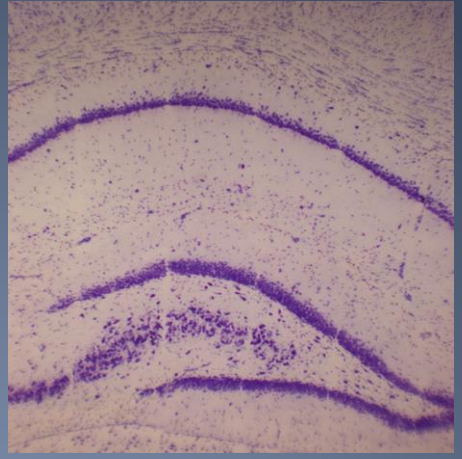
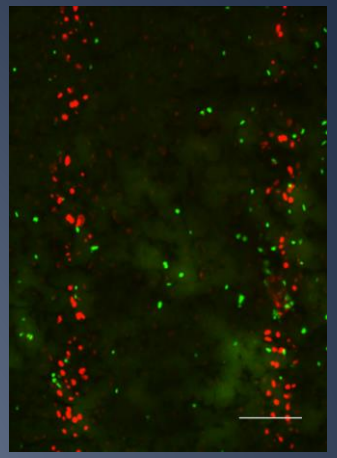
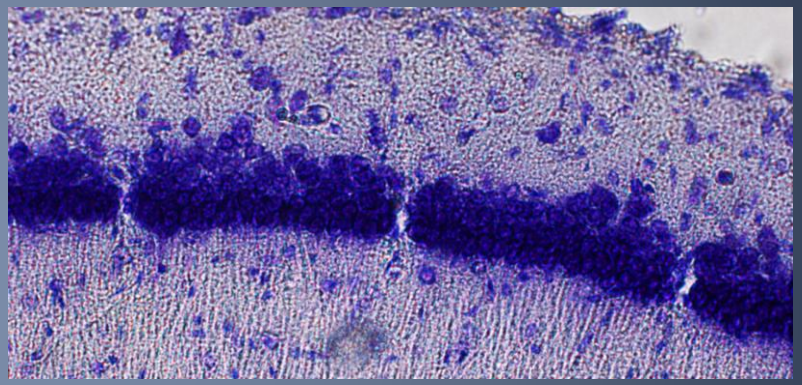




