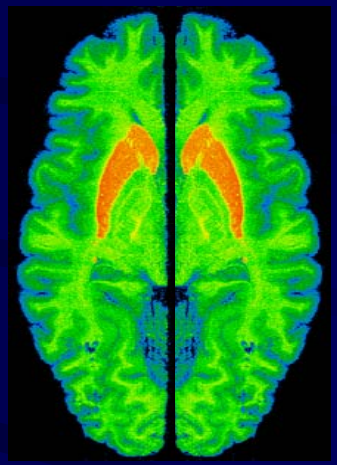
Whole Hemisphere Autoradiography in Alcoholism Research



Jari Tiihonen

Department of Forensic Psychiatry

University of Kuopio, Finland

Cloninger's model of alcoholism

Cloninger 1995

Type 1 Alcoholism

- Socially dependent character – co-operativeness
  - Emphatic, compassionate
- Social anxiety
  - Low “novelty seeking” (deliberate, rigid, orderly)
  - High “harm avoidance” (worried, fearful, pessimistic)
  - → Gradual late onset of drinking
- “Parkinsonian personality”

Type 2 Alcoholism

- Antisocial character – Unco-operativeness
  - Lack of empathy, social tolerance, compassion, moral principles
- → hostility and depression
- → aggression and suicide
- Availability of tryptophan ↓ (Virkkunen et al. 1994)
- 5-HT in platelets ↓ (Benkefat et al. 1991)
- Low 5-HIAA in CSF (Kruesi et al. 1990)

Receptor visualization techniques

- In vivo: PET, SPET
- In vitro: in situ-hybridization histochemistry, ligand-binding essays, autoradiography
- Ex-vivo

DA D_1 receptors in human alcoholics

- no studies to date
- DA D_1 receptor protein ↑ in NAC of metamphetamine users
- No change in cocaine or heroine users
  - Worsley et al. 2000
- Animal data controversial
  - Nonaka & Moroji 1990, Tomic et al. 1997, 2000

DA D_3 receptors in human alcoholics

- no studies to date
- NAC DA D_3 ↑ in cocaine overdose victims
- no change in delirium victims
  - Staley and Mash 1996
- animal data controversial
  - McBride 1997

Striatal D_2 dopamine receptor binding characteristics in vivo in patients with alcohol dependence (Hietala et al. Psychopharmacology 1994)

- [^{11}C]raclopride in PET
- 8 healthy controls (mean age 36.3)
- 9 unclassified (non-violent) alcoholics
  - abstinence 1-68 weeks (mean 20)
- Striatal D_2 receptor ratio (B_{max}/K_d) 19.7% lower (P=0.004)
- no correlation between D_2 receptors and abstinence period

Altered striatal dopamine re-uptake site densities in habitually violent and non-violent alcoholics (Tiihonen J et al. Nat Med 1995)

- [^{123}I]ω-CIT in SPET
- 19 healthy controls (mean age 34.3)
- 10 type 1 alcoholics (mean age 44.6)
  - abstinence > 2 months
  - striatal DAT ratio 25% lower (P<0.001)
- 19 type 2 alcoholics (mean age 30.5)
  - abstinence > 2 months
  - DAT ratio 8.5% higher (P<0.10)

Decreases in dopamine receptors but not in transporters in alcoholics (Volkow et al. Alc Clin Exp Res 1996)

- [^{11}C]dTMP and [^{11}C]raclopride in PET
- 17 healthy controls (mean age 47)
- 10 unclassified alcoholics (mean age 44)
  - inclusion: onset<25, exclusion: binges
  - abstinence 52±48 days
- D_2 receptor ratio 22% lower
- no differences in DAT ratio (n=5)
- no correlation to abstinence

Dopamine transporter and D_2-receptor density in late onset alcoholism (Repo et al. Psychopharmacology 1999)

- [^{123}I]PE2I and [^{123}I]epidepride in SPET
- 9 controls (mean age 46.3)
- 9 type 1 alcoholics (mean age 51.1)
  - abstinence 7-165 days (mean 43.5)
- striatal DAT ratio 21% lower ($P<0.005$)
  - no correlation between abstinence time
- no differences in D_2-receptor ratios

Dopamine transporters increase in human brain after alcohol withdrawal

(Laine P et al. Mol Psychiatry 1999)

- [^{123}I] ω-CIT in SPET
- 27 controls (mean age 37.7) and 27 unclassified alcoholics (mean age 42.2)
- DAT ratio 10% lower before detoxification
- DAT increase 14% after four week abstinence ($P<0.0001$)
- >50% committed criminal offences

Decreased striatal monoaminergic terminals in severe chronic alcoholism (Gilman S et al. Ann Neurol 1998)

- (+)[^{11}C]dihydroxytetrabenazine in PET
- labels VMAT2
- 7 controls (mean age 57)
- 7 unclassified alcoholics (mean age 52)
  - abstinence period > 2 months
- reduced binding in the caudate nucleus (8.6%; P<0.05) and putamen (6.2%)

Striatal presynaptic dopamine function measured with PET (Tiihonen et al. Mol Psychiatry 1998)

- 6-[^{18}F]-FDOPA in PET
- 8 controls (mean age 44.2)
- 10 type 1 alcoholics (mean age 47.7)
  - abstinence 3 days - 42 mo (mean 137.9 d)
- FDOPA uptake ad 28% higher (P=0.008)
- compensatory to low postsynaptic function?

