

# HCV and the Brain

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Hepatitis C is a member of the  
*Flaviviridae* family .

This includes:

- Yellow fever
- Japanese encephalitis
- Dengue Fever
- Tick borne encephalitis
- GBV-A, GBV-B, GBV-C

Many of these viruses are tropic to:

- Liver
- Brain
- Peripheral blood mononuclear cells

What is the evidence for involvement of the brain in chronic hepatitis C?

# HCV and the Brain

- Clinical studies (SF36 well being scores);
- Computer based measures of cognitive function;
- MR spectroscopy studies of cerebral metabolism;
- Virological studies of brain tissue (quasi-species studies, presence of HCV –ve strand, immunohistology, laser micro-dissection PCR studies;
- In vivo PET studies of microglial activation.

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# Neuropsychological symptoms in CHC

Fatigue, weakness, tiredness

Joint pain, muscle pains, arthritis

Memory loss and mental confusion - (Brain Fog)

Skin problems - ie: dry, itchy, rashes, spots

Depression, anxiety, irritability, mood swings

Indigestion, nausea, vomiting, gas

Sleep disturbances, insomnia

Pain or discomfort in the abdomen

Chills, sweating, hot or cold flashes

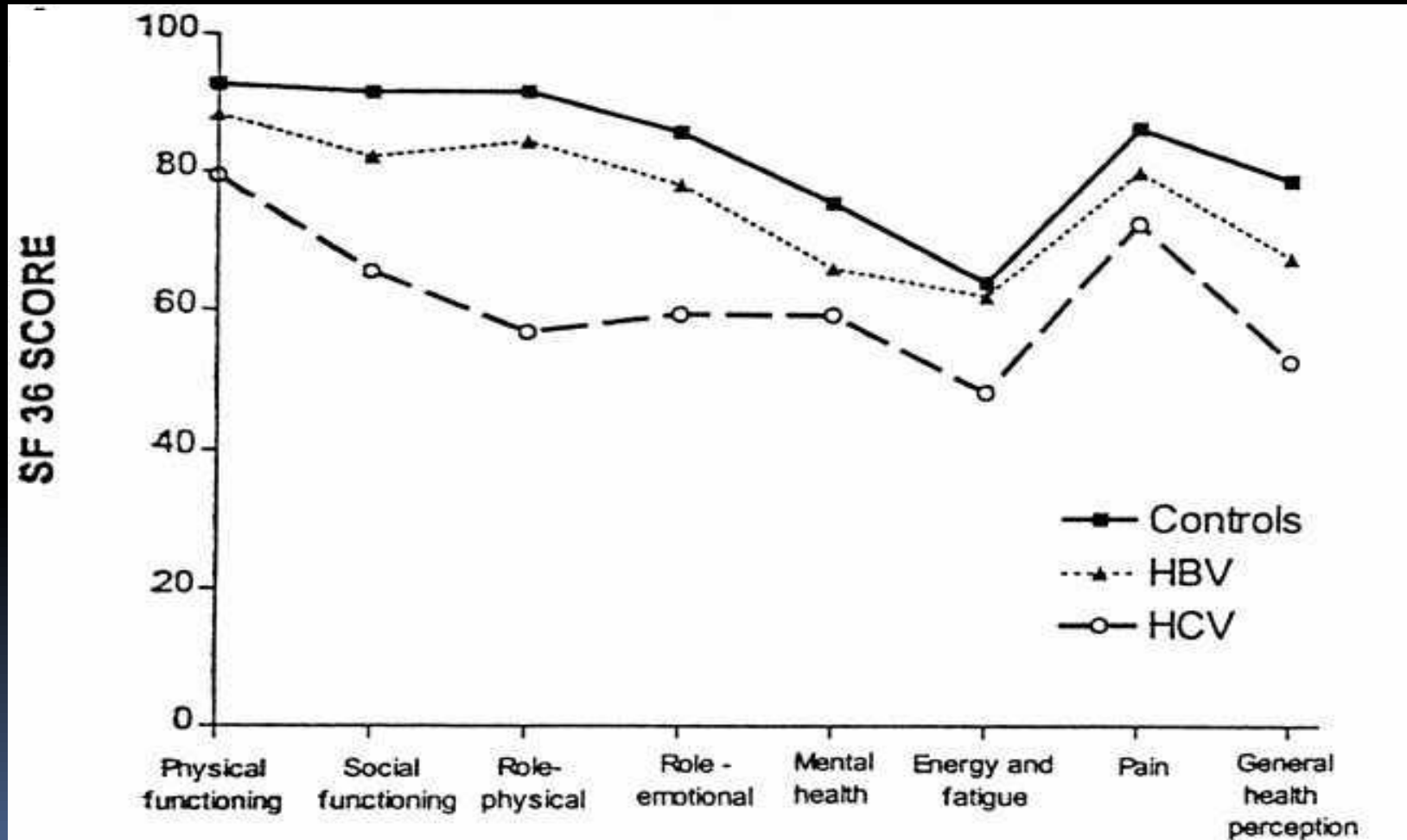
Eye or eyesight problems - (Blurred Vision/dry eyes)

Sensitivity to heat or cold, sweating

Vertigo, dizziness, coordination problems

Headaches

# SF36 well being scores



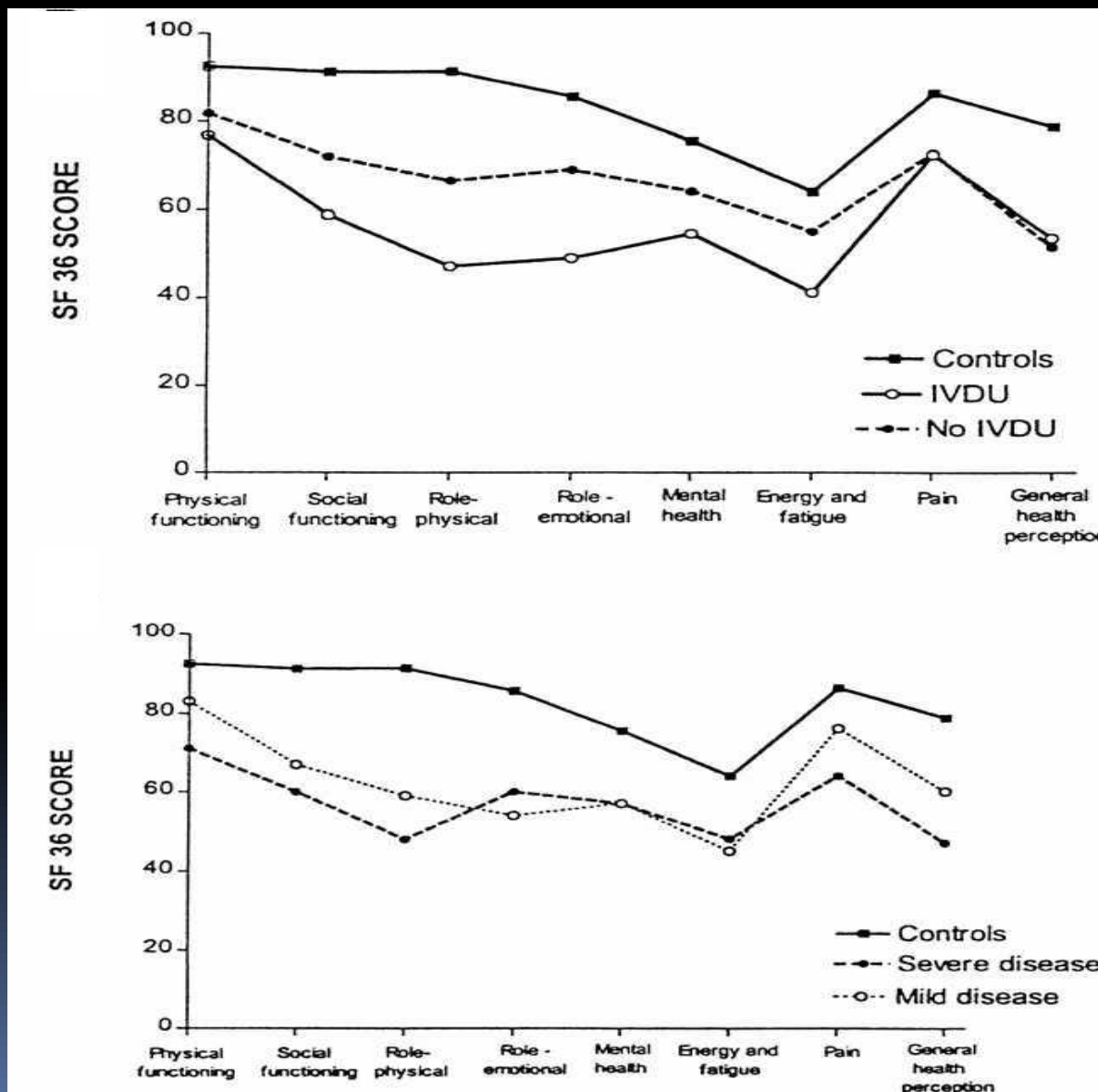
(Foster et al Hepatology 1998)

