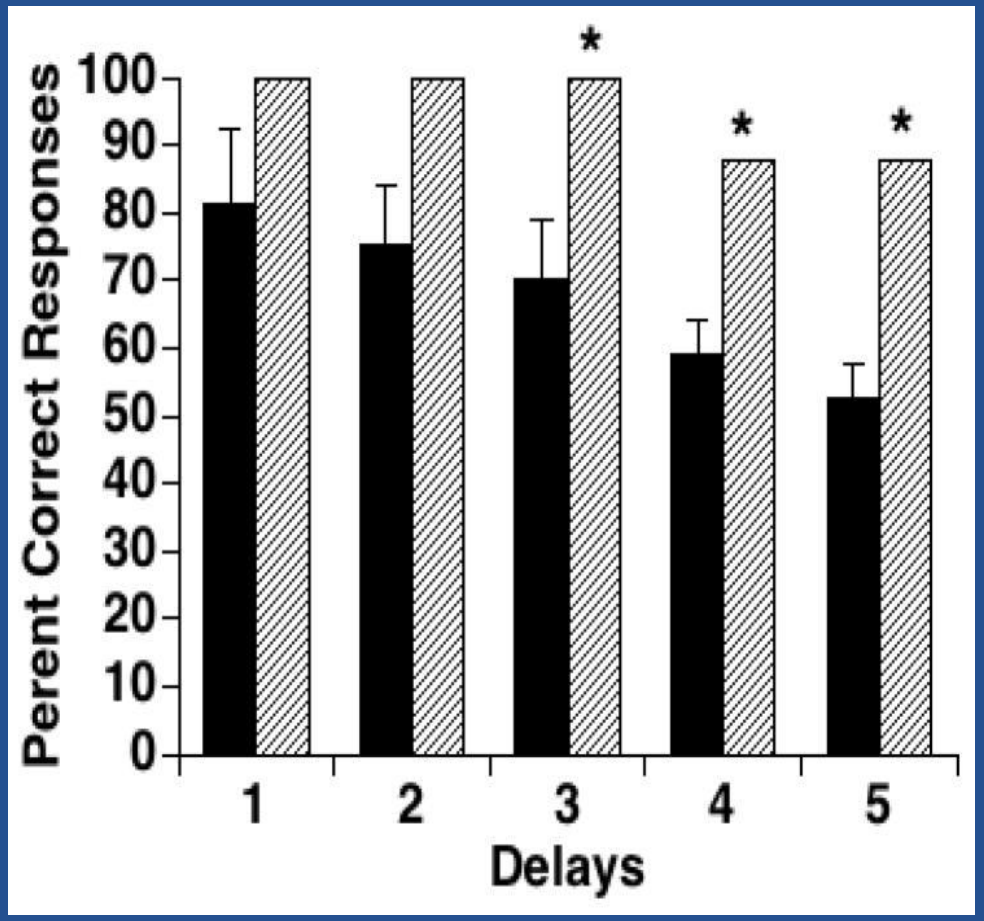
Variable Delayed Response Performance [spatial working memory]



Mn animals show impairment at intermediate to long delays

1= 2 sec
2= 5 sec
3= 10 sec
4= 30 sec
5= 50 sec

Hatched Bar: Control
Black Bar: Mn-exposed

Delays: From 2-60 sec

Summary of Results

These studies show that chronic Mn exposure in non-human primates produces deficits in fine motor control and reduces overall activity levels.

Further, there are significant deficits in spatial working memory in Mn-exposed animals relative to controls. These findings point to Mn effects on cerebral cortical structures, specifically the frontal cortex.

"Neuroimaging Studies"

T1-weighted MRI

(Mn is paramagnetic)

Positron Emission Tomography

Molecular Imaging Modalities: clinical & preclinical studies

Magnetic resonance imaging (MRI)

Magnetic resonance spectroscopy (MRS)

Positron emission tomography (PET)

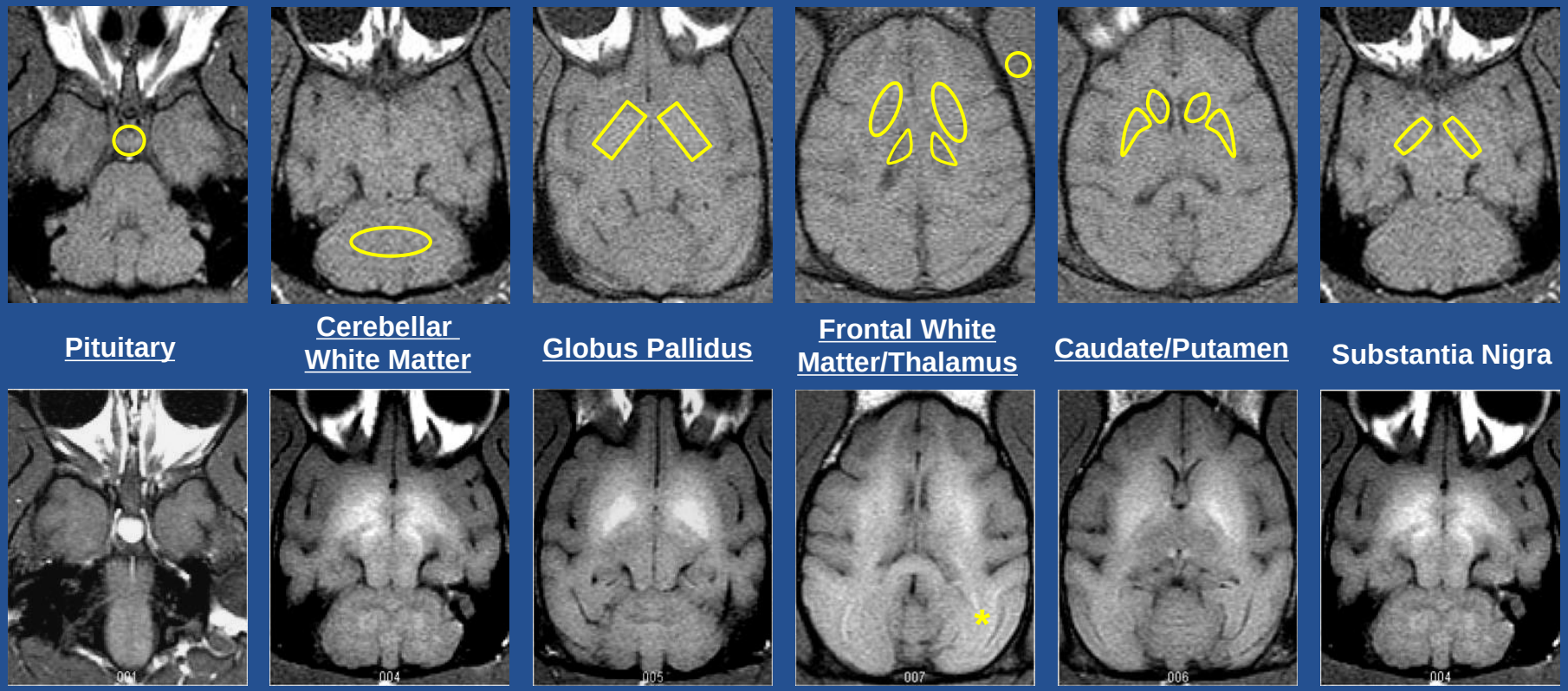
Single photon emission computed tomography (SPECT)

Functional MRI

Ultrasound

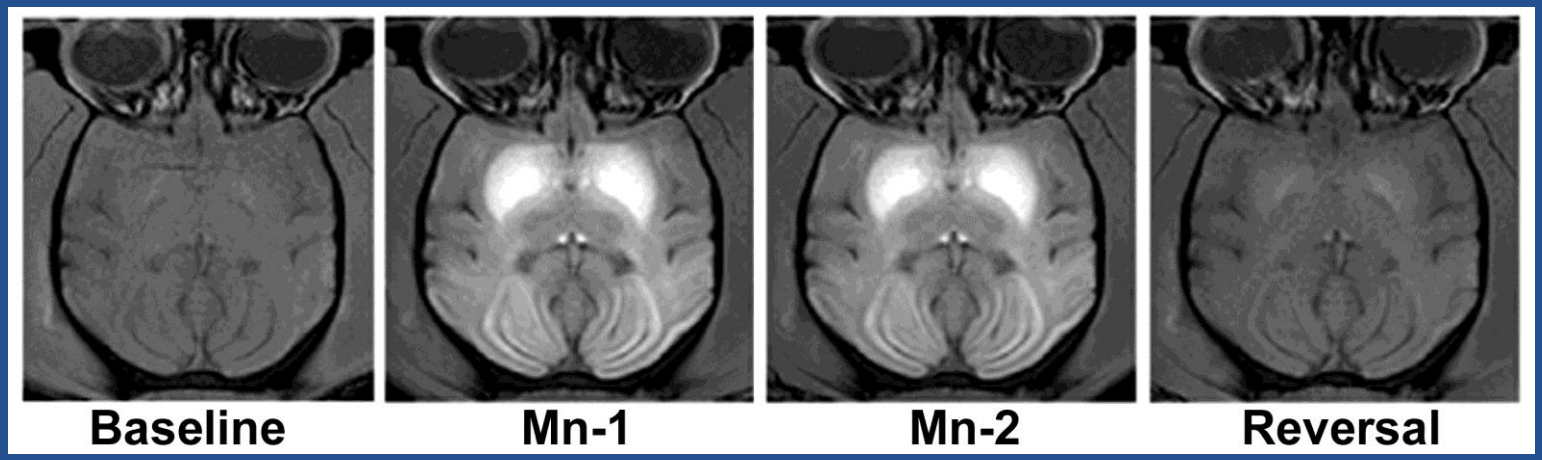
Optical imaging

T1w-MRI of monkey brain before and during manganese exposure (same animal)



Guilarte et al., Tox. Sci., 2006

Longitudinal Analysis of Manganese Levels in the Brain



Baseline	Mn-1	Mn-2	Reversal
Blood Mn= 8.9 ug/L	Blood Mn= 132 ug/L	Blood Mn= 121 ug/L	Blood Mn= 33 ug/L
No Mn	35 weeks of Mn	66 weeks of Mn	40 weeks after no Mn
T1 RT= 826 sec	T1 RT= 469 sec	T1 RT= 476 sec	T1 RT= 837 sec

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Advance Access publication April 25, 2006

Correlation of Brain Magnetic Resonance Imaging Changes with Pallidal Manganese Concentrations in Rhesus Monkeys Following Subchronic Manganese Inhalation

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Received November 23, 2005; accepted April 3, 2006

