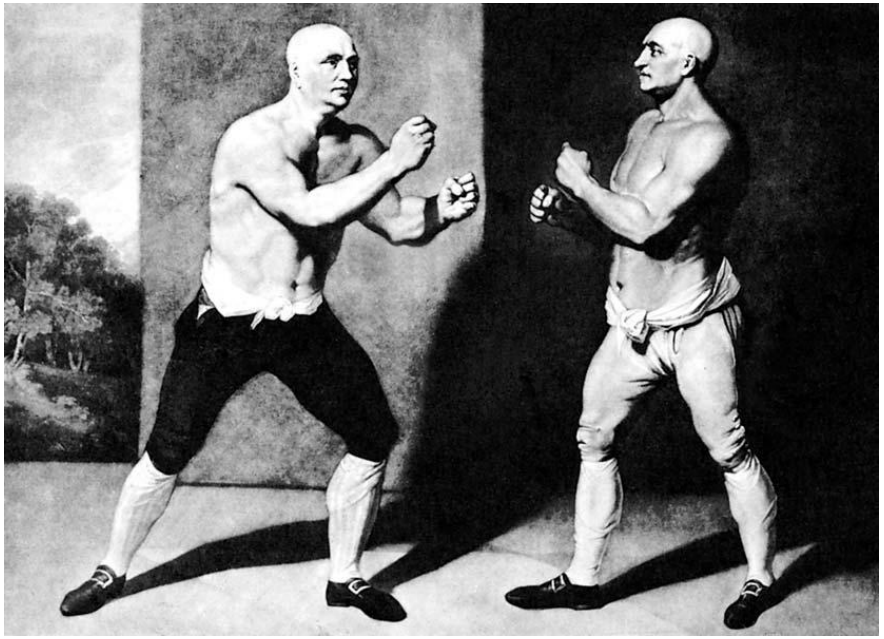
Molecular Biomarker approach to TBI and CTE

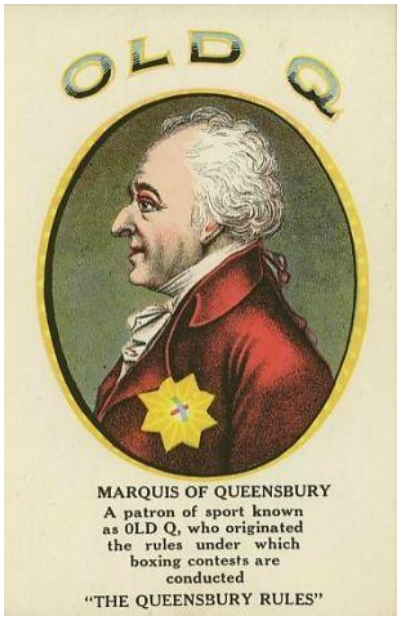
Muhammad Ali vs. Sonny Liston
1965

Ali at the opening ceremony at the Olympic Games in Atlanta
1996

Boxing – history

A





John 'Jack' Broughton (1704–1789)

- Introduced the first boxing rules in 1743
  - Broughton Rules
- Including: ring with ropes, the first type of gloves

Marquis of Queensberry

- Founded "the Amateur Athletic Club" in 1866
- Introduced the Queensberry rules
- Including: boxing gloves
  time-limited rounds,
  10 sec. count after knockout

Differences between amateur and professional boxing

4 rounds of 2 min	(4-) 10-12 rounds of 3 min
Helmet obligatory Gloves: 284 g	No helmet Gloves: 227 or 284 g
Referee stops contest (RSC) In an uneven bout Used generously	Technical knockout (TCO) In an uneven bout Not commonly used

Similarities in the rules in amateur and professional boxing

NOT allowed

Knockout

Hasim Rahman KO
by
Lennox Lewis

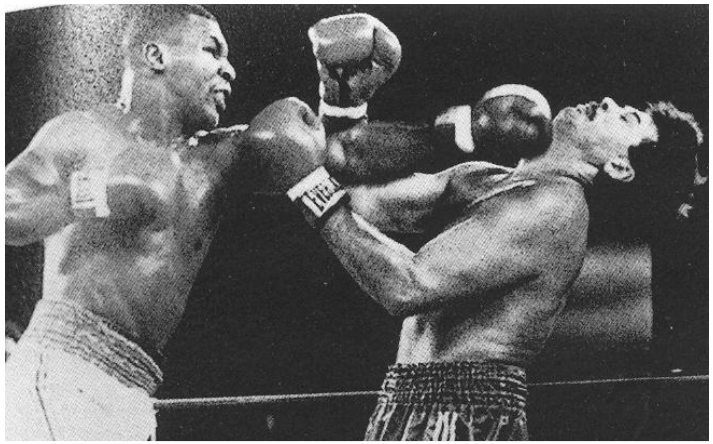
Knockout = concussion with loss of conciousness after a hit to the head

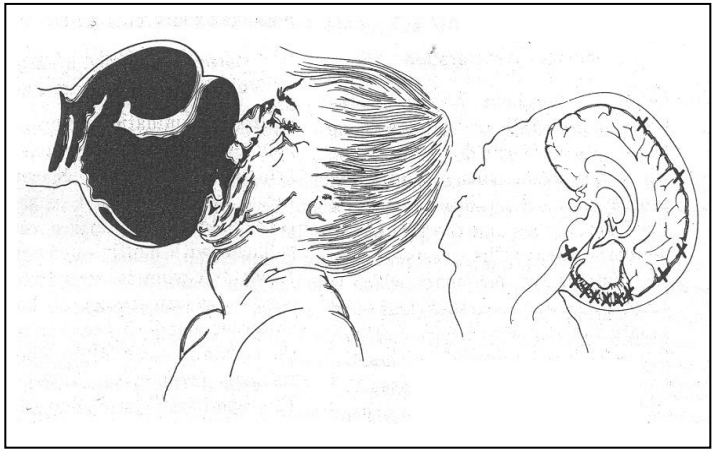
Due to a severe disturbance in nerve cell function:

- **stretching of axons**
- **deregulated flux of ions and release of excitatory neurotransmitters**
- **energy crisis: depleted energy stores / disturbed autoregulation**

Types of punches and mechanisms for brain damage

Linear acceleration

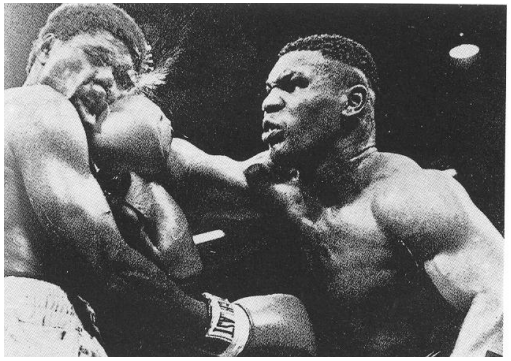




Rotational acceleration

Lampert, JAMA 1984

Chronic traumatic encephalopathy in boxers



Punch drunk syndrome	*Martland, 1928*
Dementia pugilistica	*Millspaugh, 1937*
Chronic traumatic brain injury in boxers (CTBI-B)	*Jordan, 2000*
Chronic traumatic encephalopathy (CTE)	*Omalu 2011, Stern, 2011*

- Clinical symptoms variable, but are a combination of:

  cognitive e.g. poor attention, memory problems
  neurological e.g speech problems, impaired coordination, parkinsonian symptoms
  behavioural e.g. disinhibition, aggressiveness, paranoia

- Prevalence of severe TBE in professional boxers

  17% *Roberts, 1969*
  23% *Ross, 1987*

- Professional boxers - 16% have symptoms in their daily life,
  e.g. headache, visual and hearing disturbances, shaky hands, and forgetfulness

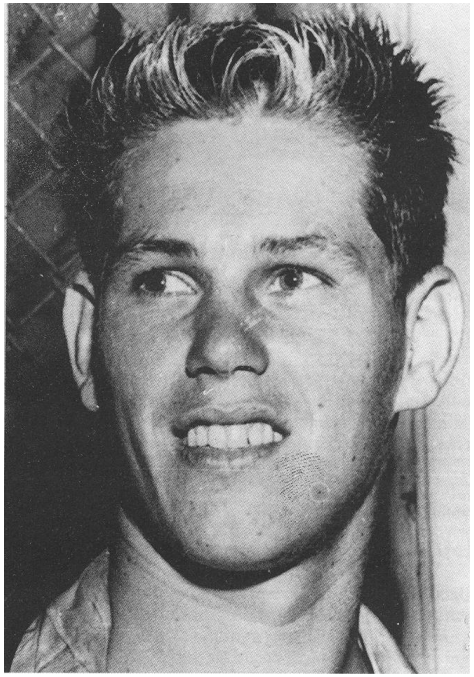
  Ohhashi, 2002

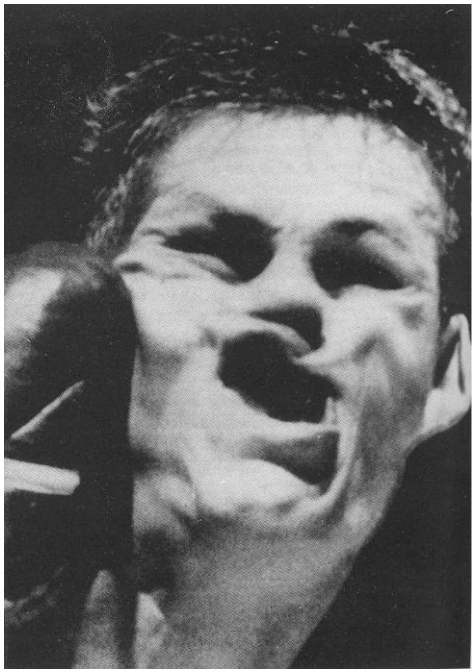
Chronic brain injury in professionals – risk factors

