

Tübingen Hearing Research Centre



University of Tübingen

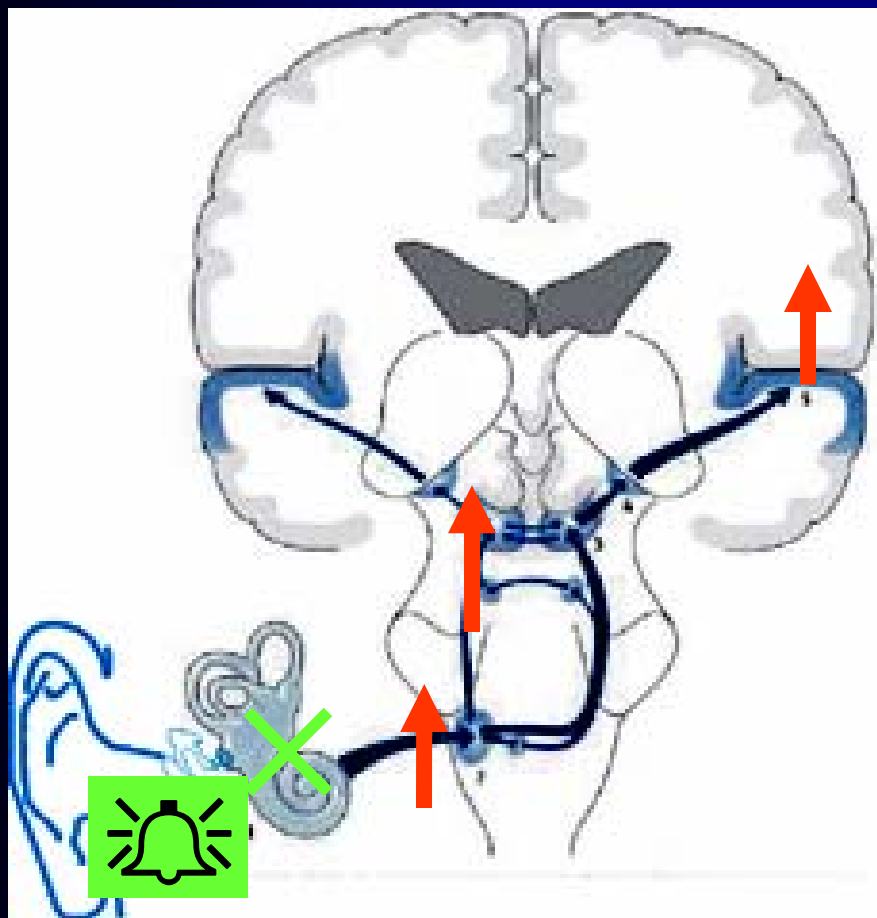


EINFLUSS HOCHFREQUENTER
ELEKTROMAGNETISCHER
MOBILFUNKKOMMUNIKATION AUF DAS
HÖRSYSTEM

*L Ruettiger**, HS Geisler, K Rohbock, I Köpschall, M Knipper*
Molecular Neurobiology, THRC

TINNITUS

Imbalance of excitatory and inhibitory inputs



AC, exhibits
enhanced spontaneous firing, subsequent to
auditory trauma.

**Cortical hyperexcitability &
Map Plasticity & Reorganisation**
associated with Tinnitus

*(Cazals, 2000; Kaltenbach and Afman, 2000; Salvi et al., 2000,
Moller, 2003; Eggermont and Roberts, 2004)*

IC, exhibit
enhanced spontaneous firing,
subsequent to auditory trauma

*(Wake et al., 1996; McFadden et al., 1998;
Cazals, 2000; Salvi et al., 2000).*

DCN, exhibits
enhanced spontaneous activity
subsequent to auditory trauma

*(Wang et al., 1996; Kimura and Eggermont, 1999; Levine, 1999;
Kaltenbach et al., 2000; 2004; Brozoski et al., 2002)*

QUESTION:

Can We Monitor Altered Neuronal Activity During Tinnitus upon Detection of **Activity Dependent Plasticity Genes** ?

STRATEGY:

Comparison of Gene Expression in the Absence and Presence of Tinnitus (Behaviour Model) Regarding only Changes that are Similar Post Various Tinnitus Paradigms (Salicylat & Acoustic Trauma h, d)

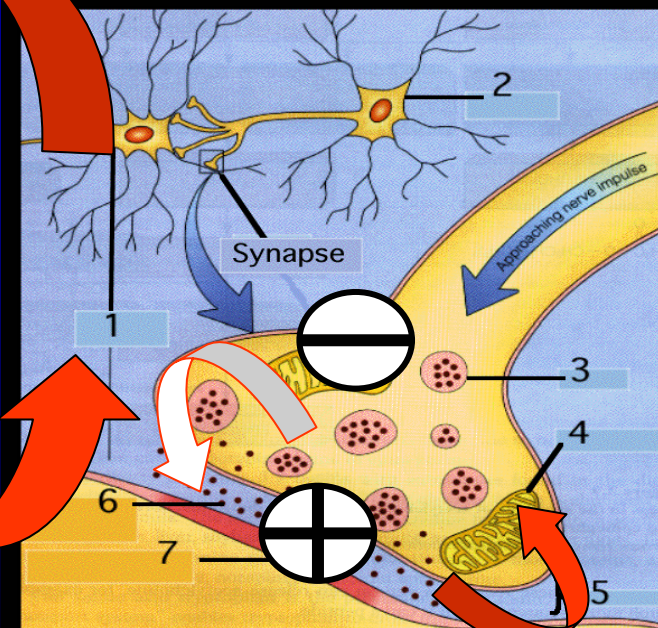
Animal Behaviour Model

(Rüttiger et al., Knipper, 2003)

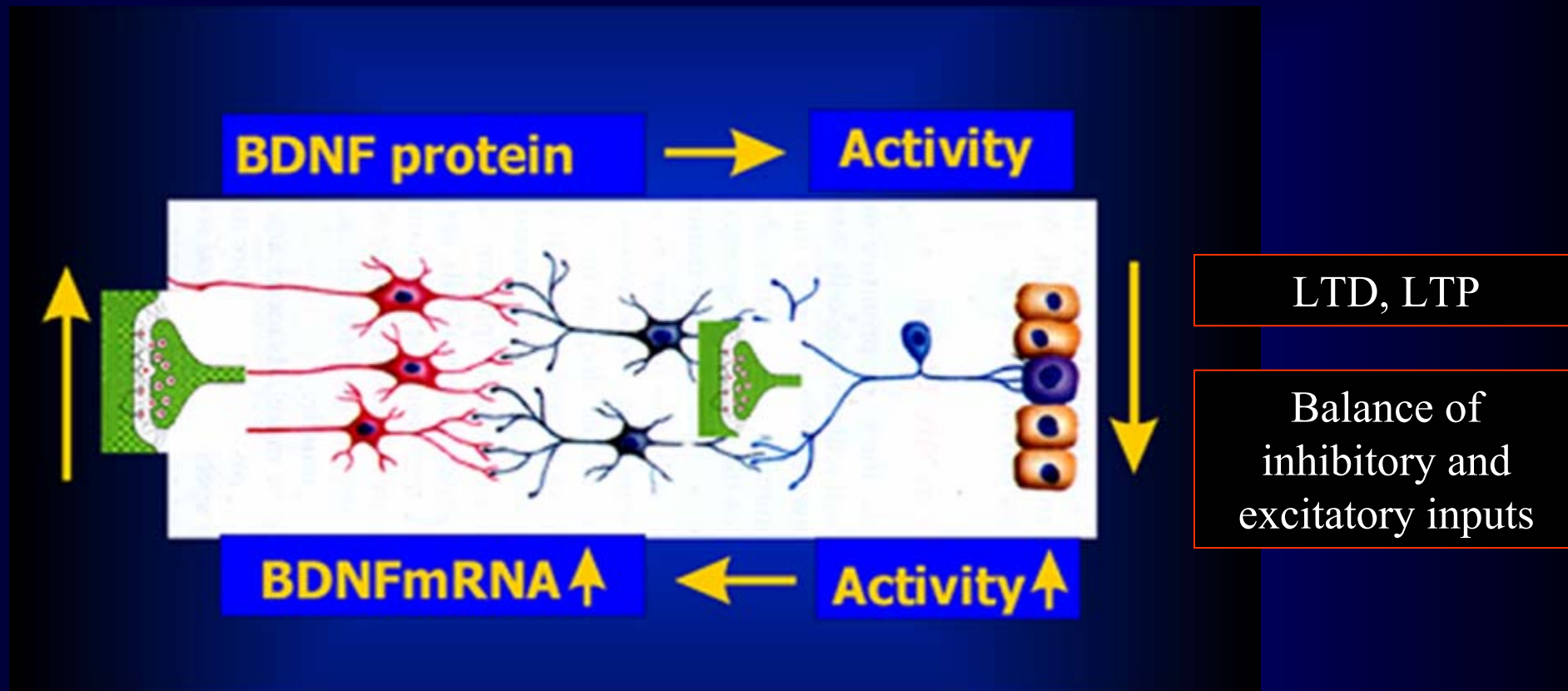


Activity-Dependent Plasticity Genes

BDNF & Arg3.1 / Arc



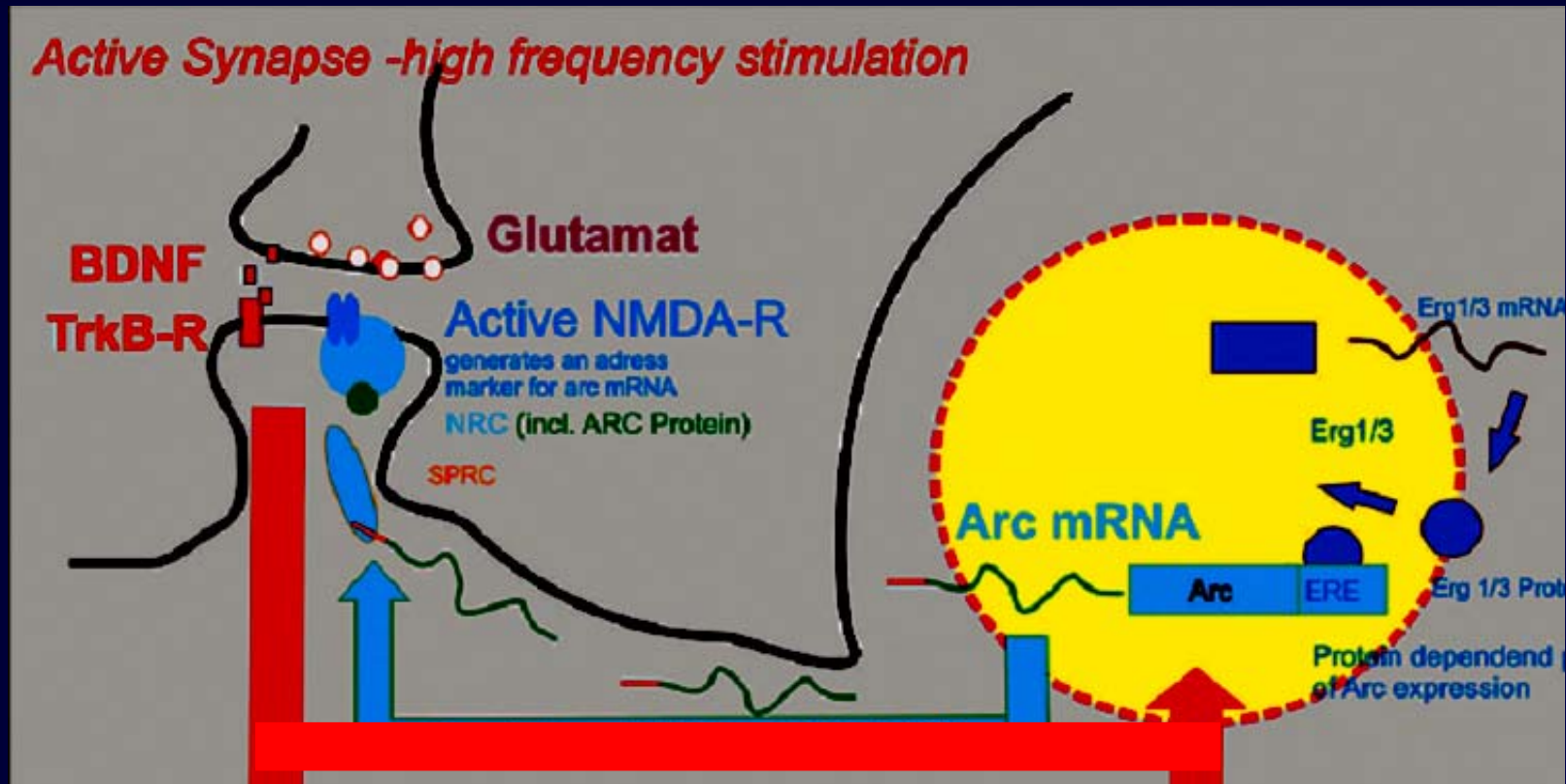
BDNF is the key regulator of neuronal plasticity...



..That Alters Efficacy of Synapses Subsequent to Activity- Dependent Transport of BDNF to Active Synapses

Poo et al., 1993, 2001; Thoenen et al., 1995, Rutherford et al., 1998; Tao et al., 1998; 2002; Desai et al., 1999; Shieh and Gosh, 1999; Knipper et al., 1993, a,b; 1994a,b; Knipper & Rylett, 1997; Chen et al., 2003; Cancedda et al., 2004; Du and Poo, 2004; West et al., 2002, Nat Rev Neurosci; Corski et al., 2003, J. Neurosci; Lu, 2003, Neuron; Pattabiraman et al., 2005, Mol Cell Neurosci

Arg3.1/Arc is a key regulator for neuronal plasticity ...