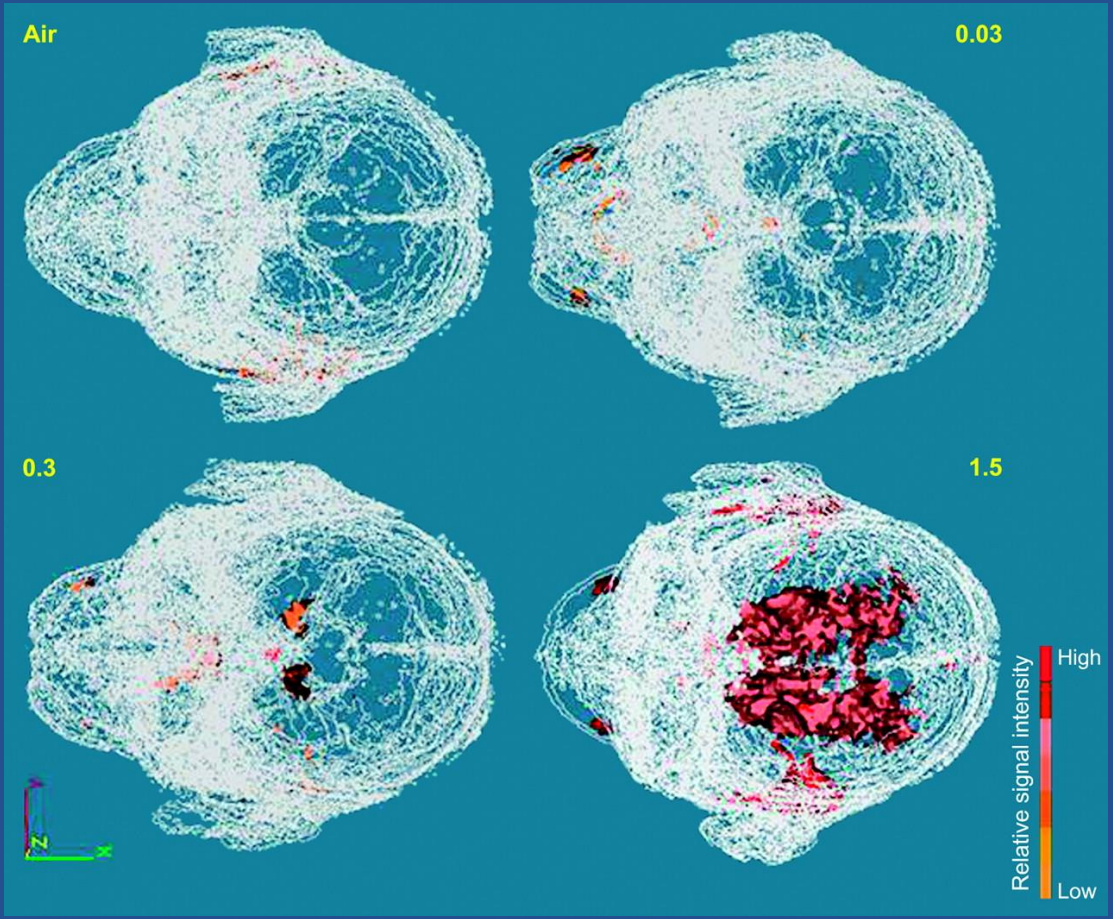
Tissue Mn Concentrations (ug Mn/g tissue wet weight) in Young Monkeys Following Subchronic Exposure to Either Air or $MnSO_4$

		Nominal $MnSO_4$ concentration (mg Mn/m^3)		
	Air	**0.06**	**0.3**	**1.5**
Olfactory epithelium[a]	0.42 ± 0.01	1.22 ± 0.15*	2.96 ± 0.46*	7.10 ± 2.01*
Olfactory bulb	0.31 ± 0.01	0.77 ± 0.04*	1.36 ± 0.15*	2.40 ± 0.18*
Olfactory tract	0.30 ± 0.06	0.43 ± 0.02	0.61 ± 0.05*	1.12 ± 0.08*
Olfactory cortex	0.19 ± 0.004	0.27 ± 0.02*	0.31 ± 0.01*	0.42 ± 0.01*
Globus pallidus[a]	0.48 ± 0.04	0.80 ± 0.04*	1.28 ± 0.15*	2.94 ± 0.23*
Putamen	0.36 ± 0.01	0.58 ± 0.04*	0.75 ± 0.05*	1.81 ± 0.14*
Caudate	0.34 ± 0.02	0.47 ± 0.04	0.69 ± 0.03*	1.72 ± 0.10*
White matter	0.17 ± 0.01	0.25 ± 0.01*	0.39 ± 0.04*	0.87 ± 0.08*
Frontal cortex	0.25 ± 0.03	0.29 ± 0.02	0.29 ± 0.01	0.47 ± 0.02*
Cerebellum[a]	0.44 ± 0.01	0.62 ± 0.02*	0.70 ± 0.04*	1.10 ± 0.11*
Pituitary	0.84 ± 0.12	1.53 ± 0.25	2.43 ± 0.13*	6.19 ± 0.61*
Blood	0.010 ± 0.001	0.015 ± 0.002	0.022 ± 0.003*	0.026 ± 0.003*
Group size (*n*)	6	6	4	4

Exposure: 6 hrs/day; 5 days/week for 13 weeks (65 exposure days)
[8hr-threshold limit value for inhaled Mn is 0.2 mg/m^3]

Dorman et al., *Toxicological Sciences*, 2006

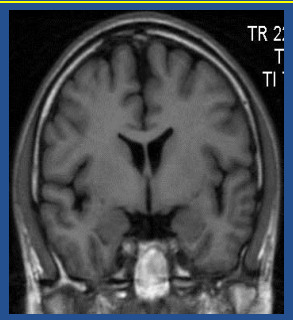
Three-dimensional Reconstruction of MRI Images from Monkeys Exposed Subchronically to Either Air or $MnSO_4$



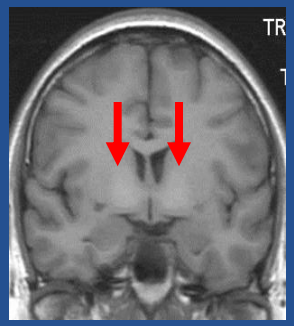
Exposure: 6 hrs/day; 5 days/week for 13 weeks (65 exposure days)

Dorman et al., *Toxicological Sciences*, 2006

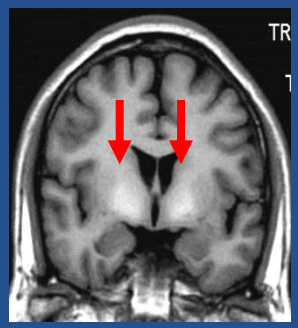
T1W-MRI –occupational exposures (inhalation)



Control T1-weighted MRI in a worker with no Mn exposure history (0.01 mgMn/m^3).



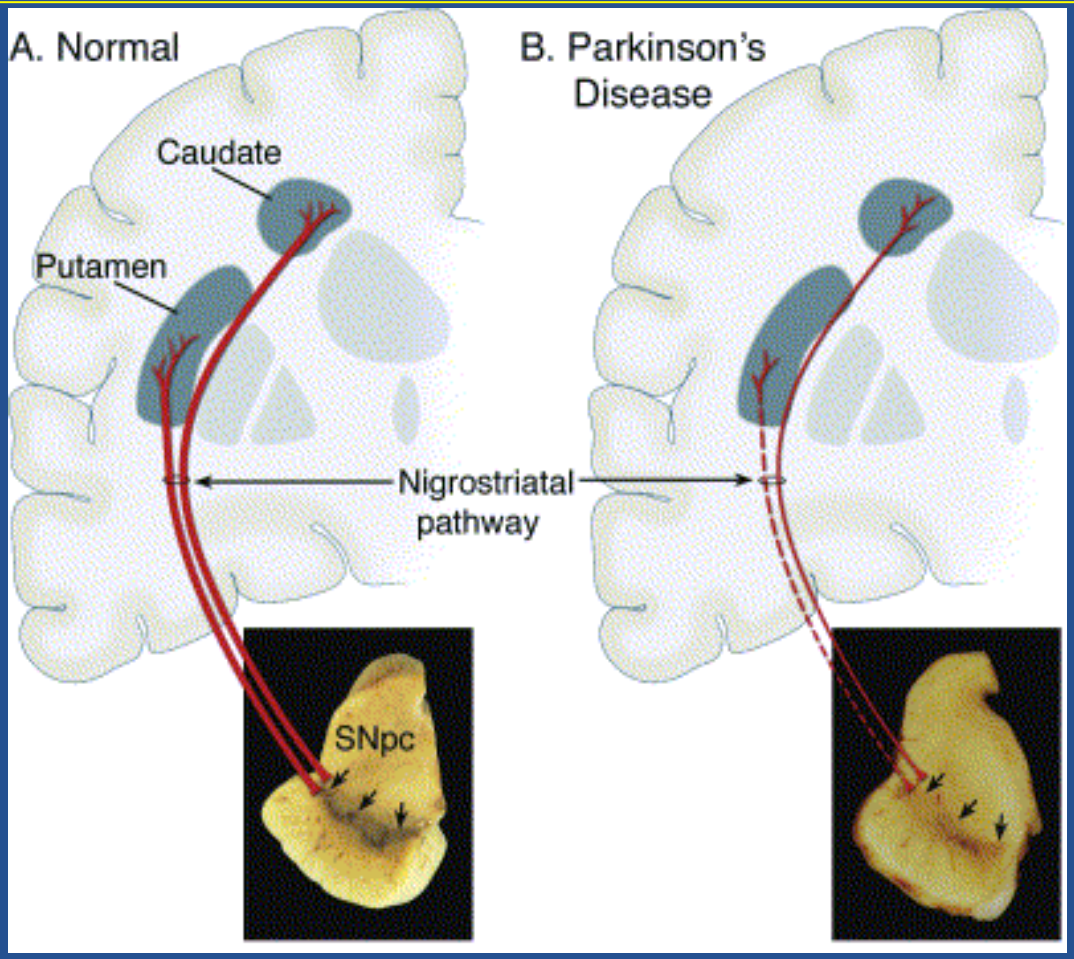
Increased T1-weighted MRI in a power distribution and monitoring worker exposed to low levels of airborne Mn (0.66 mg Mn/m^3).