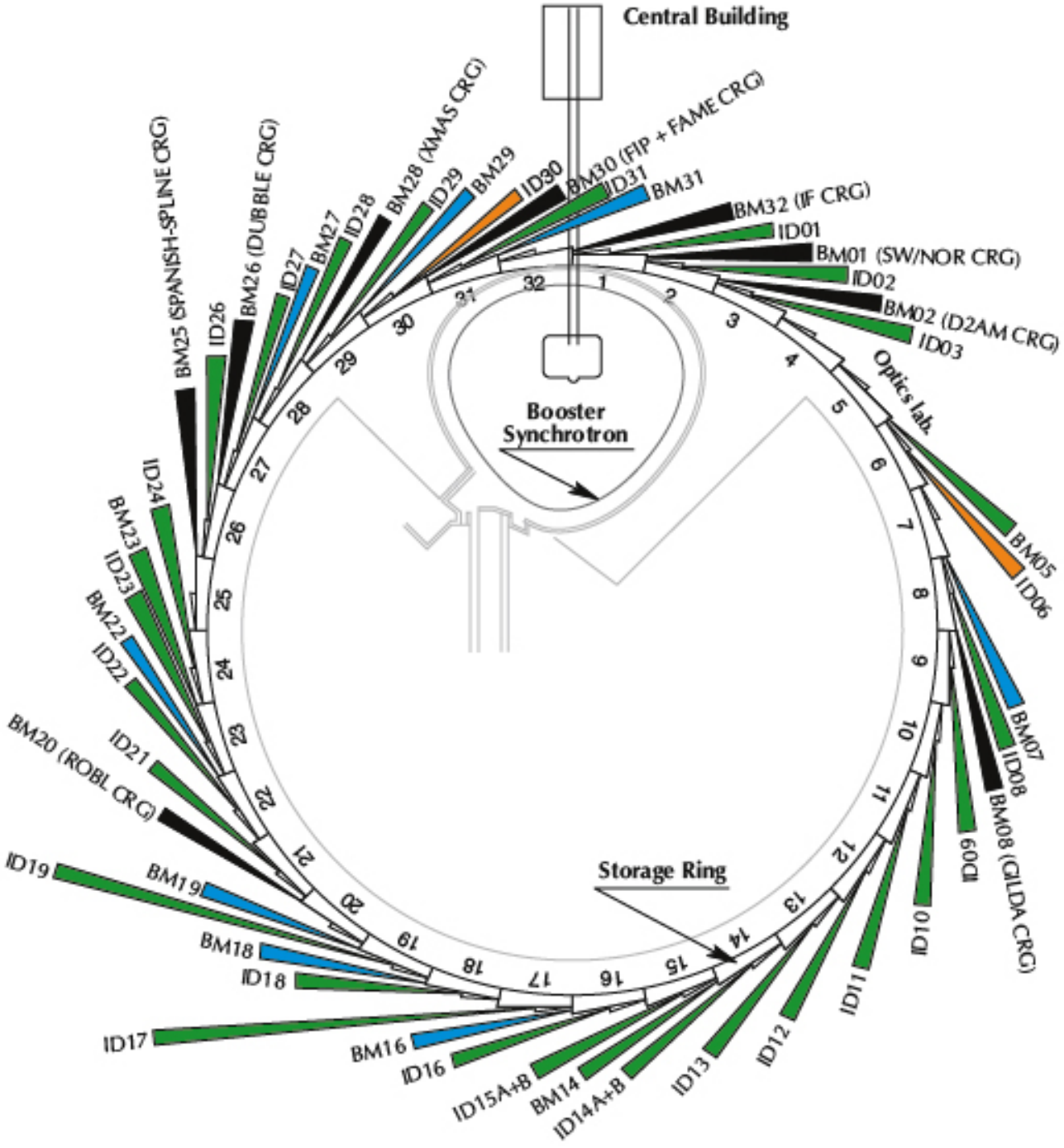


Synchrotron-generated Microbeams
A new frontier for Radiosurgery and Imaging

Pantaleo Romanelli, MD
Neurosurgery/ Brain Radiosurgery, CDI, Milano Visiting Scientist, ESRF, Grenoble