Early start of boxing career (<20 years of age)
Long boxing career (> 10 years)

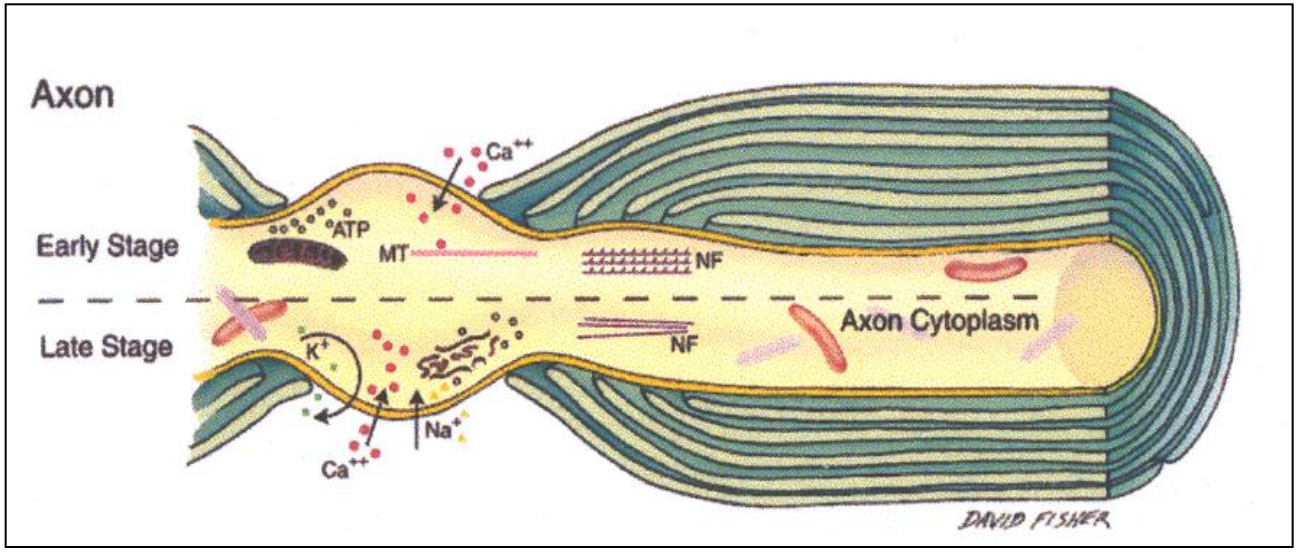
High number of bouts
High exposure to sparring

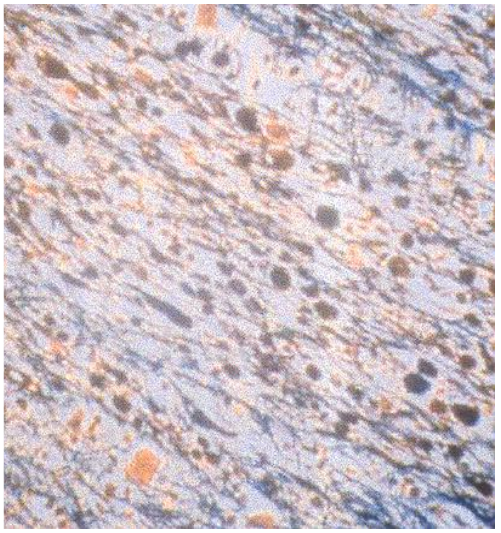
High number of knockouts
Poor performance as a boxer
Ability to take many punches without being knocked out





Central mechanism in traumatic brain injury: diffuse axonal injury (DAI)





Diffuse axonal injury

- Tearing of axons with axolemmal disruption
- Calcium influx, releae of excitatory amino acids
- Impaired metabolism, depleted energy stores
- Neurofilament compaction and microtubule disassembly
- Accumulation of transported molecules and organelles with axonal retraction balls
- Axonal disconnection and axotomy

Giza, 2001; Meythaler, 2001; Barkhoudarian, 2011

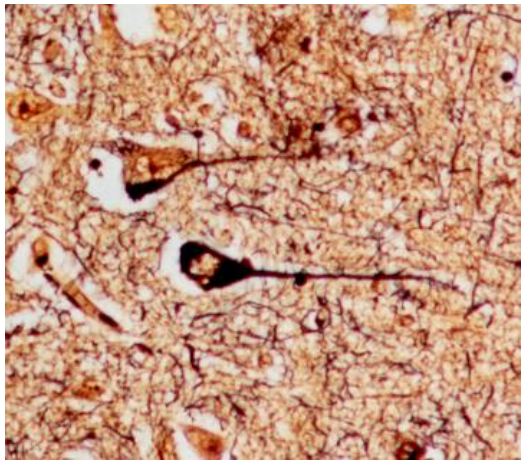
Tangles in retired professional boxers

Psychological Medicine, 1973, 3, 270-303

The aftermath of boxing[1]

J. A. N. CORSELLIS, C. J. BRUTON, AND DOROTHY FREEMAN-BROWNE[2]

From the Department of Neuropathology, Runwell Hospital, Wickford, Essex



Neuropath study on temporal cortex

20 boxers	age 22-83 years
20 controls	age 55-90 years

Temporal cortex →

Neurofibrillary tangles

Grading of tangles:

+++	35 %
++	30 %
+	20 %
No	15 %

- **Tangles and neuropil treads are structurally identical to those in AD** *Tokuda, 1991*
- **Unevenly distributed, and preferentially found in outer cortical layers** *Hof, 1992*
- **Composed of phosphorylated tau protein** *Tokuda, 1991*

β-amyloid plaques in retired boxers

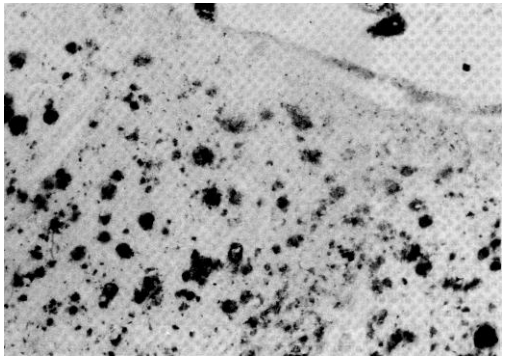
Journal of Neurology, Neurosurgery, and Psychiatry 1990;**53**:373–378

The occult aftermath of boxing

Gareth W Roberts, David Allsop, Clive Bruton

Neuropath study on temporal cortex

20 boxers age 22-83 years
20 controls age 55-90 years



Temporal kortex →

β-amyloid positive plaques

Grading of plaques:

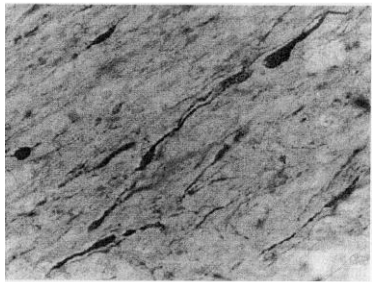
+++	**45 %**
++	**40 %**
+	**10 %**
None	**5 %**

APP and β-amyloid after traumatic brain injury

βA4 amyloid protein deposition in brain after head trauma

G. W. ROBERTS S. M. GENTLEMAN
A. LYNCH D. I. GRAHAM

Lancet 1991; **338**: 1422–23.