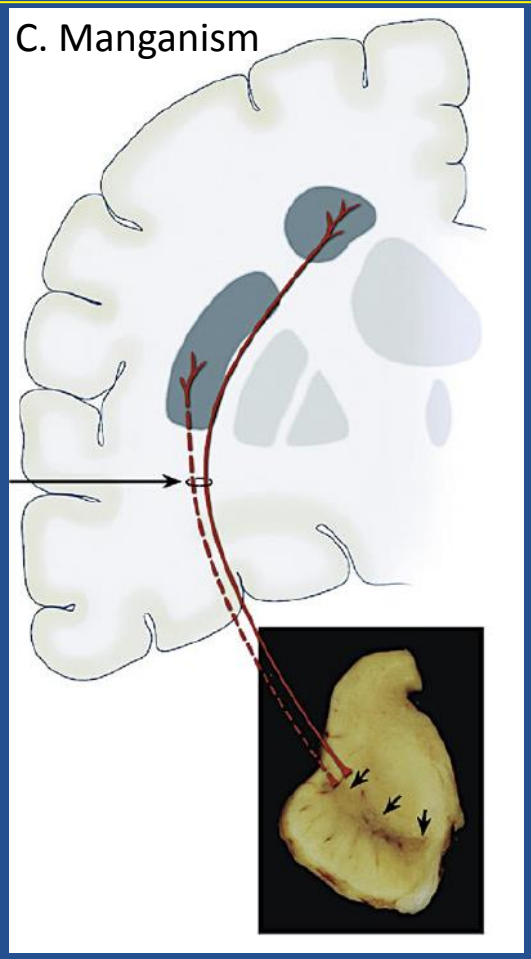


Highly increased T1-weighted MRI in a smelting worker exposed to high levels of airborne Mn (1.26 mg Mn/m^3).

Jiang et al. (2005) *Neurotoxicology* 28:126-135

Nigrostriatal Dopaminergic System in Mn-induced parkinsonism



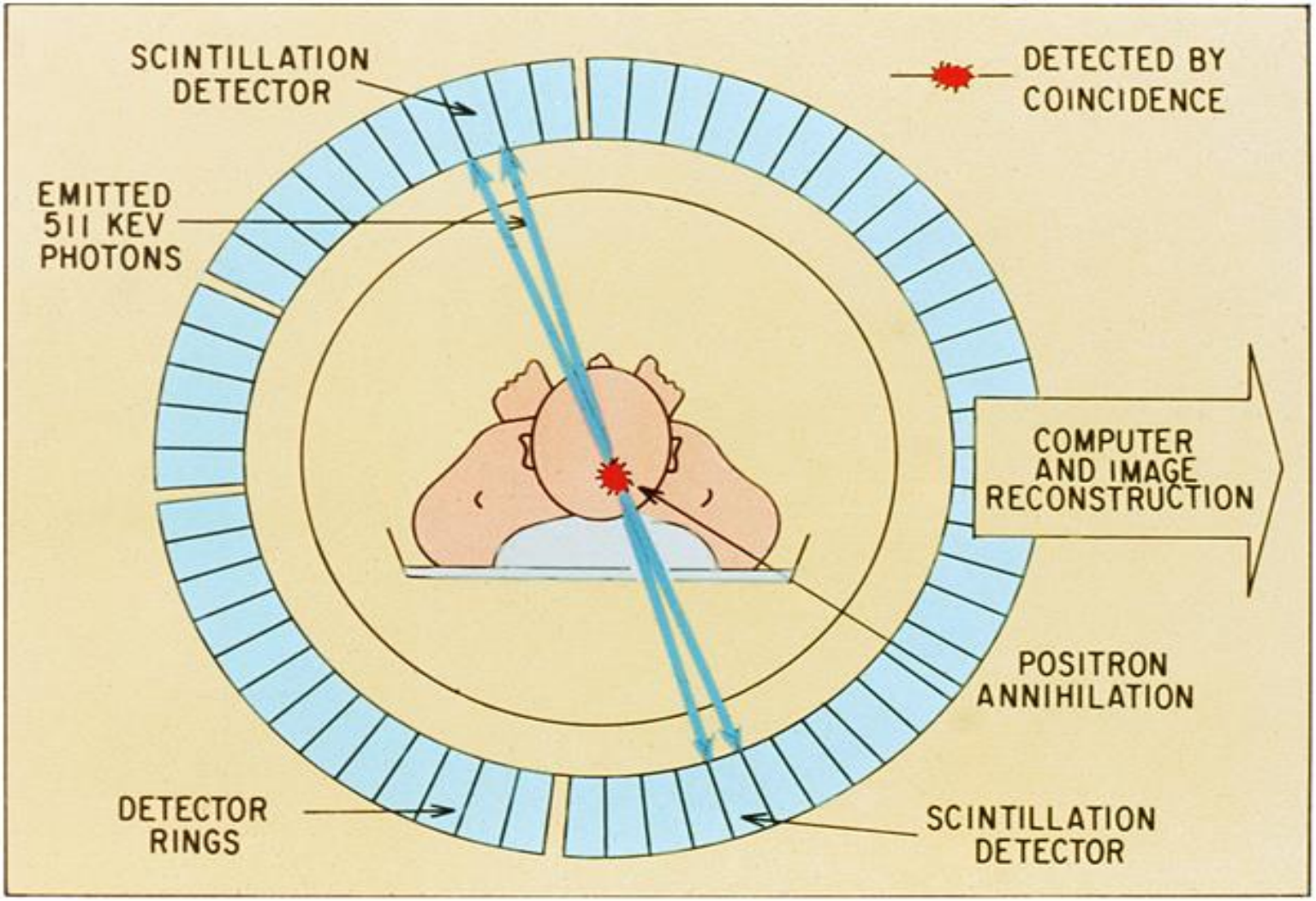


Normal

Degeneration

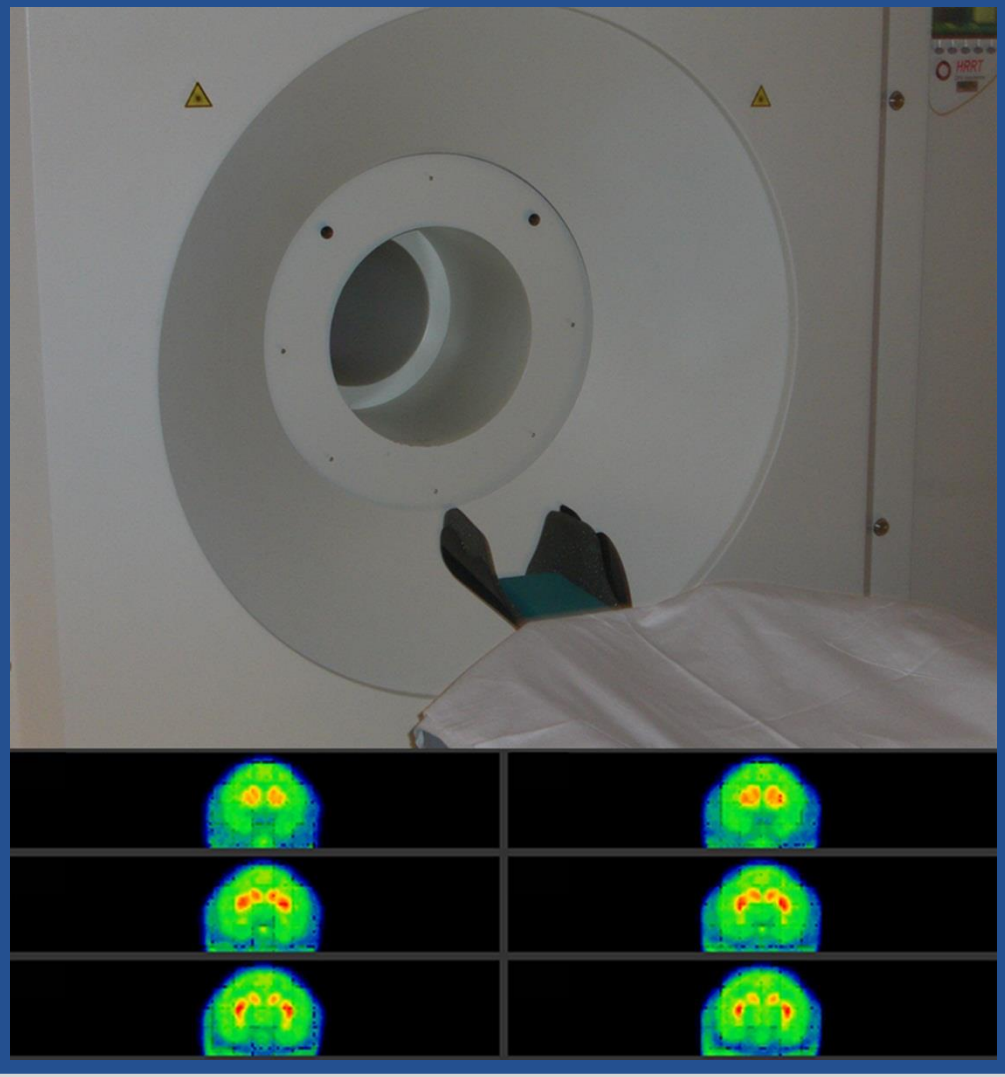
Degeneration or Dysfunction?