# Potential Confounding Factors in CHC.

Cognitive Function may be impaired by:

- cerebral effects of IVDU
- minimal hepatic encephalopathy
- effects of anxiety
- HCV infection (direct or indirect).

# SF36 Scores are not related to IVDU Use or severity of CLD?



# SF36 Scores: exclusion of confounding factors.

- Reduced SF36 scores regardless of knowledge of diagnosis

Rodger et al 1999

- SF36 scores improve after treatment

Bonkovsky et al 1999

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Patients with chronic HCV infection exhibit impaired cognitive function and have altered cerebral metabolism.

- **Formal cognitive testing shows mild impairment:**

*Forton DM et al. Hepatology 2002; 35:433*

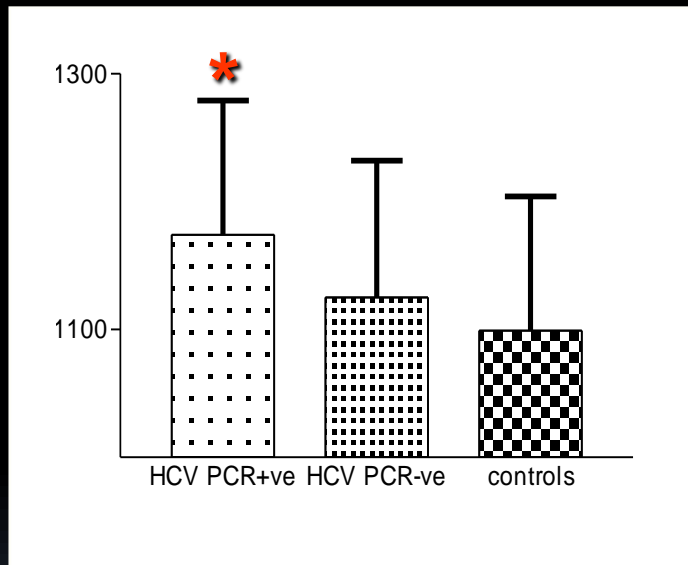
*Hilsabeck RC et al. Hepatology 2002; 35:440*

*Kramer L et al. J Hepatol 2003; 37:349*

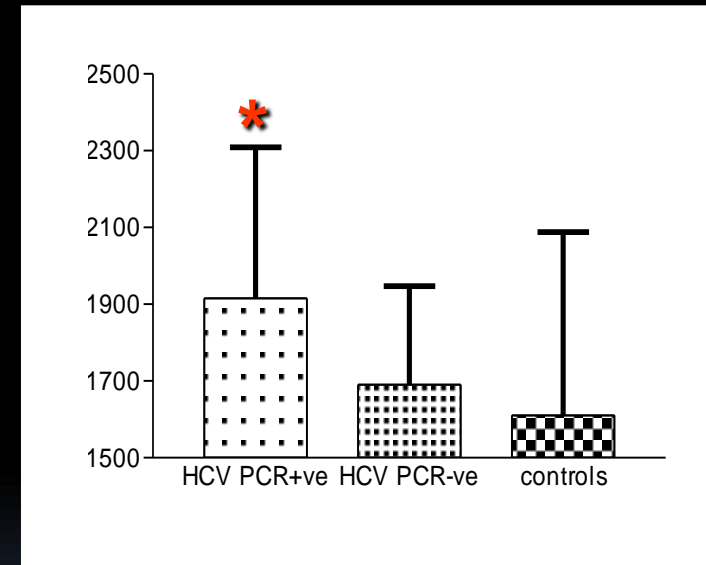
- **Abnormal cerebral metabolism using *in vivo* magnetic resonance spectroscopy**

*Forton DM et al. Lancet 2001; 7:38-9*

# HCV viraemic patients were impaired in the domains of attention, concentration and working memory



Power of concentration / ms



Speed of memory processes / ms

\*p=0.001 v controls

HCV PCR+ve patients were impaired on more cognitive tasks than PCR-ve patients: no difference between patients with and without a history of intravenous drug abuse (IVDU).

HCV PCR+ve	HCV PCR-ve	
2.15 (1.56)	1.06 (1.24)	P=0.02

IVDU+ve	IVDU-ve	
1.95 (1.83)	1.55 (1.18)	P=0.62

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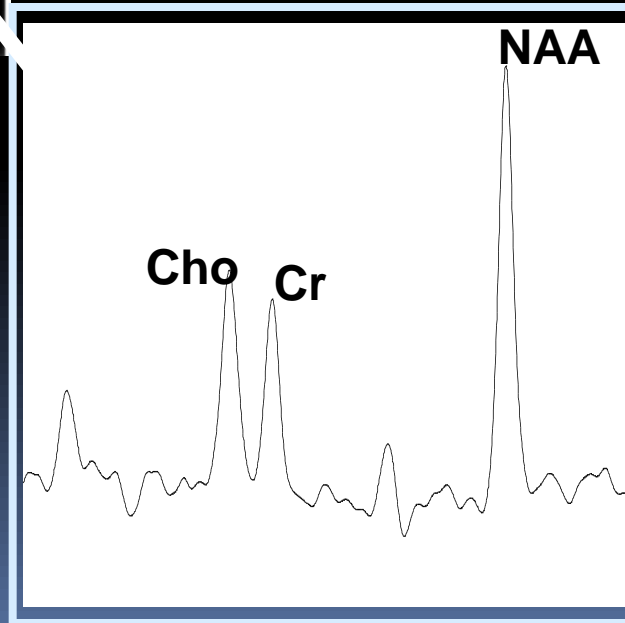
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# Normal $^1\text{H}$ MR spectrum of the Brain



