The Role of Agonal Factors in Human Postmortem CNS Research

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MEDLINE Literature Review

#	Search History	Results	Display
1	postmortem.mp. [mp=ti, ab, ot, rw, sh] Details	16188	Display
2	agonal.mp. [mp=ti, ab, ot, rw, sh] Details	315	Display
3	brain.mp. [mp=ti, ab, ot, rw, sh] Details	469207	Display
4	1 and 2 and 3 Details	41	Display

Category 4 citations were examined in detail for factors pertinent to using ADRC postmortem brain material for biochemical research

Identified Agonal Factors

- Coma, MOF, respiratory arrest, hypox.
- Brain pH *(need a standard method!)*
- PMI
- Febrile state
- Terminal medications
- Age and Gender
- Brain lobe (*regional*)

- mRNA heterogeneous factor effects add to varying stability on yield and quality
- Gene expression & postmortem CNS pH:
  - Low: depressed energy, proteolysis
  - High: elevated stress, transcription factors

Li JZ, Vawter MP, Walsh DM, Tomita H, Evans SJ, Choudary PV, Lopez JF, Avelar A, Shokoohi V, Chung T, Mesarwi O, Jones EG, Watson SJ, Akil H, Bunney WE Jr, Myers RM. Systematic changes in gene expression in **postmortem** human **brain**s associated with tissue pH and terminal medical conditions. [Journal Article] *Human Molecular Genetics. 13(6):609-16, 2004 March 15*

- **We observed a remarkable degree of natural variation among 120 samples, which represented three brain regions in 40 subjects.**
- **Individuals who suffered prolonged agonal states, such as with respiratory arrest, multi-organ failure or coma, tended to have lower pH in the brain**
- **Those who experienced brief deaths, associated with accidents, cardiac events or asphyxia, generally had normal pH.**
- **The lower pH samples exhibited a systematic decrease in expression of genes involved in energy metabolism and proteolytic activities, and a consistent increase of genes encoding stress-response proteins and transcription factors.**

Tomita H, Vawter MP, Walsh DM, Evans SJ, Choudary PV, Li J, Overman KM, Atz ME, Myers RM, Jones EG, Watson SJ, Akil H. Bunney WE Jr. Effect of **agonal** and **postmortem** factors on gene expression profile: quality control in microarray analyses of **postmortem** human **brain**. *Biological Psychiatry. 55(4):346-52, 2004*

- ***Coma*** and ***hypoxia*** do affect RNA integrity and gene expression profiles more than age, gender & postmortem factors. Propose "Average Correlation Index" to reduce specimen variability.

Many factors affect mRNA

- Preece P, Cairns NJ. Quantifying mRNA in **postmortem** human **brain**: influence of gender, age at death, **postmortem** interval, **brain** pH, **agonal** state and inter-lobe mRNA variance. [Journal Article] ***Brain*** *Research. Molecular* ***Brain*** *Research. 118(1-2):60-71, 2003 Oct 21*

  - TaqMan RT-PCR measured 7 mRNAs
  - 90 AD and 81 control brains (lobar mRNA same)
  - Females had less mRNA than males
  - Brain pH & amount of RNA (+) corr. except GFAP
  - “Agonal state a poor predictor of mRNA levels”

Cummings TJ, Strum JC, Yoon LW, Szymanski MH, Hulette CM. Recovery and expression of messenger RNA from **postmortem** human **brain** tissue. *Modern Pathology. 14(11):1157-61, 2001 Nov*

- Bryan ADRC Rapid Autopsy Program at Duke
- **10 AD + 9 Controls** (1 to 11 hr PMI)
- **19 brains** RNA integrity + mRNA gene expression (CSF pH, fever/sepsis, O_2, sudden?)
- "**All samples yield intact RNA without degradation**" ("successful gene expression may require enhanced procurement efforts")

Bissette G, Seidler FJ, Nemeroff CB, Slotkin TA. High affinity choline transporter status in Alzheimer's disease tissue from rapid autopsy. [Review] [12 refs] [Review] *Annals of the New York Academy of Sciences. 777:197-204, 1996*

- Choline transporter degrades rapidly
- Brains acquired within 2 hours of death
- Choline transporter **increased** in AD cortex compared to non-AD controls
- Putamen used as a "spared" control region