Masterclass on Steretotactic Radiosurgery (SRS/SBRT) of Brain and Spine Tumors

30th – 31st August, 2019 The Leela Ambience, Gurugram


Central Building
Booster Synchrotron
Storage Ring
Optics lab.
BM01 (SW/NOR CRG)
ID01
ID02
BM02 (D2AM CRG)
ID03
BM05
ID06
BM07
ID08
BM08 (GILDA CRG)
60CI
ID09
ID10
ID11
ID12
ID13
BM14
ID14A+B
ID15A+B
BM16
ID16
ID17
BM18
ID18
BM19
ID19
BM20 (ROBL CRG)
ID21
BM22
ID22
BM23
ID23
ID24
BM25 (SPANISH-SPLINE CRG)
ID26
BM26 (DUBBLE CRG)
ID27
BM27
ID28
BM28 (XMAS CRG)
ID29
BM29
ID30
BM30 (FIP + FAME CRG)
ID31
BM31
BM32 (IF CRG)
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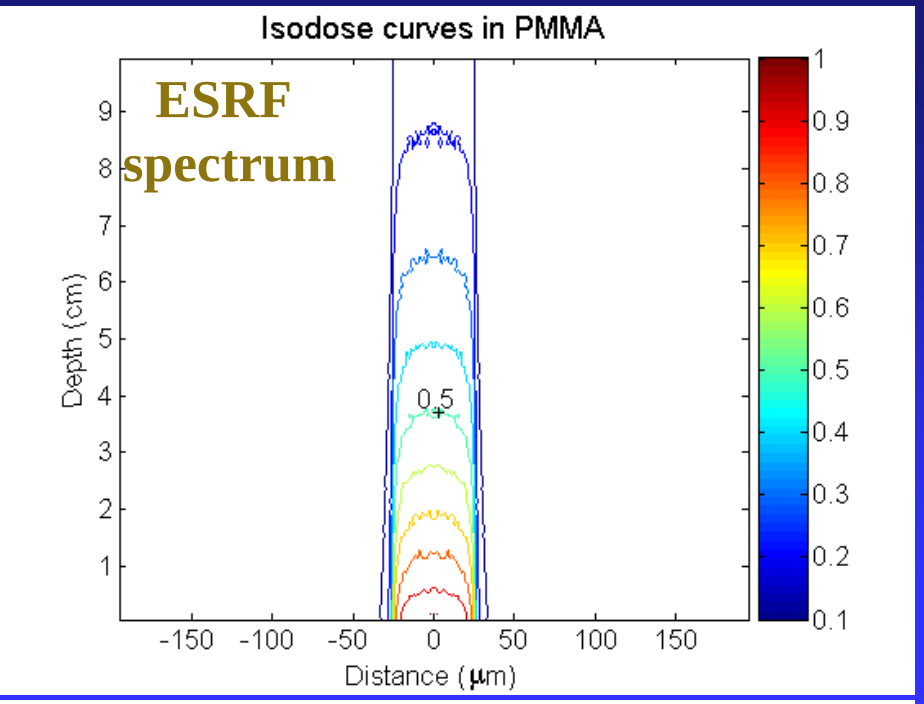
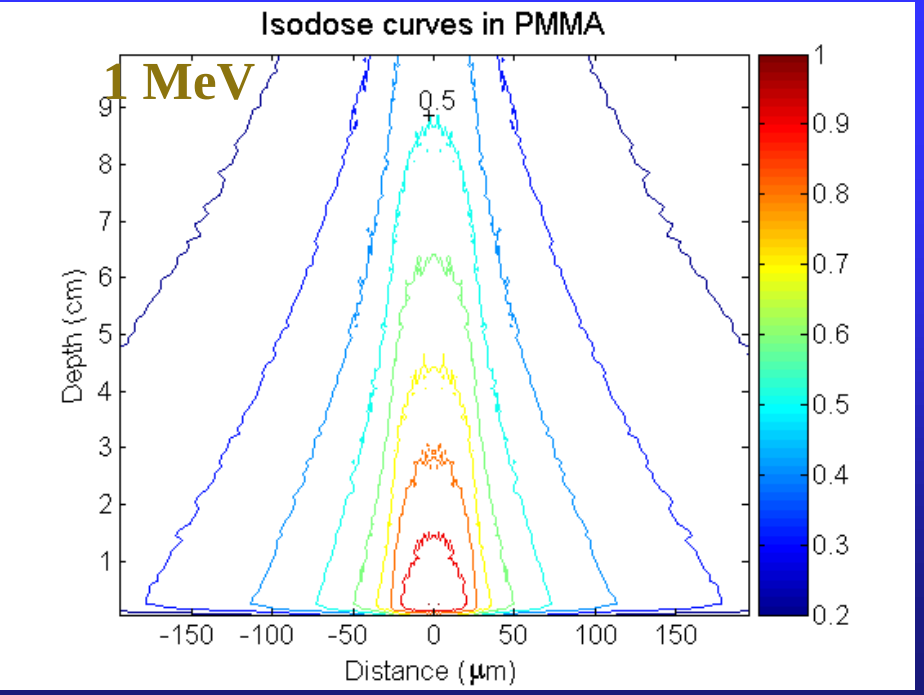
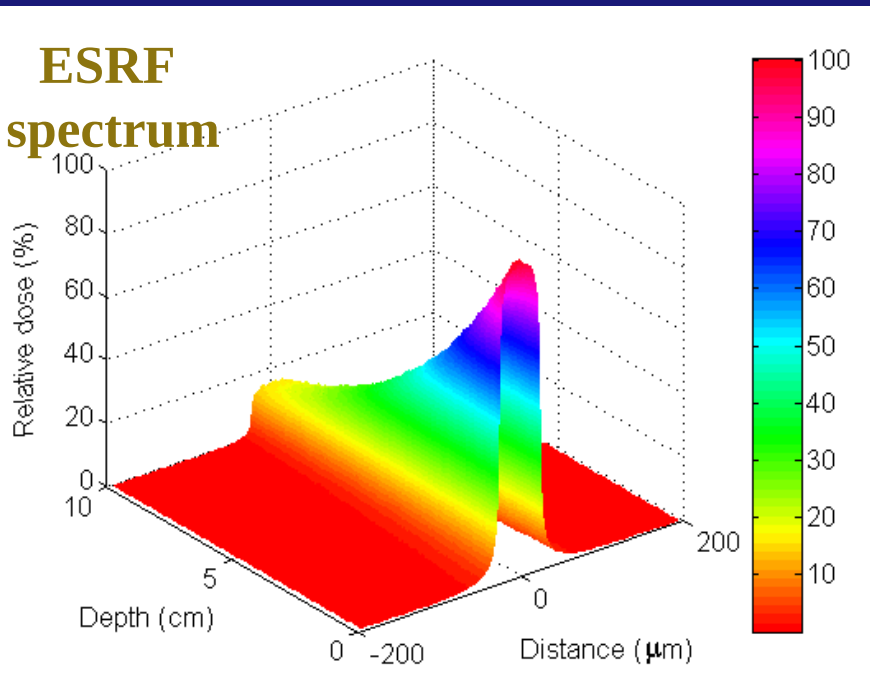
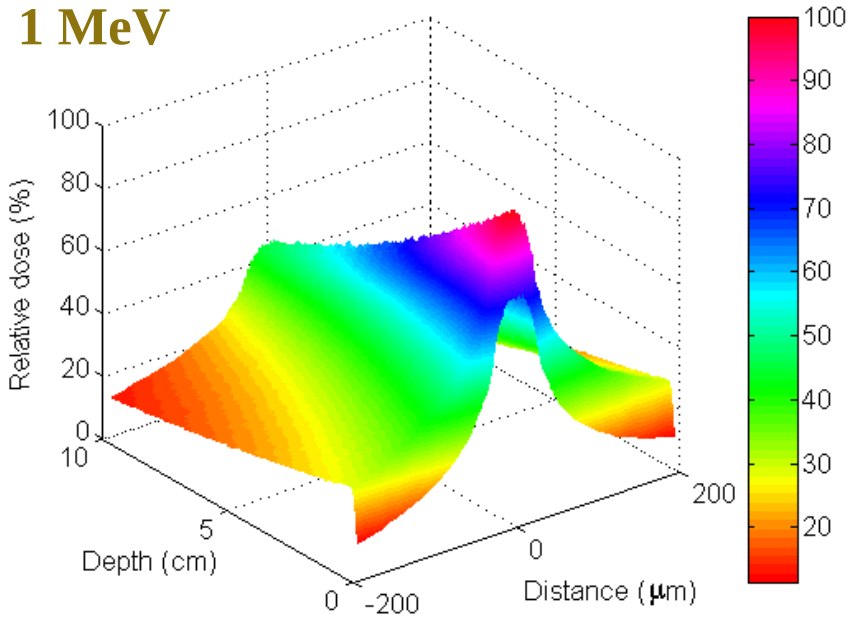
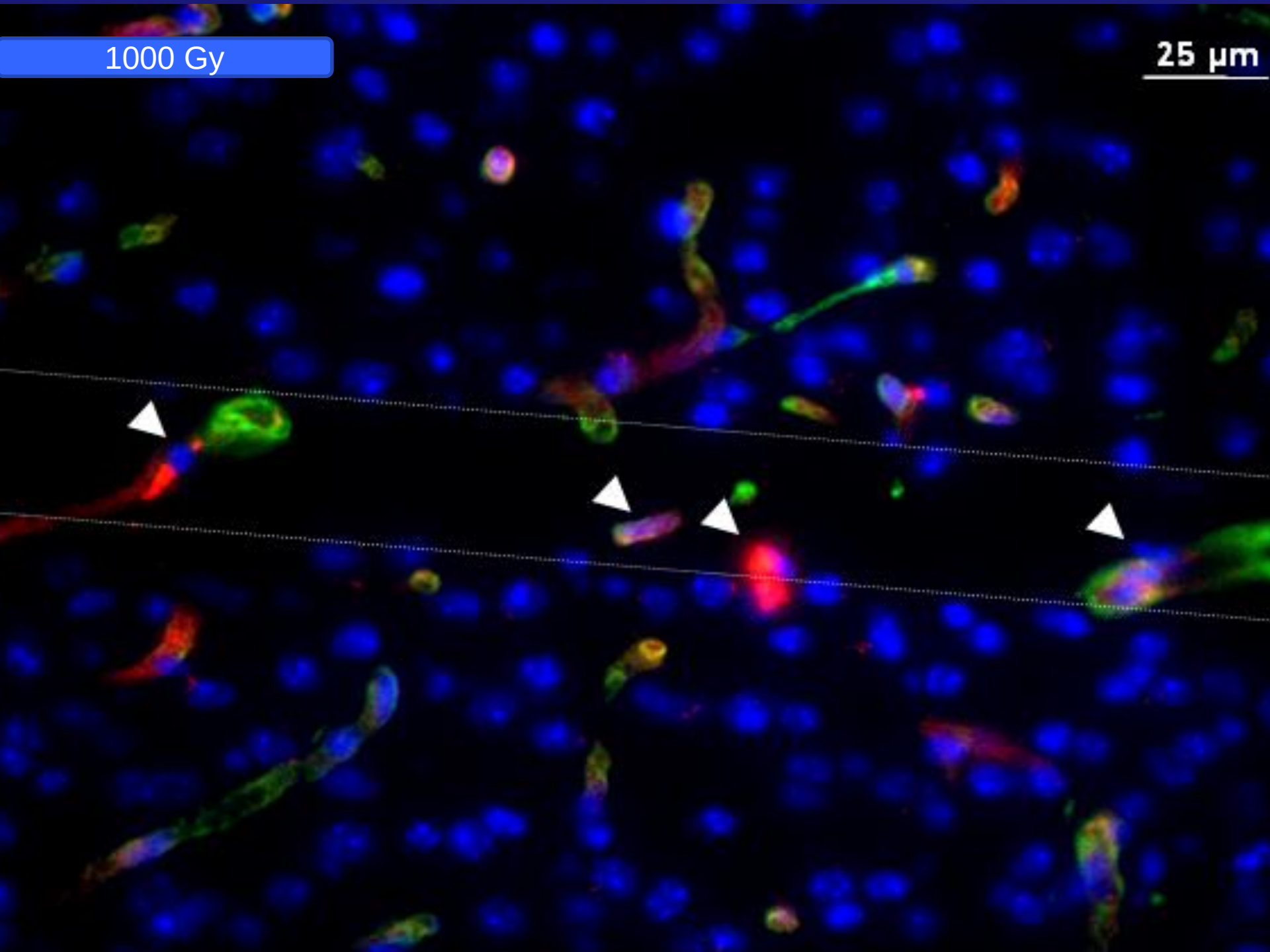