**NAA - N-acetylaspartate**

marker of neuronal integrity

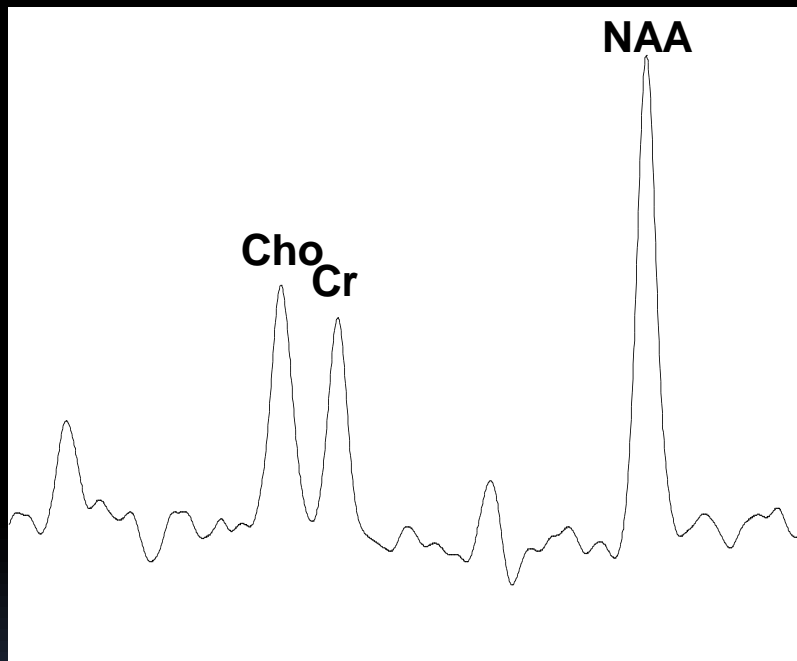
**Cho - Choline**

marker of cell membrane turnover

**Cr - Creatine**

# Hepatitis C and the Brain: evidence for metabolic abnormalities.

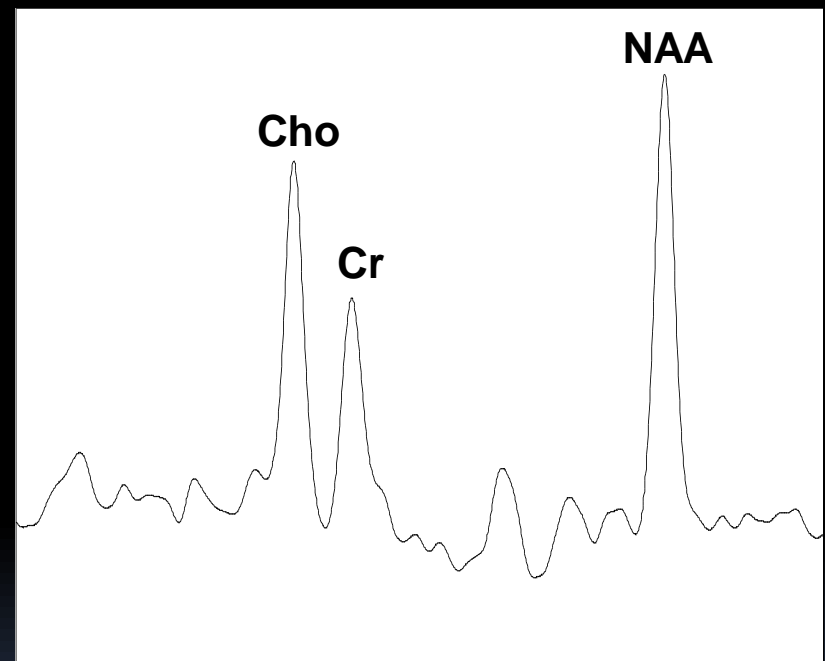
## $^1\text{H}$ - MRS results - white matter