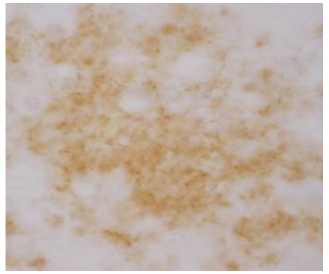


- **Increase in APP with accumulation in damaged axons**

  McKenzie, 1994; Sheriff, 1994; Gentleman, 1995
  Algren, 1996; Gleckman, 1999

- **Accumulation of BACE1 and presenilin, followed by accumulation of Aβ in damaged / swollen axons**

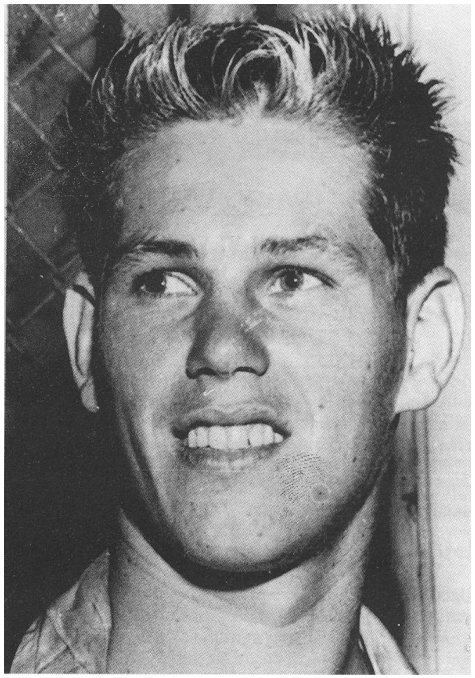
  Smith, 1999; Chen, 2004; Tran 2011

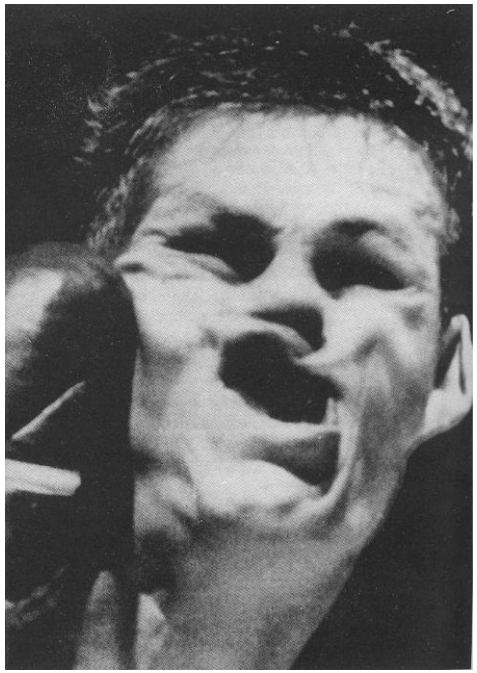


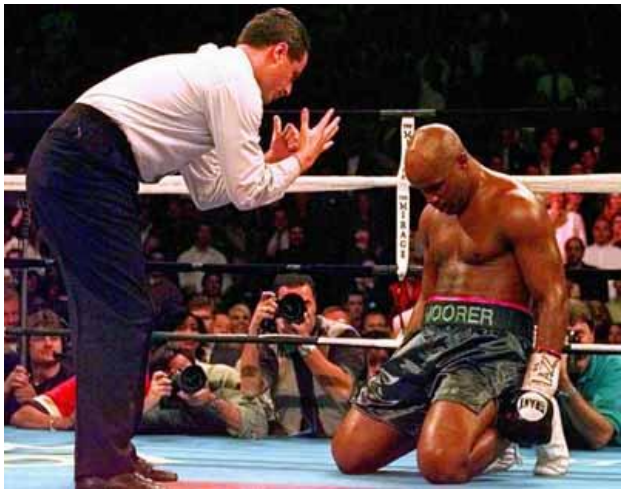
- **Release of β-amyloid from the axons, with aggregation into diffuse plaques, primarily composed of Aβ42**

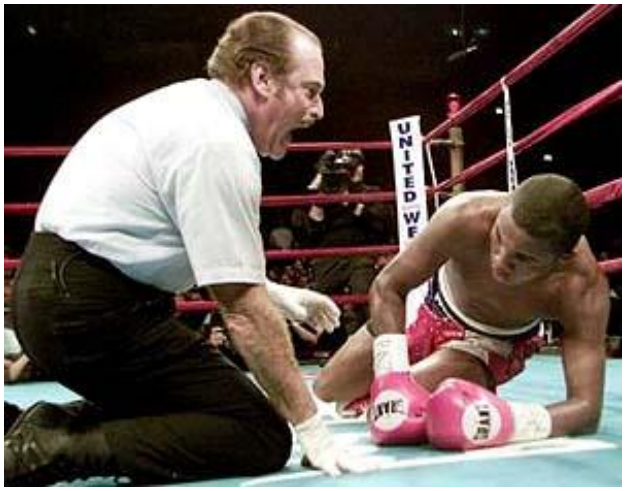
  Roberts, 1991; Gentleman, 1993; Graham, 1995;
  Horsburgh, 2000; Smith, 2003

Can acute brain damage in boxers be identified and monitored using CSF biomarkers ?

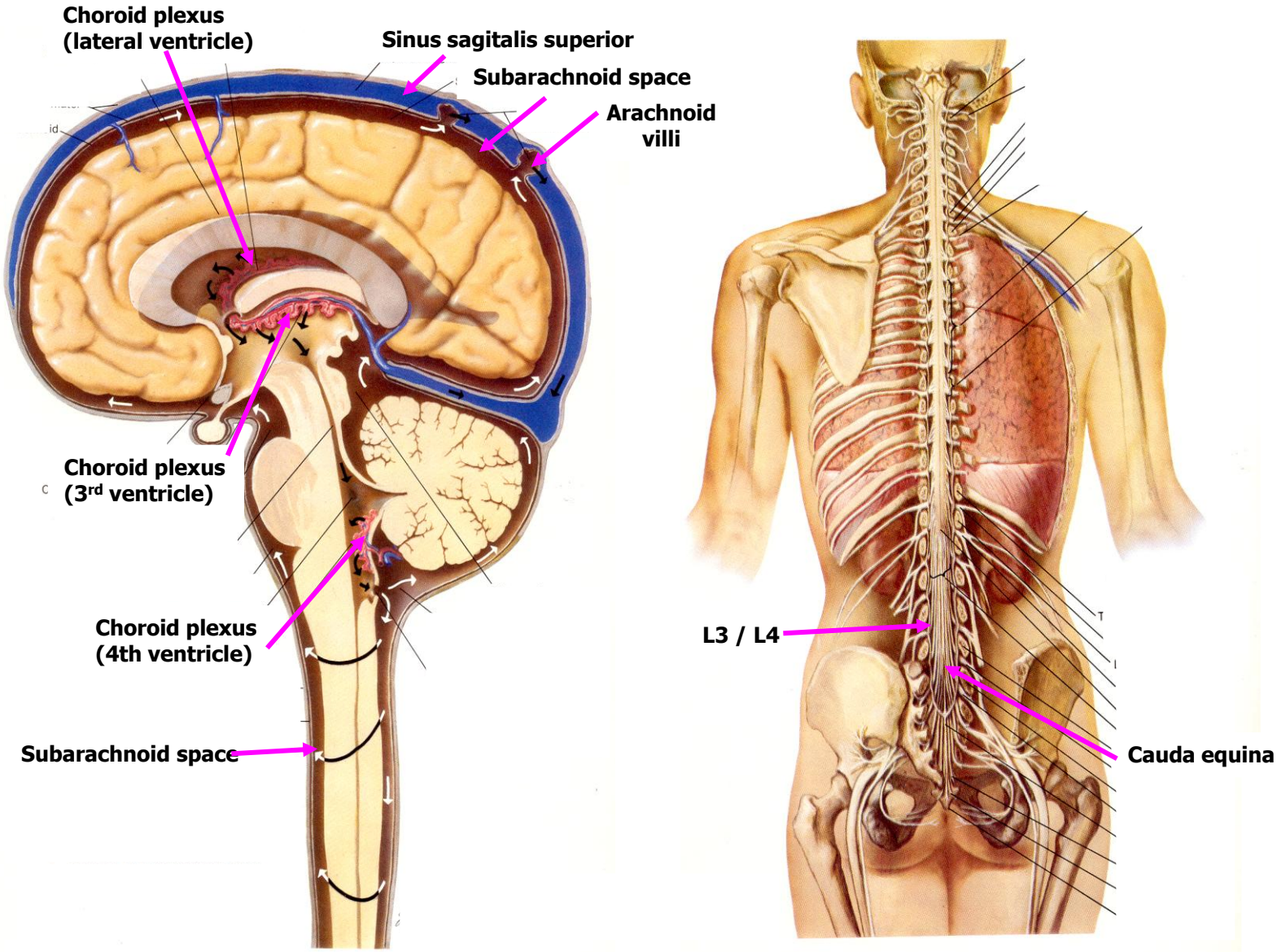




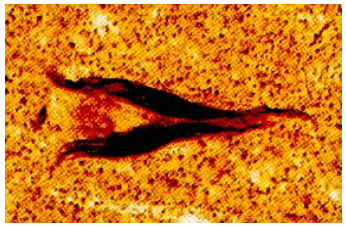




CEREBROSPINAL FLUID (CSF)