Isodose curves in PMMA
ESRF spectrum
Depth (cm)
Distance (μm)
0.5


Isodose curves in PMMA
1 MeV
Depth (cm)
Distance (μm)
0.5

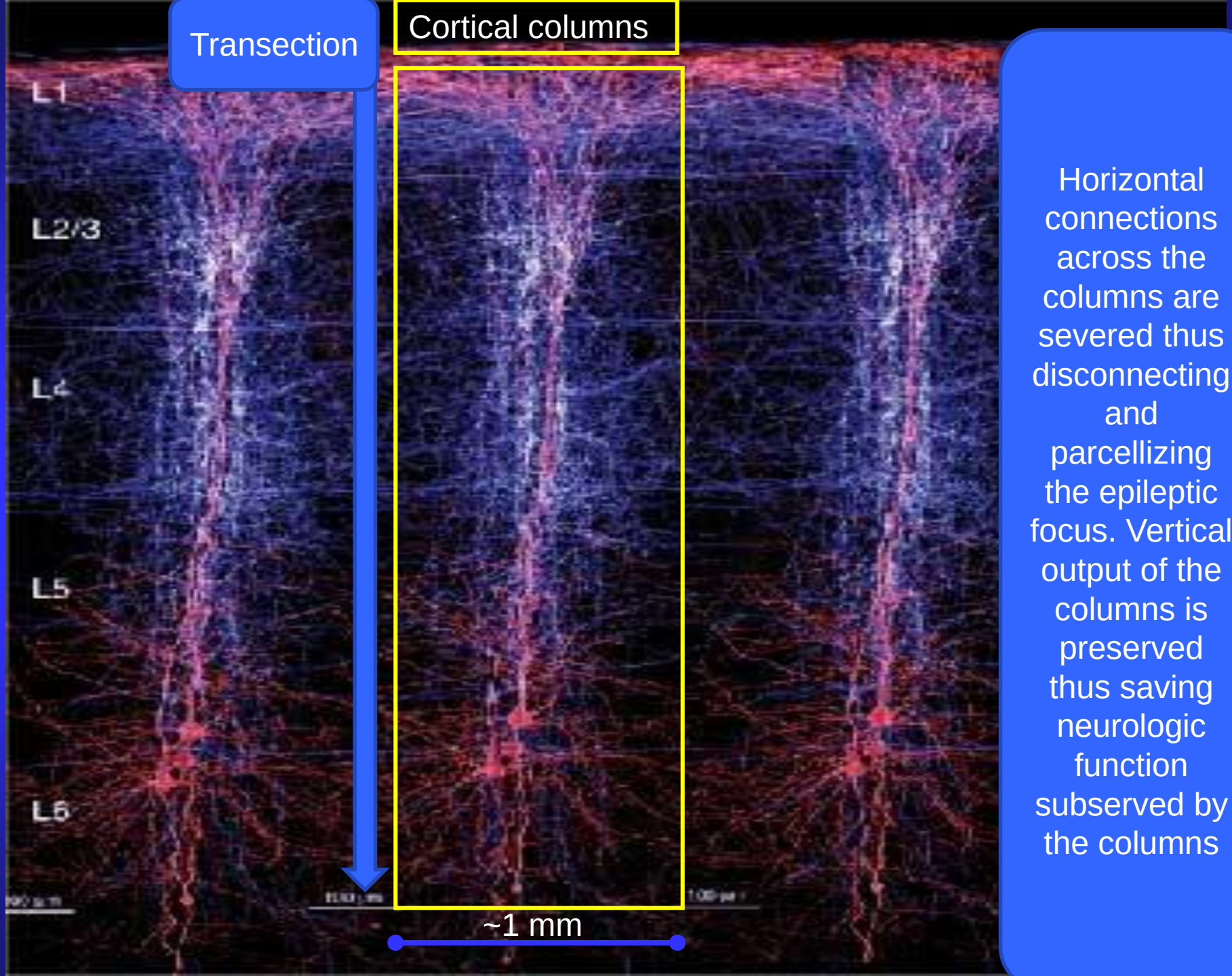

ESRF spectrum
Relative dose (%)
Depth (cm)
Distance (μm)