Positron Emission Tomography

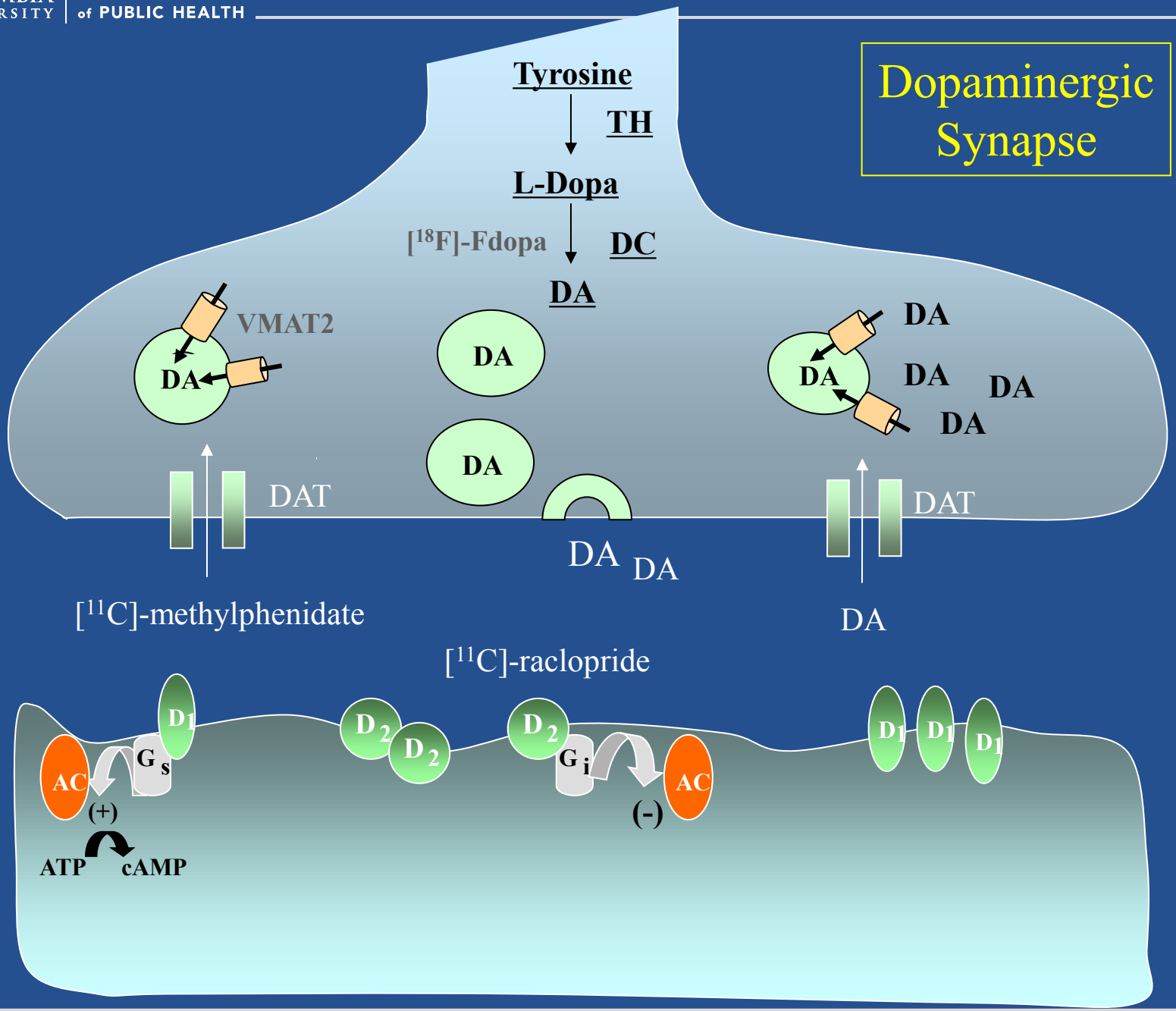


HRRT-PET scanner: High Resolution Head-Only Research Tomograph

Spatial resolution= 2.2 mm

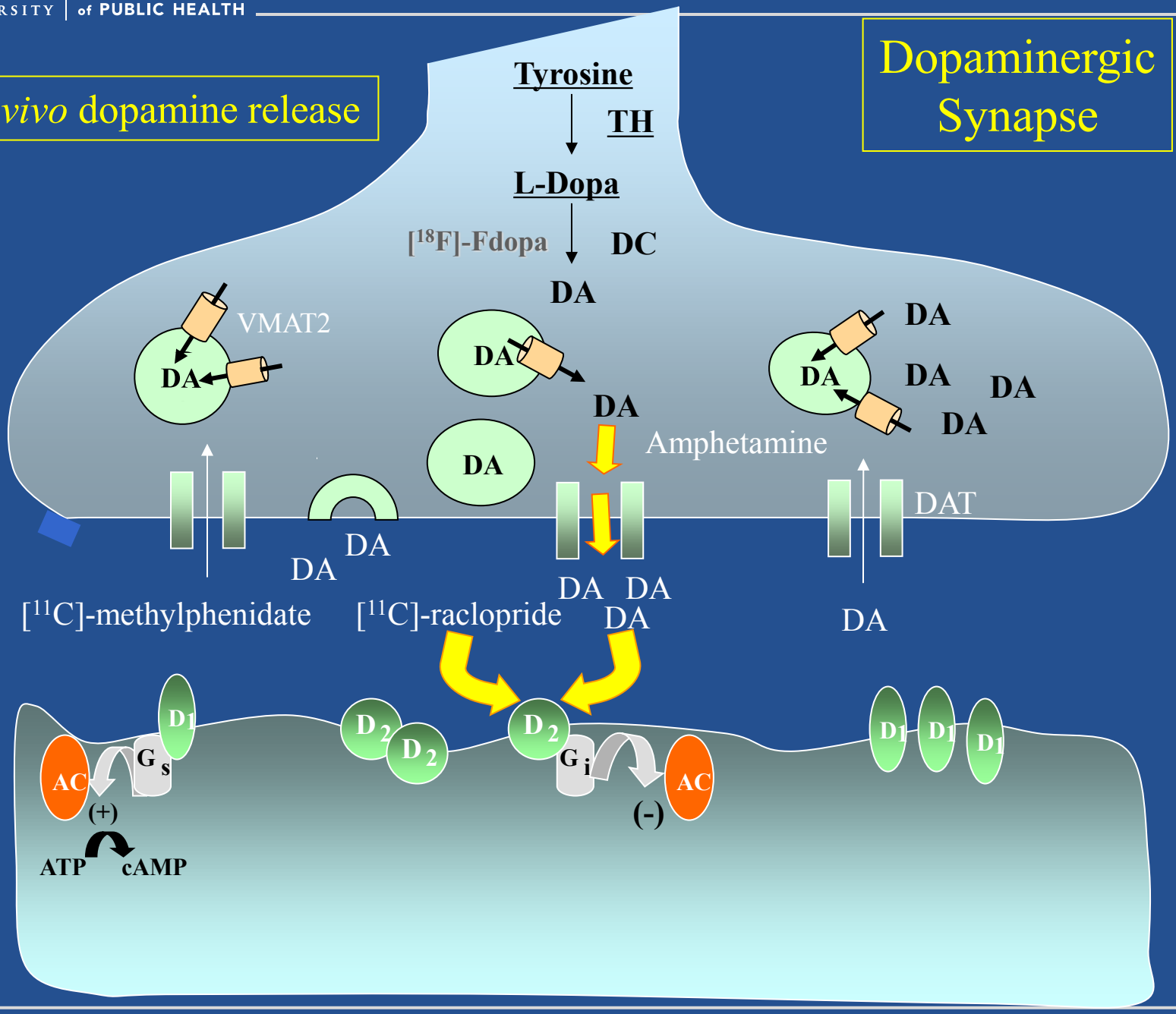


Dopaminergic Synapse



In vivo dopamine release

Dopaminergic Synapse



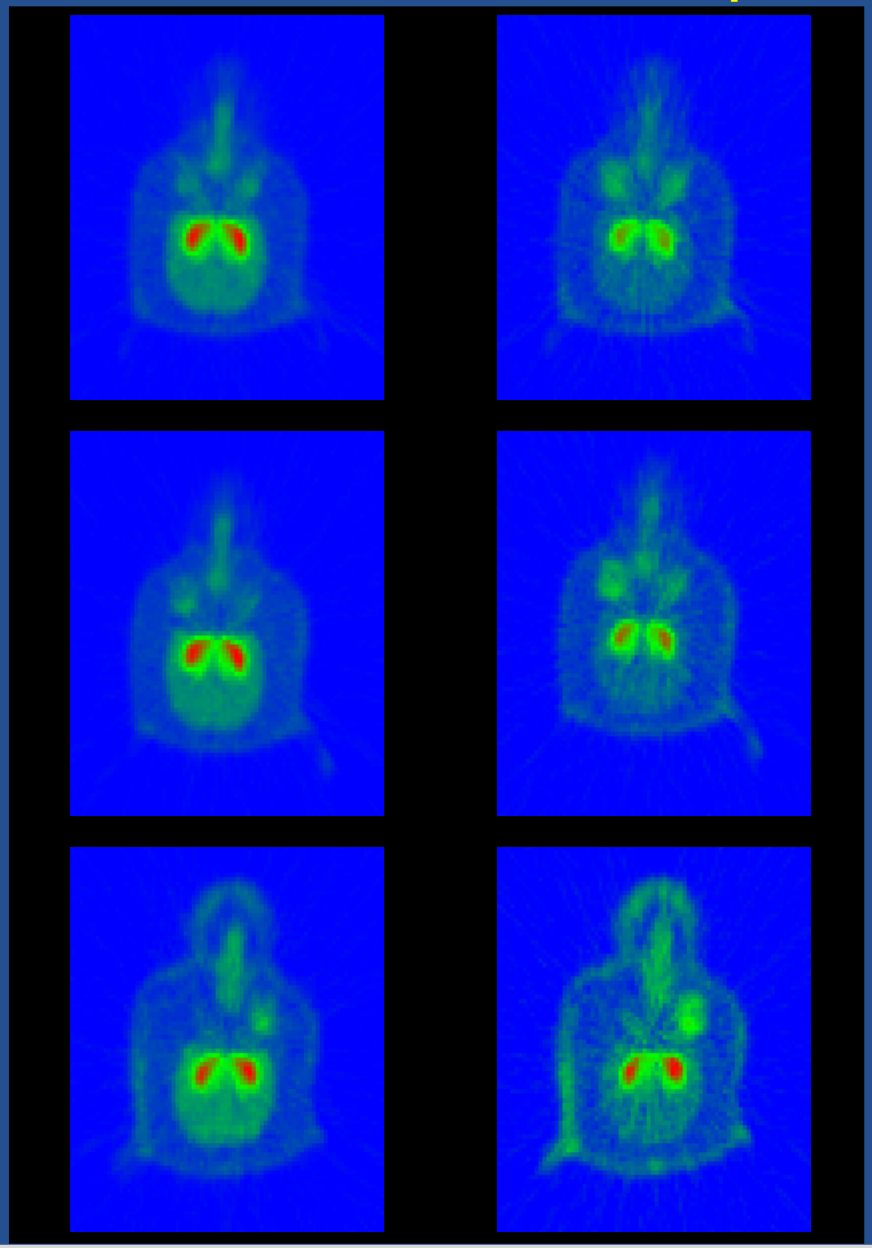
Before Amphetamine