Pros and Cons - Autoradiography

- **PROS**
- High resolution
- Quantifiable
- Pharmacological study easy
- Receptor discrimination
- WHA-whole hemisphere

- **CONS**
- Postmortem/in vitro
- Post- and antemortem effects
- Normally drug treated
- Low (?) availability
- Diagnostics

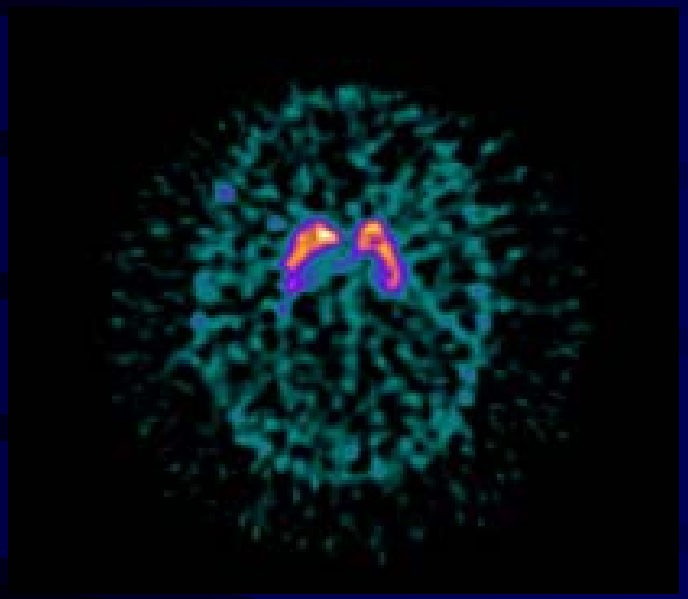
Pros and Cons - PET and SPET

- **PROS**
- In vivo
- Behavior or disease state vs. binding
- Quantifiable
- Retestable

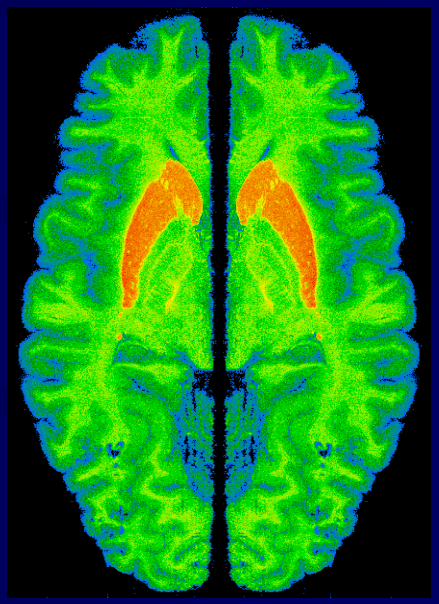
- **CONS**
- Lower resolution
- Ligand distribution, degradation
- Pharmacological studies not easy
- No (?) receptor discrimination
- Expensive

Iodinated PE2I binding in brain

Horizontal sections through the level of the basal ganglia.



[^{123}I]PE2I with SPET on a 26-year-old healthy male at 70 min after injection (150 MBq).



[^{125}I]PE2I with postmortem whole hemisphere autoradiography on a 36-year-old control subject.

Erkki Tupala, MD, PhD

Brain sampling

- Dept. Of Forensic Medicine, University of Oulu and Kuopio
- left hemispheres frozen to -75^{o}C
- medical records: cause of death, previous diseases, medical treatments
- postmortem blood chemistry for drugs incl. etOH