**Normal Subject**

Mean Cho/Cr 1.18

**P= 0.001**



**Persistent HCV**

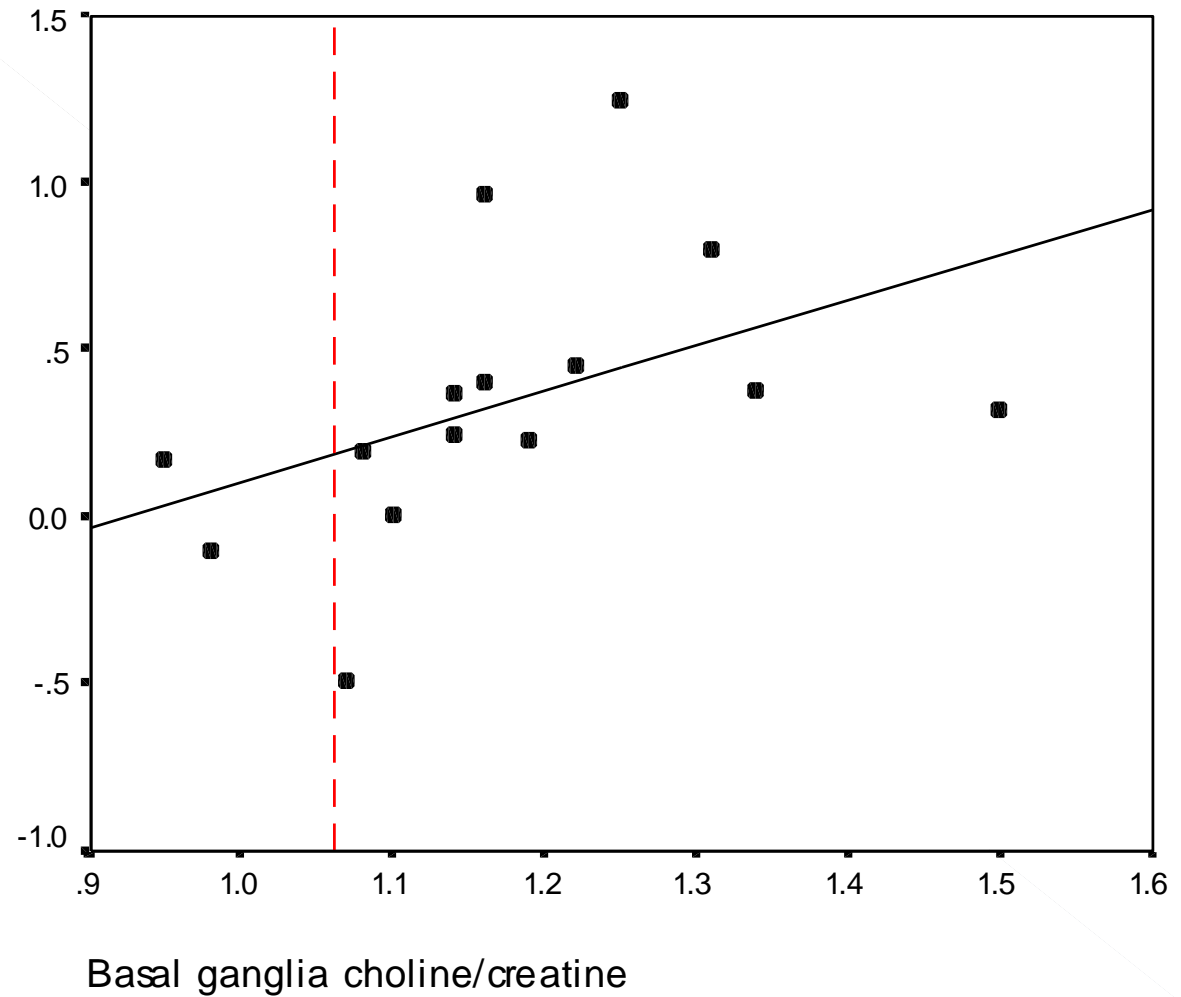
Mean Cho/Cr 1.48

**Forton et al Lancet 2001;358;38-9.**

# Hepatitis C and the Brain: MRS and Cognitive Abnormalities are linked.

$r=0.71$

$p=0.003$



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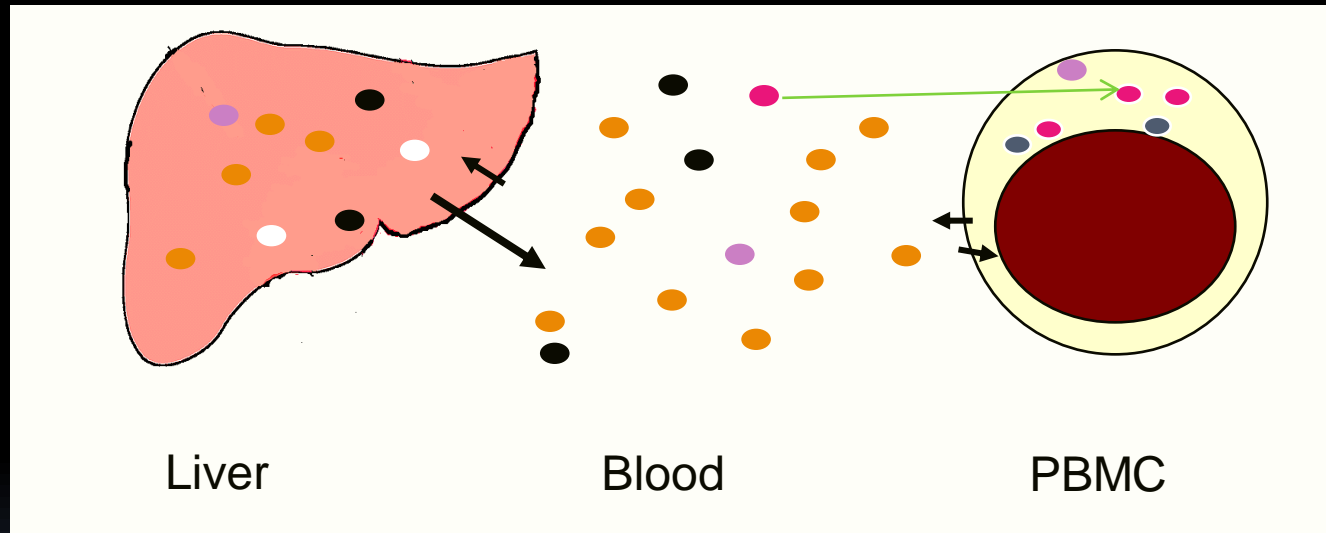
HCV sequences have been detected in brain tissue: quasi-species analyses.

- **Identification of brain-specific HVR1 variants**

*Forton D et al. Hepatology 2000; 32:269*

# Direct evidence for HCV replication in Extrahepatic sites (PBMC): quasispecies analysis.

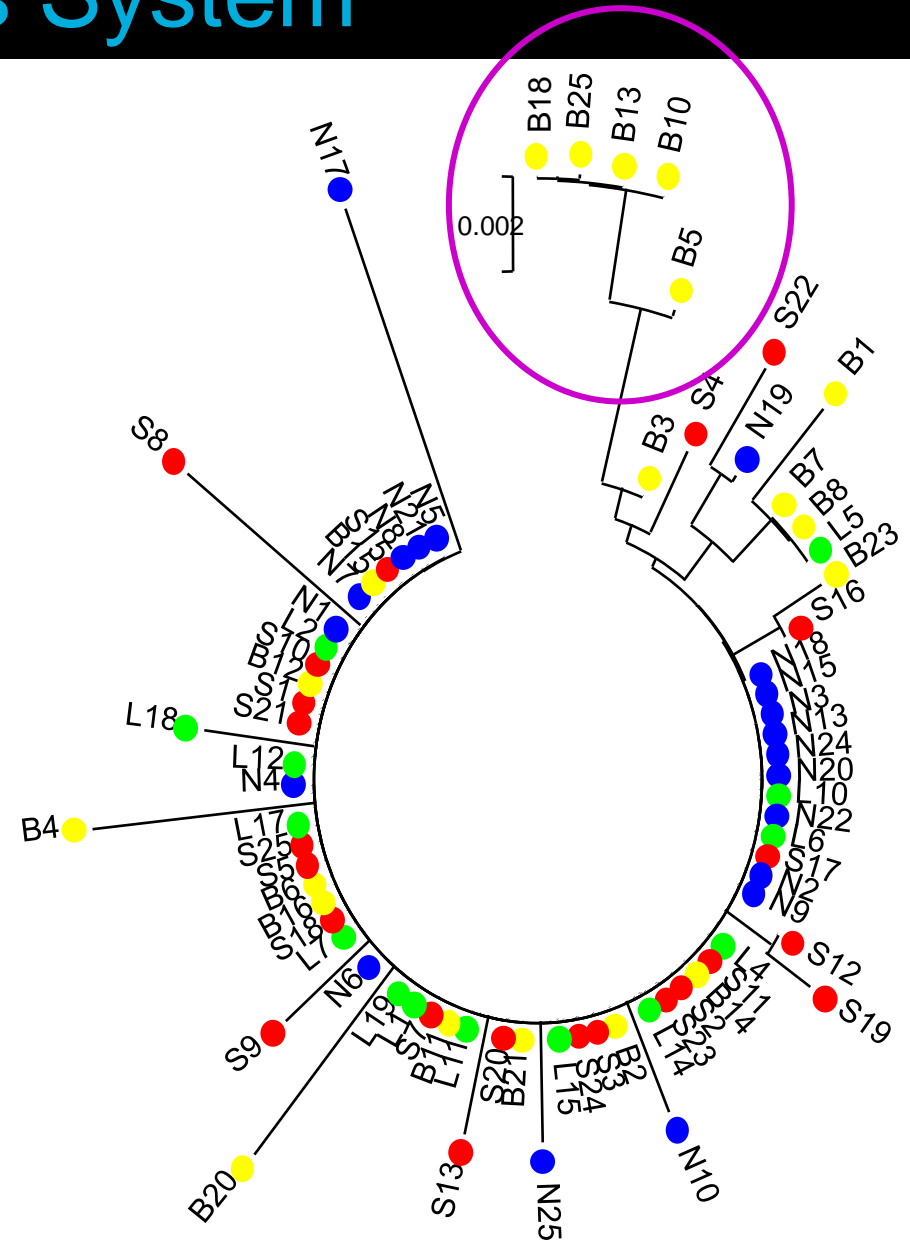
HCV generates quasispecies due to limited fidelity of viral RNA polymerase.



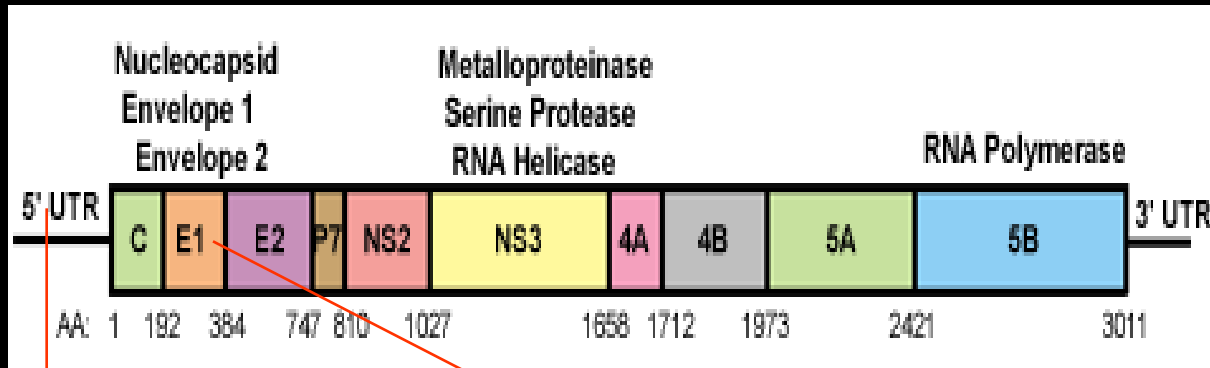
Distinct viral quasispecies present in PBMC

# Unique HCV quasispecies in the Central Nervous System

- Unique HCV IRES AND HVR1 variants were detected in the brain of 2 patients
- 40% (patient A) and 24% (patient B) of brain sequences were not seen in the other compartments
- Sequences from the brain were phylogenetically and phenetically distinct from serum-derived sequences



# HCV genetic regions of interest for quasispecies analysis.



Internal ribosomal entry site (IRES)

Mediates cap-independent translation of viral poly protein

? Role in cellular tropism

Hypervariable region 1 (HVR1)

