Whole Hemisphere Autoradiography in Alcoholism Research

Jari Tiihonen
Department of Forensic Psychiatry
University of Kuopio, Finland
Cloninger’s model of alcoholism

Temperament
- worrier (anxiety-prone)
- social dependency (dopaminergic deficit)

Character
- deliberate cautious friend
- emphatic compassionate
- impulsive risk-taking aloof
- antisocial vengeful

Alcoholism
- Type 1 alcoholic- 80%
  - adult onset
  - alternating binges
  - and abstinence
- Type 2 alcoholic-20%
  - teenage onset
  - no desire to quit
  - recurrent crime and violence

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 D₁ receptors in human alcoholics

- no studies to date
- DA D₁ receptor protein ↑ in NAC of metamphetamine users
- No change in cocaine or heroine users
  - Worsley et al. 2000
- Animal data controversial

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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 ($B_{\text{max}}/K_d$) 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)

- $[^{123}\text{I}]$-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)

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- $[^{11}\text{C}]d\text{TMP}$ and $[^{11}\text{C}]\text{raclopride}$ in PET
- 17 healthy controls (mean age 47)
- 10 unclassified alcoholics (mean age 44)
  - inclusion: onset<25, exclusion: binges
  - abstinence $52\pm48$ days
- $D_2$ receptor ratio 22% lower
- no differences in DAT ratio ($n=5$)
- no correlation to abstinence

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Dopamine transporter and $D_2$-receptor density in late onset alcoholism

(Repo et al. Psychopharmacology 1999)

- $[^{123}\text{I}]$PE2I and $[^{123}\text{I}]$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

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Dopamine transporters increase in human brain after alcohol withdrawal
(Laine P et al. Mol Psychiatry 1999)

• $[^{123}\text{I}]\ 
\text{-CIT}$ 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

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• (+)[11C]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-$[^{18}\text{F}]-\text{FDOPA}$ 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?

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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

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Iodinated PE2I binding in brain

Horizontal sections through the level of the basal ganglia.

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

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

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Brain sampling

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

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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)

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Image analysis

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

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

Control

+ 10 µM GBR 12909

Density
Low
High

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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)

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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 μm sections to gelatinized glass plates
- Air drying and storage at -25 °C before use

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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)
- $^{[125]}$IPE2I and $\mathcal{M}$-CIT in WHA
- DAT binding 35% lower in nucleus accumbens of the type 1 alcoholic group

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Dopamine transporters in nucleus accumbens

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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
- $[^{125}\text{I}]\text{PE2I (}\sim\text{-CIT})$ and $[^{125}\text{I}]$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

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Dopamine D$_2$/D$_3$ Receptors in Nucleus Accumbens and Amygdala

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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
- $[^3H]$SCH 23390 (Cis-flupenthixol)
- $[^3H]$PD 128 907 (Raclopride)
- No differences between the groups

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Dopamine Binding Sites in Controls and Type 1 Alcoholics

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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?)

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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 Hänninnen
• Kari Karkola