What Can NACC/NIA Do? [1]

- **Advertise its frozen brain resources!**
- **Broker tissue distribution requests that involve multiple ADRCs (clearing house)**
- **Encourage collaborative grants & symposia to standardize frozen tissue collection methods**
- **Explore feasibility of regional specialized brain banks (genomics, proteomics, laser capture microdissection analysis of single and pooled cells) -- *rigid acceptance criteria for specimens***

What Can NACC/NIA Do? [2]

- **Gather and distribute center-specific specimen requests and distribution data!**
- **Tabulate center-specific practical experience with using agonal factor data to facilitate research.**
- **Agonal factor use and outcome research within the ADRC community - what factors matter?**
- **Add agonal factors to the NACC database and make this data widely available to investigators**

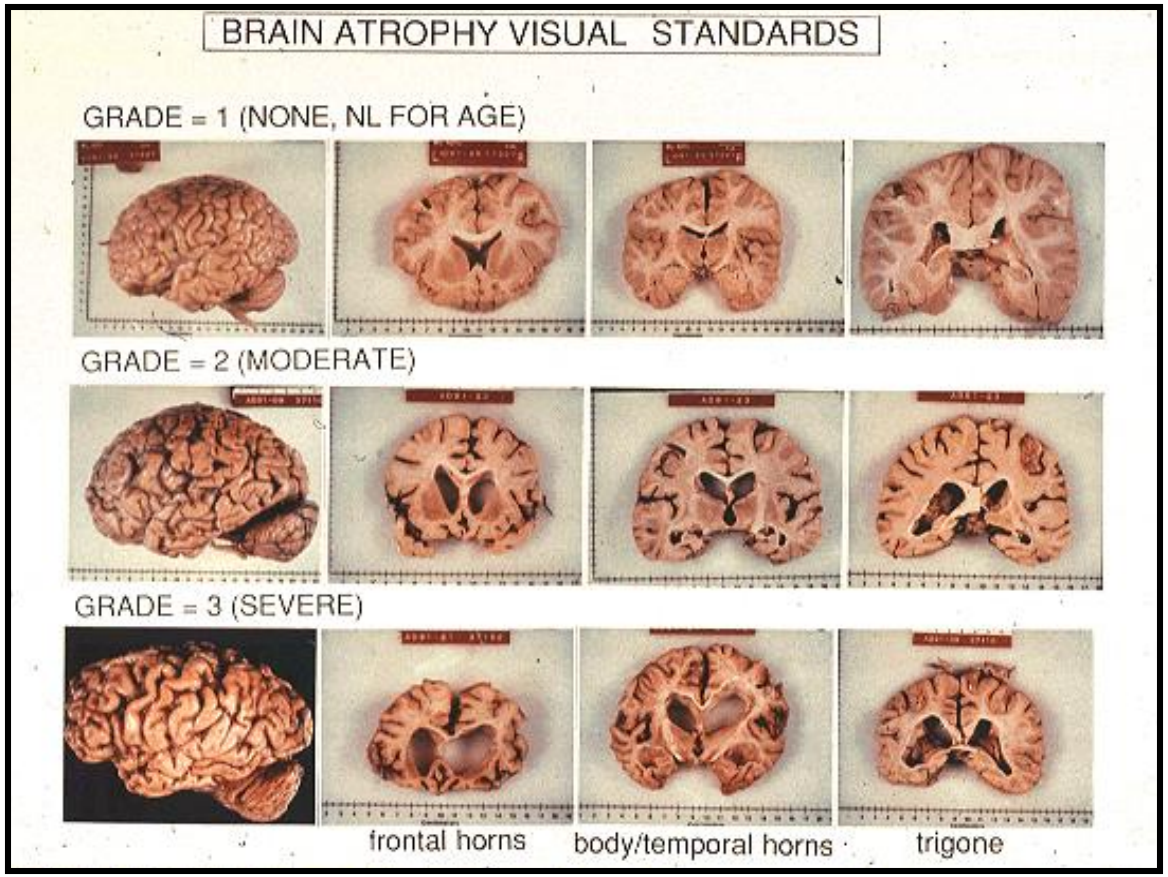
What Can ADRC Pathologists Do?