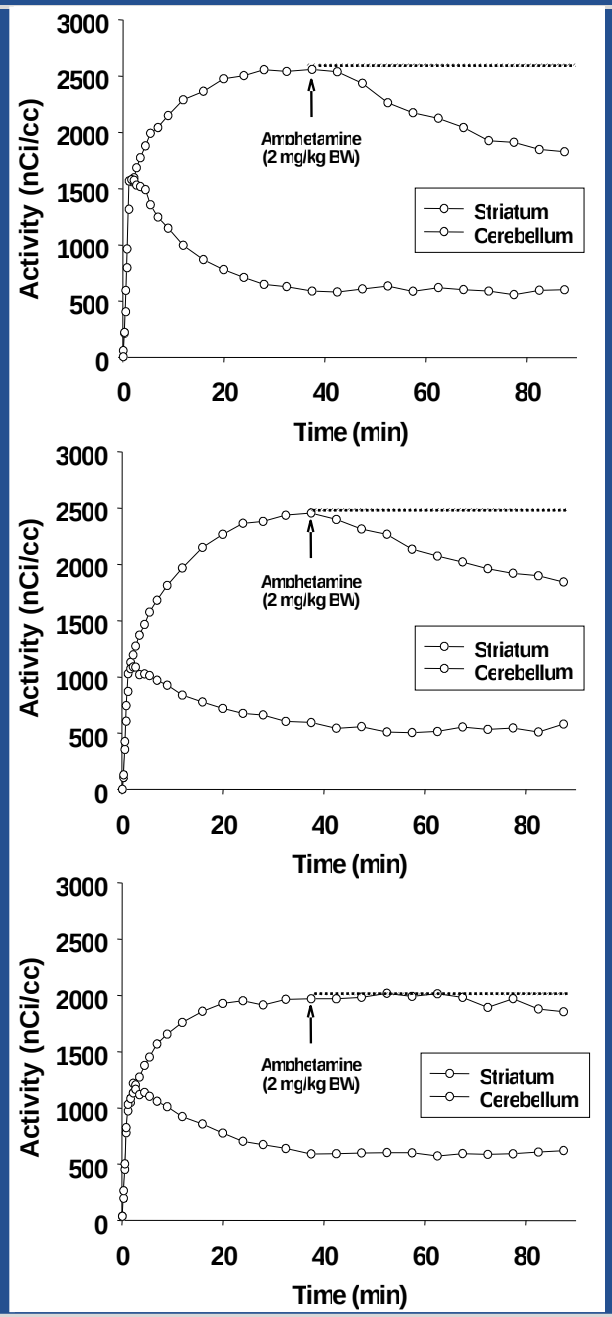
After Amph

Baseline

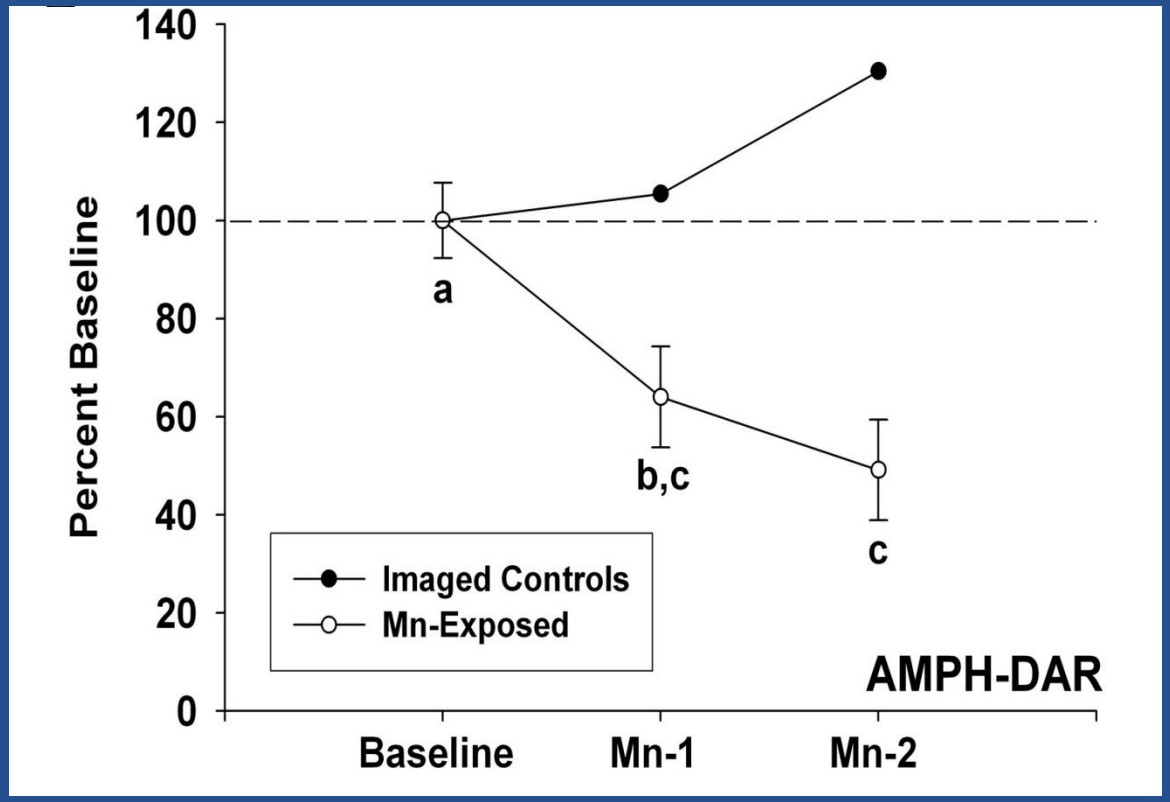
Mn-1

Mn-2



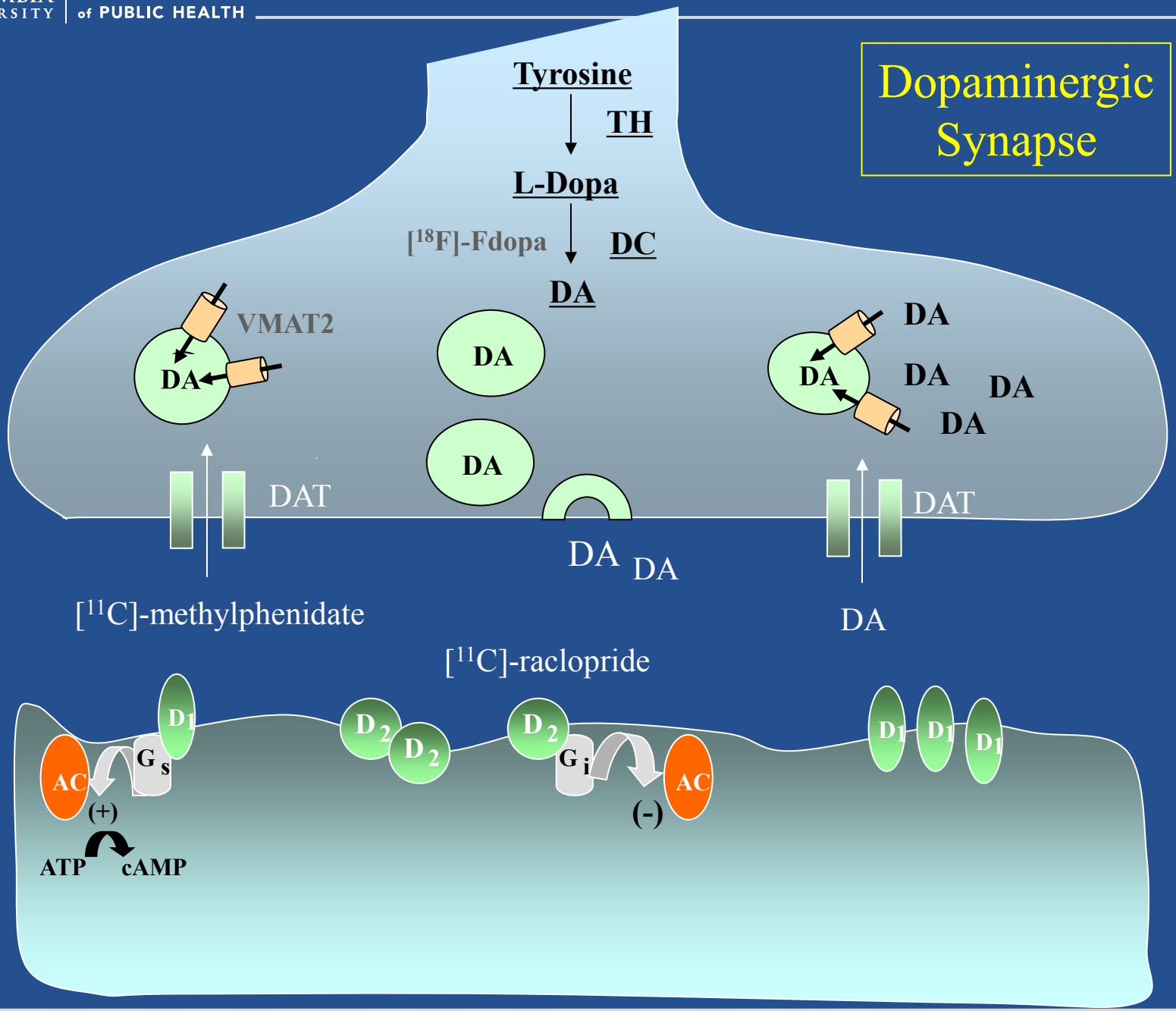


Effect of manganese exposure on *in vivo* dopamine release

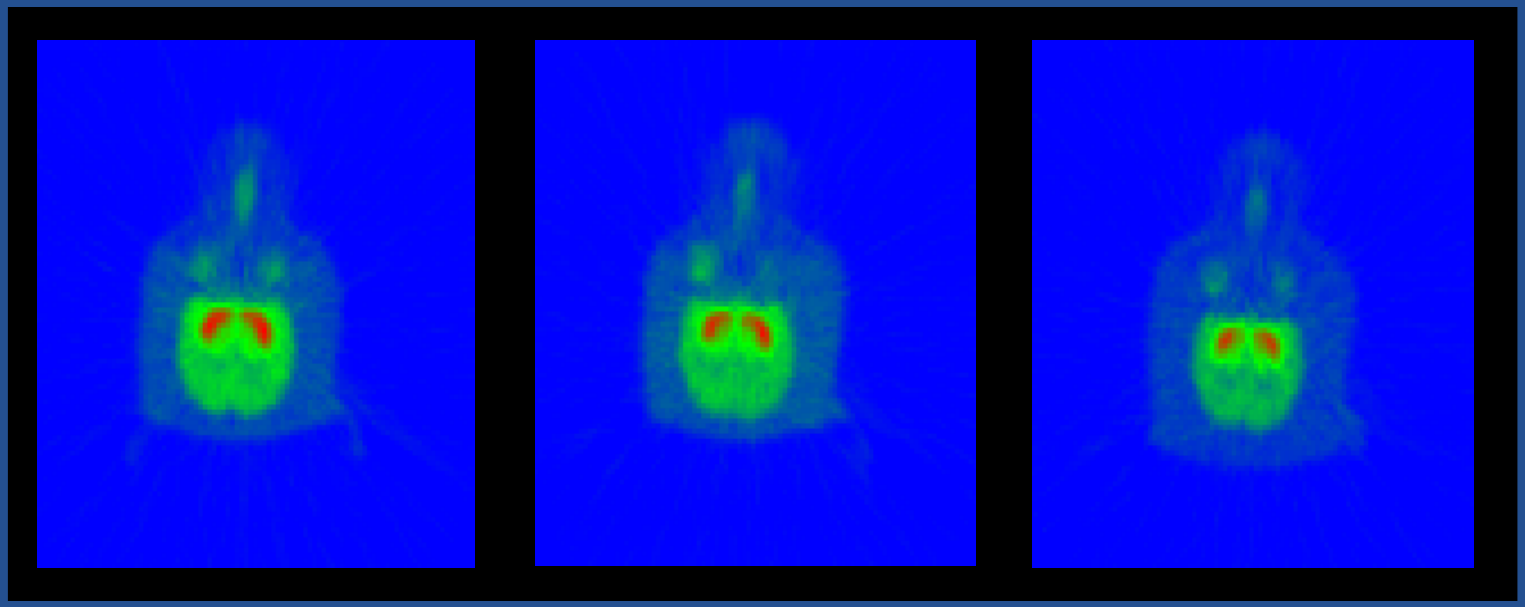


Mn exposure produced a marked, time/dose-dependent decrease in % dopamine release in the caudate/putamen.