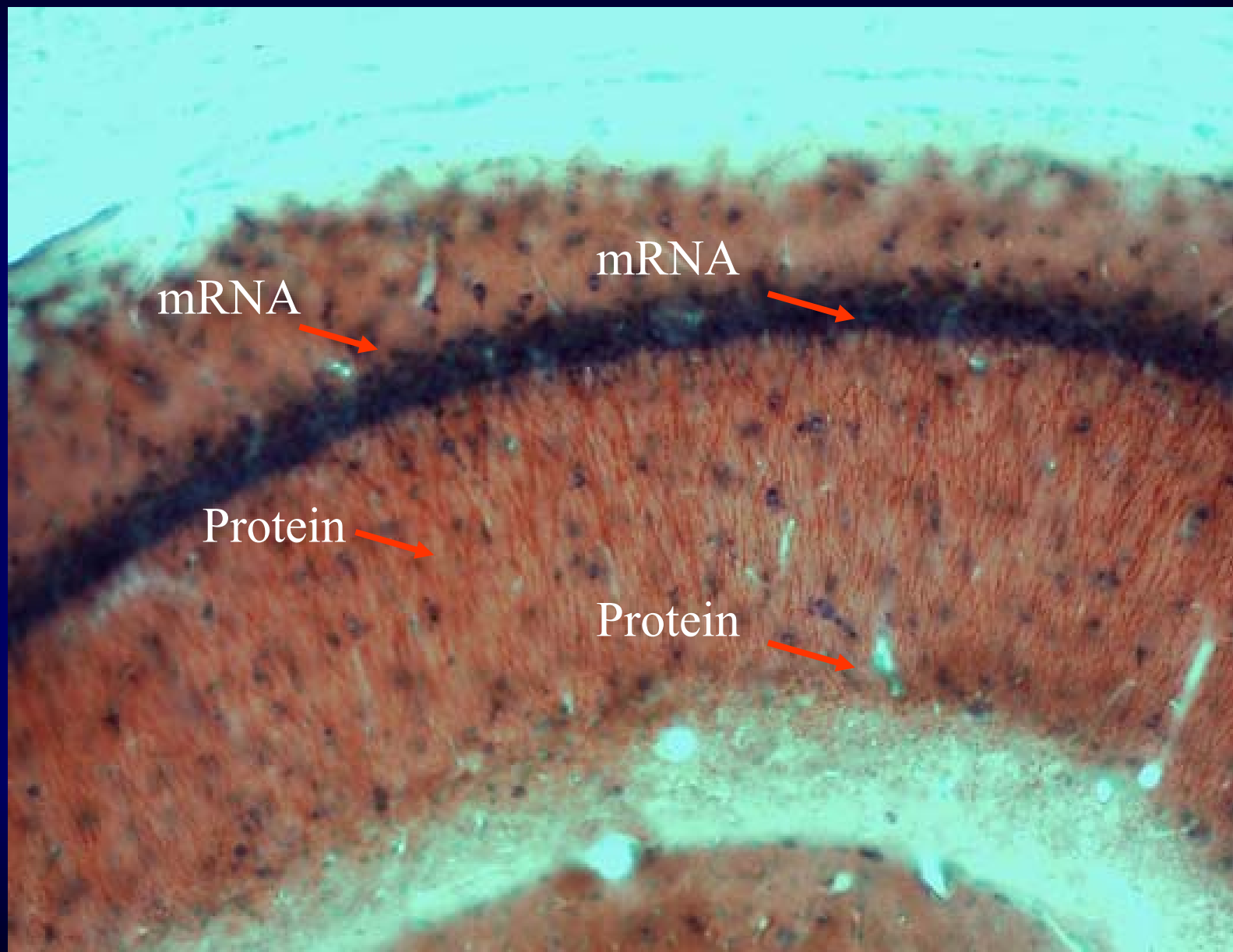


... That Provides Homeostatic Compensation for Acute Changes in Synaptic Strength Subsequent to Activity-Dependent Transport to Postsynapses → Surface AMPA Receptors

... Arg3.1/Arc ↑ after Sound; Smell, Environmental Enrichment

Link et al., 1995; Lyford et al., 1995; Yin et al., 2002; Ying et al., 2002; Montag-Sallaz et al., 1999; Guzowski et al., 2000; Waltereit et al., 2001; Bramham and Messaoudi, 2005; Mahlke & Wallhauser-Franke, 2004; Zou and Buck, 2006, Ramirez-Amaya et al., 2005, Ramirez-Amaya et al., 2005, Rial Verde et al., Neuron, 2006, Shepherd et al., Neuron, 2006).

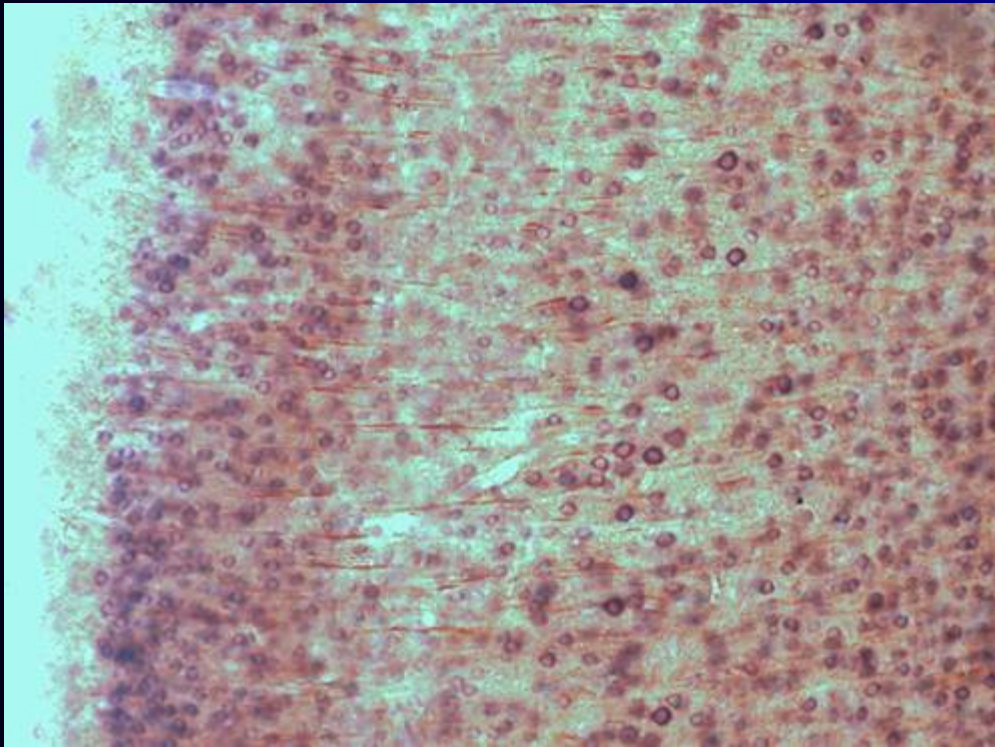
Arg3.1/Arc mRNA (blue) and Protein (red) in the hippocampus



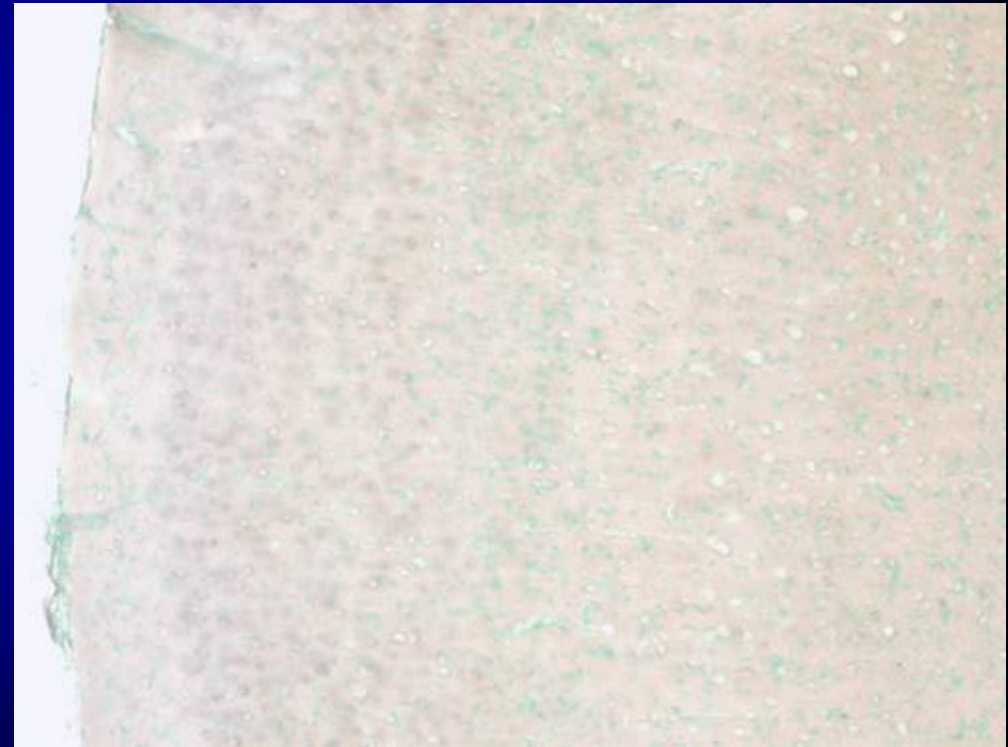
Arg3.1/Arc mRNA (blue) and Protein (red) in the auditory cortex



Antisense



Sense / without 1st AB



Arg3.1/Arc mRNA (blue) and Protein (red) in the auditory cortex

Layer II, III

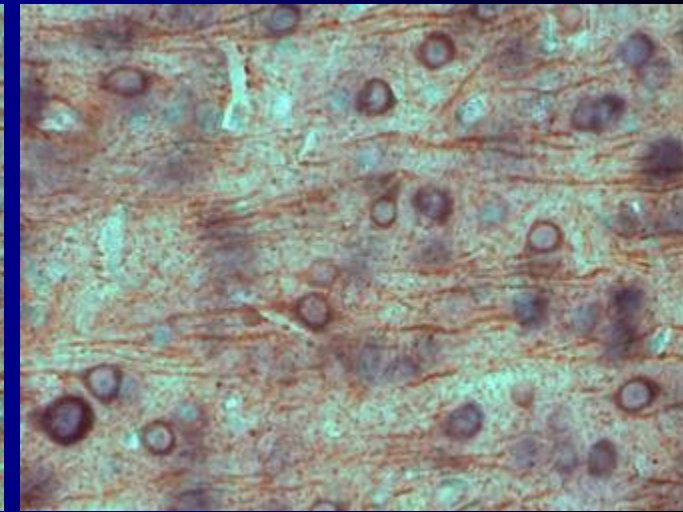
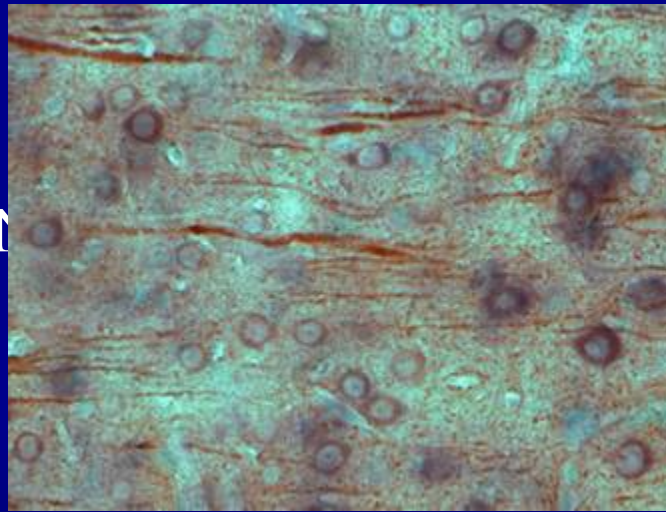
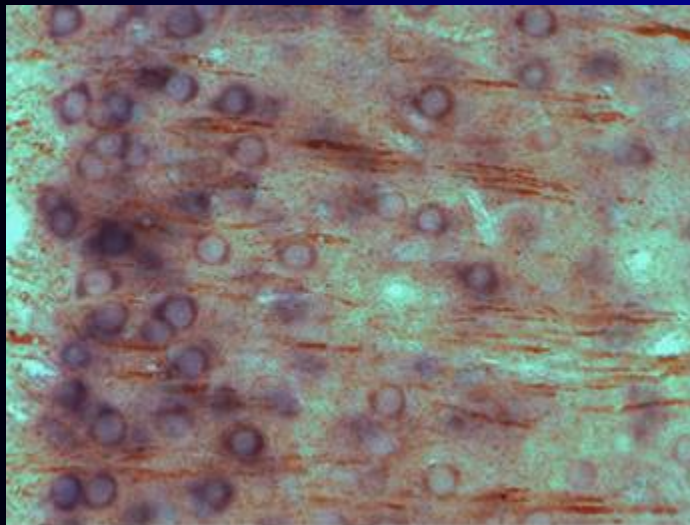
Stratum granulare (II) pyramidale (III) externum

Layer IV , V

Stratum granulare (IV) pyramidale (V) internum

Layer VI

Stratum multiforme



In a Next Step Altered BDNF and Arg3.1 Level were analysed in the Auditory System Subsequent to Tinnitus-inducing Traumata

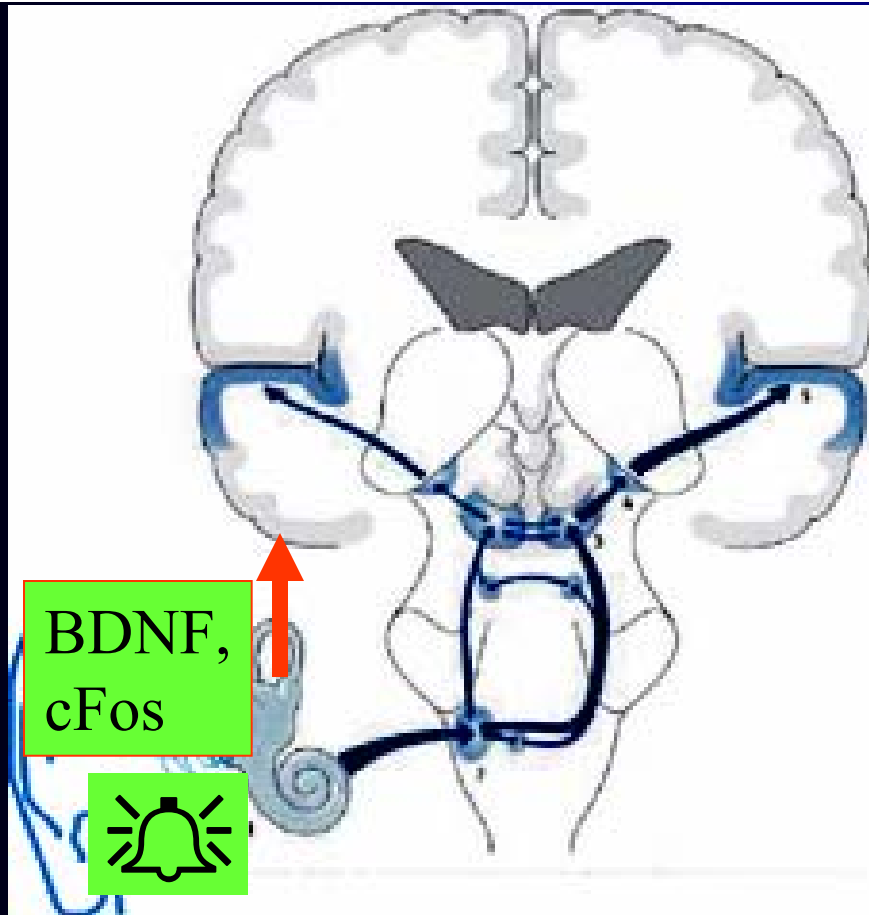
Results 6 days Post- Acoustic Trauma that induces Tinnitus (Behaviour Model) ...