- **Use existing brain banking protocols to formulate a standard protocol for all centers.**
- **Develop standard tissue block label protocols to facilitate collaborative ADRC research**
- **Adopt the McKeel-Gado visual stds-based system (*Brain Pathol 1994*) for scoring brain atrophy and ventricular dilatation at autopsy.**
- **Develop standard CSF collection protocols**
- **Add banked CNS/CSF requests received and fulfilled to the NACC-reportable data**

Frozen Human Brain Protocols

- **Nochlin D et al. (U. Washington),** *Acta Neuropathologica. 86(6):645-50, 1993*
  - **Aluminum plates chilled with dry ice (CO2)**
  - **CNS suitable for LM, EM + biochemistry**
- **Vonsattel, McKee, Hedley-Whyte et al. (Harvard),** *J Neuropathol Experimental Neurology. 54(1):42-56, 1995*
  - **Aluminum plates chilled with dry ice (CO2)**
  - **Top plate to flatten specimen (coronal slices)**
  - **Standardized block sampling protocol**

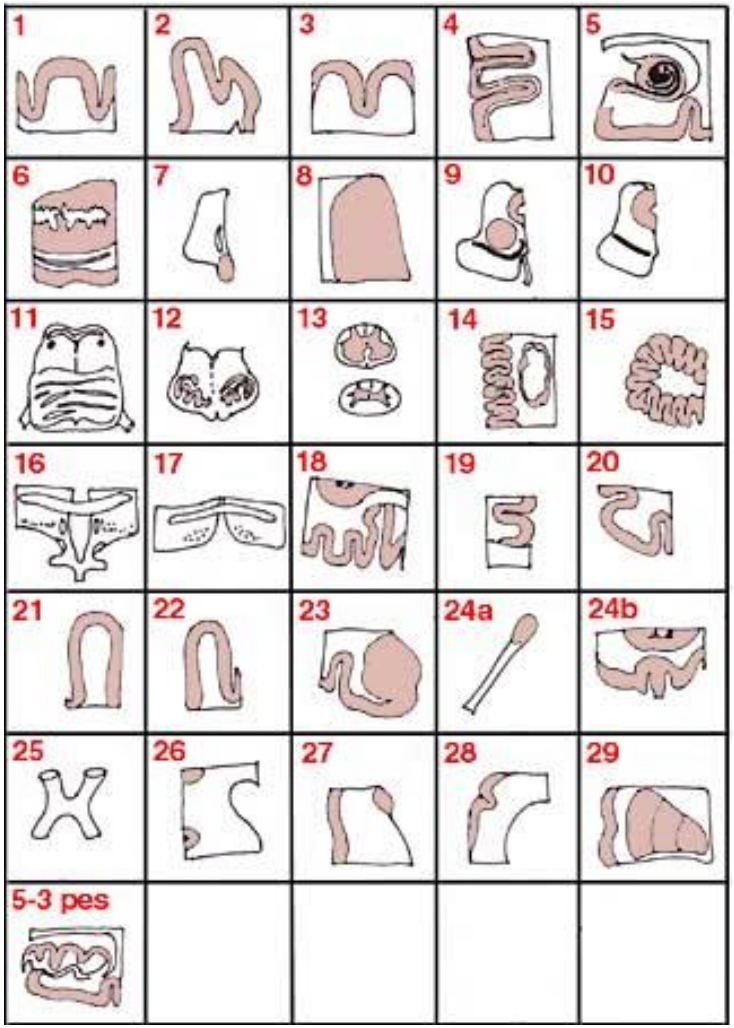
Scoring CNS Atrophy & Ventricular Size at Autopsy



McKeel DW Jr, Gado M. A visual standards based system for scoring Alzheimer and aging-related human brain atrophy at autopsy (abstr. P34-11). ***Brain Pathol 1994;4:544***

WUSM ADRC Standard Blocks

1. Frontal cortex
2. STG + MTG
3. Inf. Parietal ctx
4. Primary visual
5. Hippocampus/ERC ten levels
6. Striatum
7. Mamillary bodies
8. Thalamus
9. Nigra, rostral
10. Nigra, caudal
11. Pons, 3 levels
12. Medulla, 2 levels
13. Spinal Cord
14. Cbellum + Dent. N.
15. Cbellar vermis
16. Hypothalamus