CSF biomarkers for pathogenic processes in brain disorders

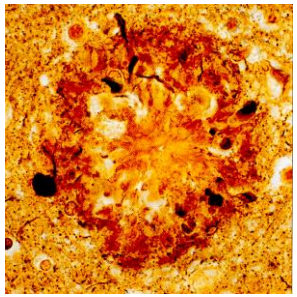


Tangles

Phospho tau

Axonal damage

NFL– long white matter axons
Tau – grey matter axons



Plaques

APP and β-amyloid

Glial damage

GFAp
S-100β

Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury

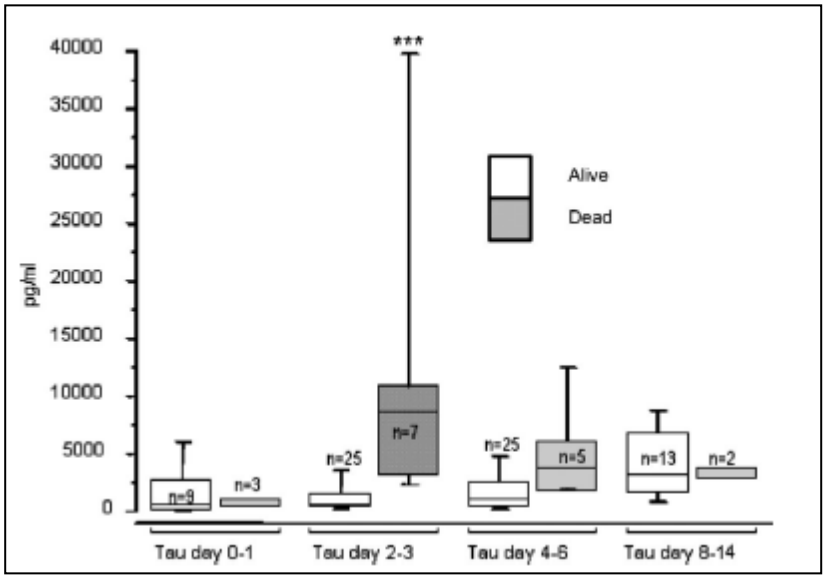
M. Öst, MD; K. Nylén, MD; L. Csajbok, MD; A. Olsson Öhrfelt, PhD; M. Tullberg, MD, PhD; C. Wikkelsö, MD, PhD; P. Nellgård, MD, PhD; L. Rosengren, MD, PhD; K. Blennow, MD, PhD; and B. Nellgård, MD, PhD

NEUROLOGY 2006;67:1600–1604

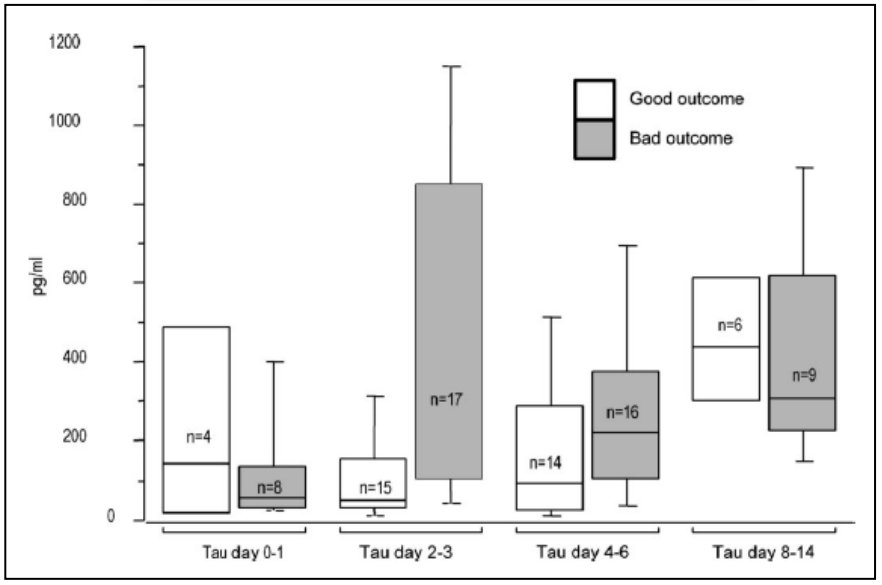
- **Study design:** 39 patients with severe TBI (aged 16-82 years)
  Glasgow Coma Scale <8 at admission
  Ventricular catheter to reduce ICP
  1-year clinical follow-up (of survivors)

V-CSF samples Day 0-1, 2-3, 4-8, and 9-14

VCSF tau in relation to acute survival



VCSF tau in relation 1-year outcome



J Neurol (2004) 251 : 870–876
DOI 10.1007/s00415-004-0451-y

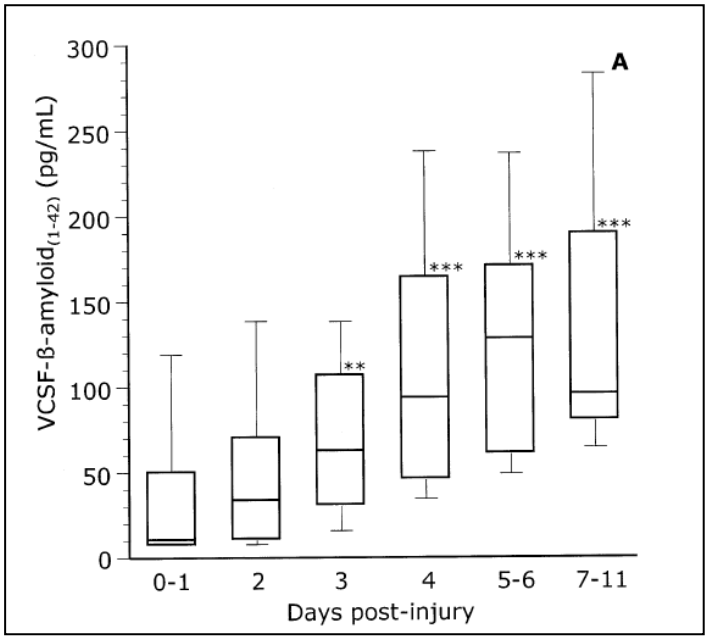
ORIGINAL COMMUNICATION

Annika Olsson
Ludvig Csajbok
Martin Öst
Kina Höglund
Karin Nylén
Lars Rosengren
Bengt Nellgård
Kaj Blennow

Marked increase of β-amyloid$_{(1–42)}$ and amyloid precursor protein in ventricular cerebrospinal fluid after severe traumatic brain injury

- **Study design:** 28 patients with severe TBI (aged 15-81 years)
  Glasgow Coma Scale <8 at admission
  Ventricular catheter to reduce ICP

V-CSF samples Daily (if possible) during 11 days



	VCSF-Aβ$_{(1–42)}$ (pg/mL)	p-Aβ$_{(1–42)}$ (pg/mL)	VCSF-α-sAPP (µg/L)	VCSF-β-sAPP (µg/L)
Day 0–1	11 (7.8–52) (n = 12)	56 (24–67) (n = 13)	18 (18–273) (n = 10)	1185 (333–2129) (n = 10)
Day 2	34 (9.9–76.5) (n = 17)	47 (28–74) (n = 9)	156 (90–318)* (n = 13)	1524 (1207–1860) (n = 13)
Day 3	63 (31–109)** (n = 21)	52 (31–68) (n = 11)	244 (219–549)* (n = 19)	1961 (1560–2205) (n = 18)
Day 4	94 (45–174)*** (n = 18)	48 (29–70) (n = 9)	221 (127–536)* (n = 14)	1813 (1342–2236) (n = 16)
Day 5–6	129 (60–171)*** (n = 20)	57 (37–68) (n = 11)	262 (150–663)* (n = 15)	1887 (1437–2202)* (n = 20)
Day 7–11	96 (79–196)*** (n = 20)	44 (28–67) (n = 14)	366 (180–579)** (n = 17)	1865 (1676–2174)* (n = 18)

Study on CSF biomarkers in boxers

ORIGINAL CONTRIBUTION

Neurochemical Aftermath of Amateur Boxing

Henrik Zetterberg, MD, PhD; M. Albert Hietala, MD, PhD; Michael Jonsson, MD; Niels Andreasen, MD, PhD; Ewa Styrud, BSN; Ingvar Karlsson, MD, PhD; Åke Edman, MD, PhD; Cornel Popa, MD; Abdullah Rasulzada, MD; Lars-Olof Wahlund, MD, PhD; Pankaj D. Mehta, MD, PhD; Lars Rosengren, MD, PhD; Kaj Blennow, MD, PhD; Anders Wallin, MD, PhD