Cochlea:

Tinnitus-inducing acoustic trauma →

BDNF ↑

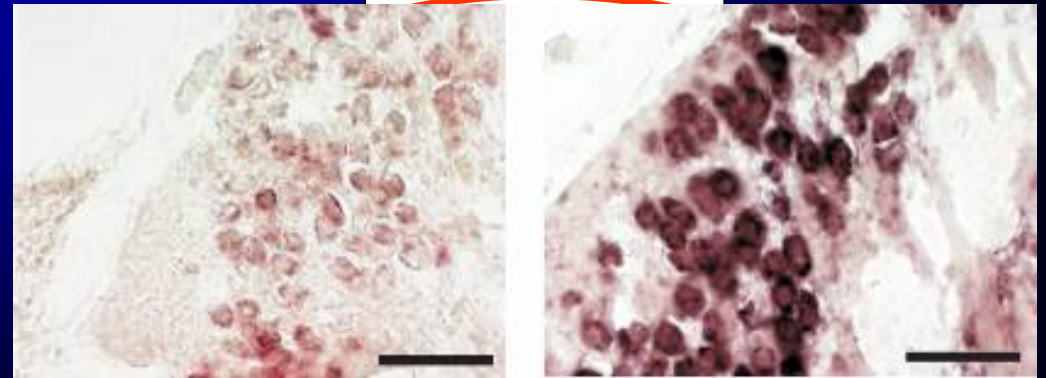
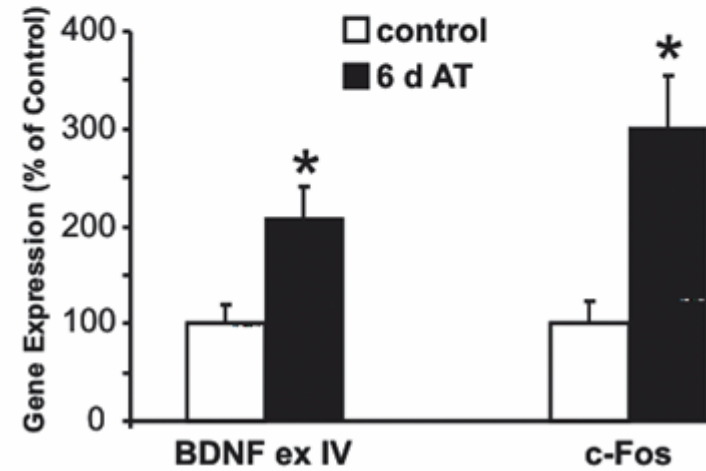
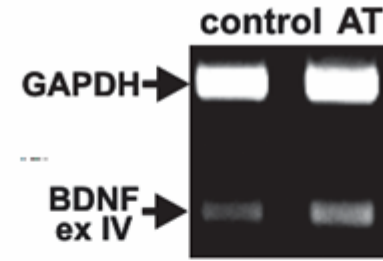
6 days after acoustic trauma (120 db, 10 kHz, 2h)



Tan et al., Knipper, Neurosci 2007

A

Cochlea



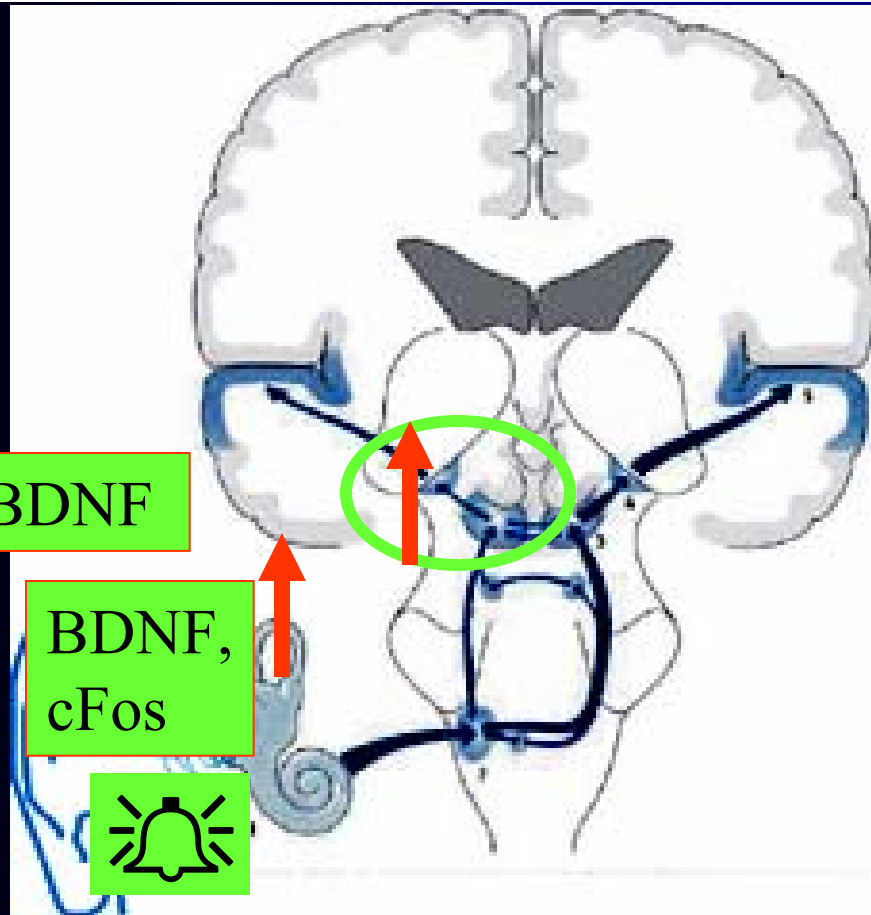
Spiral ganglia neurons exhibit enhanced BDNF ex IV (cFos)

Inferior Colliculus:

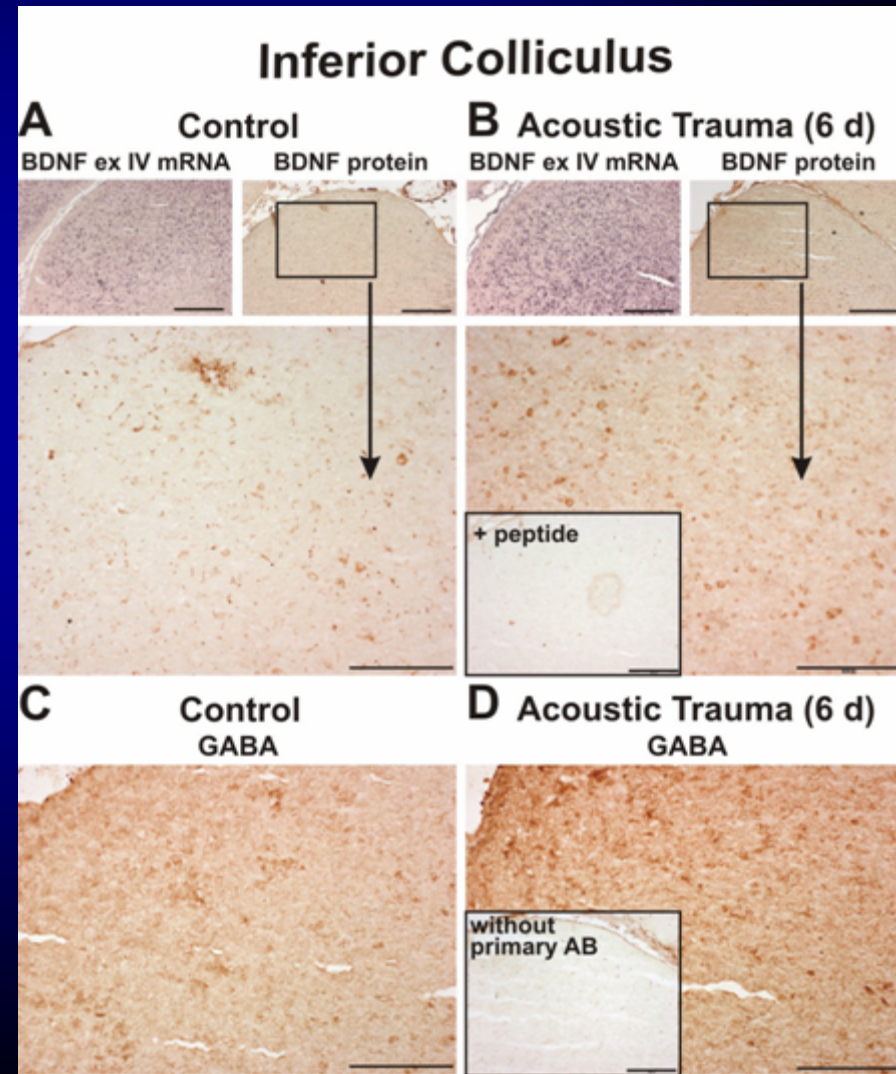
Tinnitus-inducing acoustic trauma →

BDNF ↑

6 days after acoustic trauma (120 db, 10 kHz, 2h)



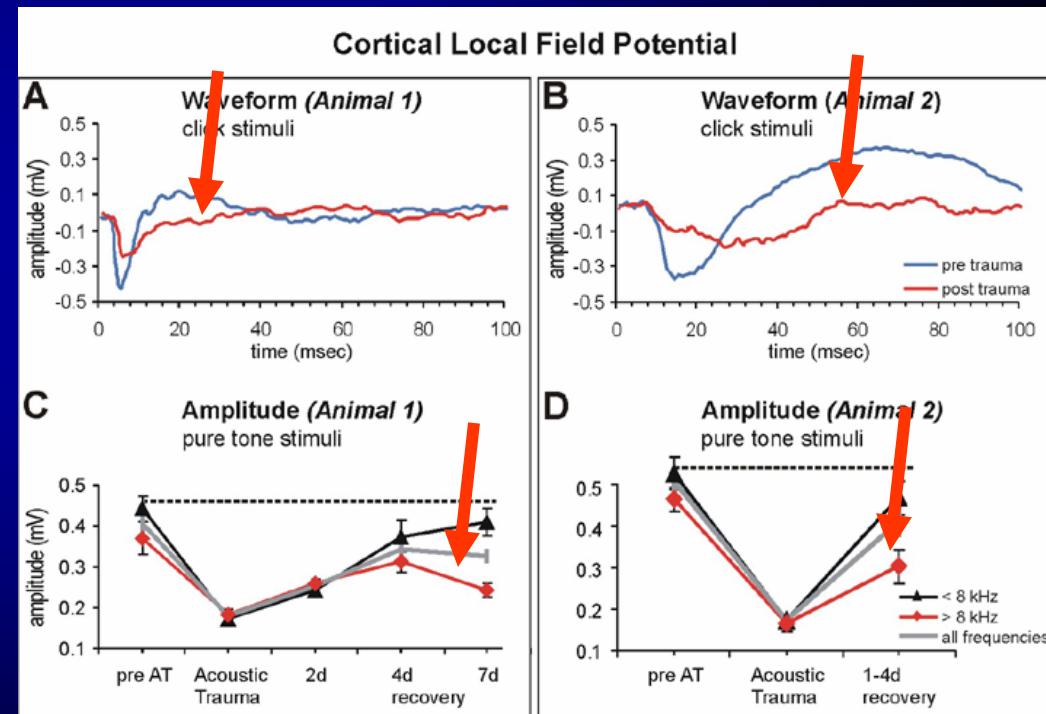
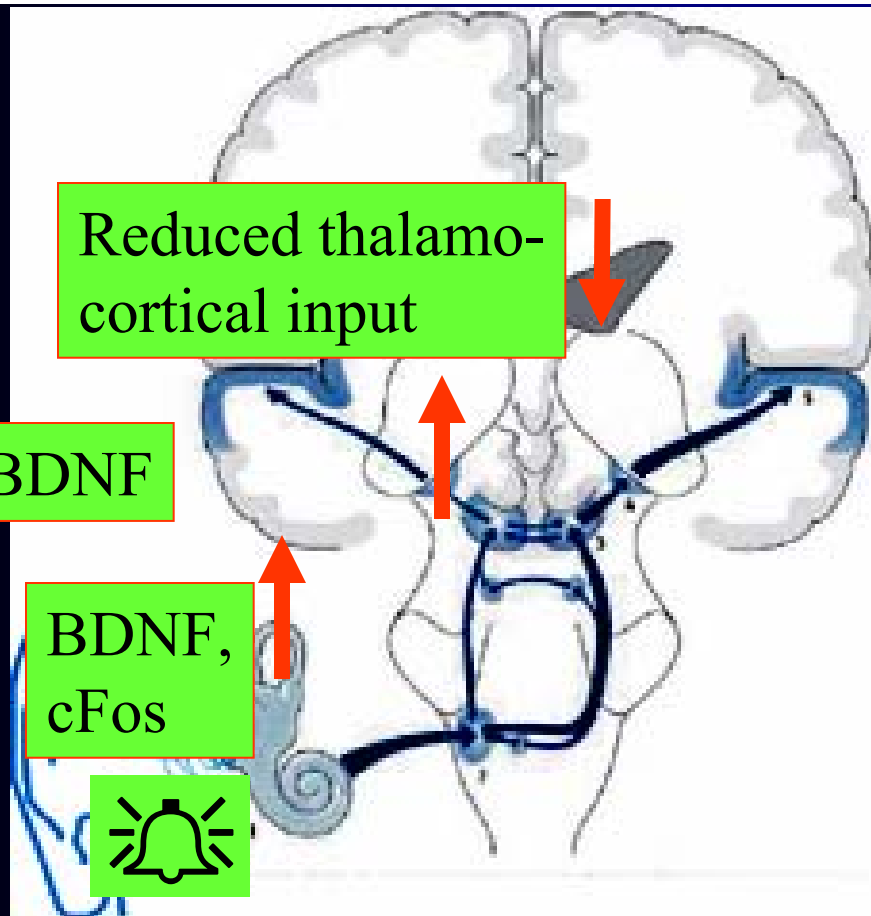
Tan et al., Knipper, Neurosci 2007



Medial Geniculate Body:

Tinnitus-inducing acoustic trauma →
thalamocortical input ↓

6 days after acoustic trauma (120 db, 10 kHz, 2h)



Chronical Multi-Electrode Recording AI (for 7days)