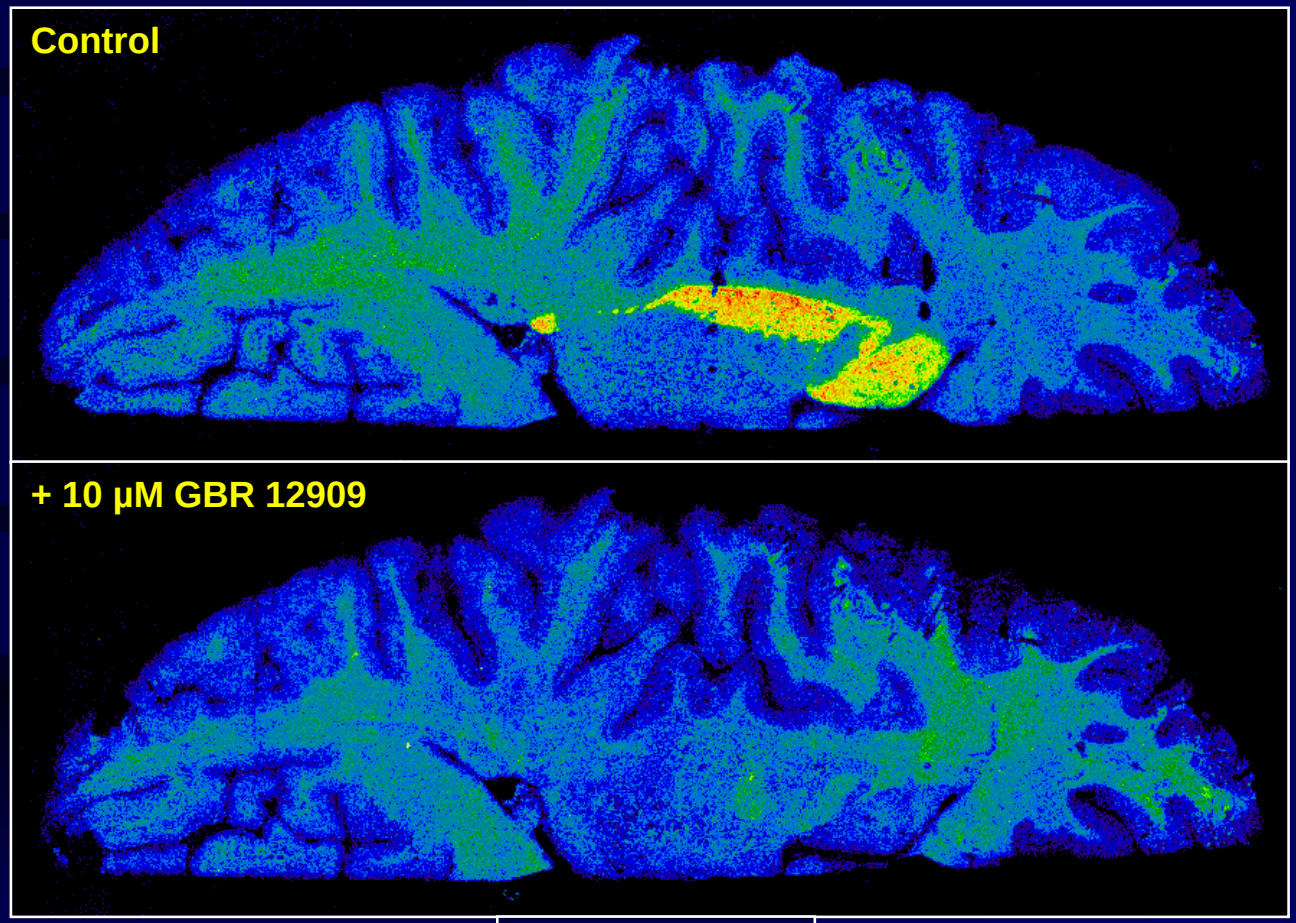
Autoradiography

- ^{125}I- and ^{3}H- labeled ligands
- Competing substance
- Incubation, normally 1 h
- Washing - unbound ligand
- Air-drying
- Exposure to radiation-sensitive film (2d-12 wk)

Image analysis

- Computerized densitometry
- Scanner
- Adobe Photoshop, Scionimage for Windows
- Commercial calibration scales
- Double-Blind
- Cresyl-violent staining - anatomical correlate