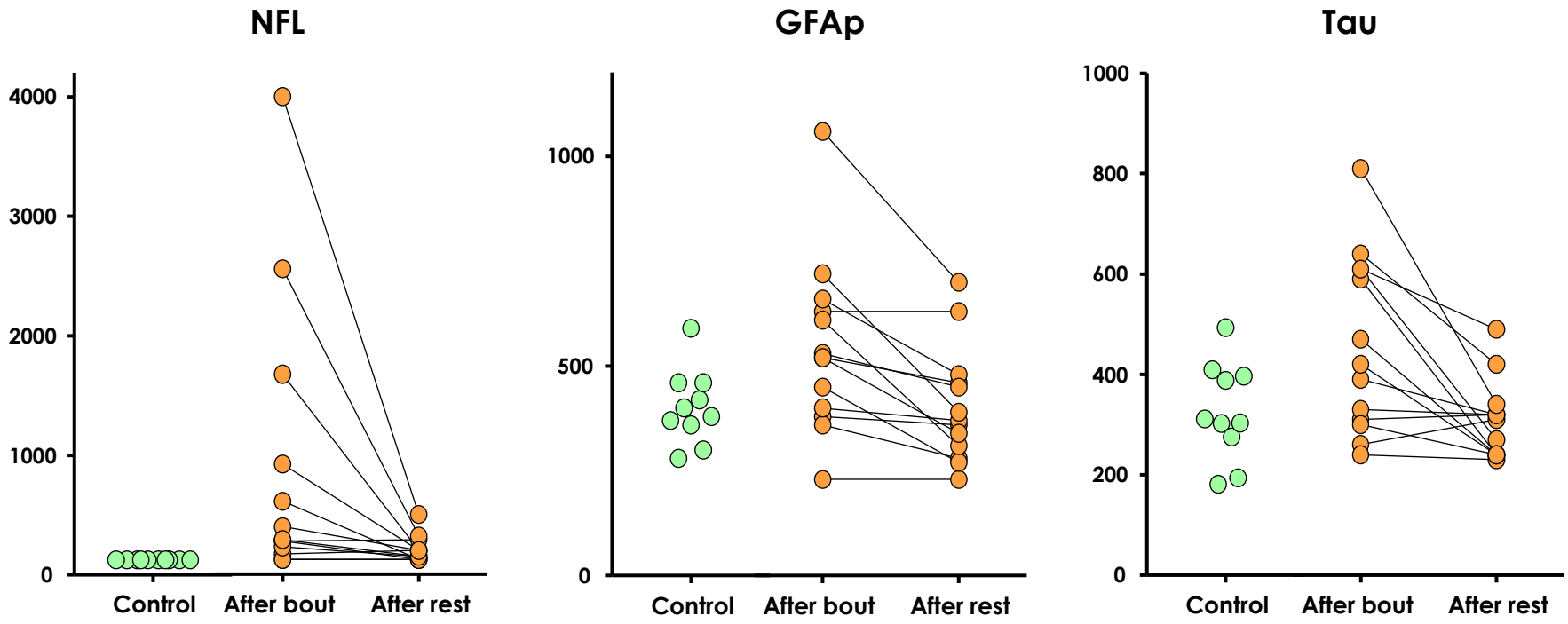
Arch Neurol. 2006;63:1277-1280

- **Study design:** 14 amateur (olympic) boxers
  10 healthy age-matched controls (non-athletes)

CSF samples A) After bout (7-10 days)
B) After a rest period (3 months)

Individual CSF biomarker values in boxers after a bout and after a period of rest from boxing

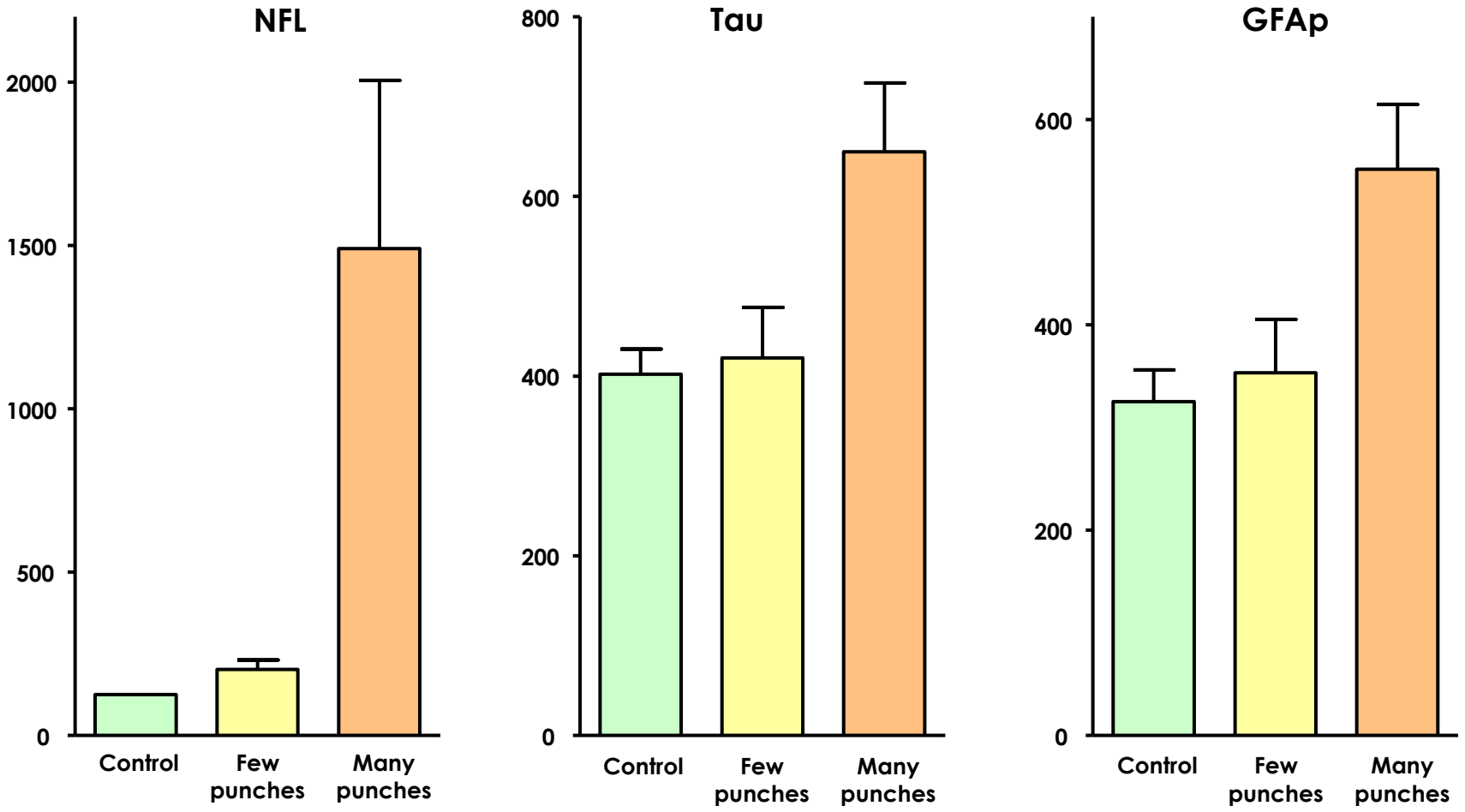
- After bout = 7-10 days after bout
- After rest = > 3 months rest from sparring + bouts



Zetterberg et al, Arch Neurol 2006

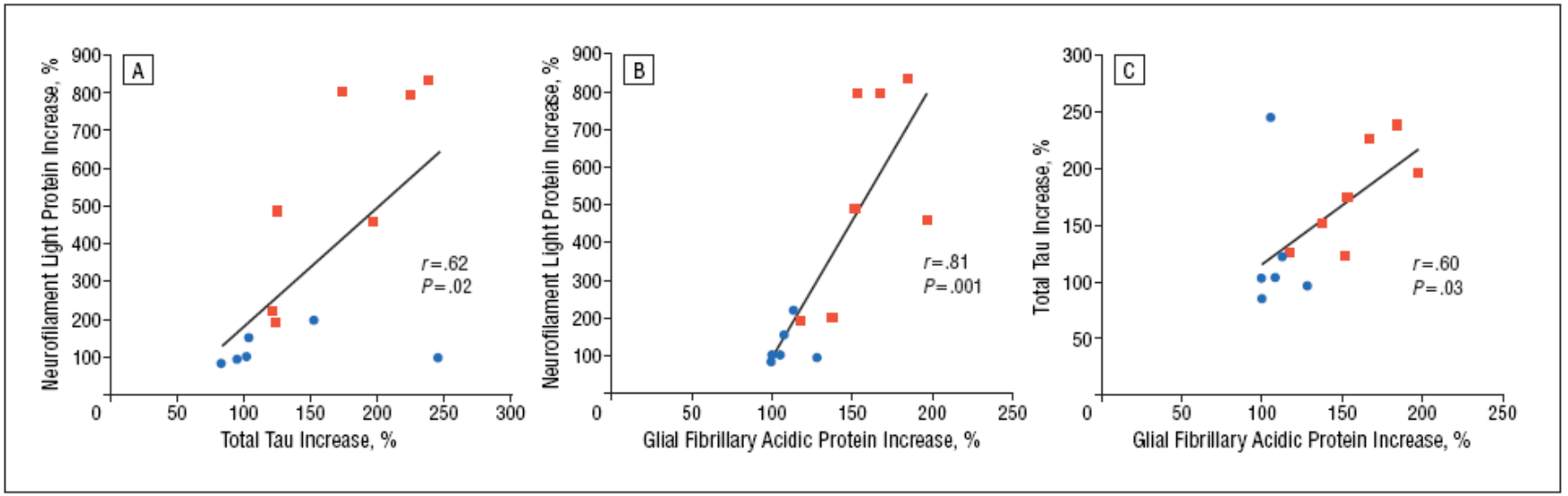
Increase in CSF biomarkers after bout correlate with number/severity of punches