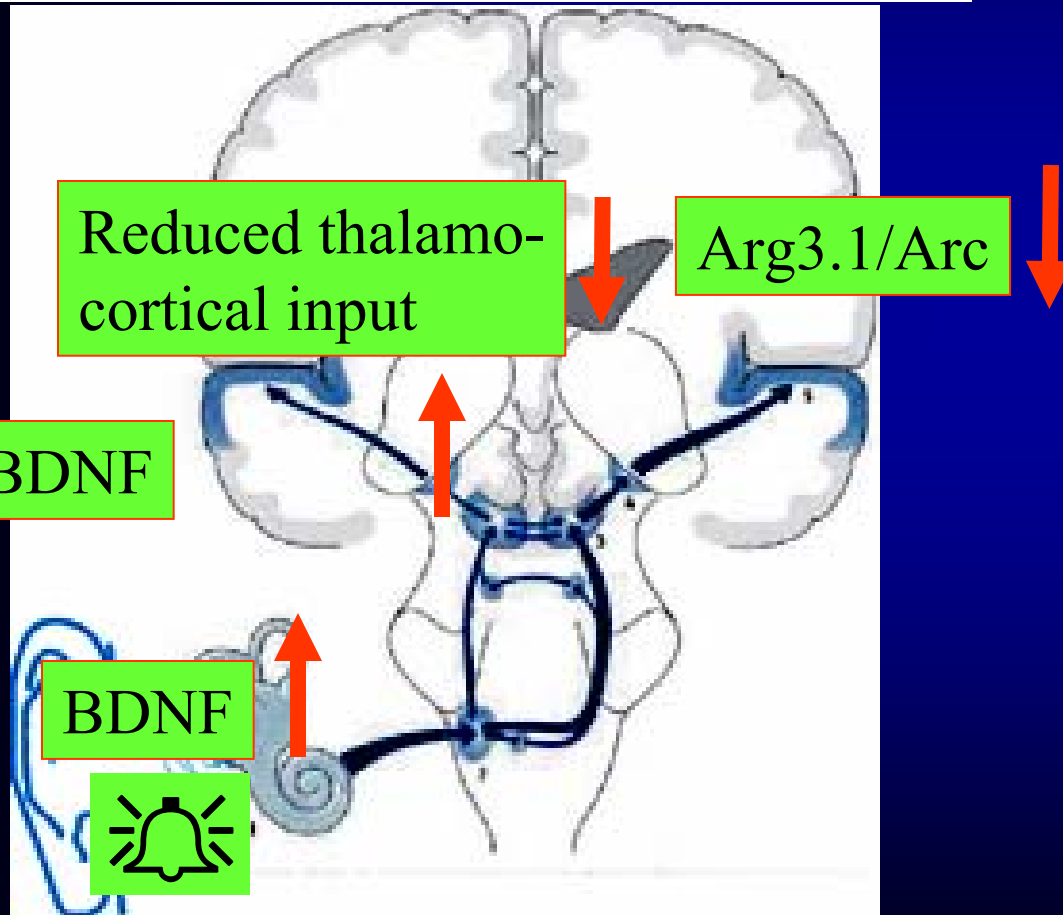
Tan et al., Knipper, 2007

Auditory Cortex, A1:

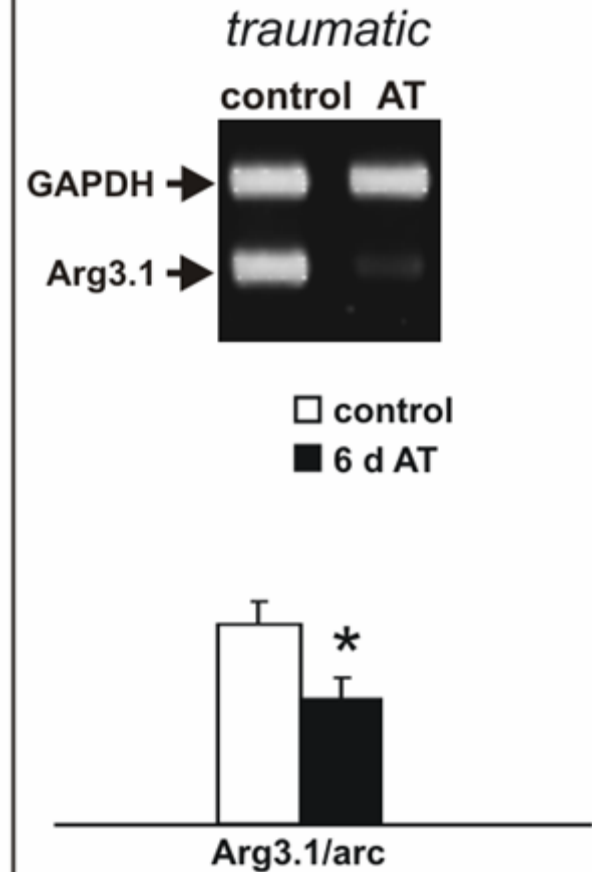
Tinnitus-inducing acoustic trauma →

Arg3.1/Arc ↓

6 days after acoustic trauma (120 db, 10 kHz, 2h)



Auditory Cortex



IN CONCLUSION

Comparison of Gene Expression in the Absence and Presence of Tinnitus (Behaviour Model) and Changes in Activity Dependent Genes can be Used to Monitor Induction of Tinnitus

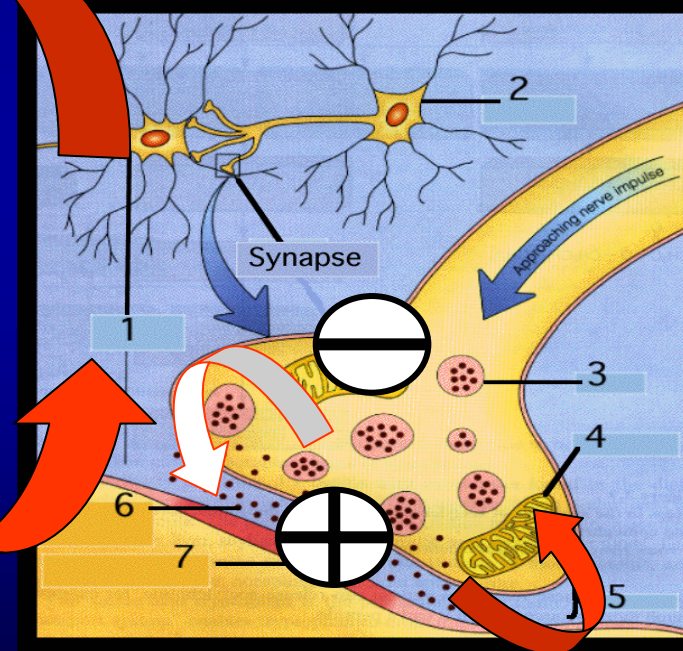
Animal Behaviour Model

(Rüttiger et al., Knipper, 2003)



Activity-Dependent Plasticity Genes

BDNF & Arg3.1 / Arc



Rüttiger et al., Knipper, 2003;
Tan et al., Knipper, Neurosci 2007

Assignment: Sept 2005

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38201 Salzgitter

D

The Direct Correlation of the Potential Trigger to Induce Tinnitus upon HF Electromagnetic Fields Should be determined upon Detection of Thresholds for 4 Different HF Exposition Intensities on

- (1) Tinnitus Induction in Behaviour Animal Models
- (2) Altered Expression of Activity Dependent Genes in IC and AC

Design:

1.3 Übersicht über die geplanten Experimente im 24 monatigen Zeitverlauf

(VH = Verhaltensmodell; MB = Molekularbiol Ansatz, BF = Befeldung)

Block A,B 6 Monate	Block C Messreihe I 3 Monate		Block D Messreihe II 3 Monate		Block E Messreihe III 3 Monate		Block F Messreihe IV 3 Monate		Block G,H 6 Monate
Literatur Dosimetrie/Temperatur Halteung/Käfigsystem	BF <u>20 x</u> <u>Tiere</u> im VH	Hörmessung, Konditionierung Gruppe II	BF <u>20 x</u> <u>Tiere</u> im VH	Hörmessung, Konditionierung Gruppe III	BF <u>20 x</u> <u>Tiere</u> im VH	Hörmessung, Konditionierung Gruppe IV	BF <u>20 x</u> <u>Tiere</u> im VH		Entblindung der Versuchsgruppen Auswertung wie unten beschrieben bis hin zu Publikationreife
		BF <u>20 x</u> <u>Tiere</u> im MB		BF <u>20 x</u> <u>Tiere</u> im MB		BF <u>20 x</u> <u>Tiere</u> MB		BF <u>20 x</u> <u>Tiere</u> im MB	Auswertung der Hörmessung und Verhaltensdaten
Hörmessung, Konditionierung Gruppe I	Verhalten 1) vor, 2) akut u 3) 2 Wochen nach Befeldung		Verhalten 1) vor, 2) akut u 3) Wochen nach Befeldung		Verhalten 1) vor, 2) akut u 3) Wochen nach Befeldung		**Optional: Wirkung auf Tinnitus, n=4 Verhalten 1) vor, 2) während, 3) akut u 4) Wochen nach Befeldung		Statistik und Erstellung graphische Darstellung Publikation
Mol-Ribosonden Synthese Bestellung von AK/Kits	Mol <u>akut</u> , Cochlea, Hirn Präparieren mRNA, RT PCR		Mol <u>2 Wochen</u> , Cochlea, Hirn Präparieren mRNA, RT PCR		Mol <u>akut</u> , Cochlea, Hirn Präparieren mRNA, RT PCR		Mol <u>2 Wochen</u> , Cochlea, Hirn Präparieren mRNA, RT PCR		RT-PCR Densitometrie Statistik, Auswertung
MB erste Gruppe von <u>20 Tieren</u> ohne Befeldung	Cochlea /Hirn schneiden, In situ Hybridisierung		Cochlea /Hirn schneiden, In situ Hybridisierung		Cochlea /Hirn schneiden, In situ Hybridisierung		Cochlea /Hirn schneiden, In situ Hybridisierung		Fotographie, Quantifizierung Auswertung

Design:

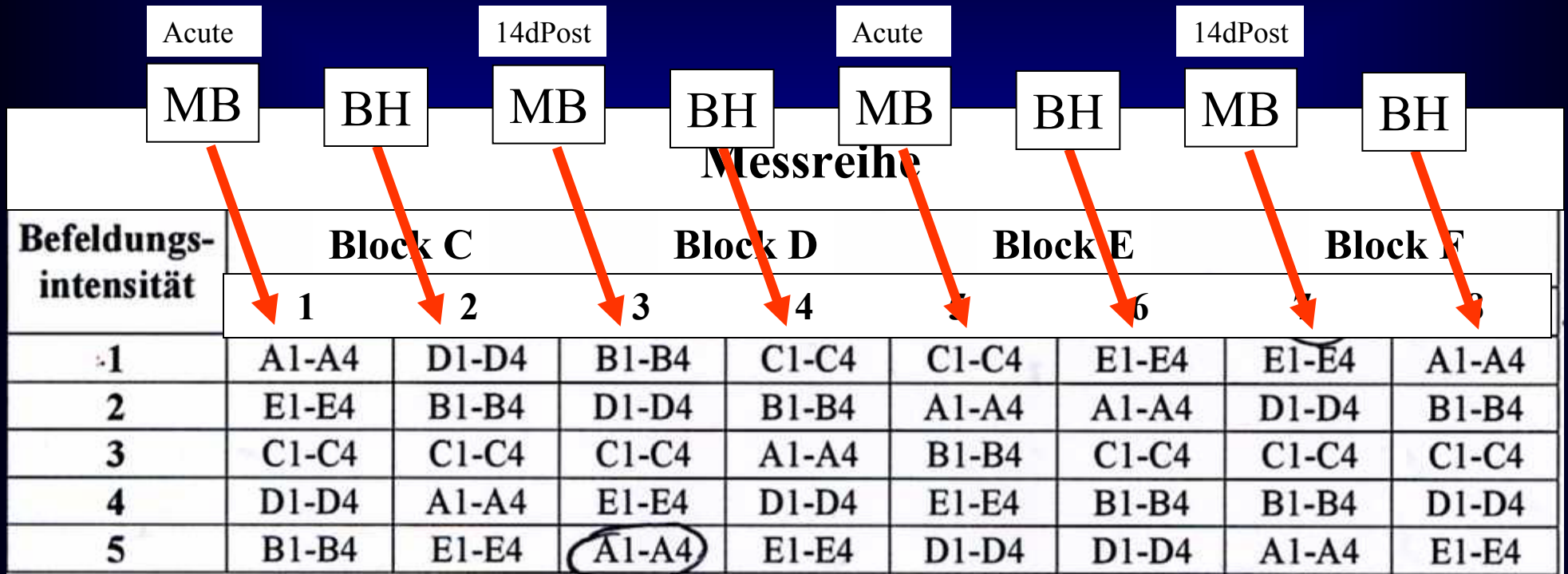
D

Radiation occurs over 4 weeks (5days,2h a day), 4 animals with

- (1) 0.02W/kg
- (2) 0.2 W/kg
- (3) 2.0 W/kg
- (4) 20 W/kg
- (5) Control group in a seperated room, identical conditions artifical radiation

Design:

D



(1) Analysis of Tinnitus Induction Using Activity Dependent Gene Expression

Design:

Organ Preparation: Co, IC, AC (Block B (unexposed) C,E=acute;D,F=14dpost



RNA Isolation (Aliquot 100ng /Re-Transcription)



cDNA Reverse Transcription (SensiScript-Quiagen)



RT-PCR (Cyclophilin = house keeping gene; Arg3.1/Arc; BDNF ex IV, cFos)



Densitometry (Cell-F; OSiS Olympus Soft Imaging System)

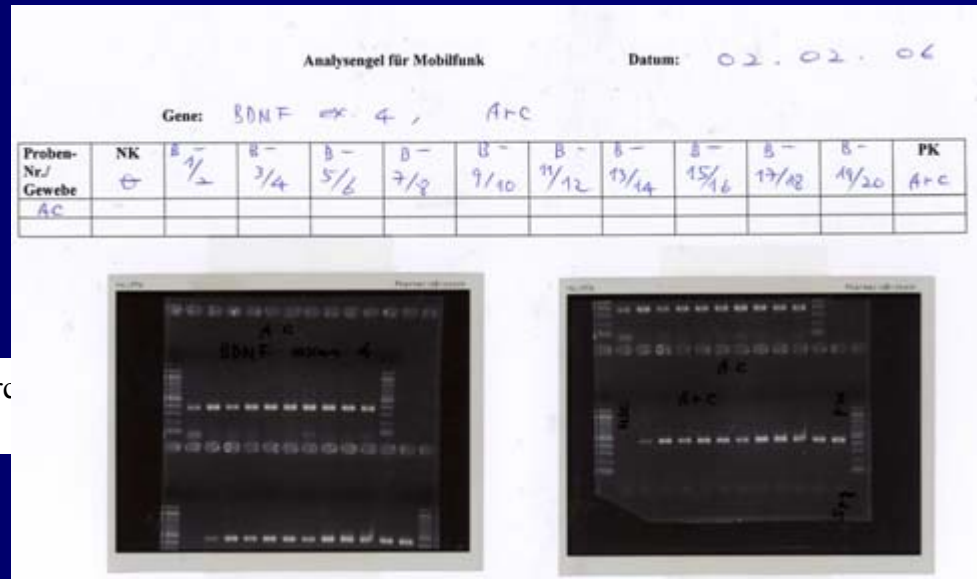


Statistic (Students-test; SD, unpaired; $p < 0.05$)