Dopamine Transporters in WHA using [^{125}I]PE2I



Erkki Tupala, MD, PhD

Diagnostics

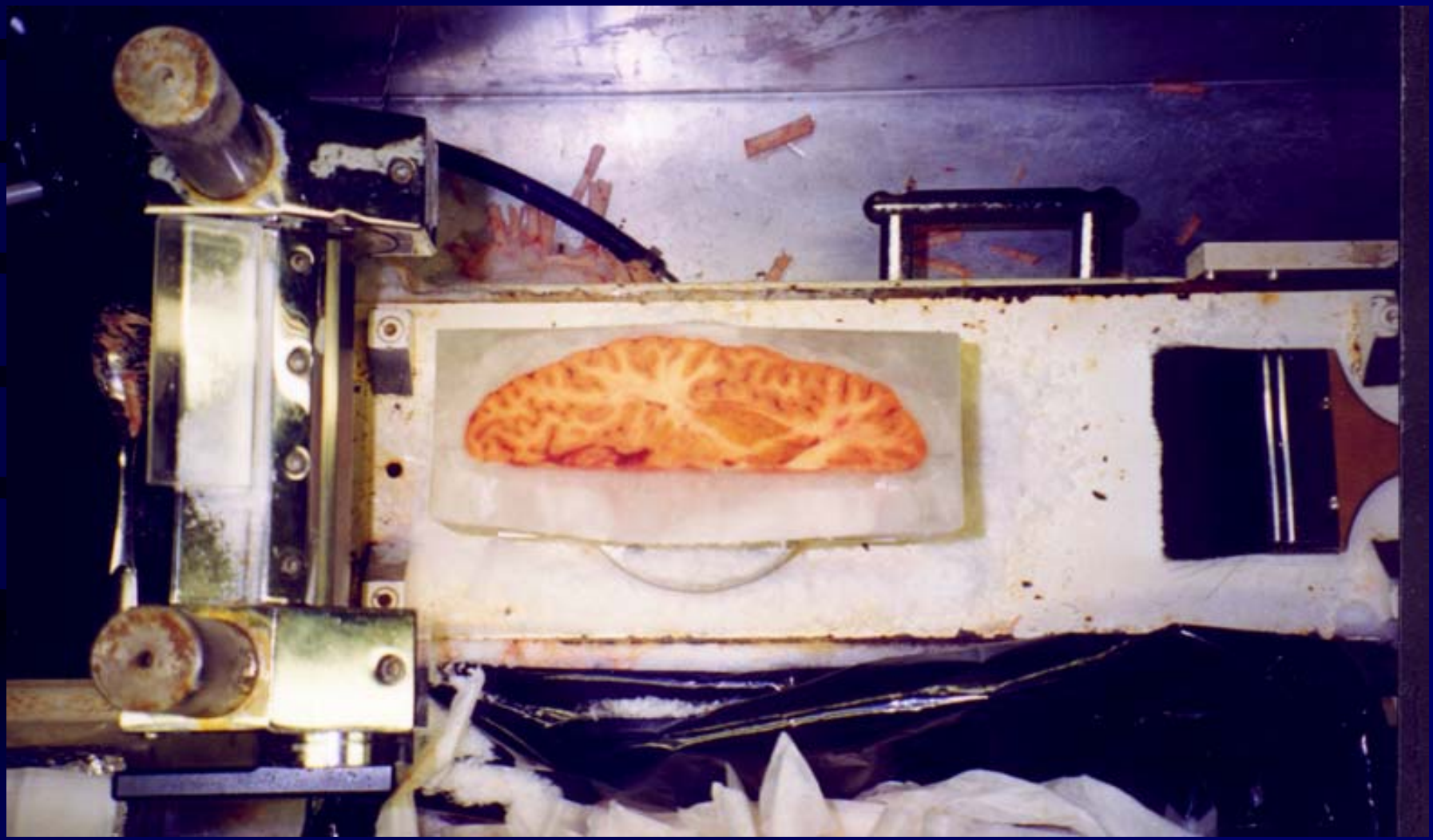
- DG by two physicians independently
- Mental disorders according to DSM III-R
- Alcoholic subtypes according to Cloninger (1981)
- Kappa coefficient 0.9 regarding alcoholism
- Other CNS disorders ruled out
- CNS medications excluded (Benzodiazepines)

Study subjects

- White caucasians
- 10 controls (8 males, 2 females; age 53.5)
- 9 type 1 alcoholics (8 males, 1 female; age 52.7)
- 8 type 2 alcoholics (males; age 34.6)
- postmortem interval < 24 h
- alcoholics intoxicated at the time of death (-one type 1 and two type 2 alcoholics)
- 1 control intoxicated at the time of death

Cryosectioning

- Dept. of Pharmacology and Toxicology, University of Kuopio, Finland
- CMC block
- Canto-meatal cutting plane
- Heavy-duty cryomicrotome
- 100 💻m sections to gelatinized glass plates
- Air drying and storage at -25 ^{o}C before use