- Few punches = < 15 punches
- Many punches = > 15 punches or grogginess during or after the bout



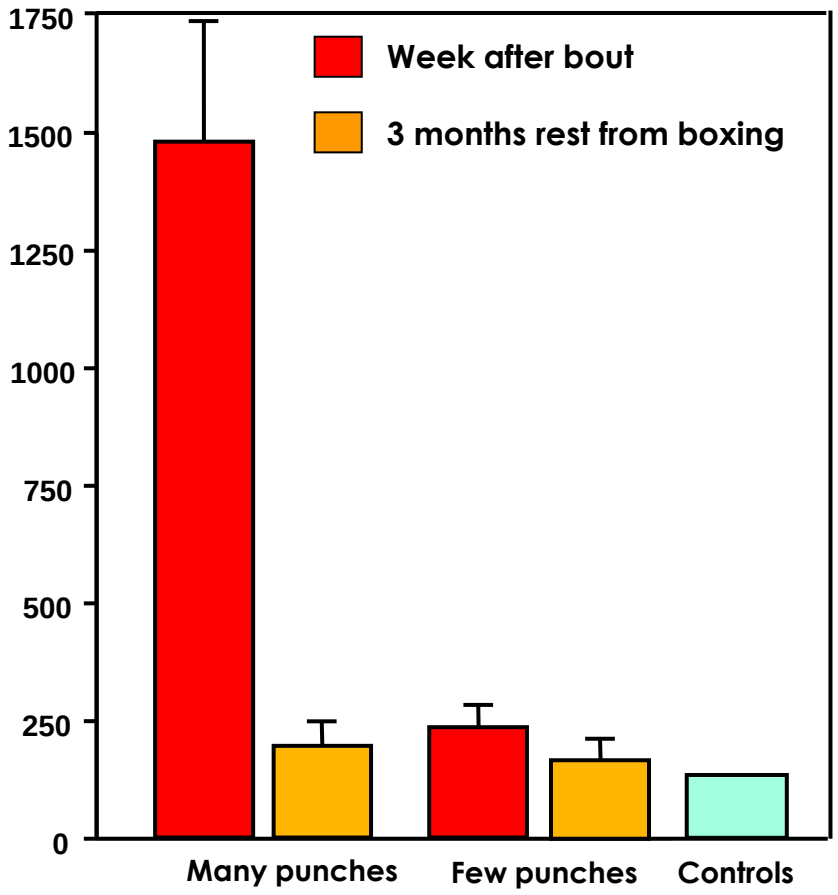
Zetterberg et al, Arch Neurol 2006

Changes in CSF biomarker in boxers are highly correlated



Zetterberg et al, Arch Neurol 2006

CSF biomarkers to monitor axonal damage in boxing

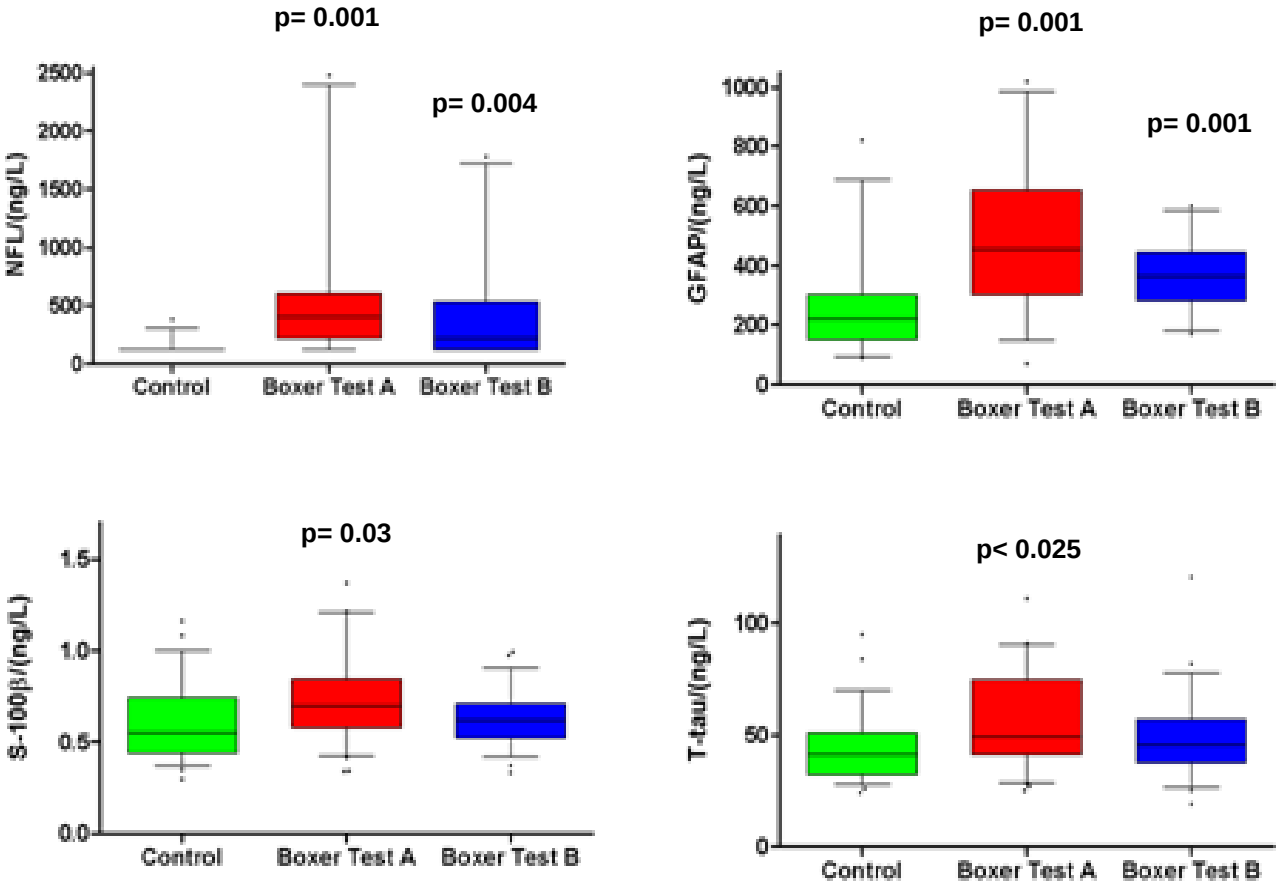


- Amateur boxing is associated with acute axonal and glial damage
- Distinct increase in CSF NFL despite no knock outs
- CSF NFL returns to normal after a rest period of 3 months
- Severity correlate with severity / number of punches

CSF biomarkers in olympic boxing
- Study by the Medical Committee of the Swedish Boxing Association

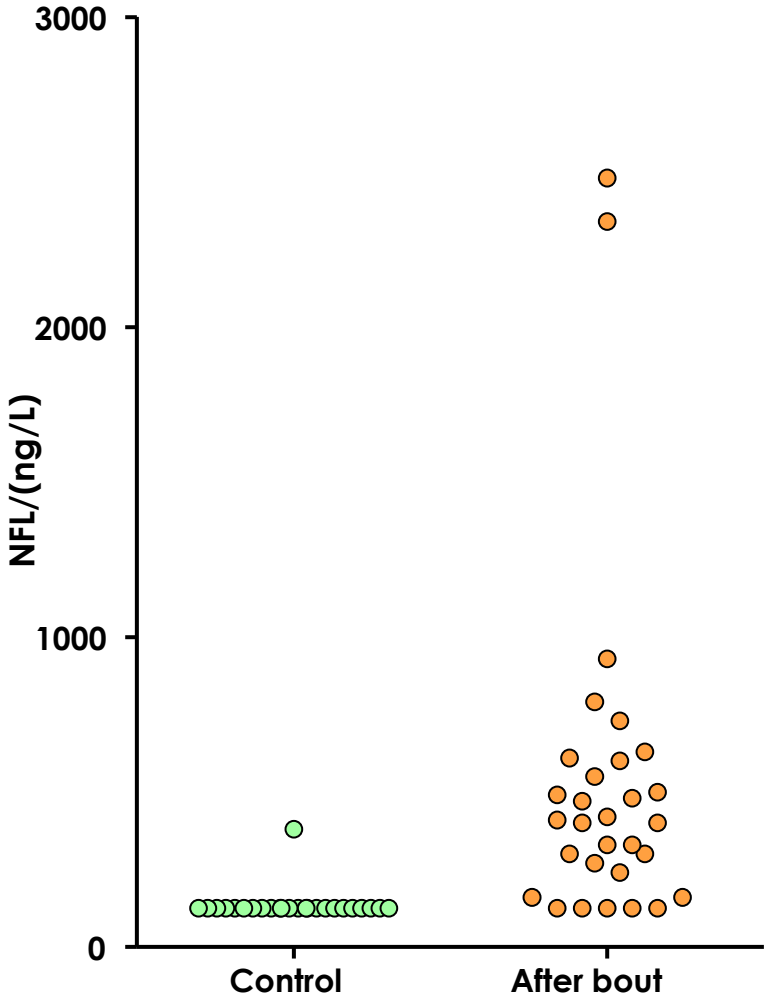
• Study design:	30 amateur (olympic) boxers 25 healthy age-matched controls
CSF samples	A) After bout (1-6 days) B) After a rest period (> 2 weeks)