RT-PCR: Example

D

RT-PCR



Arg3.1/Arc
450 bp

DNF Exon VI
377 bp

Cyclophilin

Cyclophilin

Cyclophilin
300 bp

Cyclophilin=
300 bp



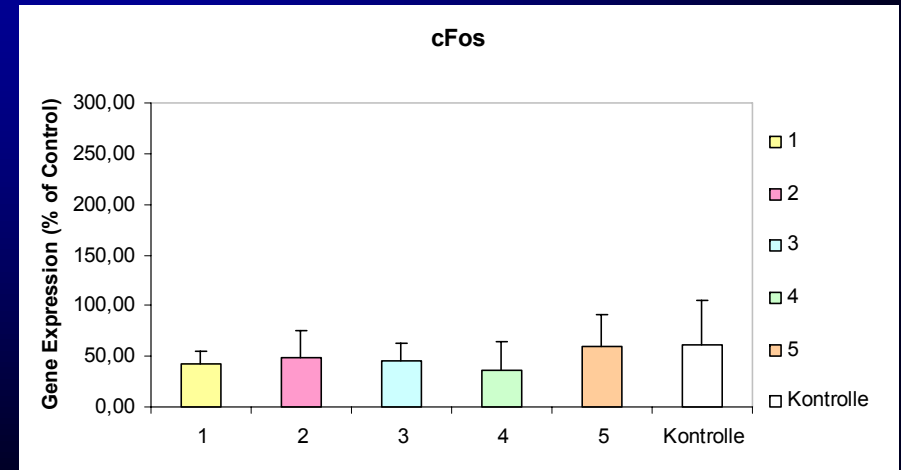
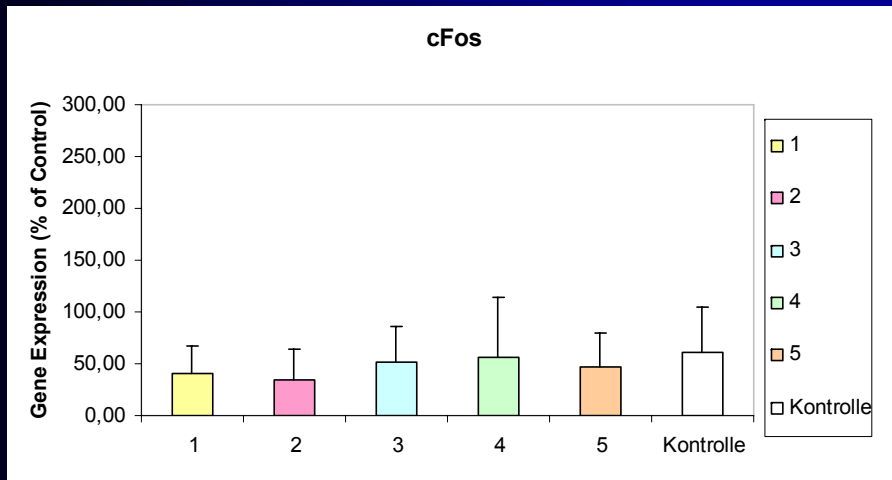
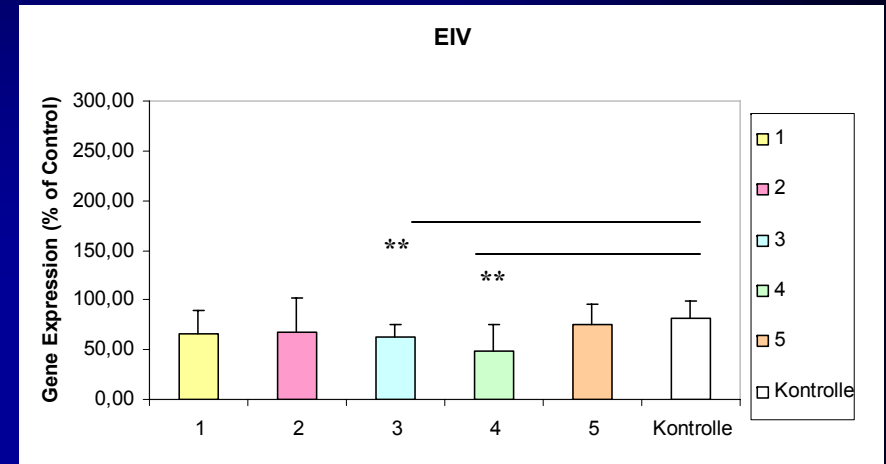
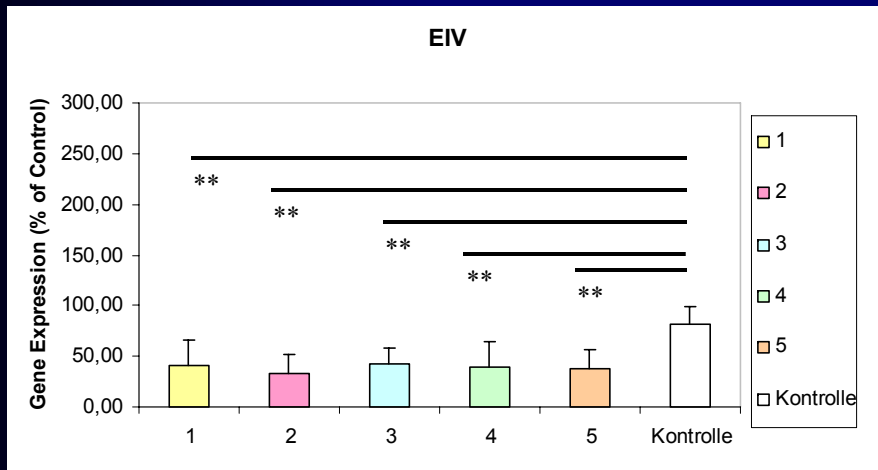
RT-PCR: Result

D

Statistic: BDNF Ex IV, cFos in CO

Acute

14 d Post-Radiation



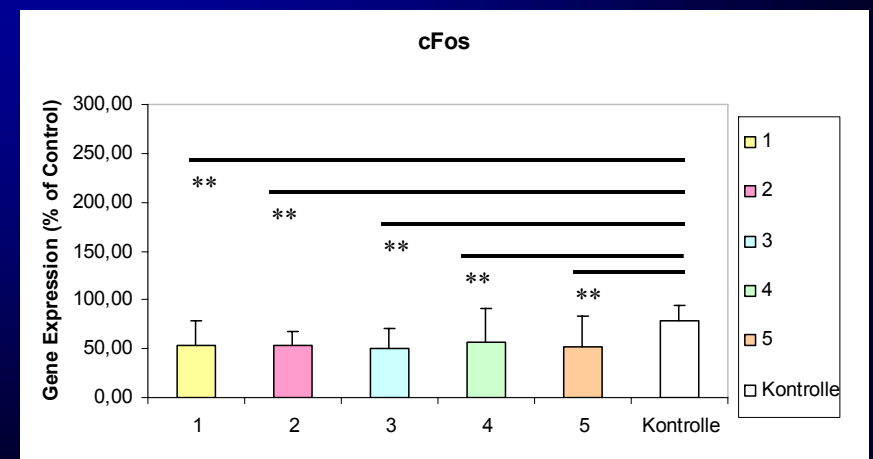
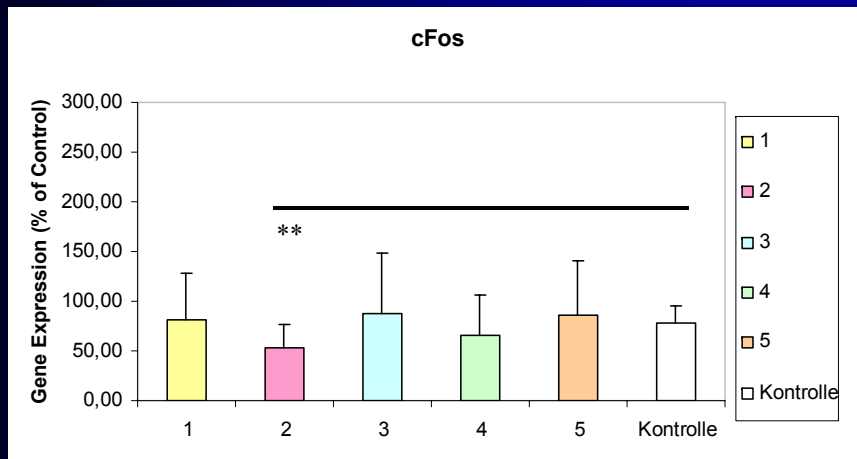
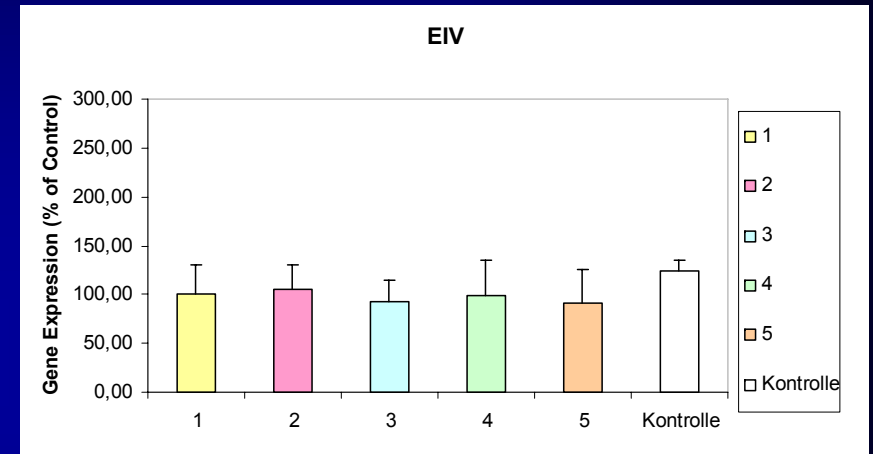
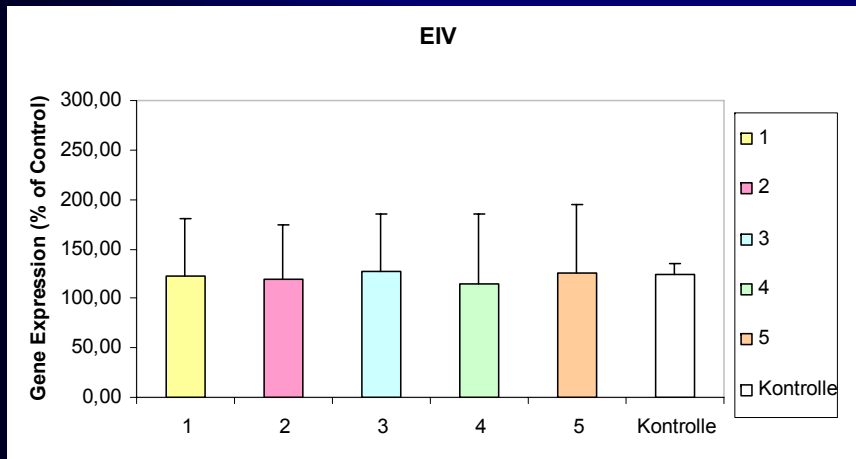
RT-PCR: Result

D

Statistic: BDNF Ex IV, cFos in IC

Acute

14 d Post-Radiation



RT-PCR: Result

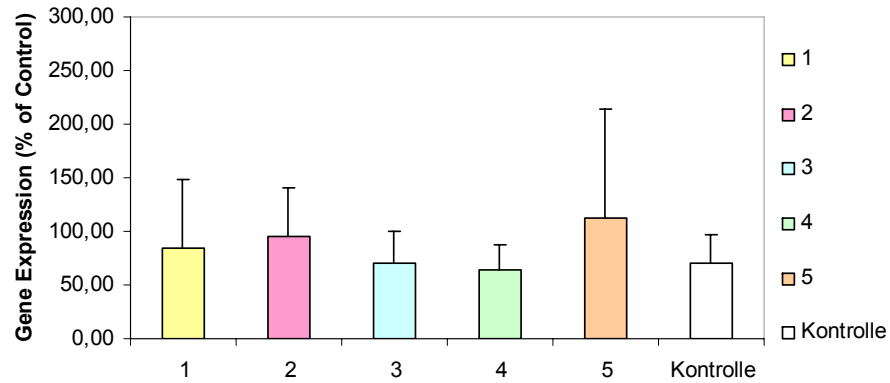
D

Statistic: Arg3.1/Arc in AC

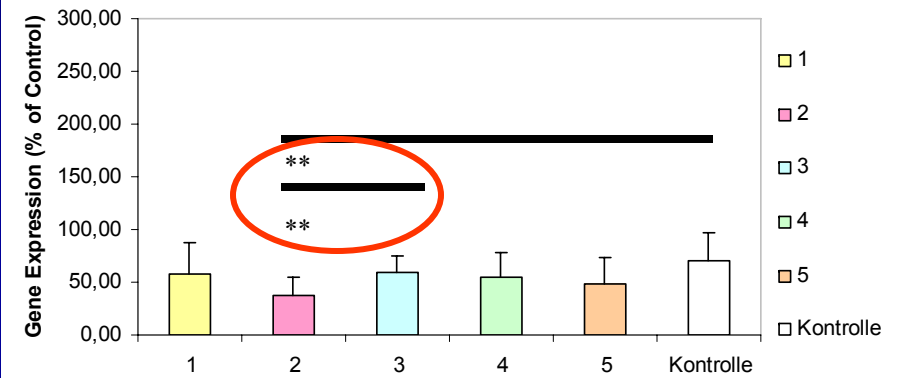
Acute

14 d Post-Radiation

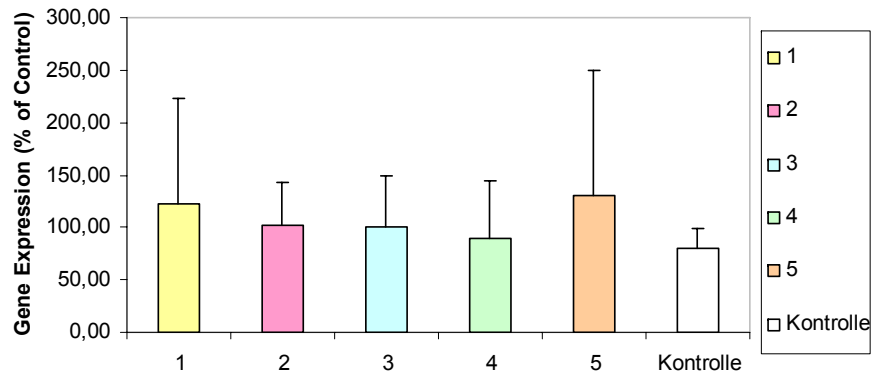
Arg3.1/Arc



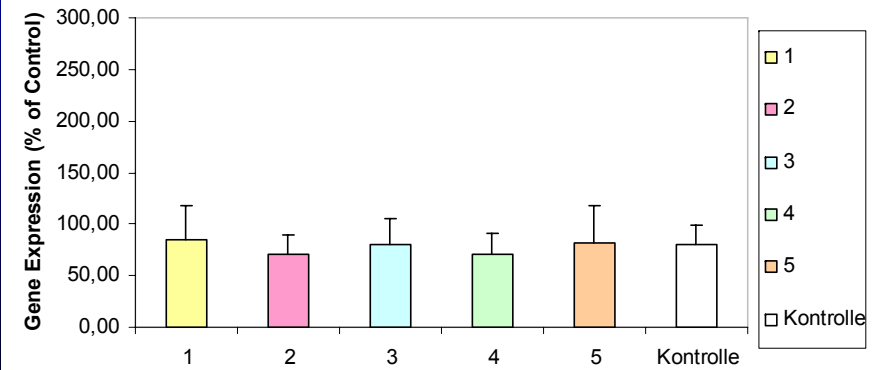
Arg3.1/Arc



EIV



EIV



(2) Analysis of Tinnitus Induction Using Animal Behavioral Model

Tinnitus Behavior - Design

Hearing Measurement

ABR Measurement

Conditioning

4-8 Week Training for 4 days/ Week (20 animals x 8 h a day)