Dopaminergic Synapse



[^{11}C]-methylphenidate PET - dopamine transporter (DAT)

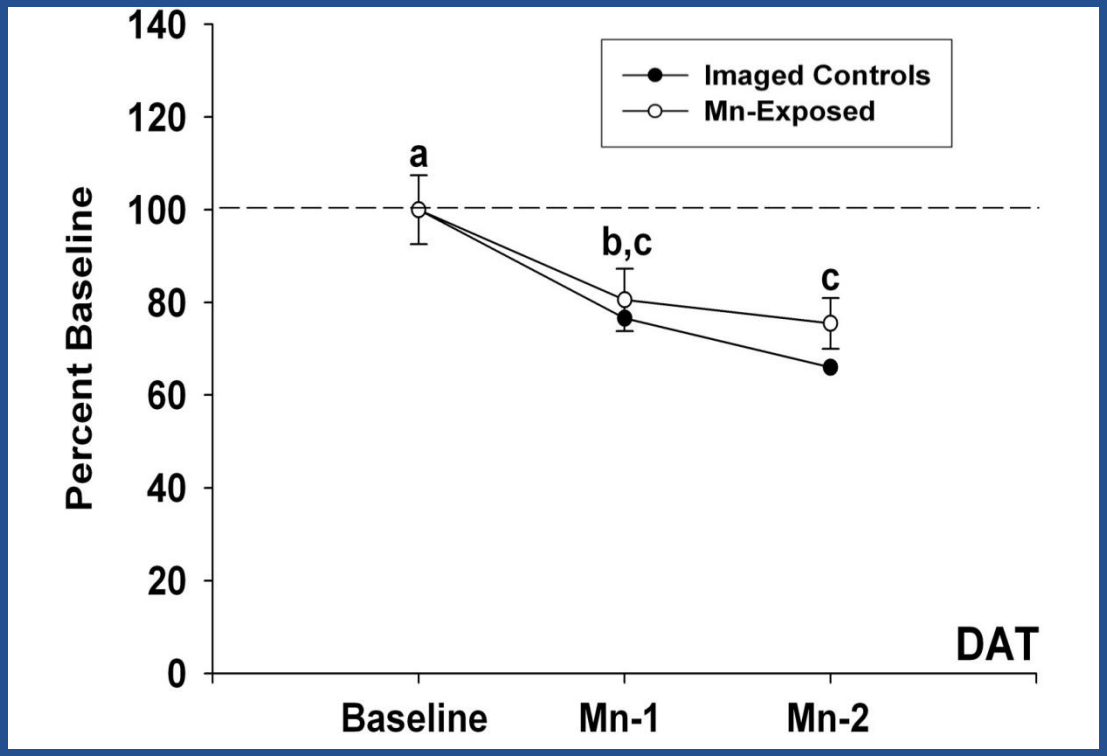


Baseline Mn-1 Mn-2

All three PET scans are on the same animal

Guilarte et al., Exp. Neurol., 2006; J Neurochem 2008

Effect of manganese exposure on dopamine transporter levels as measured by [^{11}C]-methylphenidate PET



No Significant Differences Between Mn-treated and Imaged-controls

Results indicate that dopamine terminals do not degenerate

Summary of Results

Our PET studies show that animals chronically exposed to Mn express a marked inhibition of *in vivo* dopamine release in the absence of terminal degeneration (based on DAT levels) in the striatum.

These findings suggest that Mn-induced movement abnormalities are not associated with dopamine neuron degeneration as in idiopathic Parkinson's disease, but result from dopamine neuron dysfunction, i.e. inability to release dopamine.

These findings have now been confirmed in rodent models and in human studies

"Neuropathological Studies"

Gene array

Diffuse β-amyloid plaques

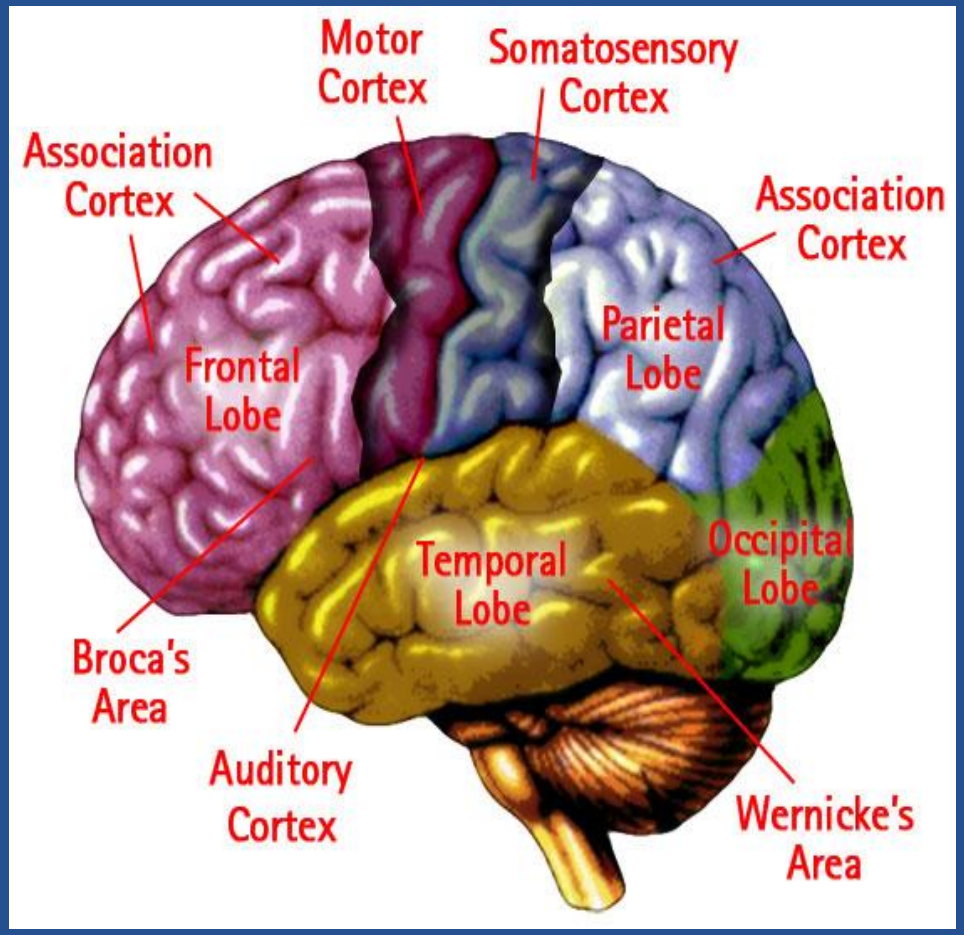
Neurodegeneration

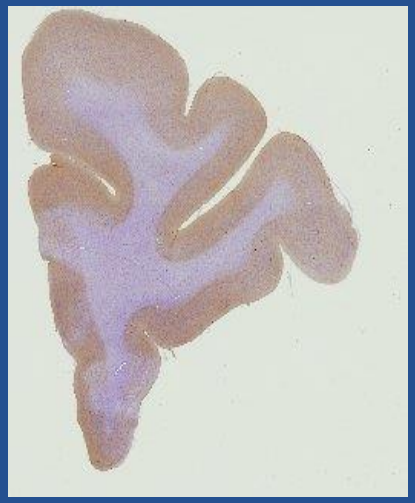
Frontal Cortex & Working Memory

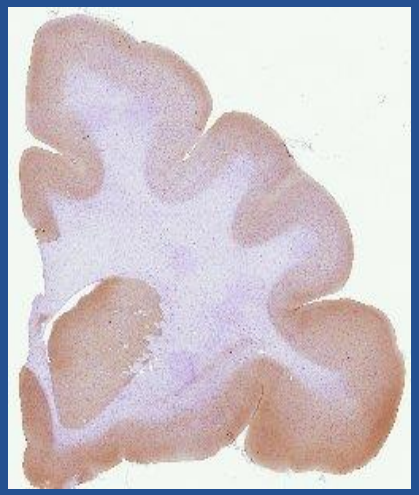
The frontal cortex is involved in executive functions, for example working memory. Working memory is a central cognitive domain that has been defined as the ability to temporarily maintain and manipulate information on line. Also called short-term memory.

Since working memory was impaired in Mn-exposed non-human primates, we performed gene array and neuropathological studies in the frontal cortex.