1 MeV
Relative dose (%)
Depth (cm)
Distance (μm)


1000 Gy
25 µm

Cellular-scale Radiosurgery

- ESRF-based microbeam irradiation can deliver doses of 1000s Gy to tissue slices as small as 25 μm
- Irradiation time @ESRF <0.1 sec
- This extremely fast , highly stereoselective irradiation modality generates irreversible tissue damage spatially restricted to the cells within the beam path while adjacent cells are not damaged, thus providing the ability to perform radiosurgery on a cellular scale
- Microbeam Radiosurgery is unique for its ability to restrict the delivery of extremely high radiation doses to microscopic volumes, thus generating a microsurgical incision which can be 10 times smaller than any knife
- Microbeam Cortical Transections have been developed to offer a new non-invasive way to perform an extremely challenging procedure(Multiple Subpial Transections) used to treat refractory epilepsy foci located over eloquent cortex.



Horizontal connections across the columns are severed thus disconnecting and parcellizing the epileptic focus. Vertical output of the columns is preserved thus saving neurologic function subserved by the columns

Cortical and Hippocampal Transections

- Transections over the sensorimotor cortex and visual cortex are well tolerated without neurological damage*
- Sensorimotor cortex transection induce seizure control without neurologic damage in rats with status epilepticus induced by focal injection of kainic acid in sensorimotor cortex**
- Hippocampal transections are well tolerated as well***

*Nature Sci Rep 2017,Phys Med 2015
**PlosOne 2013
***Nature Sci Rep 2018

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Rat sensorimotor cortex tolerance to parallel transections induced by synchrotron-generated X-ray microbeams

Erminia Fardone[1,6], Alberto Bravin[1], Alfredo Conti[2], Elke Bräuer-Krisch[1], Herwig Requardt[1], Domenico Bucci[3], Geraldine Le Duc[1], Giuseppe Battaglia[3] & Pantaleo Romanelli[4,5]

Microbeam radiation therapy is a novel preclinical technique, which uses synchrotron-generated X-rays for the treatment of brain tumours and drug-resistant epilepsies. In order to safely translate this approach to humans, a more in-depth knowledge of the long-term radiobiology of microbeams in healthy tissues is required. We report here the result of the characterization of the rat sensorimotor cortex tolerance to microradiosurgical parallel transections. Healthy adult male Wistar rats underwent irradiation with arrays of parallel microbeams. Beam thickness, spacing and incident dose were 100 or 600 µm, 400 or 1200 µm and 360 or 150 Gy, respectively. Motor performance was carried over a 3-month period. Three months after irradiation rats were sacrificed to evaluate the effects of irradiation on brain tissues by histology and immunohistochemistry. Microbeam irradiation of sensorimotor cortex did not affect weight gain and motor performance. No gross signs of paralysis or paresis were also observed. The cortical architecture was not altered, despite the presence of cell death along the irradiation path. Reactive gliosis was evident in the microbeam path of rats irradiated with 150 Gy, whereas no increase was observed in rats irradiated with 360 Gy.