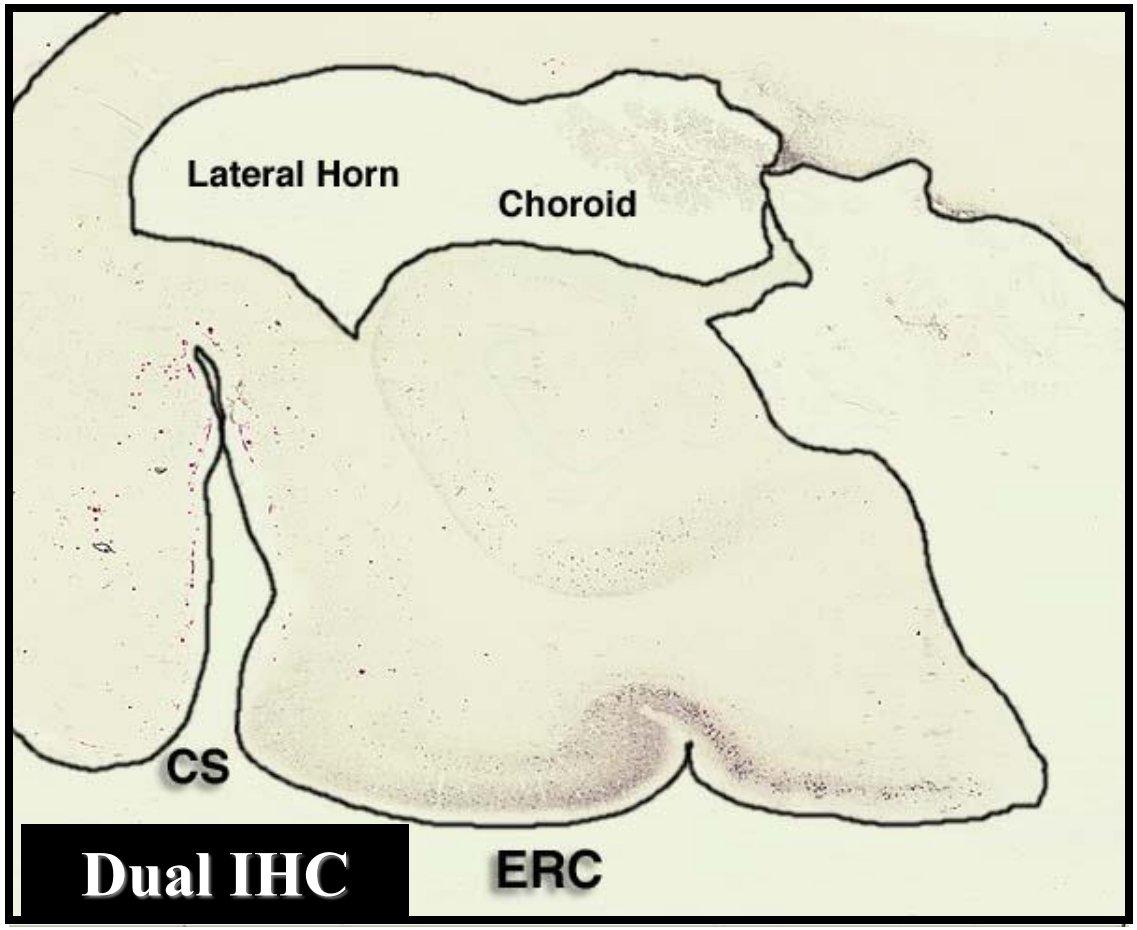
17. Nucleus basalis
18. Orbitofrontal ctx
19. Ant. Cingulate
20. Inf. Temporal ctx
21. Primary motor ctx
22. Primary sensory ctx
23. Amygdala
24. Olfact. Tract & Bulb & ant. olf. nucleus
25. Optic chiasm & nerve
26. WM, deep frontal
27. WM, mid portion
28. WM, occipital
29. Caudate, putamen & globus pallidus
30. Posterior cingulate