Tinnitusinduction

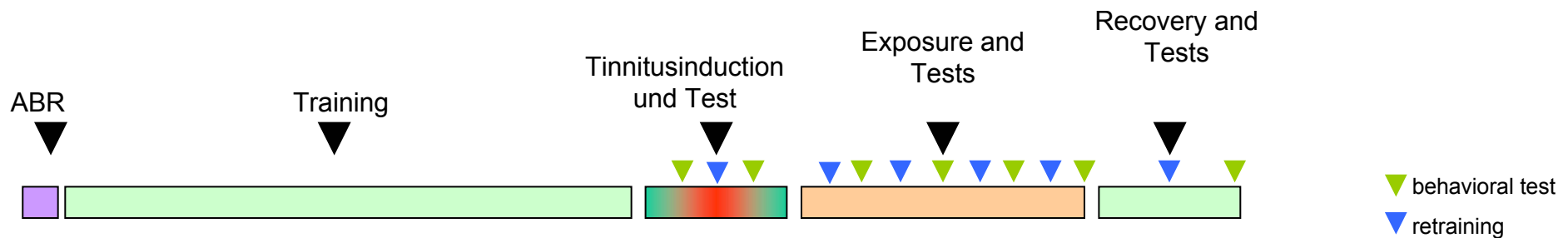
Next 2 Weeks Tinnitus Induction using Salicylat/Saline

Radiation

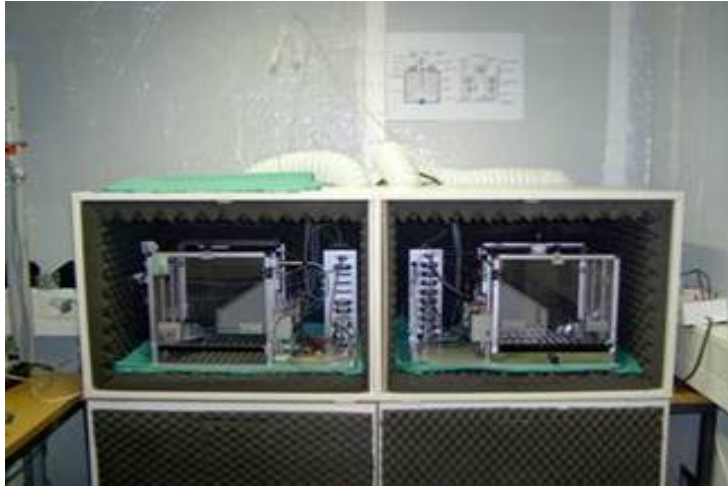
For 4 Weeks at 5 days/Week, including 1 Behavioral Test at the End of the Week

Recovery

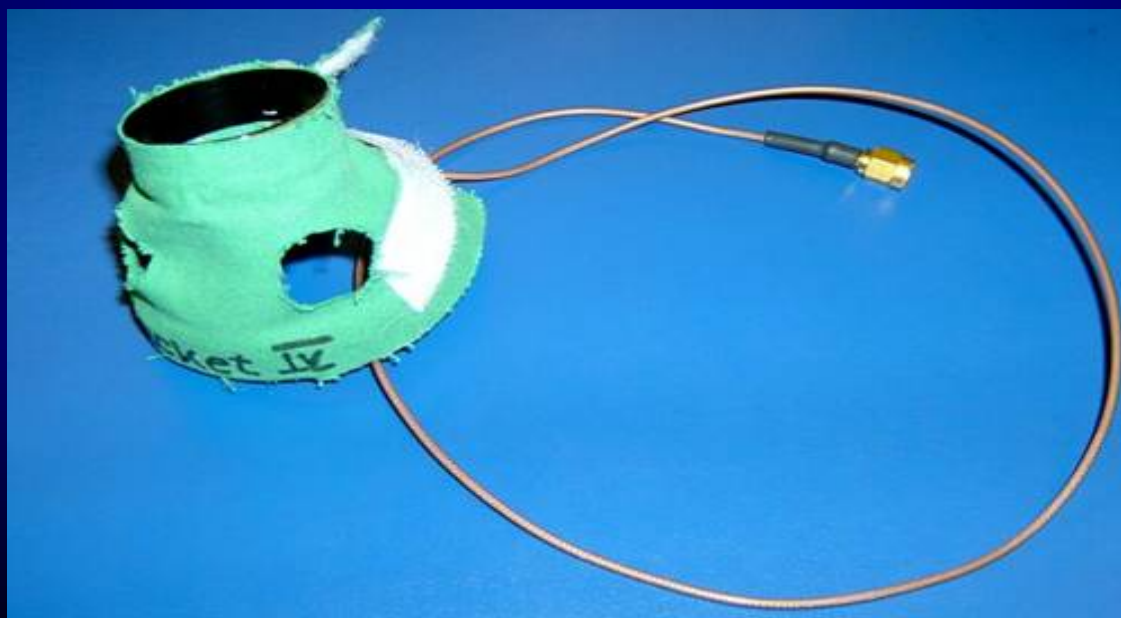
Behavioral Test 2 Weeks after the End of the Radiation



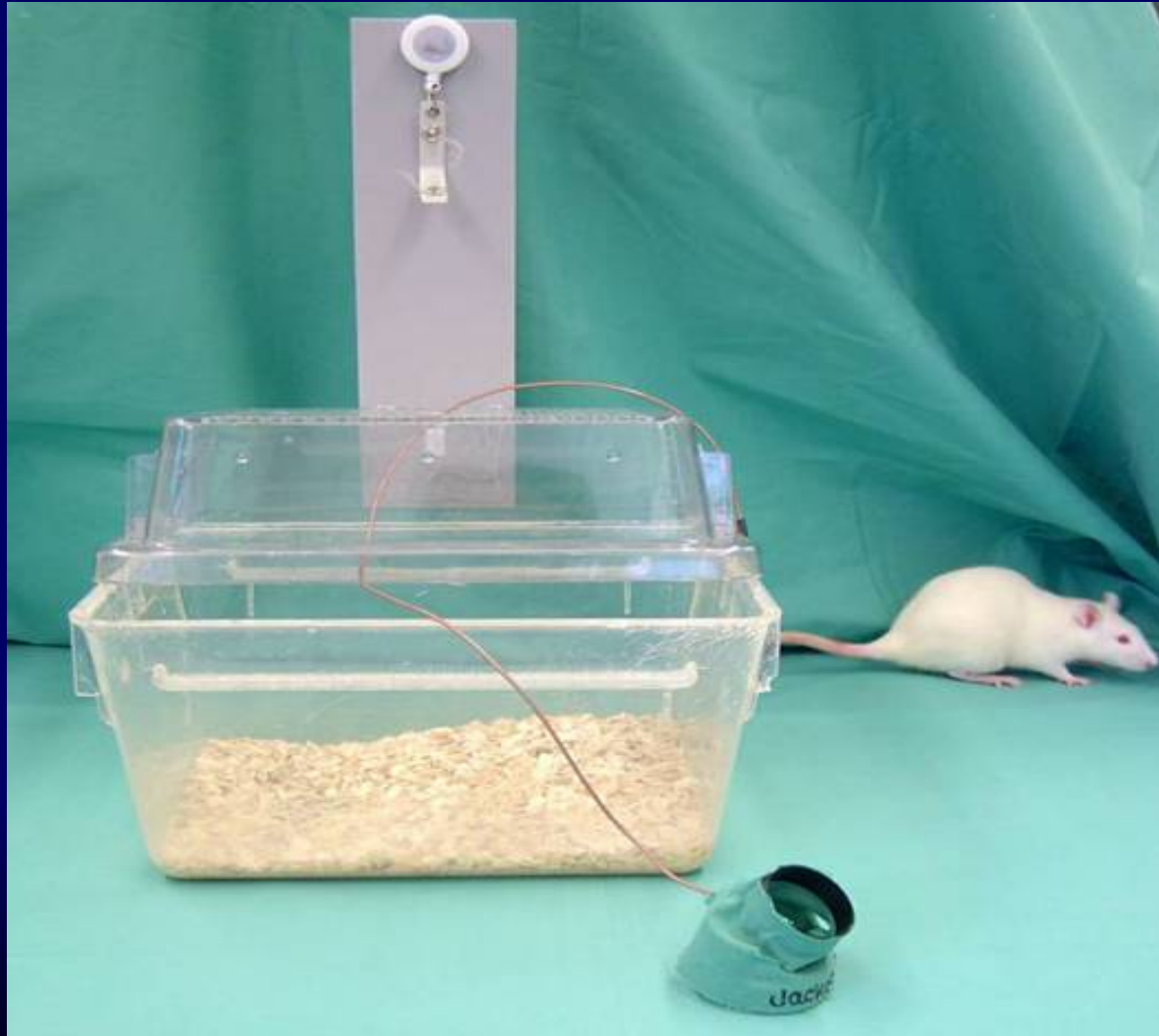
Behavior System



Aerial



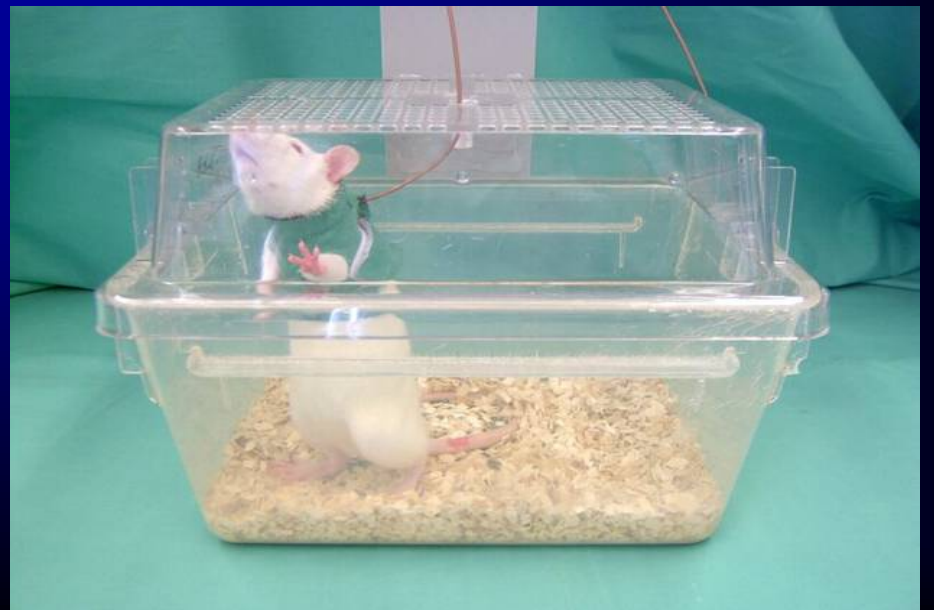
Cage



Positioning of the Aerial



Behavior in the Cage



Exposure



Exposure



Exposure Software

The screenshot displays a Windows XP desktop environment. The desktop background is blue. On the left side, there is a vertical column of icons: Arbeitsplatz, Netzwerkumgebung, Internet Explorer, CamRecorder, Letzte Befehung, PanTilt, CamRecording-Befel..., Backup_Befehung, and Bitte lesen. In the top right corner, there is a logo for 'Measurement & Automation'.

The central window is titled 'RATEX'. It contains several control panels:

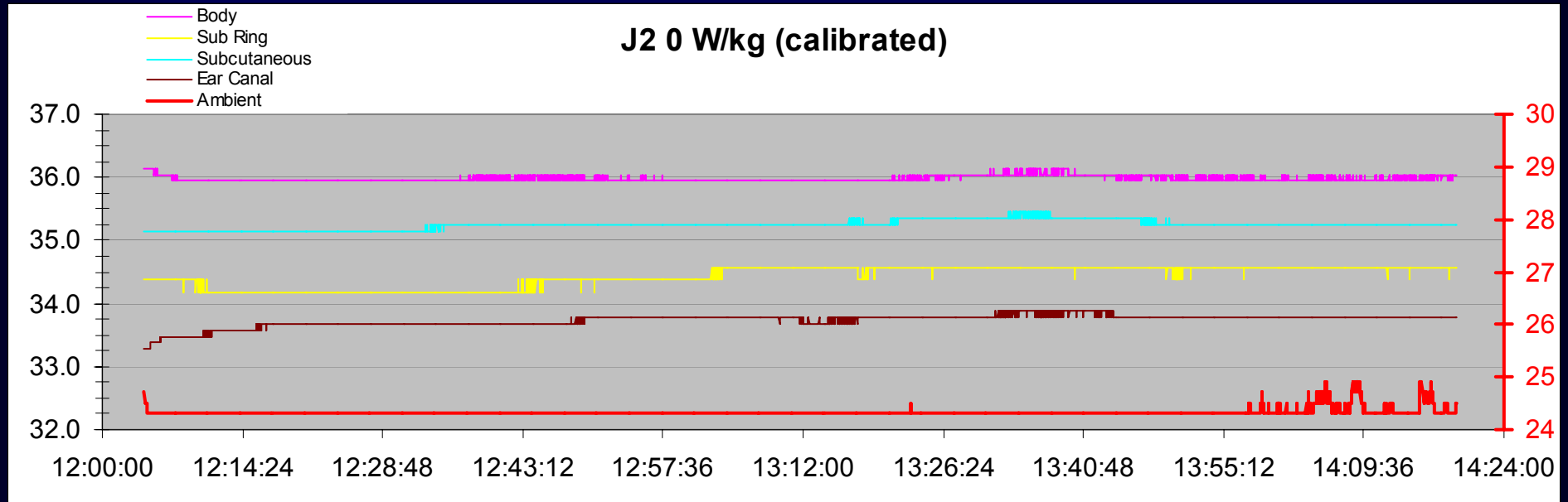
- Antennenrel:** Includes 'Start' and 'Stop' buttons, a 'Test' button, a 'Testdauer (Min.)' dial (0-120), and a 'MRCD' dial (0.0-1.0).
- Meldung:** A text box containing the word 'Exposition'.
- Exposition:** Includes 'Start' and 'Stop' buttons, a 'Pause' button, and a 'Dauer (Min.)' input field set to '15'.
- Yonst für:** Includes 'An' and 'Aus' buttons, and three dials for 'Hauptwert', 'Baleidung th.', and 'Säzung'.