OPEN ACCESS Freely available online

PLOS ONE

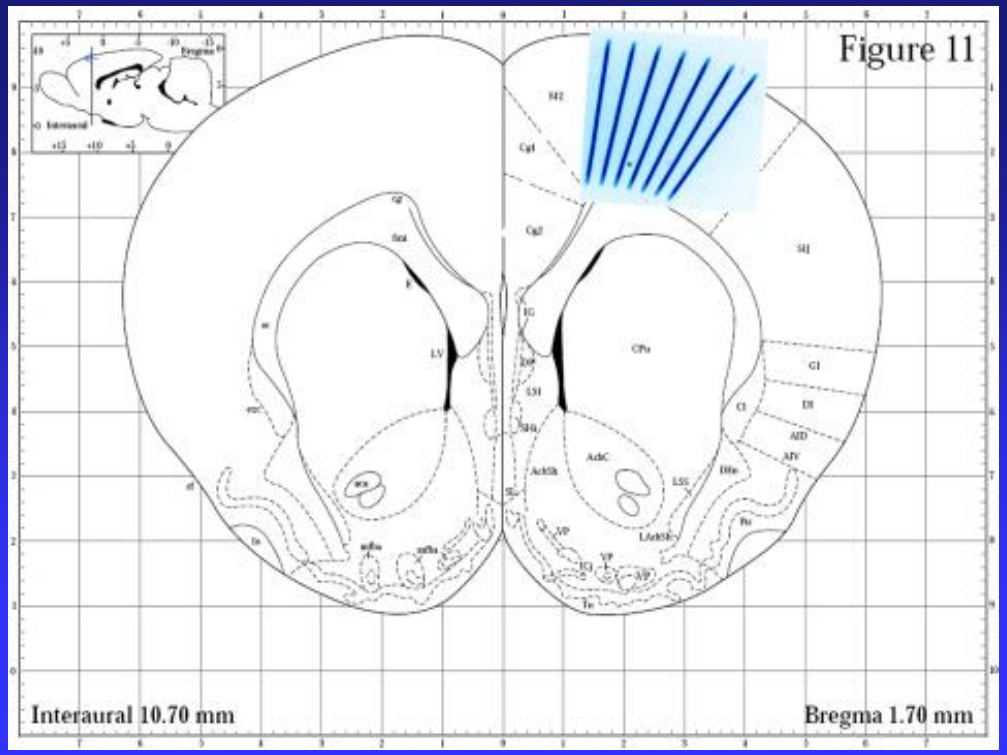
Synchrotron-Generated Microbeam Sensorimotor Cortex Transections Induce Seizure Control without Disruption of Neurological Functions

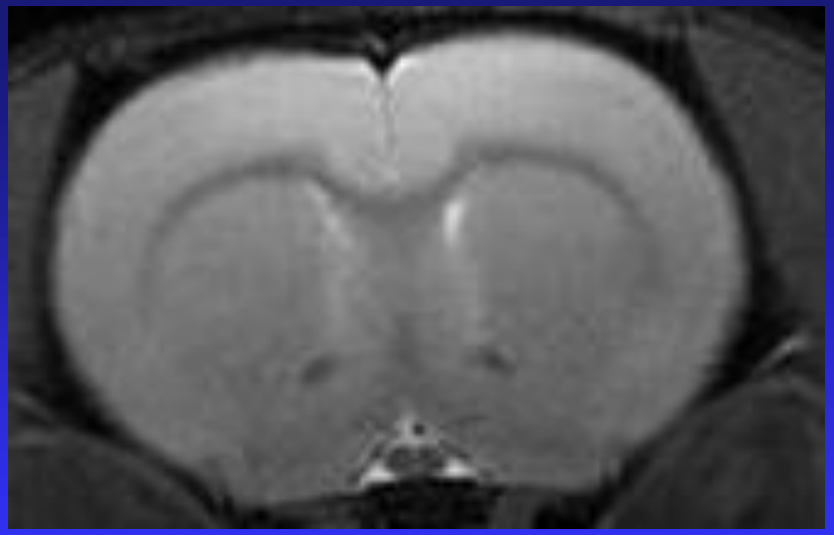
Pantaleo Romanelli[1,2,3]*, Erminia Fardone[3], Giuseppe Battaglia[4], Elke Bräuer-Krisch[3], Yolanda Prezado[3], Herwig Requardt[3], Geraldine Le Duc[3], Christian Nemoz[3], David J. Anschel[5], Jenny Spiga[6], Alberto Bravin[3]

1 Centro Diagnostico Italiano, Brain Radiosurgery, Cyberknife Center, Milano, Italy, **2** AB Medica, Lainate, Italy, **3** European Synchrotron Radiation Facility, BP220, Grenoble, France, **4** Istituto Di Ricovero e Cura a Carattere Scientifico Neuromed, Località Camerelle, Pozzilli, Italy, **5** Comprehensive Epilepsy Center of Long Island, St. Charles Hospital, Port Jefferson, New York, United States of America, **6** Department of Physics, University of Cagliari and Istituto Nazionale di Fisica Nucleare, Monserrato, Italy