Frontal Lobe/Cortex







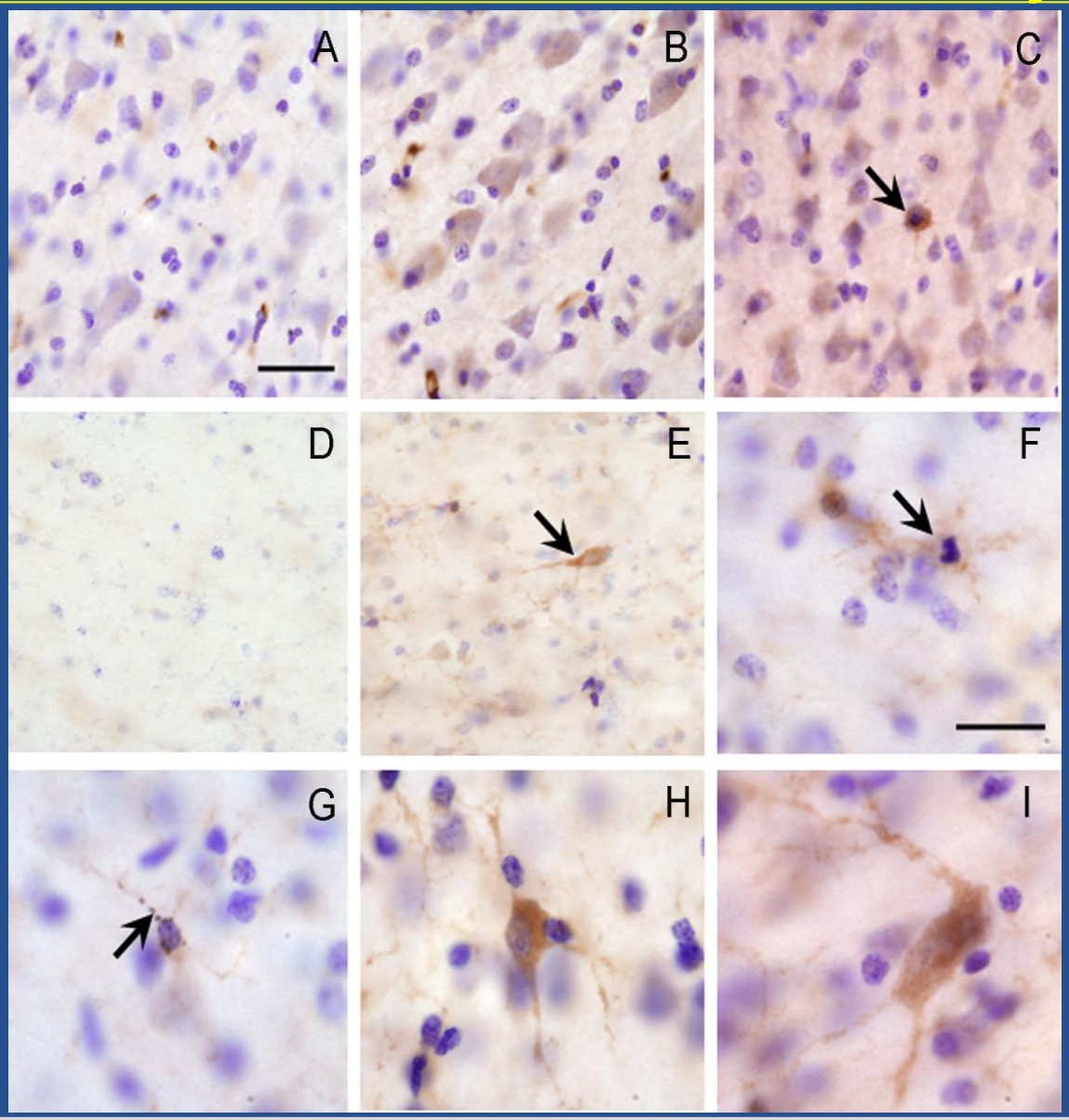
Frontal Cortex Microarray Results

Gene Symbol	Function	Z-ratio
APP Family		
APLP1	**APP shedding, copper reg.**	**2.90**
Apoptosis		
RRAGA	metalloprotease,TNF interacdtion	2.72
PAWR	tumor suppressor	1.96
CYCS (cytochrome c)	ETC; apoptosis	1.76
C4BPB	C4b binding on apoptotic cells	-1.53
Cholesterol		
IDI1	cholesterol biosynthesis	2.34
APOA1	cholesterol efflux	1.89
PMVK	cholesterol biosynthesis	1.76
Synaptic Function		
STAU2	mRNA transport, spine formation	2.48
DYNC2LI1	dynein subunit	2.37
SNX9	EGFR degradation, vesicle recycling	2.31
AGMAT	polyamine biosynthesis	2.18
ARL1	vesicle regulation	2.02
KLC4	kinesin light chain	1.96
CLIC3	cell/dendrite growth regulation	1.85
VTI1B	vesicle transport	1.68
Proteasome/ Ubiquitin / Protein Folding / Turnover		
HERPUD2	ER stress; protein folding	2.40
RNF40	E3 ubiquitin ligase	2.37
PSMA4	proteasome subunit	1.96
FKBP14	protein folding	1.83
IDS	lysosomal degradation	1.62
PSMA3	proteasome subunit	1.52
PPIE	protein folding	-1.59
UBE2N	E2 ubiquitin conjugation	-1.68

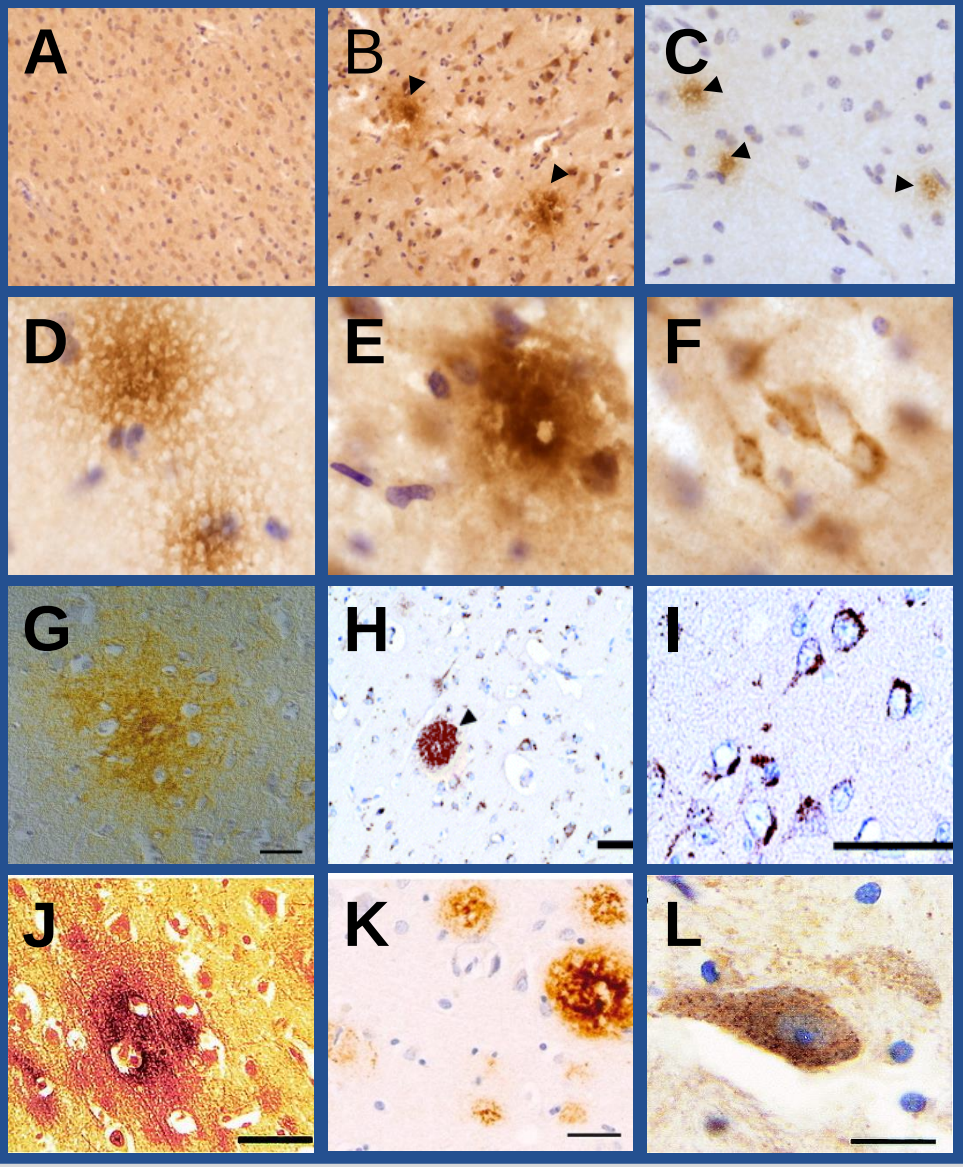
APLP1 gene expression also increased in the thalamus

Gene Symbol	Function	Z-ratio
Inflammation		
CXCL5	neutrophil activation	2.57
LAT2	T&B cell activation	2.32
ZNF3	immune cell activation	2.06
IFNGR1	immune response	1.84
TGFA	mitogenic response	1.74
SPP1	immune response	1.74
Cell Cycle regulation / Transcription / DNA repair		
ZMYM6	DNA binding	2.72
ERCC2	DNA damage, apoptosis act.	2.55
ZPBP	transcription	2.36
CCDC44	DNA integration	2.31
NFYB	p53-dependent transcription	2.25
ZNF397	transcription	2.18
IMPDH2	guanine biosynthesis	2.06
PLAGL2	recognizes DNA	1.96
XRN2	RNA metabolism	1.95
EGLF9	EGF homology	1.93
CDK7	cell cycle regulation	1.89
IGFBP4	prolongs insulin growth factors	1.73
NME1	nuceloside kinase	-1.53
Other		
GRAMD1A	glycosyltransferase	2.86
ANXA2	cytoskeletal stability	2.58
MMACHC	vitamin B12 metabolism	2.43
EPM2A	glycogen metabolism	2.33
NOL6	actin binding	2.13
DPM1	GPI anchoring	2.06
SEH1L	nuclear pore	2.05
EPHX1	epoxide detoxication	2.03
GYPA	glycophorin A	1.96
IDS	lysosomal degradation	1.62
CYHR1	cysteine rich, binds galectin-3	1.56
PIGY	GPI biosynthesis	-1.59

APLP1 Immunohistochemistry



β-amyloid Immunohistochemistry-6E10



A: Control animal

B-F: Mn-exposed animals

G: Aged canine brain (from: Czasch & Baumgartner, *Neurobiology of Disease* 27: 293, 2006)

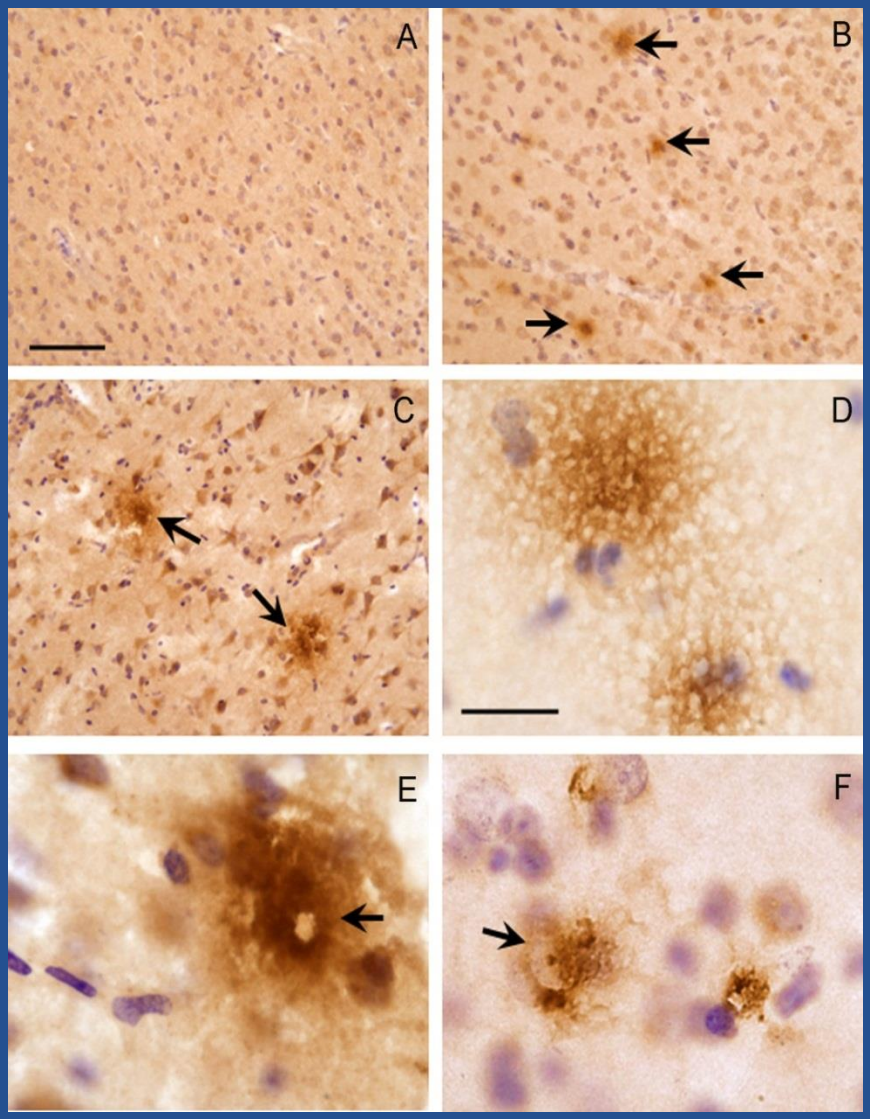
H & I: Aged monkey brain (from: Kimura et al., *Neuropath Appl Neurobiol* 31: 170, 2005)