CSF biomarkers in olympic boxers



Neselius et al, PLoS ONE 2012

Main finding: increase in the axonal protein NFL after bout



- High CSF NFL (> 125 pg/mL)

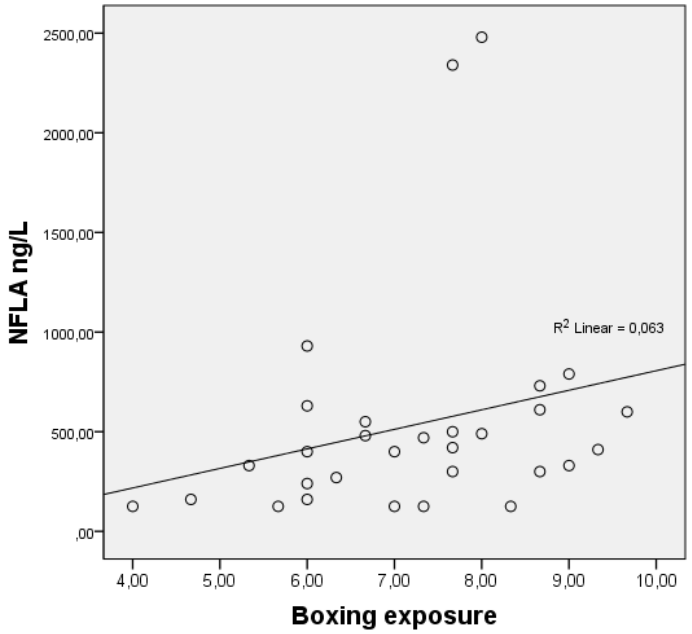
Boxers after bout	25/30	83 %
Boxers at follow-up	13/25	52 %
Controls	1/25	4 %

Neselius et al, PLoS ONE 2012

Axonal damage as measures by CSF NFL correlates with boxing exposure

- **Boxing exposure score:**

Bouts the week before test	(1 – few, 2 – intermediate, 3 – many)
Grading of bouts by boxer	(1 – easy, 2 – intermediate, 3 – tough)
Grading of bouts by expert	(1 – easy, 2 – intermediate, 3 – tough)

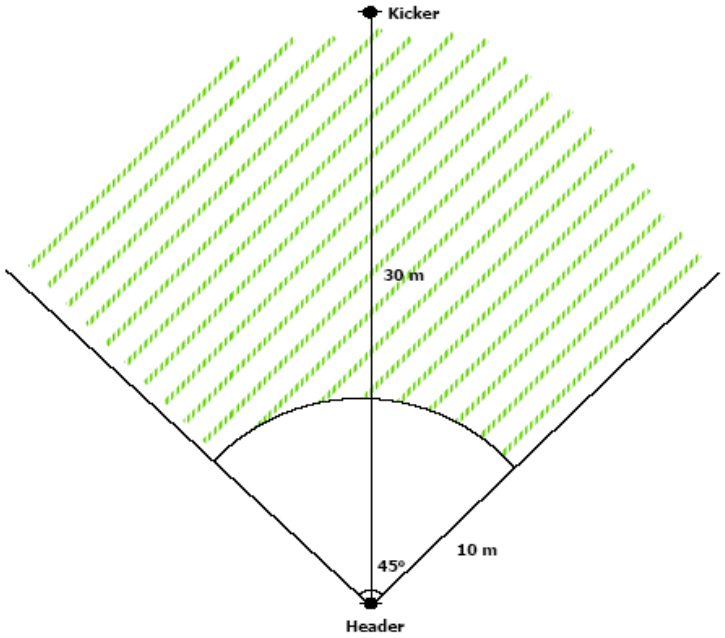


Conclusions:

- Amateur boxing is associated with acute axonal and glial damage
- Distinct increase in CSF NFL despite no knock outs
- Severity correlate with boxing exposure / severity of bout

Neselius et al, PLoS ONE 2012

Does headings in soccer cause brain damage ?



Study design: Soccer players n= 23
MD students (controls) n= 10

Headings from 30 meter (goalkeeper kick)
Zero – 15 -30 correct headings

CSF tap 1 week after headings

Zetterberg et al, Br J Sports Med 2007

CSF and serum biomarkers for brain damage in soccer players

ORIGINAL ARTICLE

No neurochemical evidence for brain injury caused by heading in soccer

Henrik Zetterberg, Michael Jonsson, Abdullah Rasulzada, Cornel Popa, Ewa Styrud, Max Albert Hietala, Lars Rosengren, Anders Wallin, Kaj Blennow

Br J Sports Med 2007;**41**:574–577. doi: 10.1136/bjsm.2007.037143

Table 1 Demographic and biochemical variables in soccer players and controls*

Variables	Soccer players with 10 approved headings (n = 10)	Soccer players with 20 approved headings (n = 13)	Controls (n = 9†)
Age (years)	26 (19–32)	23 (20–28)	24 (22–27)
Total number of headings	14 (11–20)	23 (20–57)	0
Albumin ratio	4.1 (2.4–9.3)	3.9 (2.0–8.7)	4.1 (2.5–6.3)
NF-L (ng/l)	<125	<125	<125
T-tau (ng/l)	315 (170–400)	250 (190–420)	320 (120–540)
GFAP (ng/l)	265 (180–510)	260 (190–330)	280 (190–460)
S-100B in CSF (μg/l)	0.87 (0.71–1.2)	0.82 (0.48–1.3)	1.1 (0.77–1.2)‡
S-100B in serum (μg/l)	0.060 (0.030–0.12)	0.040 (0.010–0.07)	0.040 (0.030–0.060)

*Data are presented as median (range).
†One of the 10 controls was excluded because of bleeding caused by the lumbar puncture.
‡p = 0.049 for controls vs players with 10 approved headings and p = 0.008 for controls vs players with 20 approved headings.

- **Mental health problems are common among soldiers returning from war**

The health of UK military personnel who deployed to the 2003 Iraq war: a cohort study

Matthew Hotopf, Lisa Hull, Nicola T Fear, Tess Browne, Oded Horn, Amy Iversen, Margaret Jones, Dominic Murphy, Duncan Bland, Mark Earnshaw, Neil Greenberg, Jamie Hacker Hughes, A Rosemary Tate, Christopher Dandeker, Roberto Rona, Simon Wessely

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JANUARY 31, 2008 VOL. 358 NO. 5

Mild Traumatic Brain Injury in U.S. Soldiers Returning from Iraq

Charles W. Hoge, M.D., Dennis McGurk, Ph.D., Jeffrey L. Thomas, Ph.D., Anthony L. Cox, M.S.W., Charles C. Engel, M.D., M.P.H., and Carl A. Castro, Ph.D.

NEUROPATHOLOGY

A Battle No Soldier Wants to Fight

- **Post traumatic stress disorder (PTSD)**

or

- **Mild traumatic brain injury (TBI) due to exposure to high-impact blasts from weapons (without direct head injruy)**

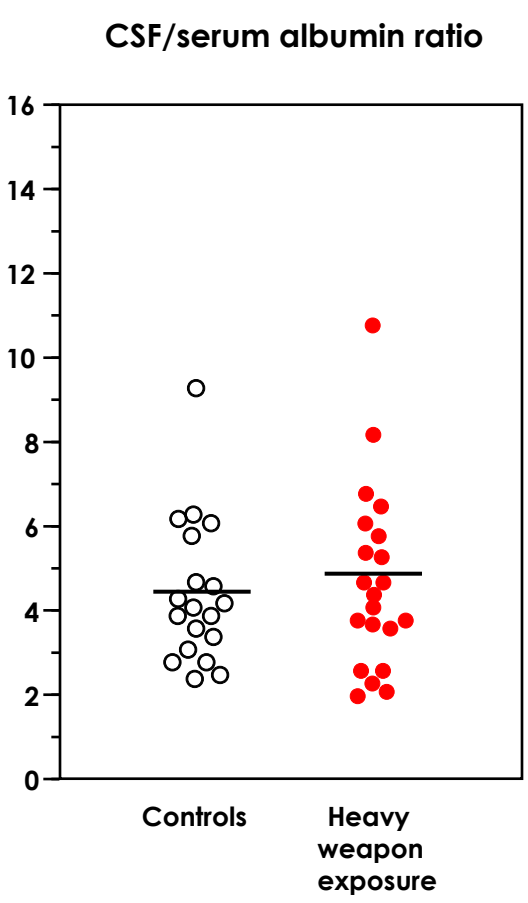
Controlled study on blast overpressure by firing heavy weapons