? Selective adsorption

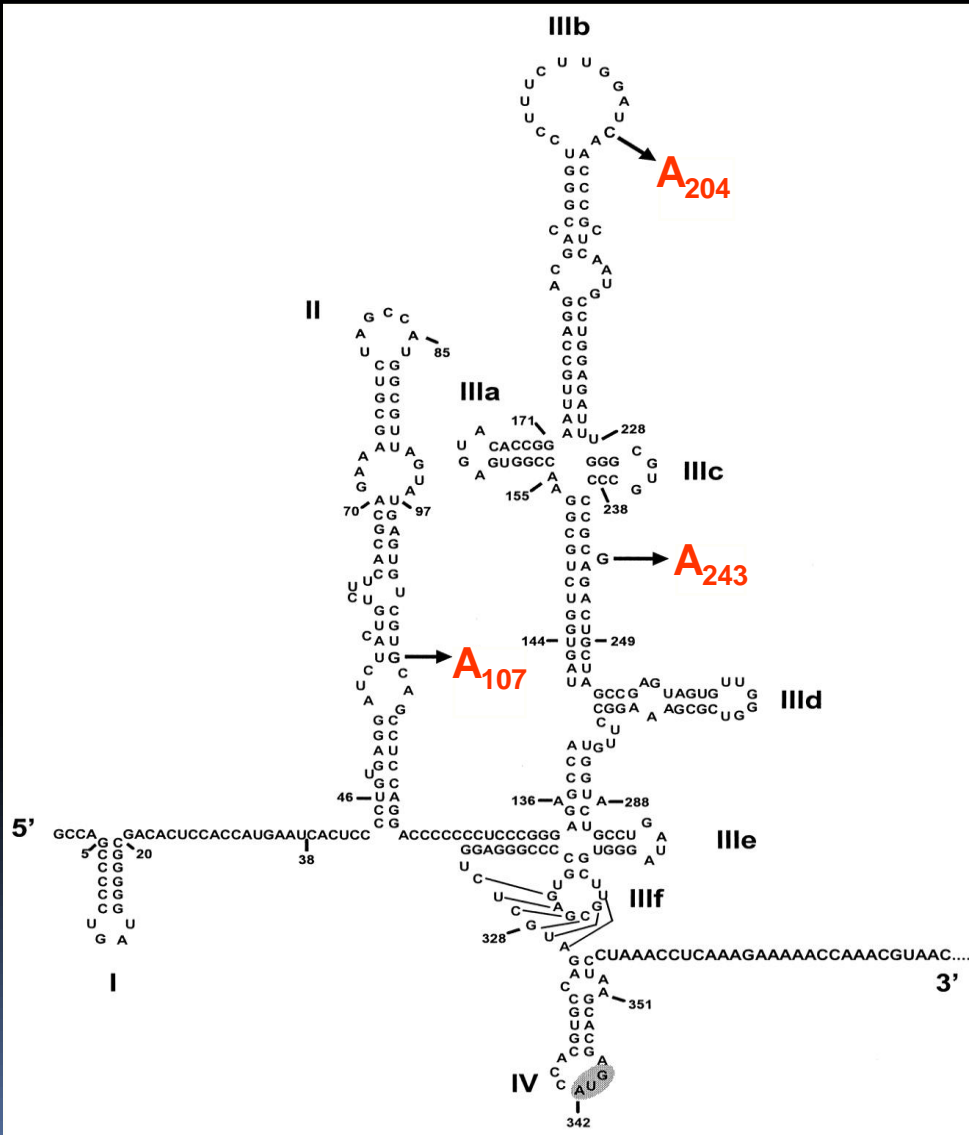
# Data indicating that HCV replicates in the brain.

- **Brain specific HCV mutations** were detected in HVR1 & a highly conserved region of the HCV genome (IRES).
  - **24% (patient 1) and 40% (patient 2) of sequences from the brain were not seen in the other compartments**, strongly suggesting that these sequences did not arise from blood contamination.

**Findings are consistent with HCV infection of CNS**

- **5% of the brain sequences contained the C/A (204) G/A (243) mutation, previously associated with lymphotropism** and enhanced translation in PBMC and lymphoid lines. (Forton et al 2004)

# Mutations in IRES derived from brain are same as those found in PBMC.



- **AAA** selected during viral passage through lymphoblastoid line

*Nakajima et al. J Virol. 1996;70:3325-3329*

- **AAA** confers increased translational efficiency in lymphoblastoid lines

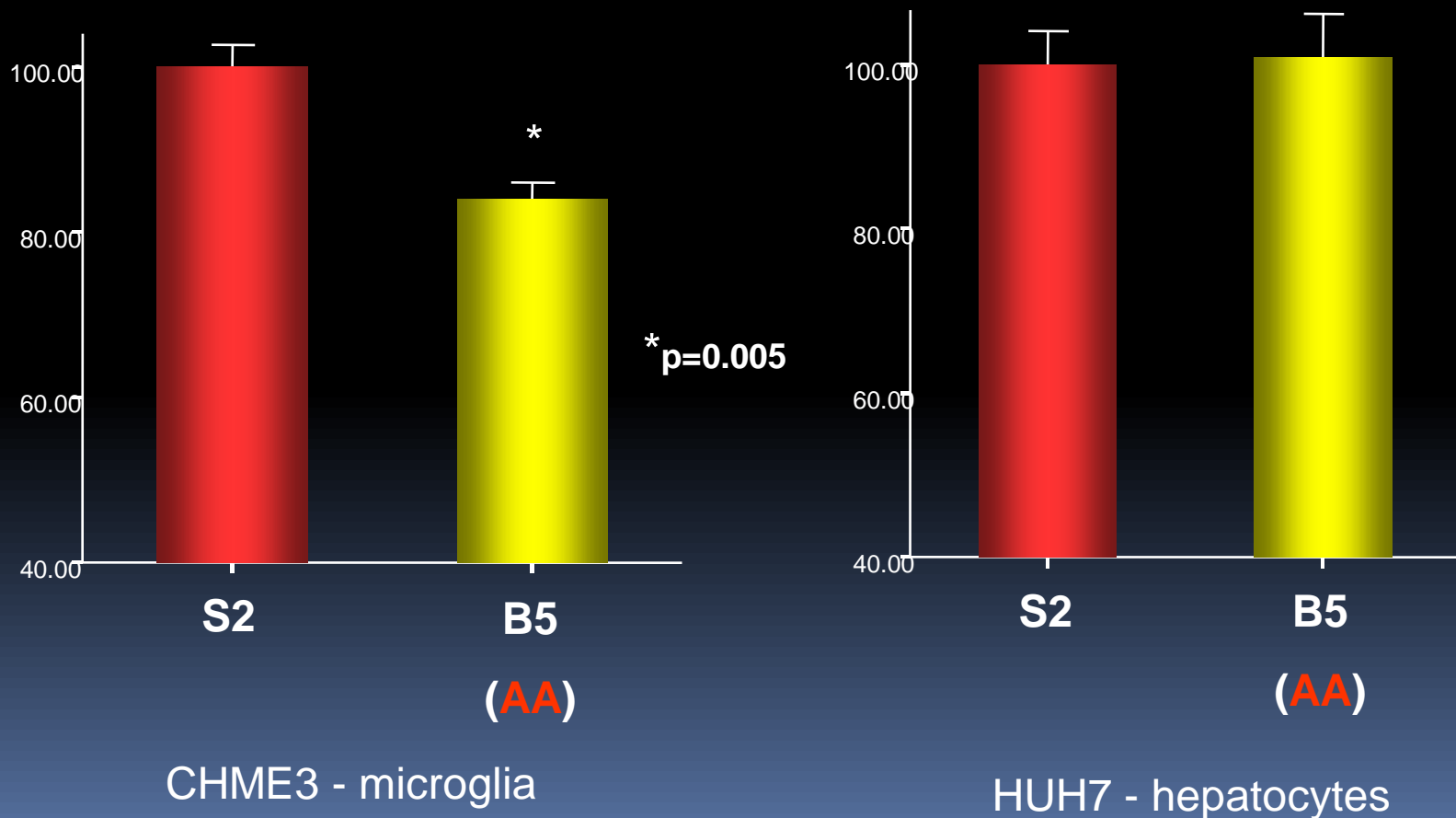
*Lerat et al. J Virol 2000;74:7024-31*

- **-AA** sequences detected in PBMC and dendritic cells

*Laskus et al. J Infect Dis 2000;181:442-8*

*Laporte et al. Blood 2002 epub*

# Brain derived IRES variant (A(204) A(243)) is functional in hepatocytes & microglia.



HCV sequences have been detected in brain tissue and quasi-species analysis and presence of negative strand suggest replication in the CNS.