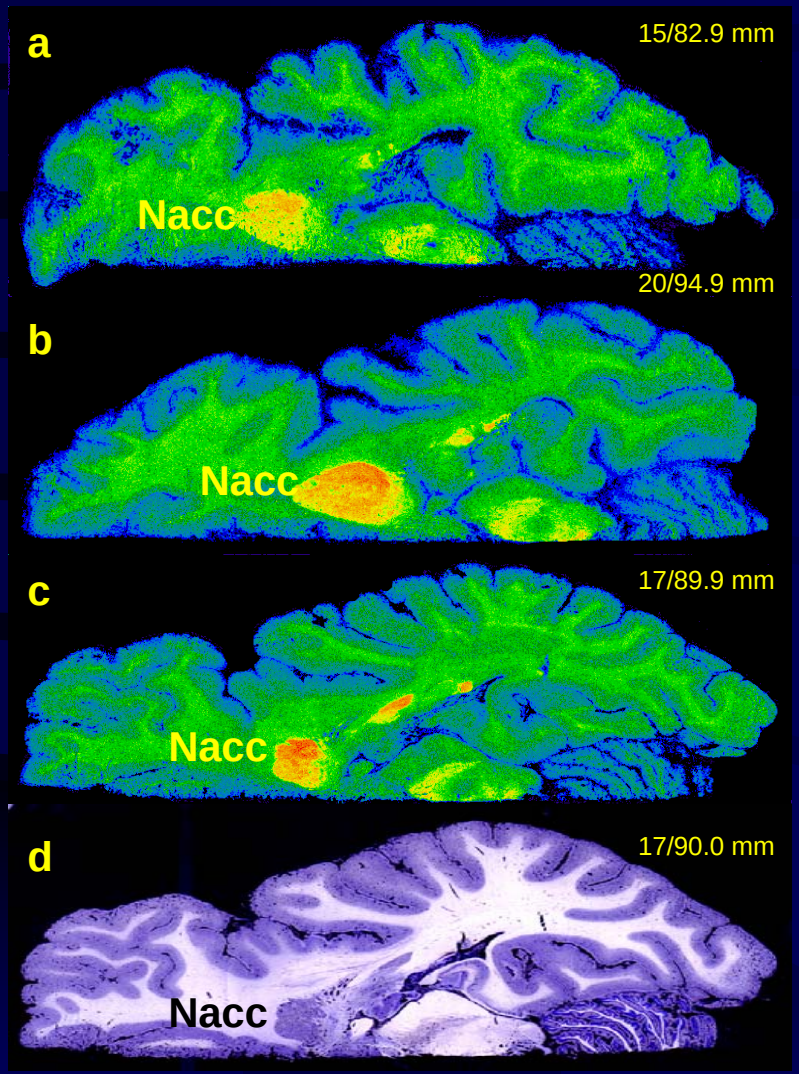
Erkki Tupala, MD, PhD

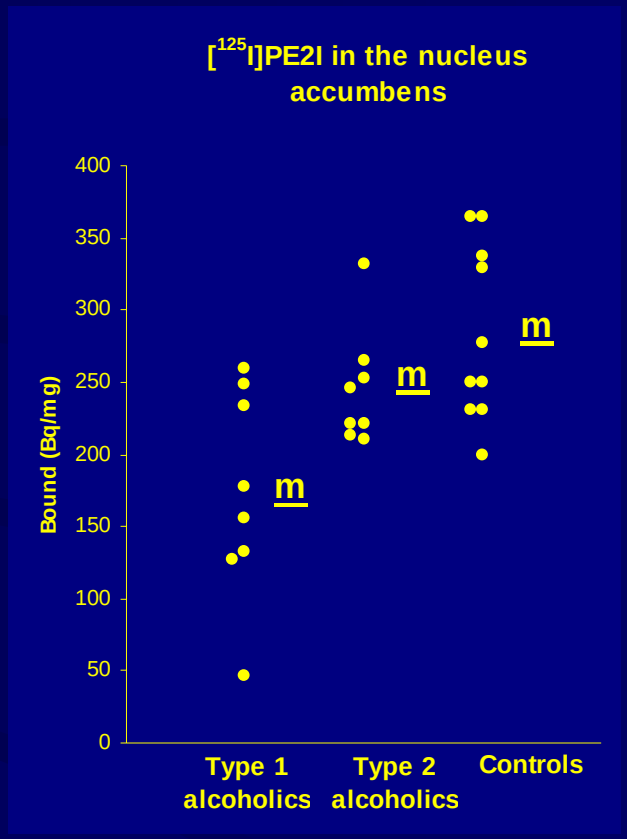
Dopamine Transporter Density in Nucleus Accumbens of Type 1 alcoholics

(Tupala et al. Lancet 2000)

- 7 type 1 alcoholics: (6 men, mean age 50.6 years)
- 7 controls: (5 men, mean age 54.3 years)
- [^{125}I]PE2I and ω-CIT in WHA
- DAT binding 35% lower in nucleus accumbens of the type 1 alcoholic group