J: 17 year old Down Syndrome (from: Gyure et al., *Arch Pathol Lab Med* 125: 489, 2001)

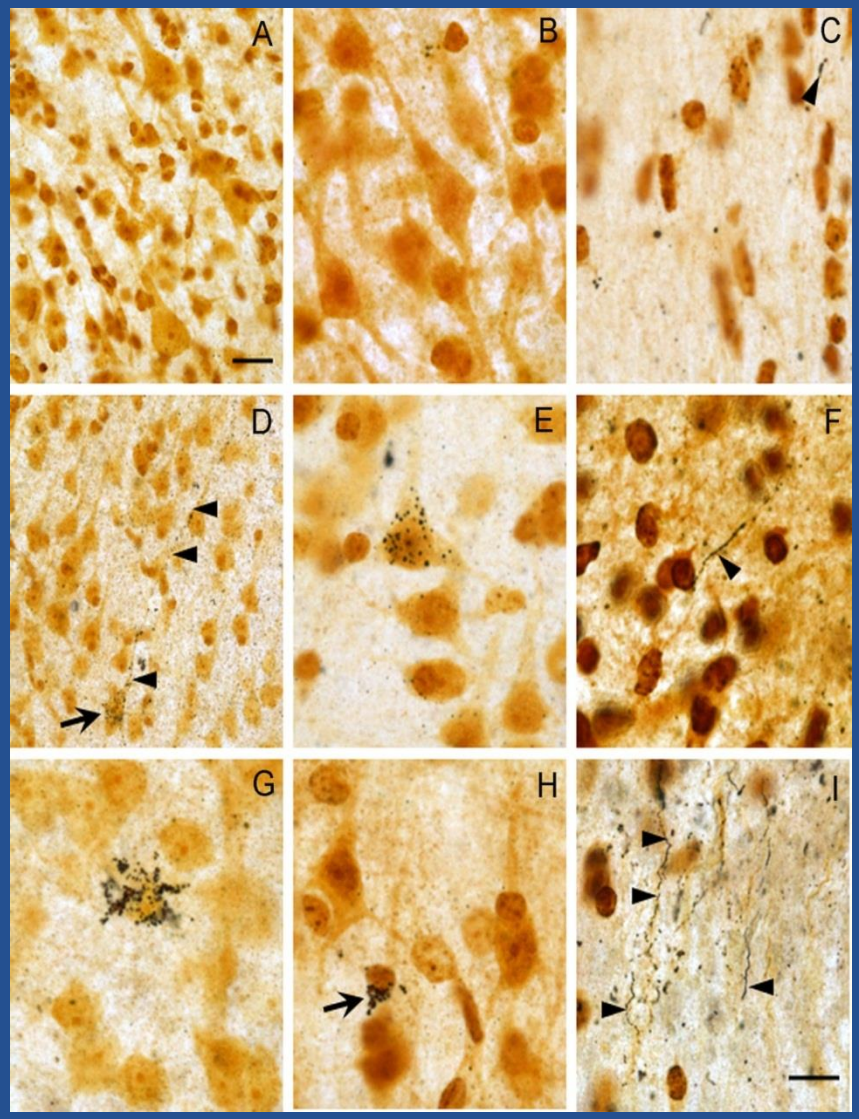
K: AD patient (from: Alafuzoff et al., *J Neuropath Exp Neurol* 65: 740, 2006)

L: neuron from a 20 month old Down Syndrome that developed Aβ plaques (Gyure et al., *Arch Pathol Lab Med* 125: 489, 2001)

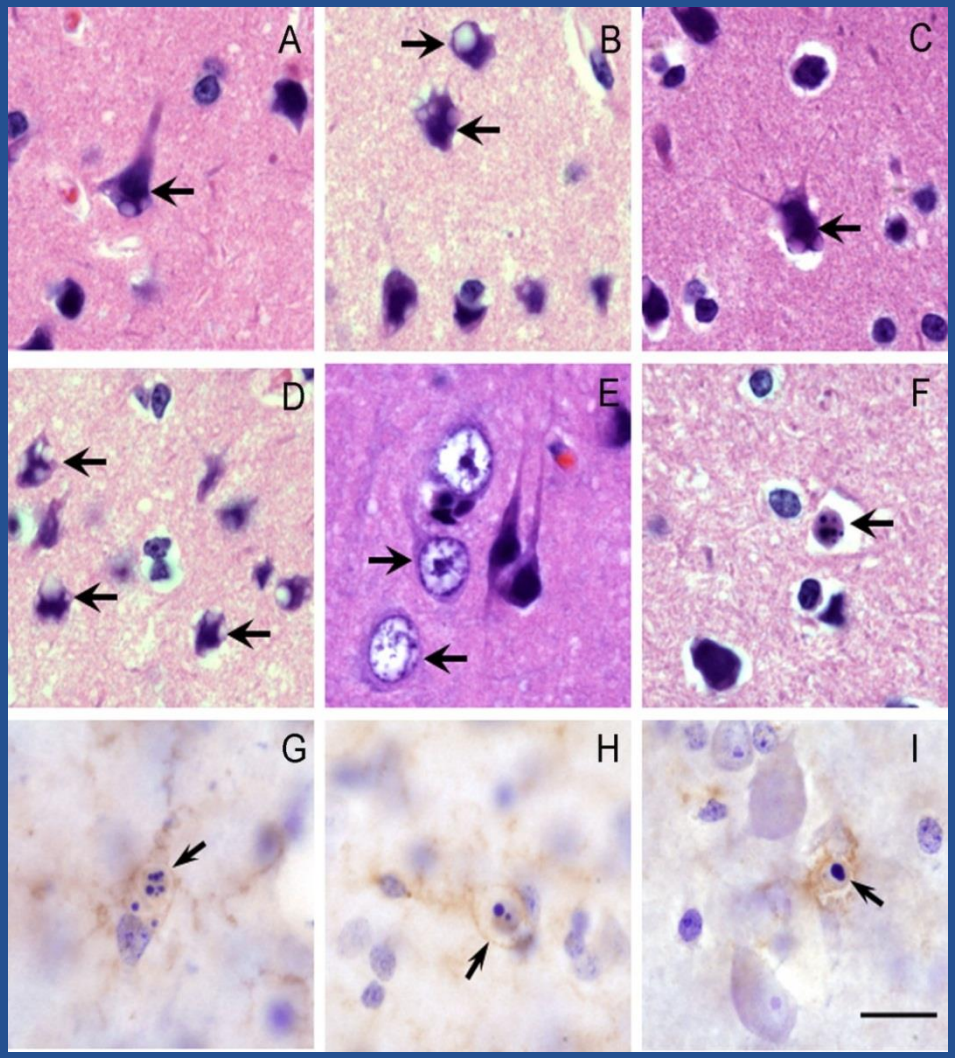
Aβ Immuno-6E10



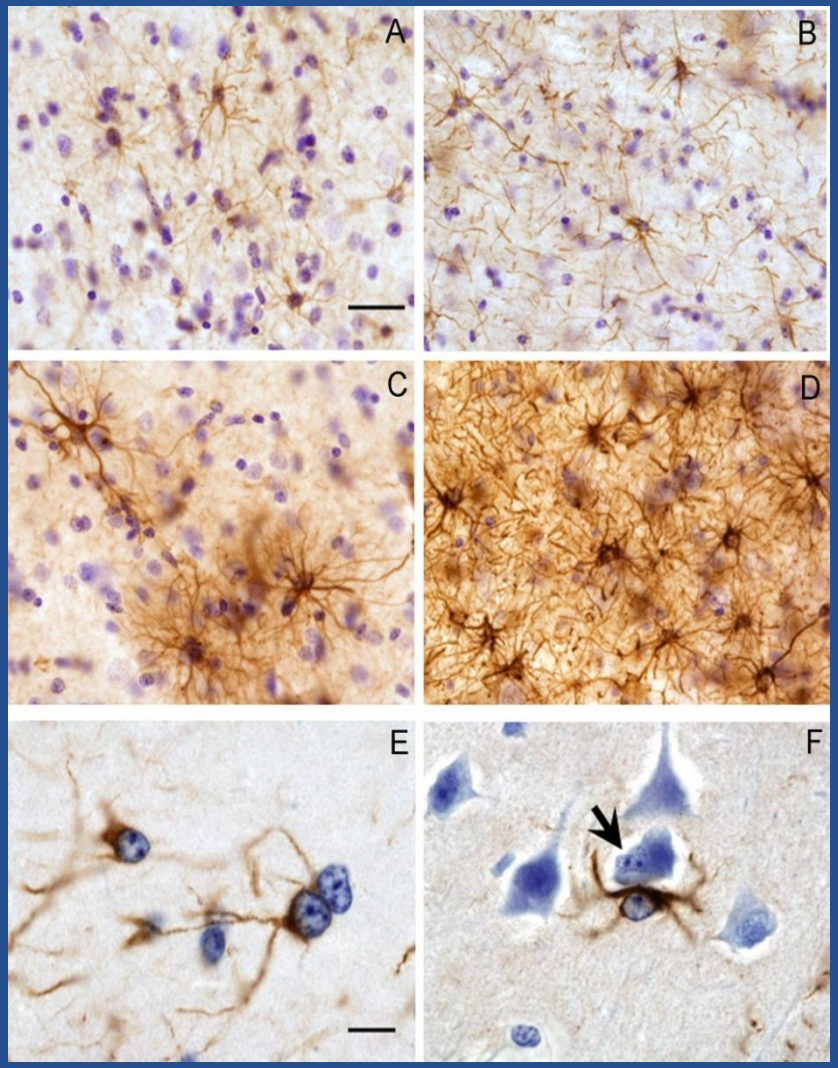
Silver Staining



Nissl Stain



GFAP Immuno



Manganese Neurotoxicity-Frontal Cortex

These studies indicate that Mn exposure produces an increase in APLP1 gene and protein expression in the frontal cortex.

Exposure to Mn results in diffuse β-amyloid plaques in the grey and white matter in the frontal cortex.

Neurodegenerative changes were observed in the frontal cortex grey and white matter using Silver and Nissl stains and these included apoptotic cells. These degenerative changes were associated with glial activation.

These neurodegenerative changes may be responsible for the working memory deficits observed in these animals.

Recent human studies have also implicated the frontal cortex in Mn-induced neurotoxicity.

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Thank You!!

Questions??