- **Identification of brain-specific HVR1 variants**

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- **Detection of HCV negative-strand sequences in the CNS**

*Radkowski M et al. J Virol 2002; 76:600-8.*

- **Microdissection of microglial cells reveals negative strand HCV**

*Wilkinson et al 2010*

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# Post-mortem Tissue Studies.

- **Immuno-histology:**

HCV in astrocytes and microglia of HIV co-infected subjects (Letendre et al; J Infect Dis 2007 196 368-370).

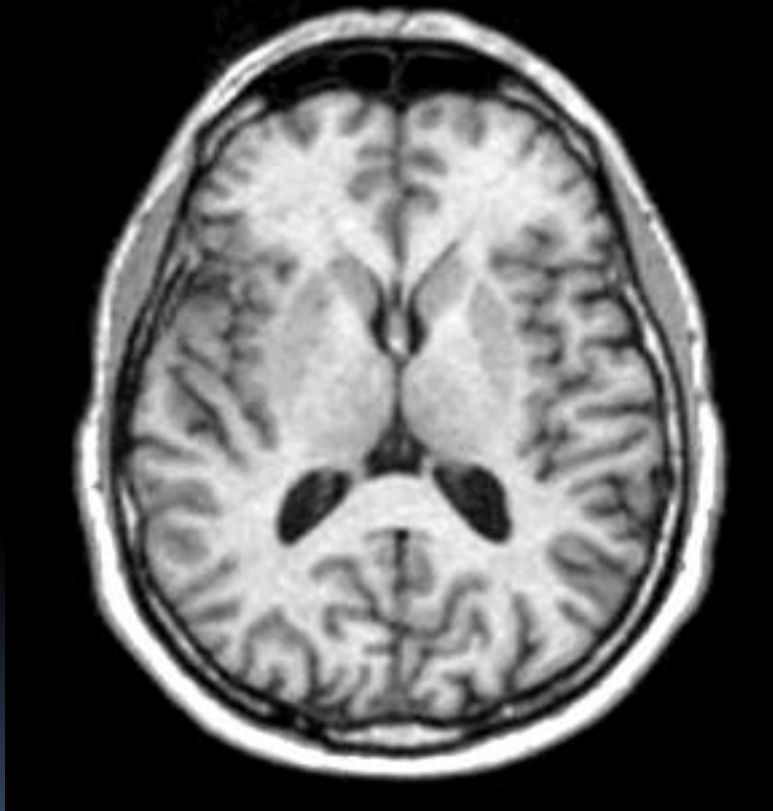
- **Micro-dissection RT-PCR studies:**

negative-strand HCV RNA has been detected in microglia/macrophages derived from autopsy brain tissue of HCV-infected patients (J Wilkinson et al 2010).

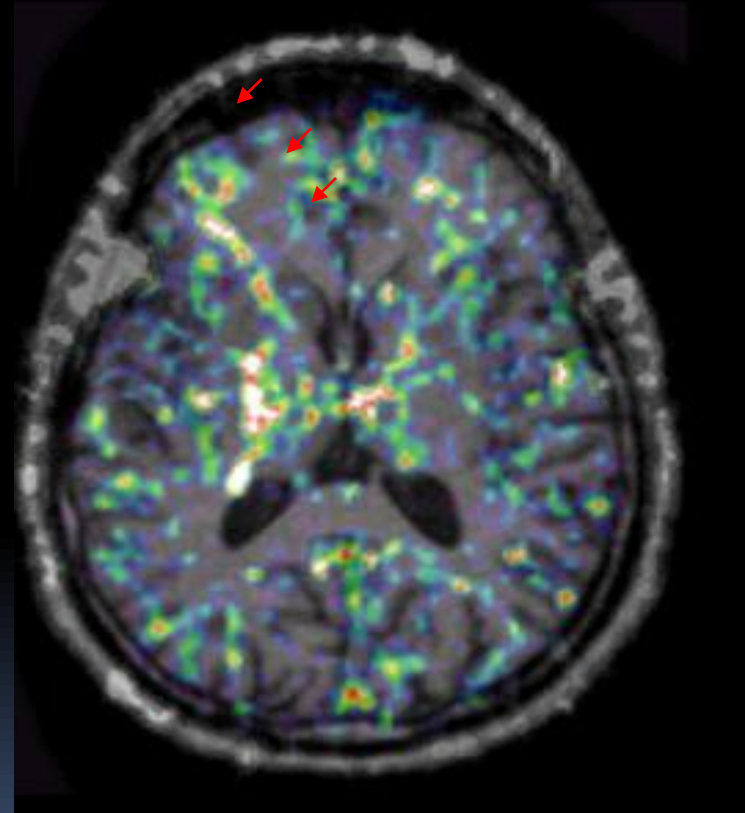
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# PET Scans of Brain using Ligand that binds to Activated Microglia in CHC.