Dopamine transporters in nucleus accumbens



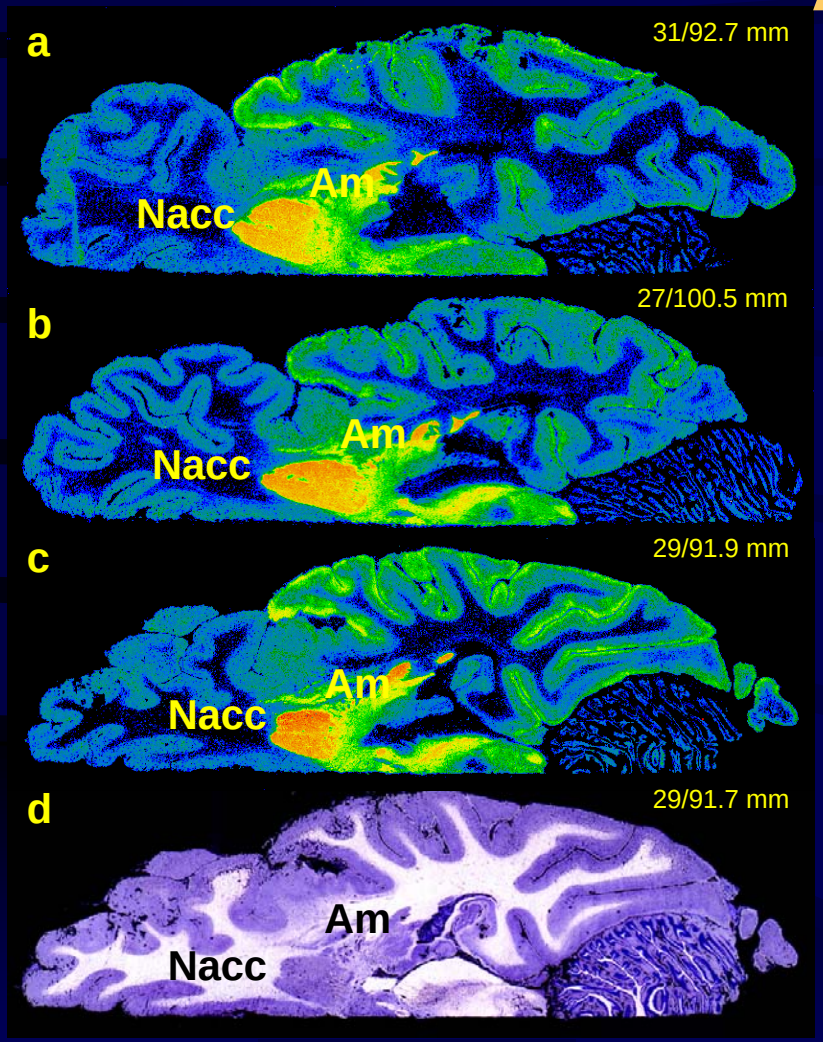


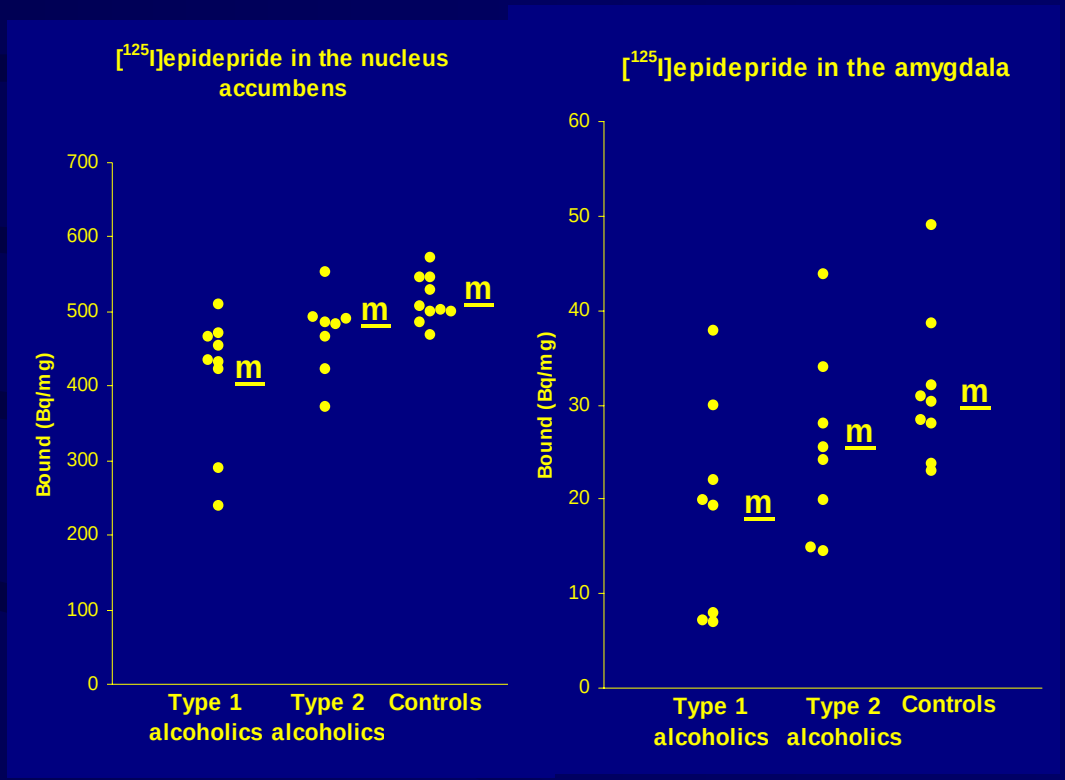
Erkki Tupala, MD, PhD

Dopamine D_2/D_3-receptor and Transporter Densities in Nucleus Accumbens and Amygdala of Type 1 and 2 Alcoholics (Tupala et al. Mol Psychiatry 2001)

- 9 type 1 alcoholics, 7 men, mean age 52.9 years
- 8 type 2 alcoholics, all men, mean age 34.6 years
- 10 controls, 8 men, mean age 53.5 years
- [^{125}I]PE2I (ω-CIT) and [^{125}I]epidepride (cis-flupenthixol)
- D_2/D_3 receptors 25% ↓ in nucleus accumbens and 41% ↓ in amygdala among type 1 alcoholics
- type 2 alcoholics at the same level with controls