At the bottom, two 'WebCam2000' preview windows are open, both showing a live video feed of a laboratory setup with several glass containers on a circular platform. The left window title is 'WebCam2000 - Creative WebCam Live! Mat...' and the right is 'WebCam2000 - Microsoft WDM Image Captu...'. Both windows have 'View Preview' and 'Enable Web Server' checked. The status bar at the bottom shows the Start button, taskbar icons for 'Pan and Tilt', 'WebCam2000 - Creat...', 'rates', and 'WebCam2000 - Micro...', and a system tray with the time '11:33'.

Behavior during exposure

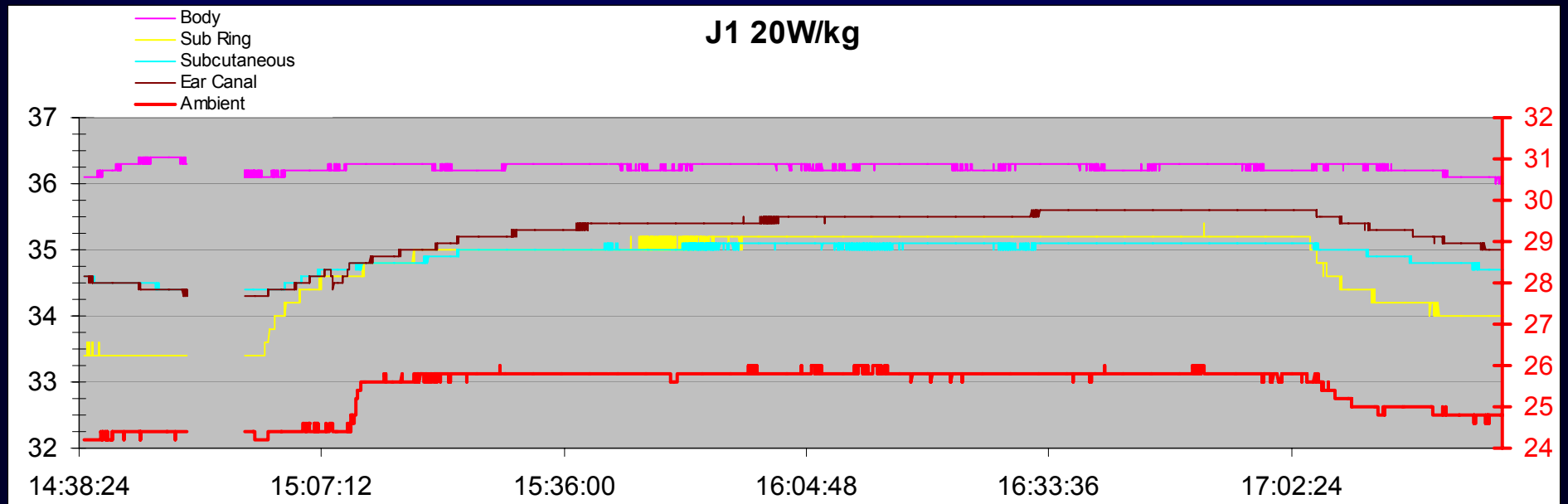


Body temperature (under anesthesia): sham exposure



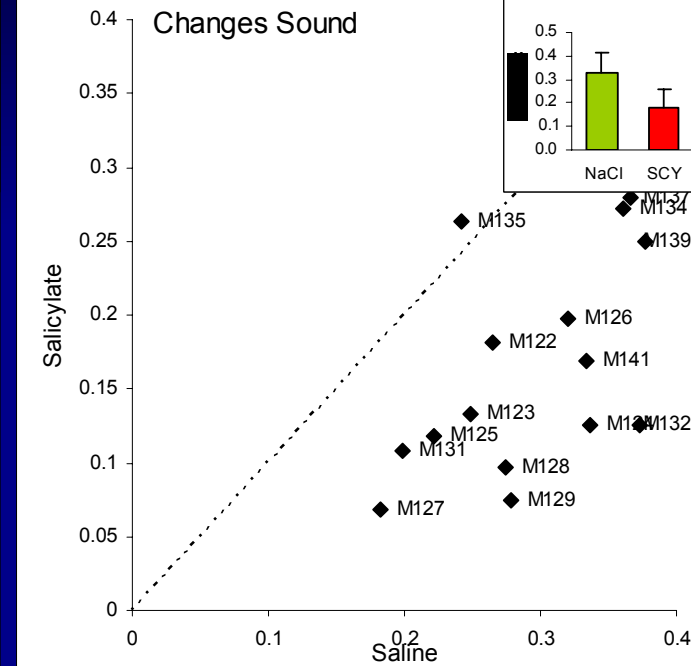
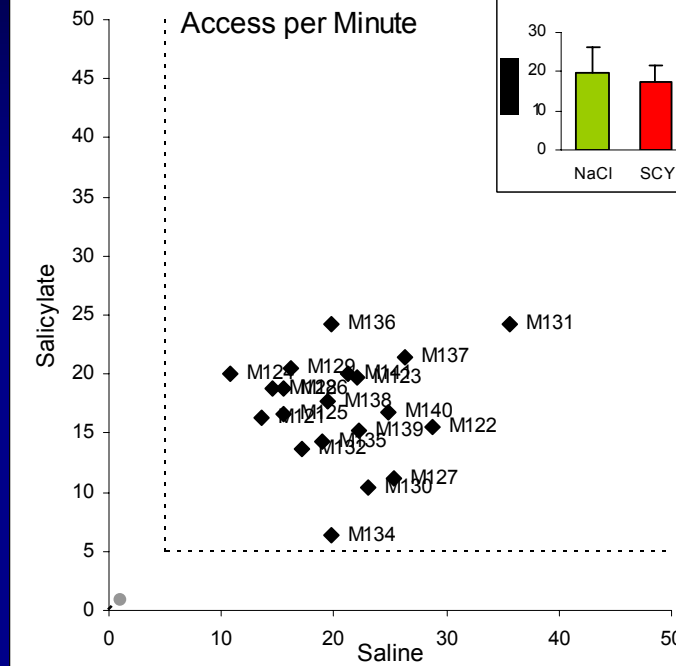
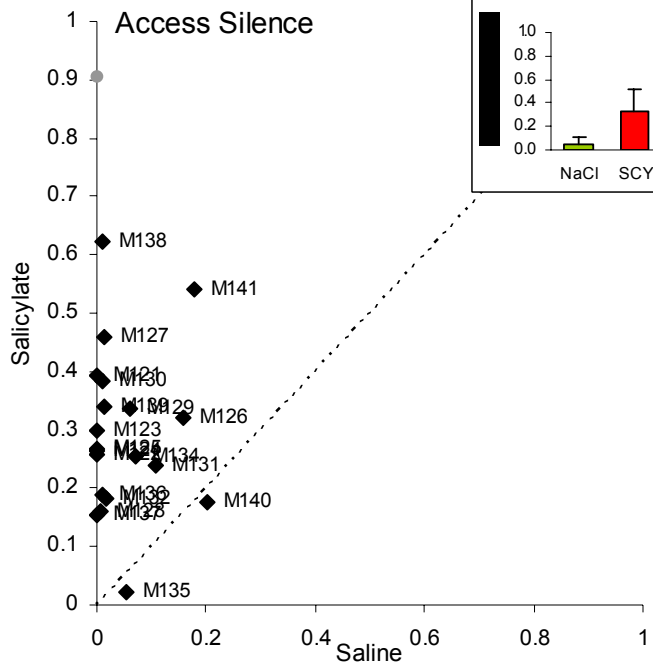
- stable body temperature
- slight temperature rise under antenna ring and in ear canal
- constant ambient temperature

Body temperature (under anesthesia): 20 W/kg exposure



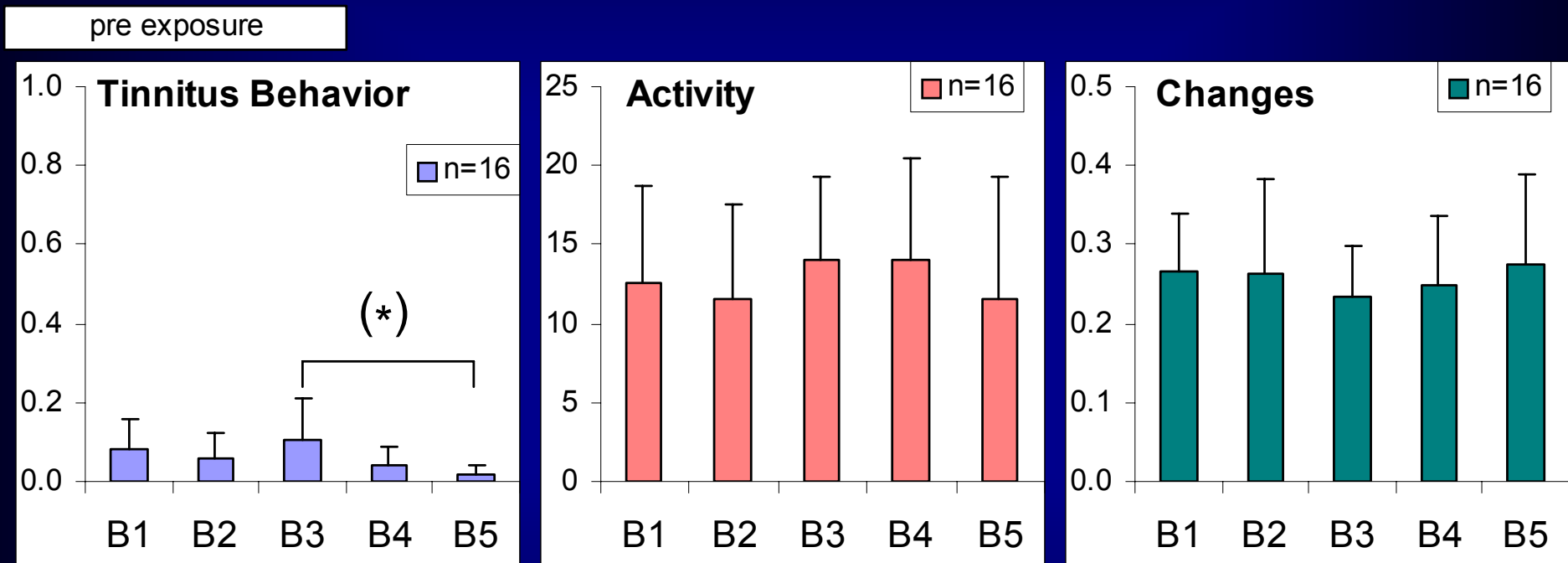
- stable body temperature
- temperature rise under antenna ring, in ear canal and subcutaneously
- delayed rising ambient temperature (convection of warm air ?)

Analysis of Tinnitus Induction Using Salicylate



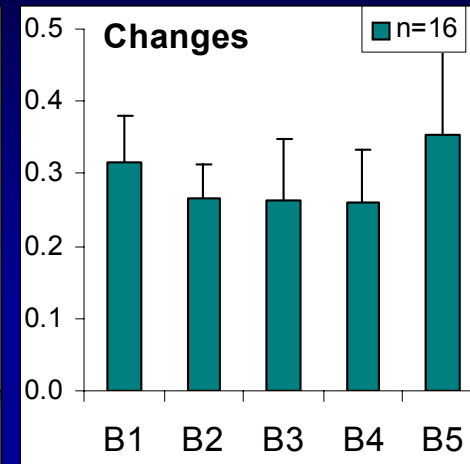
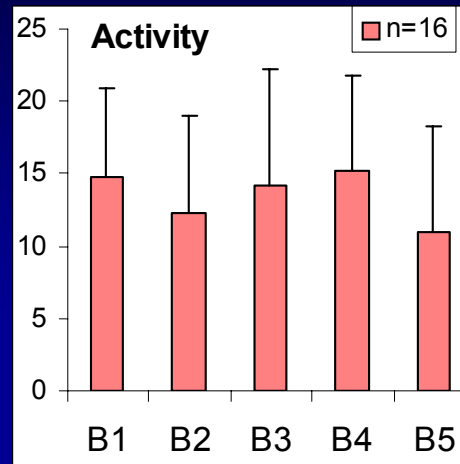
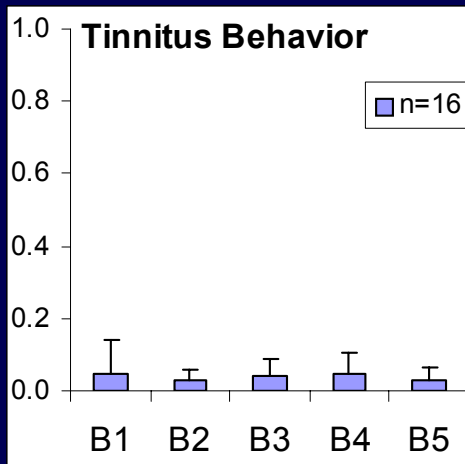
- more accesses during silence after Salicylate injection
- similar activity with Salicylate induced Tinnitus
- fewer side changes in the behavior cage during sound phase