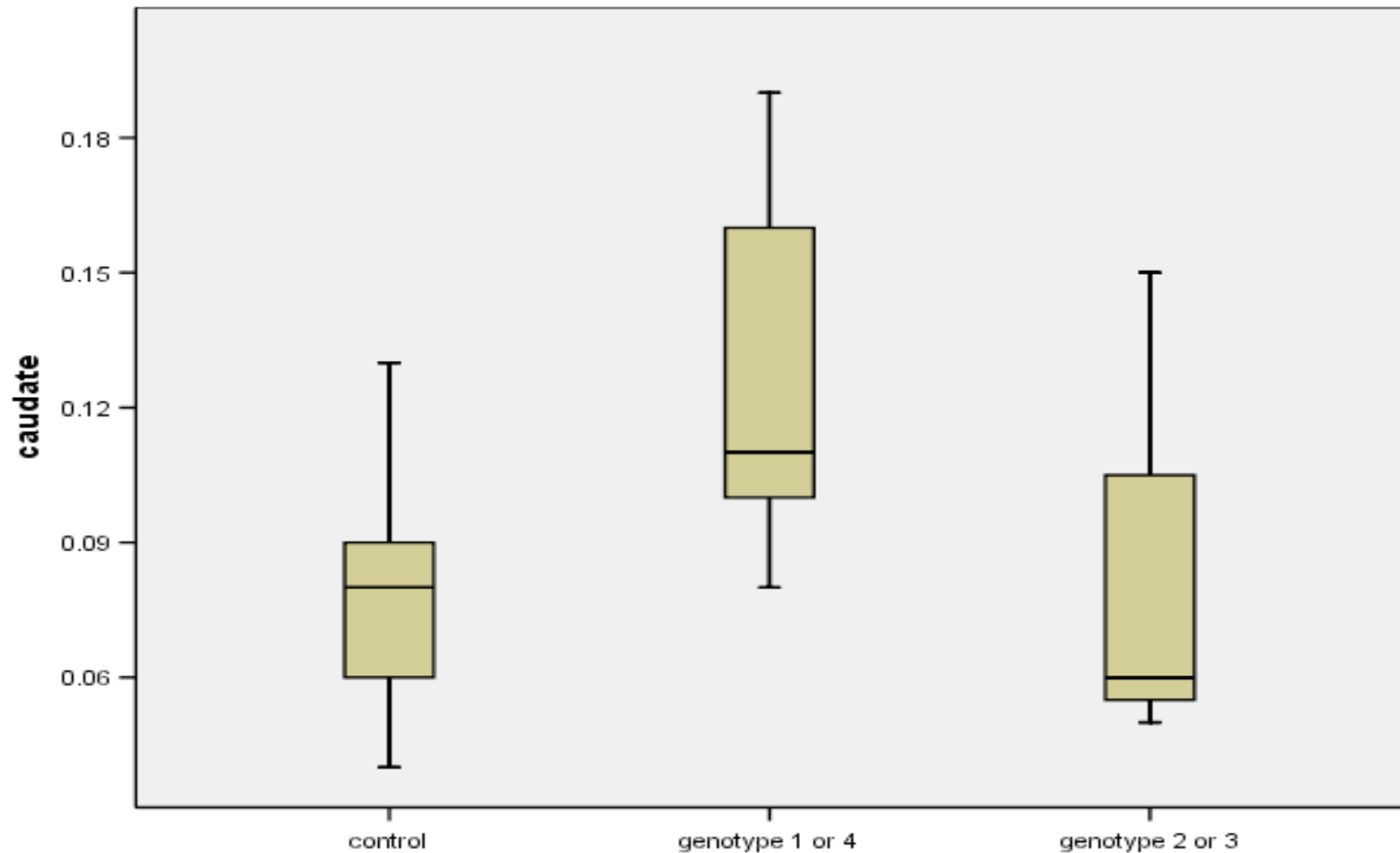


T1-weighted MRI



PK11195-PET  
superimposed on MRI

# HCV and the Brain: PET scans for PBL Receptor Up-regulation.



# Which CNS cells can be infected?

## Studies in Cell lines.

- **Neuroepithelioma** (SK-N-MC; SK-PN-DW)) cell lines **do** express CD81, SR-B1, Claudin-1 and occludin, to allow viral entry and support HCV replication (NF Fletcher et al Gut 2010 139: 1366-1374).
- **Neuroblastomas, gliomas, astrocytomas do not** express claudin-1, do not allow viral entry and do not support viral replication.
- **Microglial** cell lines **do not** express occludin and cannot be infected.

# Possible Explanations of Differences between in vivo and in vitro data.

- Inability to infect microglia in vitro may be due to de-differentiation of microglial cells in culture, resulting in loss of appropriate receptors for HCV entry;

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## Possible Explanations of Differences between in vivo and in vitro data.

- Inability to infect microglia in vitro may be due to de-differentiation of microglial cells in culture, resulting in loss of appropriate receptors for HCV entry;
- Monocytes and microglia may take up HCV by a different mechanism to hepatocytes (FcR uptake);

# Potential Implications of Uptake of HCV via Fc Receptors.

- *Fc receptors are present on monocytes, dendritic cells and microglia* and may allow uptake of anti-HCV coated HCV;

**Note:** Anti-envelope (HVR1 &2) antibodies are produced late in acute infection (after 3 months) but are present throughout chronic infection and therefore

**if uptake of the virus into monocytes (and microglial cells in the brain) does occur by Fc receptors, infection of the brain will not occur in acute but will occur in chronic infection.**

# What is the clinical significance of HCV Replication in the Brain?

- **In chronic infection:** is relapse after IFN monotherapy due to infection of the brain?
- **In acute HCV infection:** is the infrequency of relapse after monotherapy with interferon (Jaeckel et al 2001) due to the absence of HCV infection of the brain?

# Does Ribavirin prevent relapse by Inhibiting HCV Replication in Monocyte Lineage Cells?

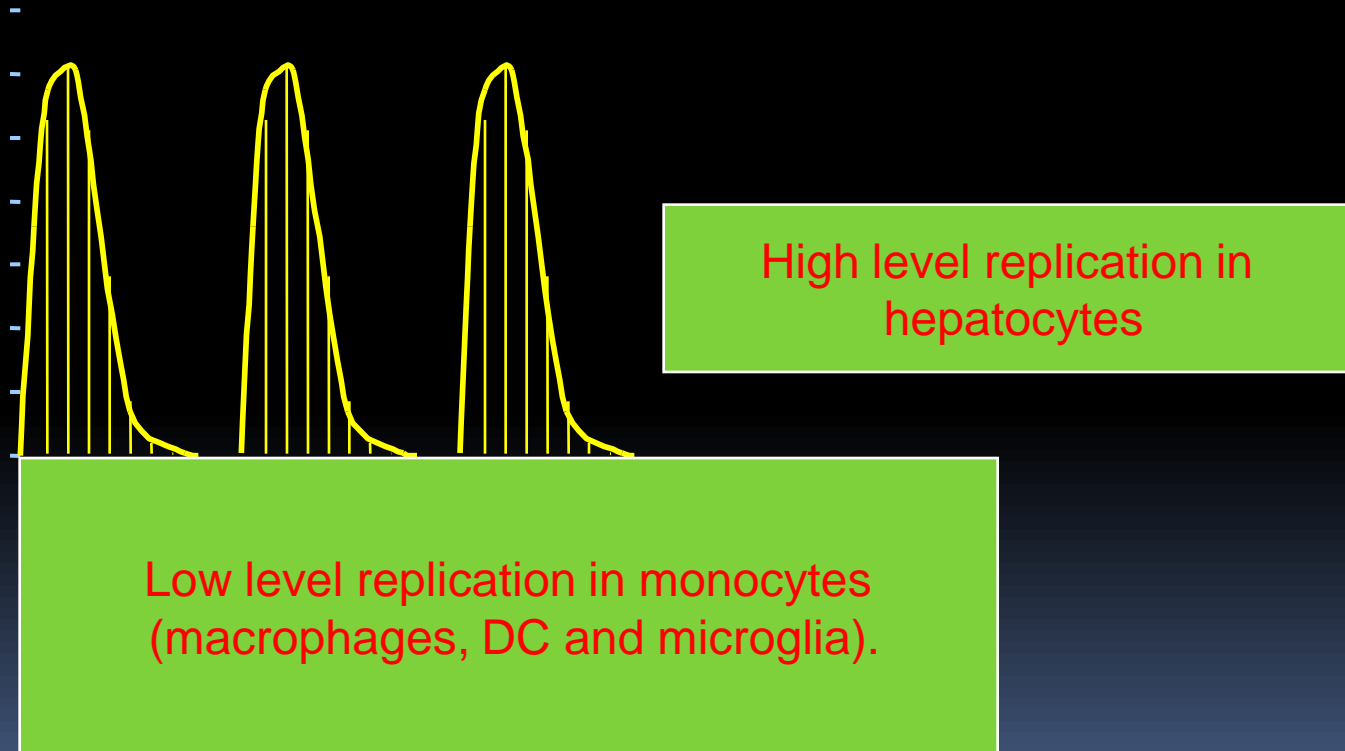
- **Inosine monophosphate dehydrogenase (IMPDH)** is the rate limiting enzyme controlling the synthesis of guanosine in the monocyte and lymphoid lineages but not in the hepatocyte;
- **Ribavirin is an IMPDH inhibitor** and because of its high lipid solubility may enter the basal ganglia controlling HCV replication in this site thereby preventing relapse;
- **Why did the other IMPDH inhibitors not work** –possible issues around bioavailability in the brain?
- **Viramidine** which is activated in the liver is not effective in preventing relapse (Poordad et al 2010)
- **Guanosine is readily available in the liver and therefore de novo synthesis by IMPDH is not rate limiting for HCV replication at this site, where most of the virus is produced.** Thus the IMPDH inhibitory activity of ribavirin has little effect on levels of total HCV viraemia (Dusheiko et al 1996).