31ff - Pathologic lesions

Standardized Immunohistochemistry

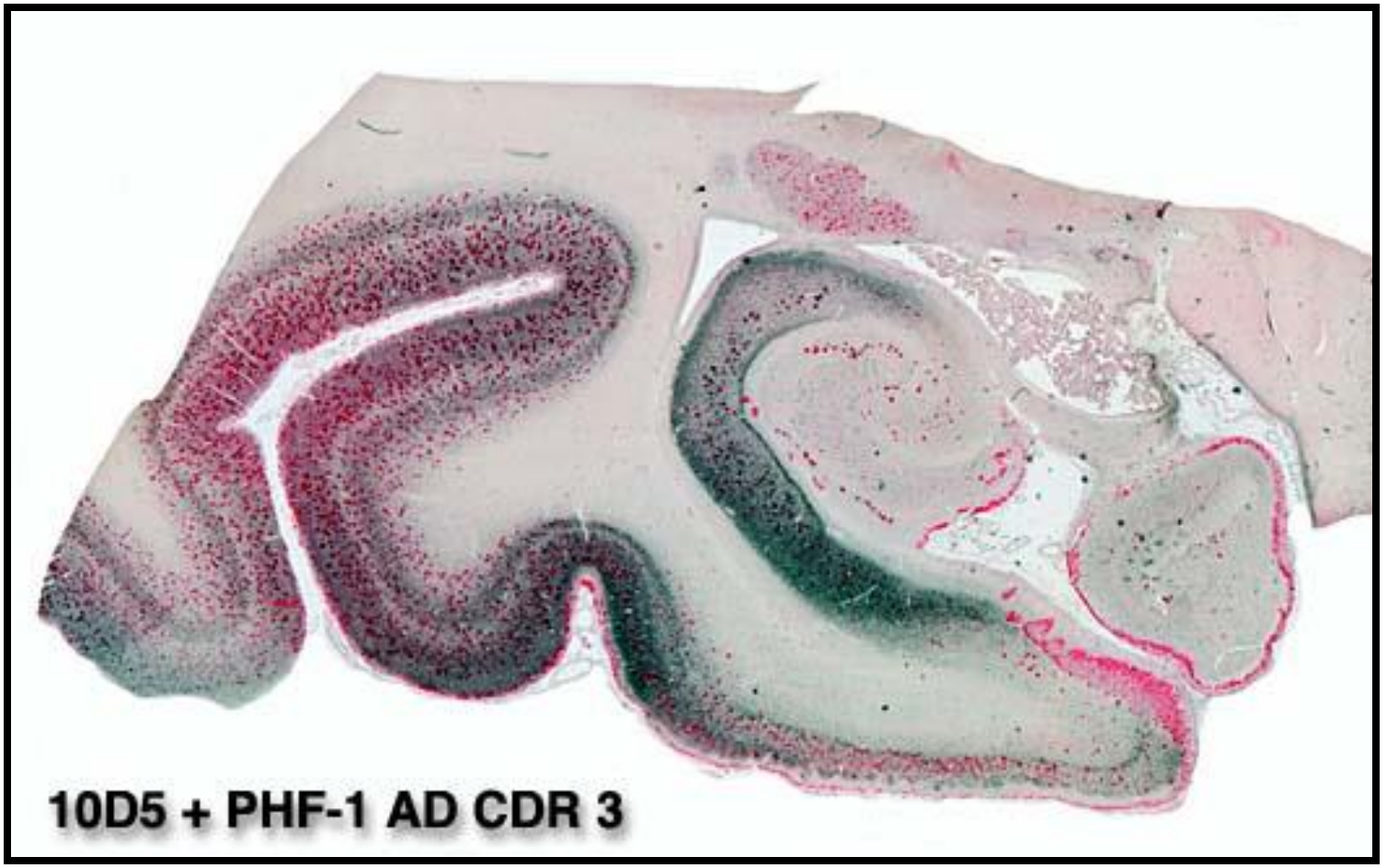
- At present no standardization exists in IHC methodology among ADRCs
- Includes fixation, embedding materials, pretreatment protocols, reagent sources, antibody working titers, substrates used, etc.
- Hence results vary non-systematically and adversely affect comparisons among results obtained at various centers.

CDR 0 Hipp: 10D5 Aß + PHF1



Braak & Braak Neurofibrillary STAGE III

CDR 3 AD Hipp: 10D5 Aß + PHF1



Braak Stage VI

There is lots of Work to do!

Standardization now will yield major dividends in the future.