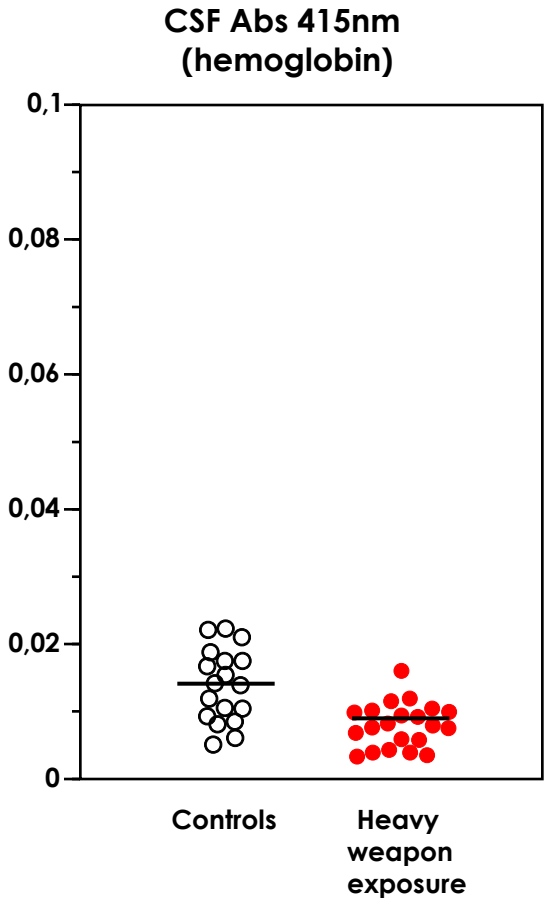
- Officers from the Swedish Armed Forces (n=6)
- Howitzer – Haubits FH77B
- 3 shots – 15.5 cm shells, charge no. 9
- Blast: 184 dB, B-duration 38 ms (MIL STD 1474D)

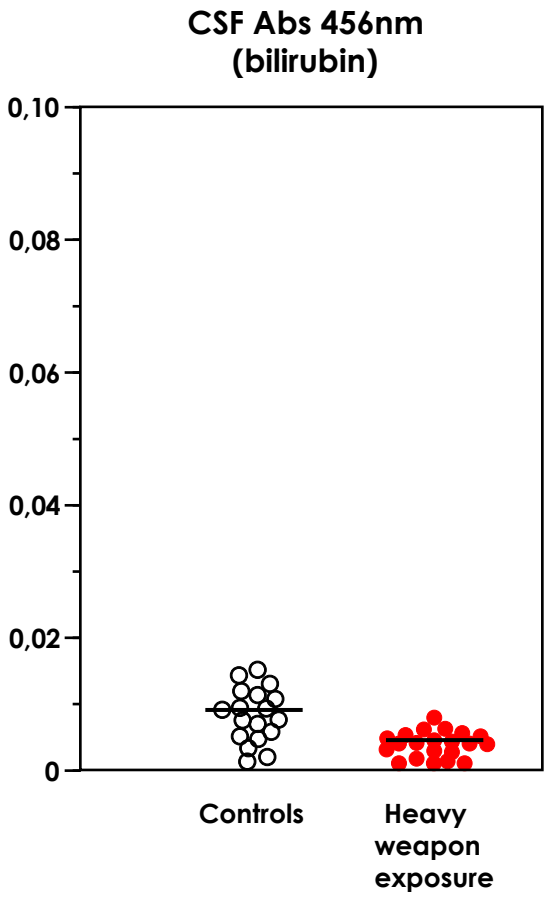
- Officers from the Swedish Armed Forces (n= 15)
- Bazooka – P-skott 86, SAAB Bofors
- 6 shots – 8.4 cm High-explosive Anti-tank grenade
- Blast: 182 dB, B-duration 13 ms (MIL STD 1474D)

- LP taken 8 days after exposure
- Analysis of: blood-brain barrier function
  spectrofotometry (haemorrhage)
  axonal markers: T-tau, NFL
  glial markers: S-100β, GFAP

Controlled study on blast overpressure by firing heavy weapons



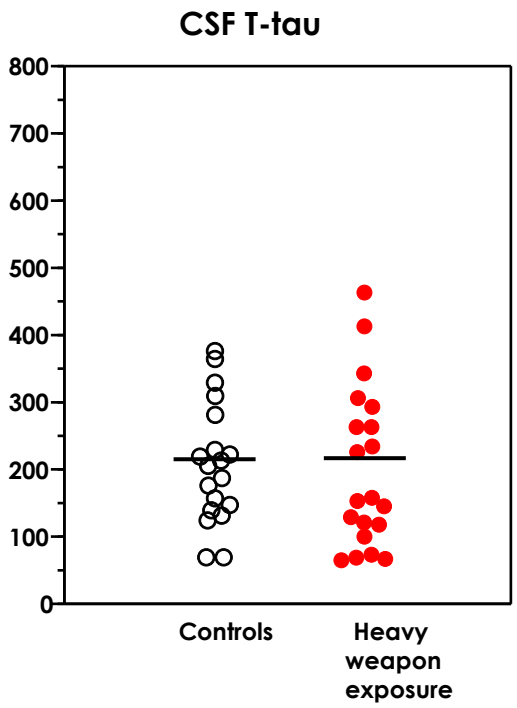


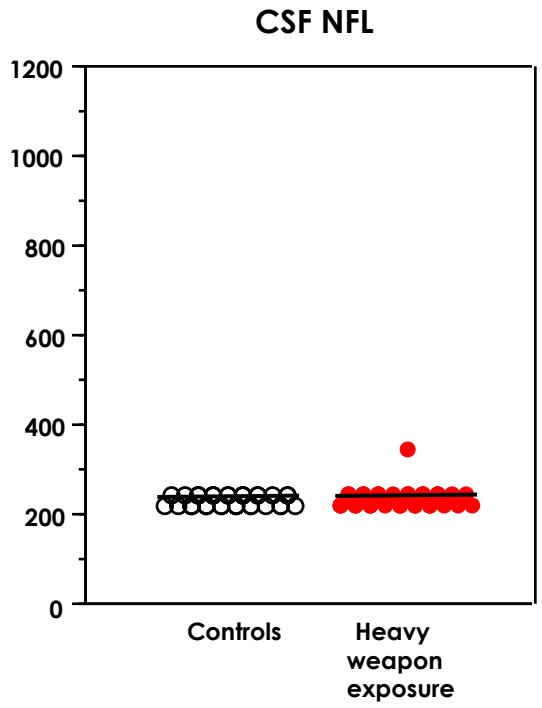


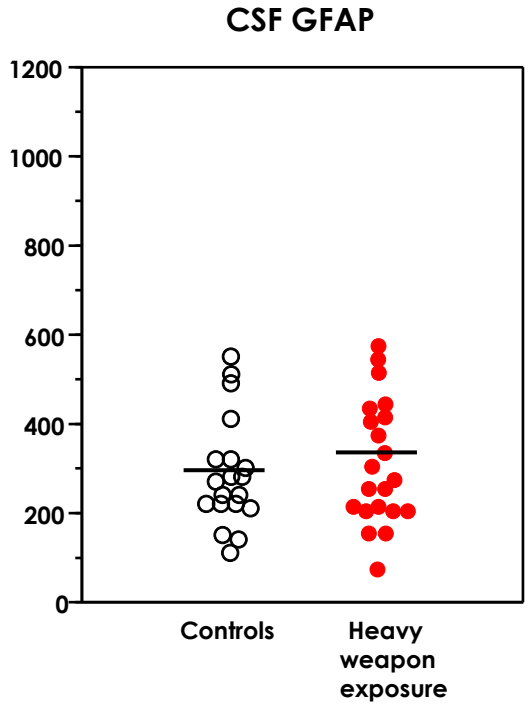
No evidence of blood-brain barrier damage

No evidence of brain haemorrhage

Controlled study on blast overpressure by firing heavy weapons







No evidence of neuronal / axonal damage

No evidence of glial damage

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No neurochemical evidence of brain injury after blast overpressure by repeated explosions or firing heavy weapons

K. Blennow[1], M. Jonsson[2], N. Andreasen[3], L. Rosengren[4], A. Wallin[2], P. A. Hellström[5], H. Zetterberg[1]

No neurochemical evidence of:

- **Blood-brain barrier damage**
- **Brain haemorrhage**
- **Axonal damage**
- **Glial cell damage**

after exposure to severe blast overpressure

CSF biomarkers and boxing

CSF biomarkers may give guidelines for boxing physicians:

- **Use CSF biomarkers to identify and quantify axonal damage after bouts with knock-out or many head punches**
- **CSF NFL level may determine the time-point for return to play**
  - **normal levels – immediate return**
  - **very high levels – extended period without sparring / bouts**
- **Normalization of CSF biomarkers in repeat sample before sparring / bouts?**