Dopamine D_2/D_3 Receptors in Nucleus Accumbens and Amygdala



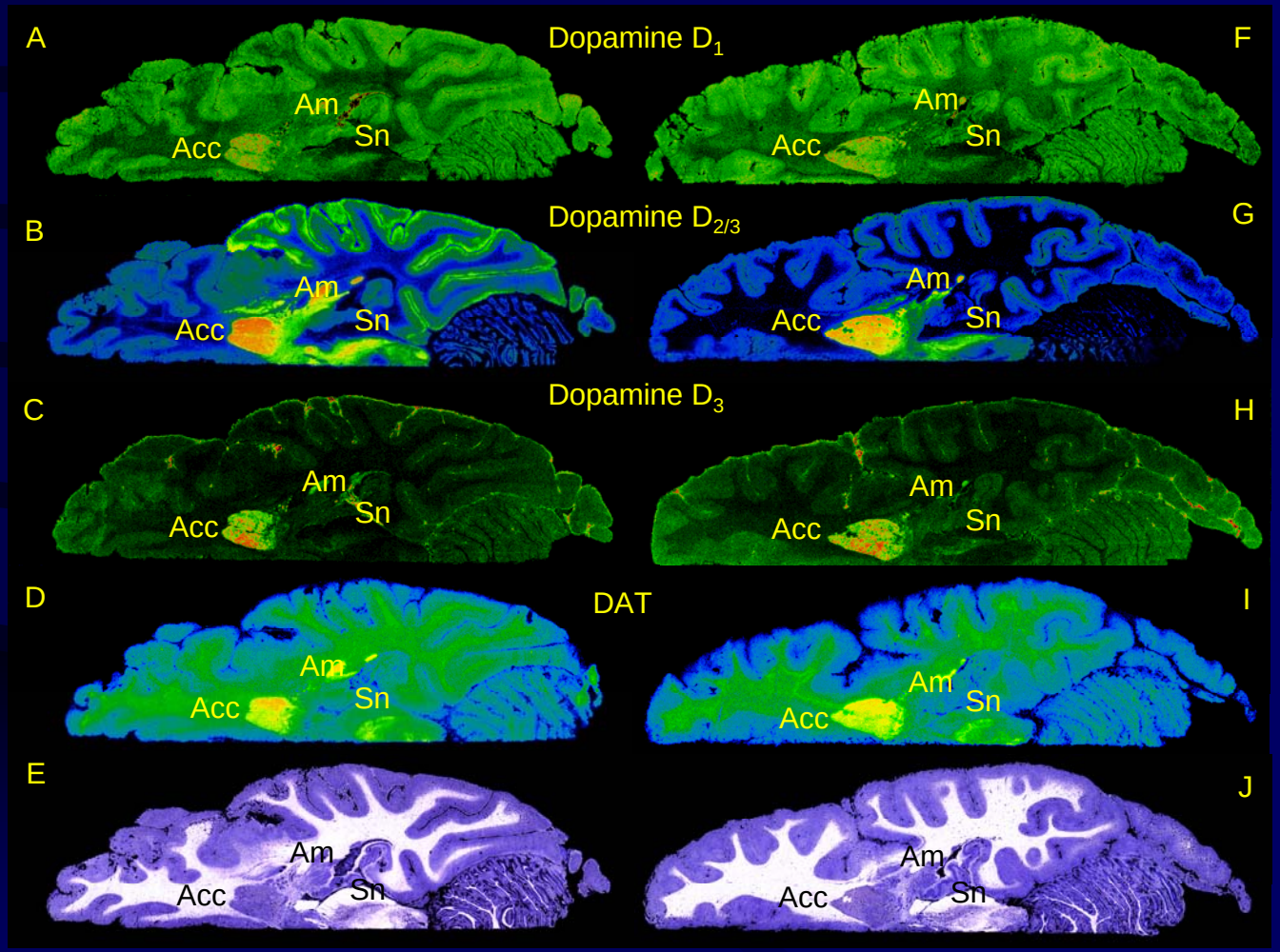


Erkki Tupala, MD, PhD

Dopamine D_1 and D_3 Receptors in Alcoholics

- 9 type 1 alcoholics, 7 men, mean age 52.9 years
- 8 type 2 alcoholics, all men, mean age 34.6 years
- 10 controls, 8 men, mean age 53.5 years
- [^{3}H]SCH 23390 (Cis-flupenthixol)
- [^{3}H]PD 128 907 (Raclopride)
- No differences between the groups

Dopamine Binding Sites in Controls and Type 1 Alcoholics



Erkki Tupala, MD, PhD

Am J Psychiatry 2002; 159: 599-606

Serotonin Transporter Distribution and Density in the Cerebral Cortex of Alcoholic and Nonalcoholic Comparison Subjects: A Whole-Hemisphere Autoradiography Study

Tuija Mantere, M.D.

Erkki Tupala, M.D. Ph.D.