Behavior before exposure

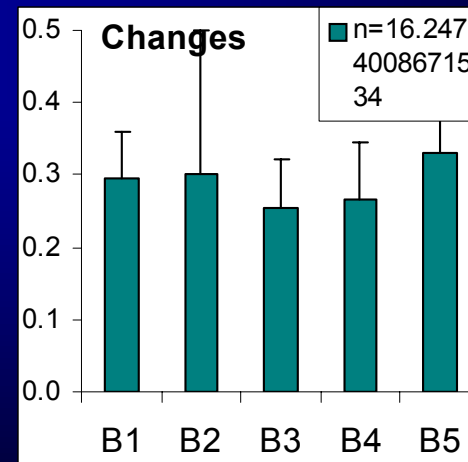
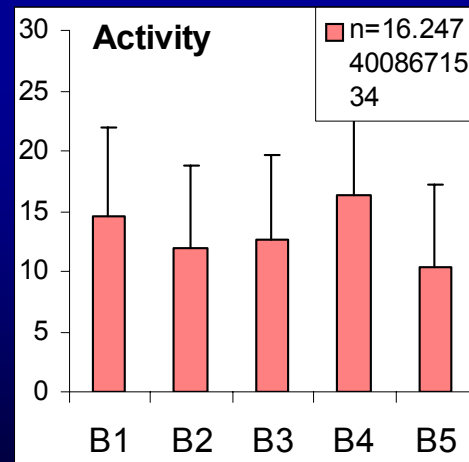
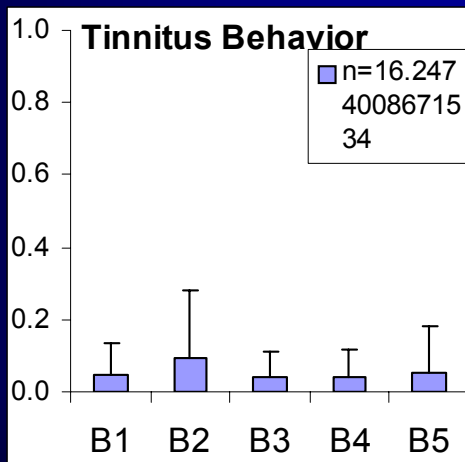


Behavior during exposure period

1 week exposure

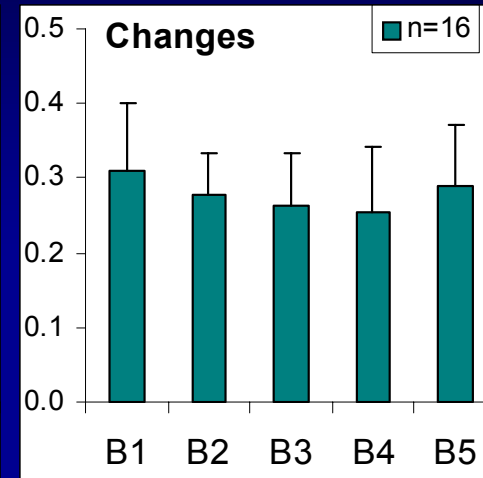
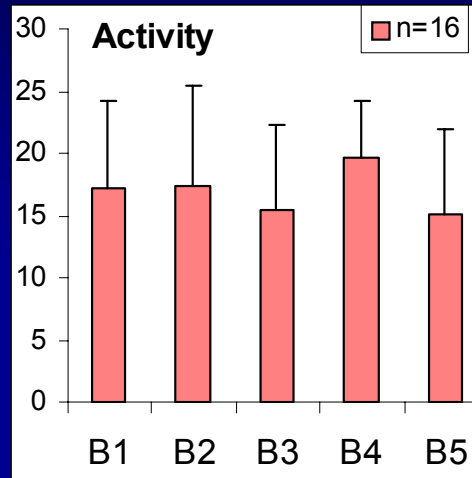
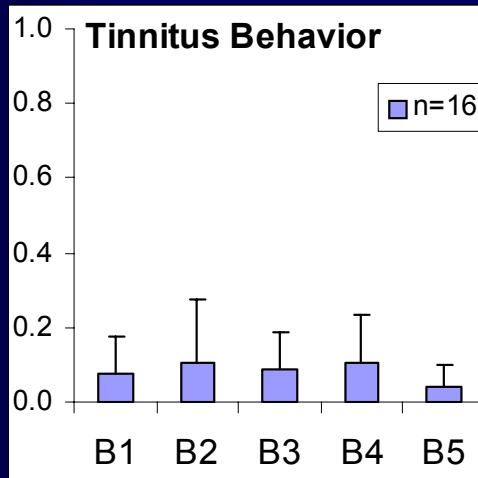


2 weeks exposure

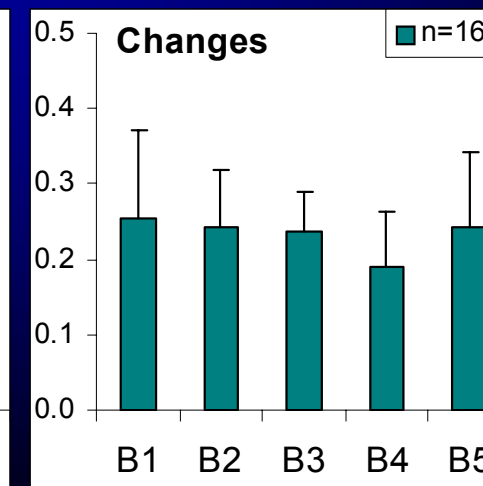
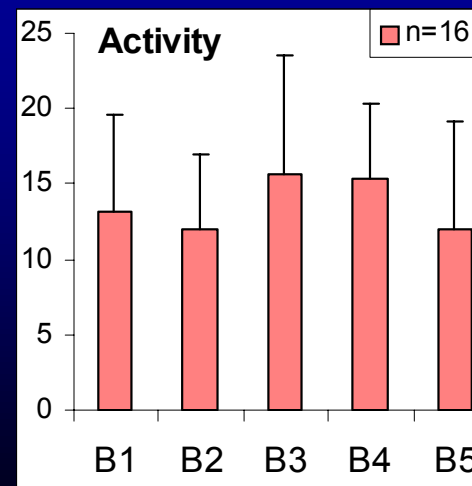
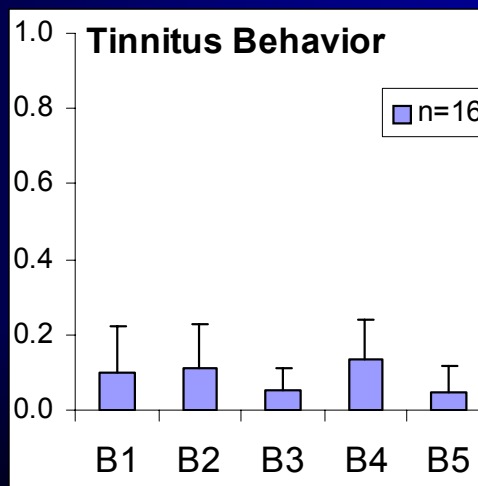


Behavior during exposure period 2

3 weeks exposure

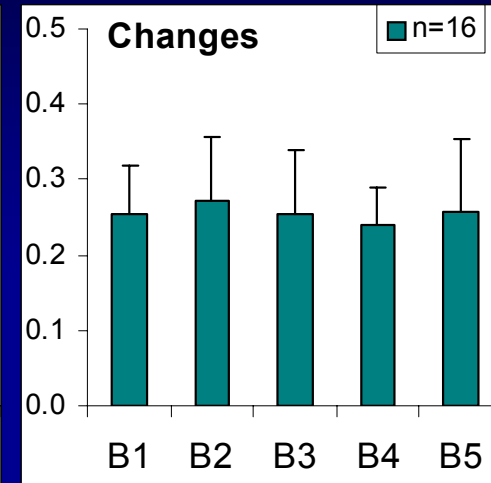
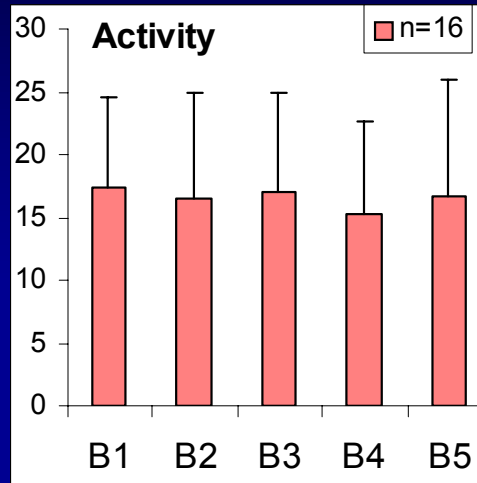
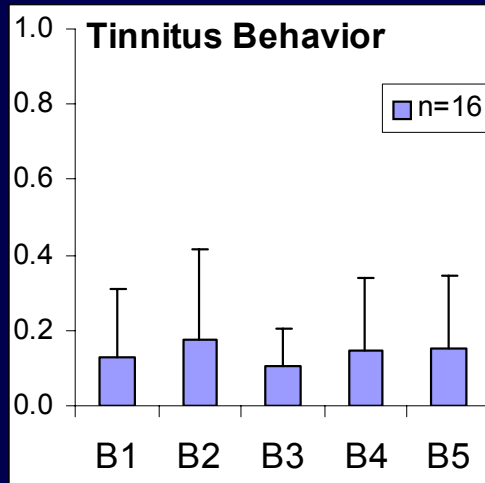


4 weeks exposure

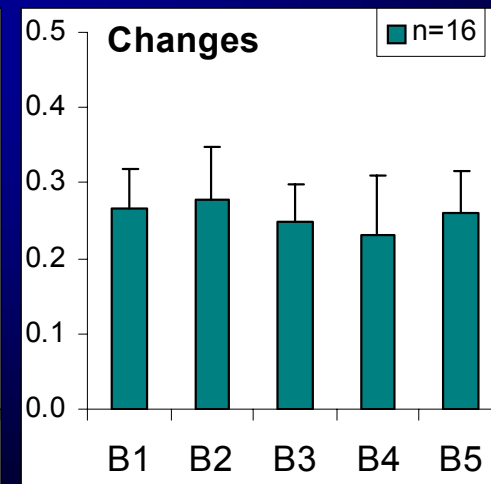
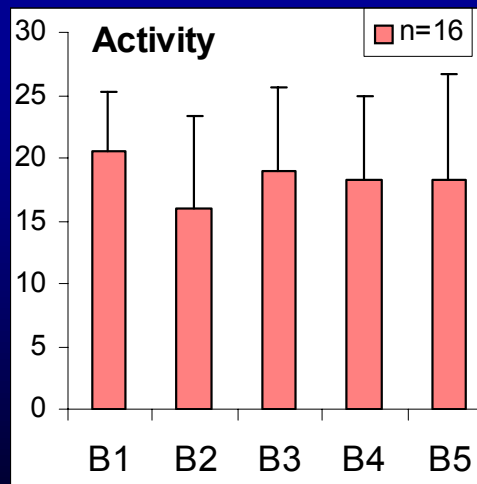
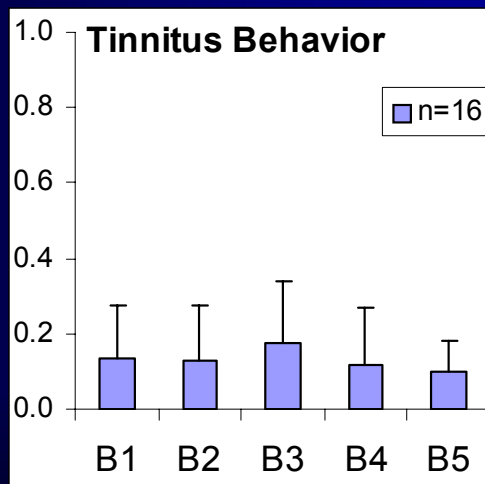


Behavior after exposure (recovery)

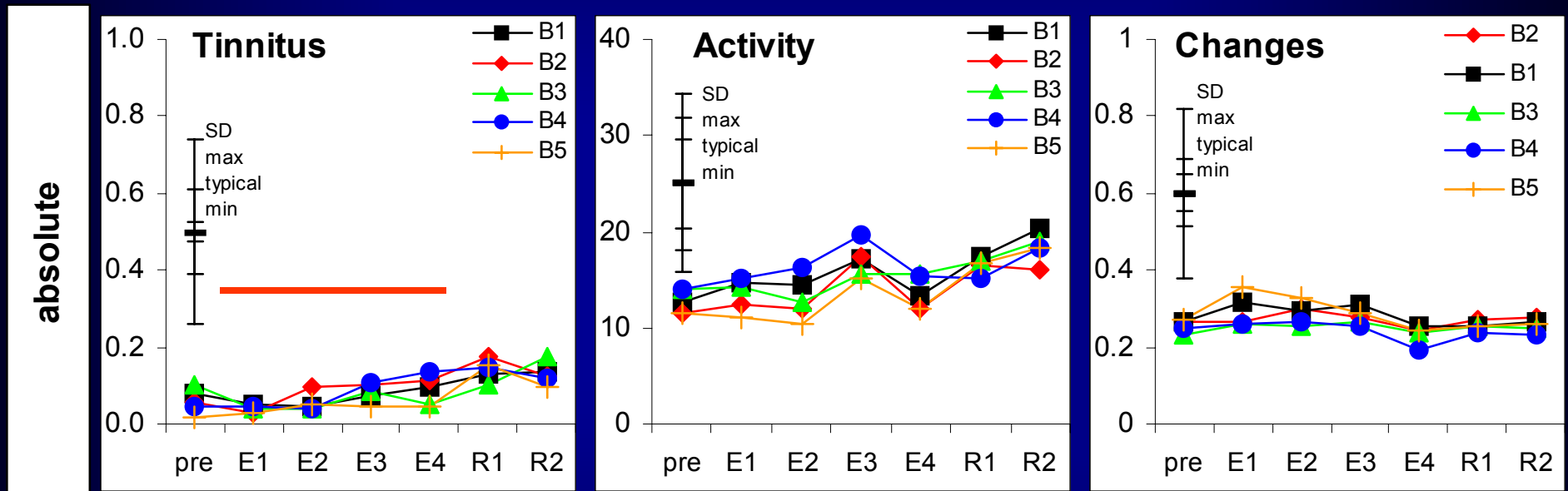
1 week recovery



2 weeks recovery



Behavior : time course



Activity Dependent Genes: Summary

1) First statistical approaches (t-test) did not reveal significant effects between cFos, BDNF Ex IV, Arg3.1/Arc of different exposed animals in CO, IC, AC

Behavior : summary

2) First statistical approaches (t-test) did not reveal important significant effects of exposure on tinnitus sensation

- exposure with 20 W/kg has mild local temperature effects, but does not lead to a rise of body temperature
- little unlearning effects on behavior

Behavior : outlook

- unblinding of exposure groups
- dose dependent correlation analyses and nonparametric statistics of tinnitus behavior & AG