# Questions to be Answered.

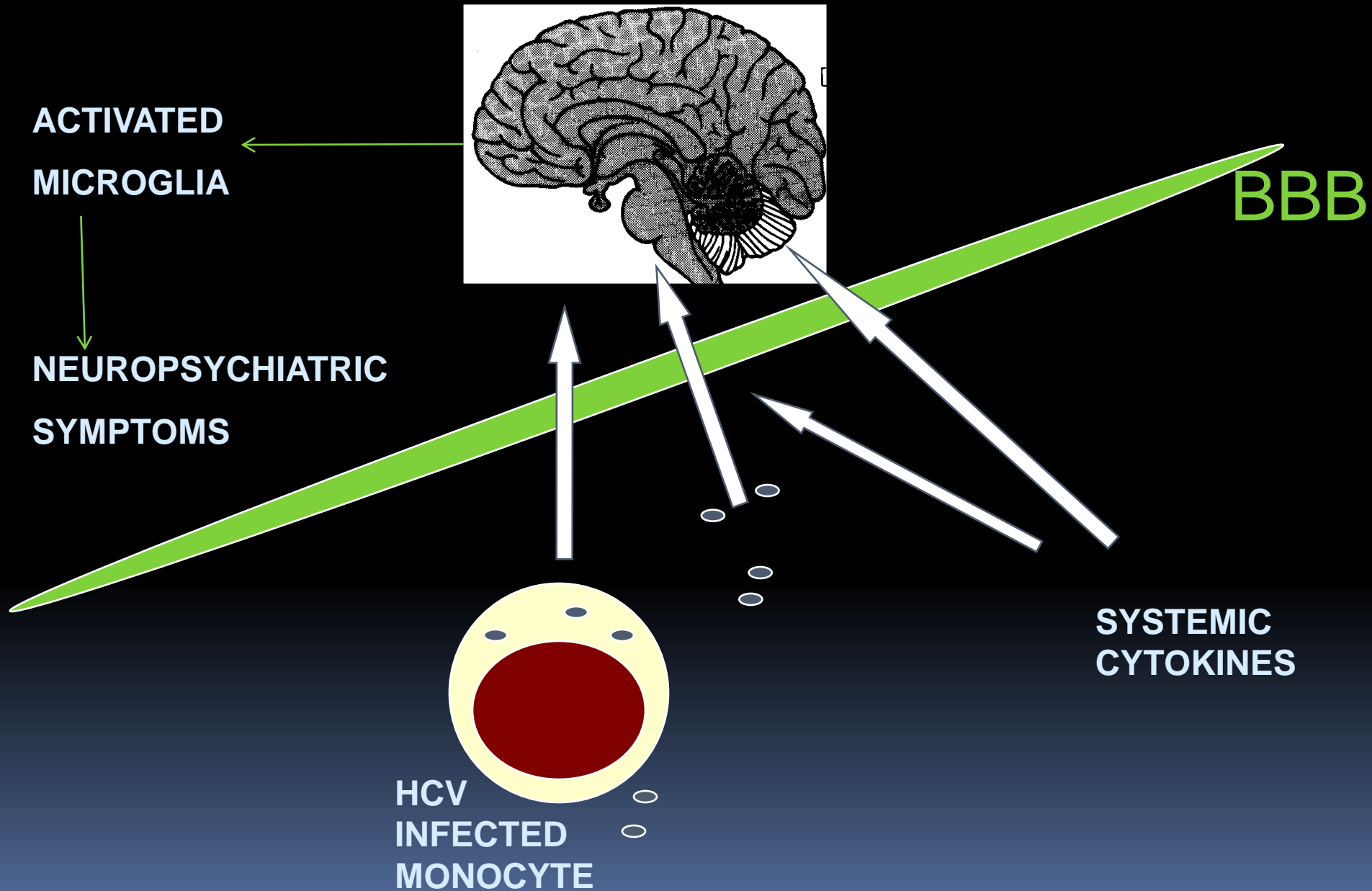
- Does HCV infection of the brain occur in chronic but not acute HCV infection? We need PET studies in acute hepatitis C.
- Does ribavirin, an IMPDH inhibitor, prevent relapse after IFN treatment of CHC because it is lipophylic, crosses the BBB, and inhibits HCV replication in the brain? We need drug distribution studies in man.

If this is the case, ribavirin will be necessary for oral IFN-free DAA therapy to prevent relapse (Lok et al 2011 AASLD Abstract; Gane et al 2011 AASLD Abstract 34)

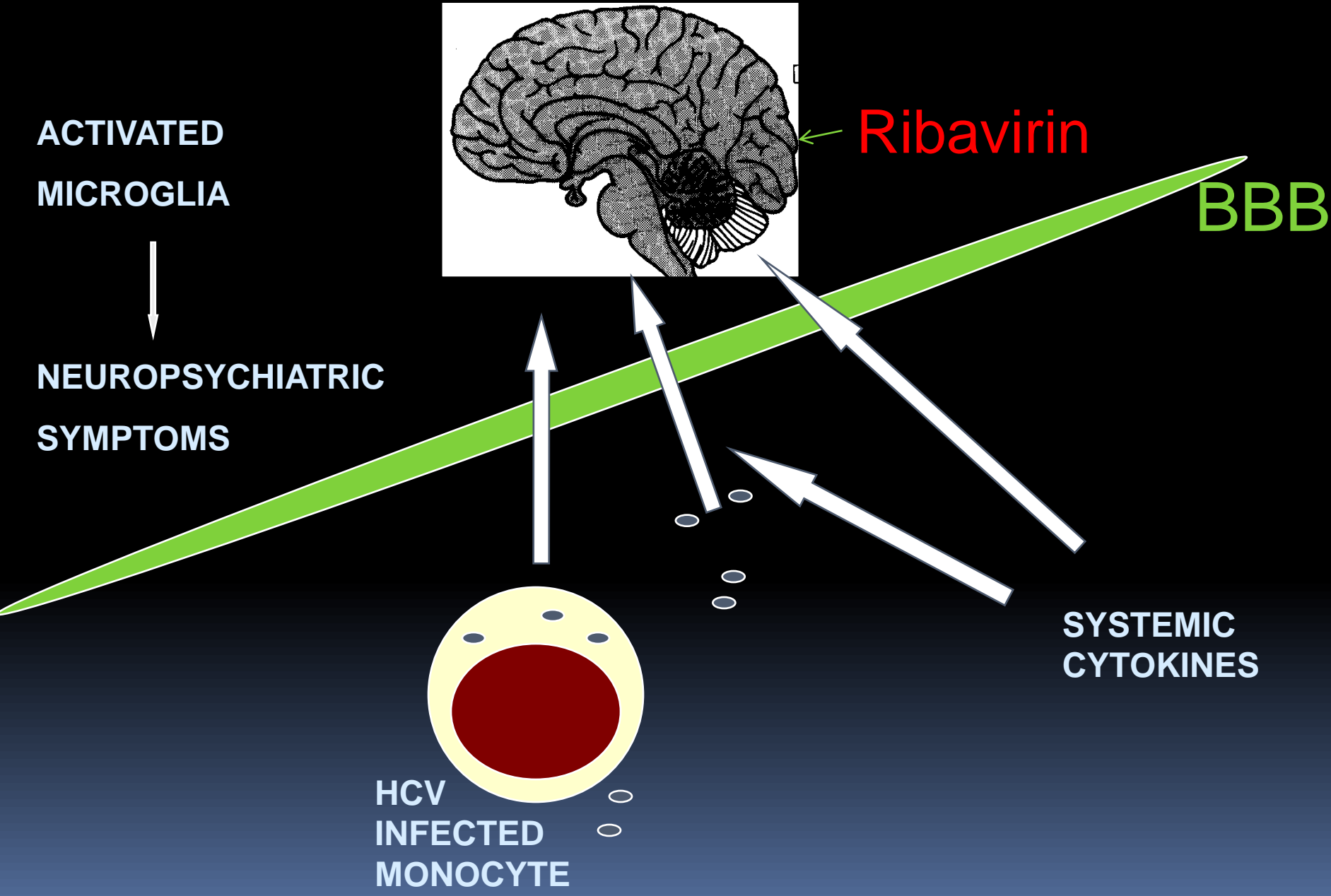
# Model of HCV replication in hepatocyte and monocyte lineages.



# CHC: HCV Infects Brain Microglial Cells.



# Does Ribavirin Treat HCV Infection in Brain?



# Acknowledgements

- Imperial College:
  - Dan Forton
  - Peter Karayiannis
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  - Bob Grover