Håkan Hall, Ph.D.

Terttu Särkioja, M.D., Ph.D.

Pirkko Räsänen, M.D., Ph.D.

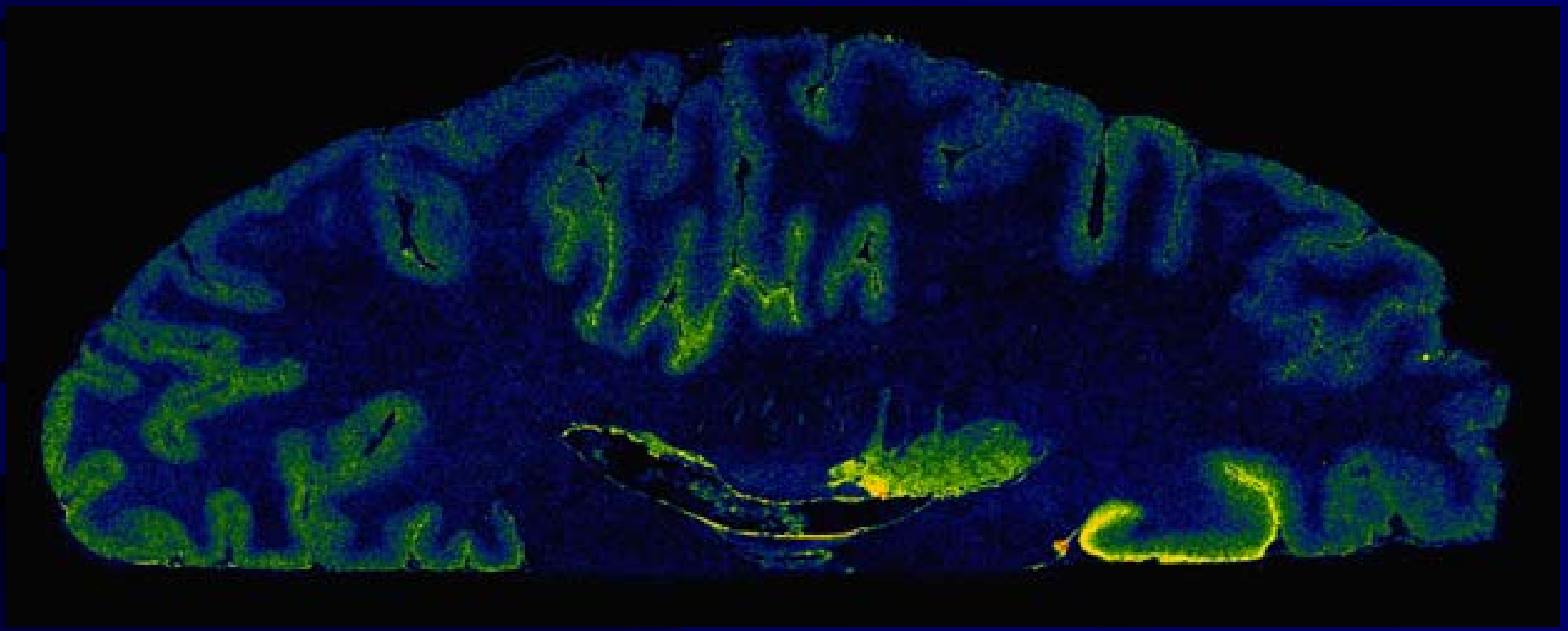
Kim Bergström, Ph.D.

James Callaway, Ph.D.

Jari Tiihonen, M.D., Ph.D.

RESULTS: In the human cerebral cortex, serotonin transporter binding sites were concentrated in the perigenual anterior cingulate cortex. Substantially sparser serotonin transporter density (up to 35%) was observed in the perigenual anterior cingulate cortex of alcoholic subjects in relation to nonalcoholic comparison subjects. After adjustment for age and postmortem delay, this finding remained statistically significant. **CONCLUSIONS:** A lower serotonin transporter density among the alcoholic subjects was observed, specifically in the so-called "affect" region, suggesting an association between ethanol addiction and dysfunctional serotonergic neurotransmission in this area.

Autoradiogram Showing [3H]Citalopram Binding



Tuija Mantere, MD

Conclusions

- The results support Cloninger's model of alcoholism
- DA deficit may be selective to D_2 receptor and type 1 alcoholism
- Alcoholics should be classified when DA system is studied/treatment strategies are applied
- Type 1 alcoholics may benefit from drugs that enhance DA activity (partial agonists?)

Acknowledgements

- Erkki Tupala
- Håkan Hall
- Terttu Särkioja
- Pirjo Halonen
- Kim Bergström
- Jyrki Kuikka
- Pirkko Räsänen
- Tuija Mantere
- Esa Korpi
- Jarmo Hietala
- Jukka Hiltunen
- Jouko Vepsäläinen
- Synnöve Eriksson
- Eila Hyvärinen
- Pirjo Hänninen
- Kari Karkola