Abstract

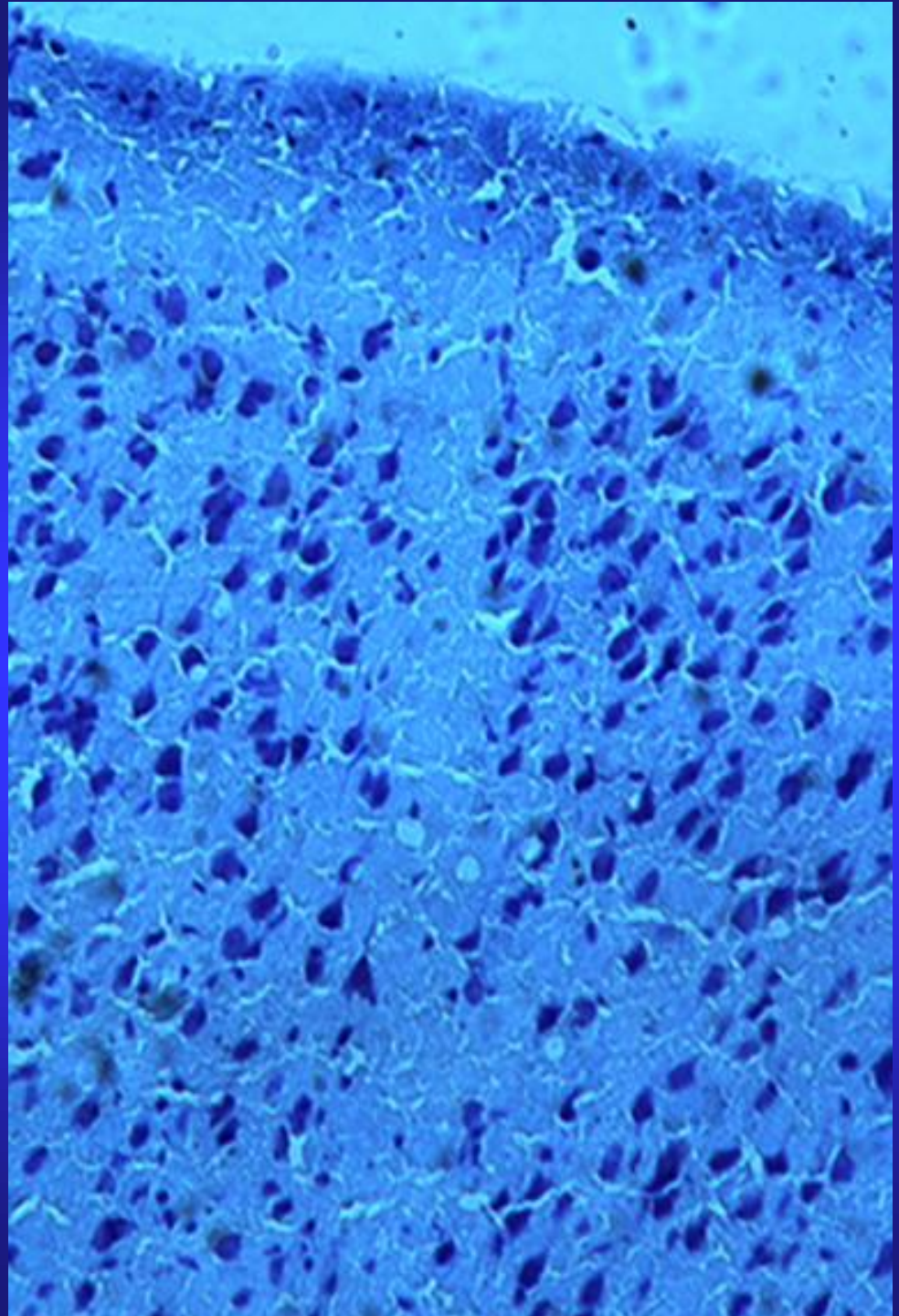
Synchrotron-generated X-ray microplanar beams (microbeams) are characterized by the ability to deliver extremely high doses of radiation to spatially restricted volumes of tissue. Minimal dose spreading outside the beam path provides an exceptional degree of protection from radio-induced damage to the neurons and glia adjacent to the microscopic slices of tissue irradiated. The preservation of cortical architecture following high-dose microbeam irradiation and the ability to induce non-invasively the equivalent of a surgical cut over the cortex is of great interest for the development of novel experimental models in neurobiology and new treatment avenues for a variety of brain disorders. Microbeams (size 100 µm/600 µm, center-to-center distance of 400 µm/1200 µm, peak entrance doses of 360-240 Gy/150-100 Gy) delivered to the sensorimotor cortex of six 2-month-old naïve rats generated histologically evident cortical transections, without modifying motor behavior and weight gain up to 7 months. Microbeam transections of the sensorimotor cortex dramatically reduced convulsive seizure duration in a further group of 12 rats receiving local infusion of kainic acid. No subsequent neurological deficit was associated with the treatment. These data provide a novel tool to study the functions of the cortex and pave the way for the development of new therapeutic strategies for epilepsy and other neurological diseases.



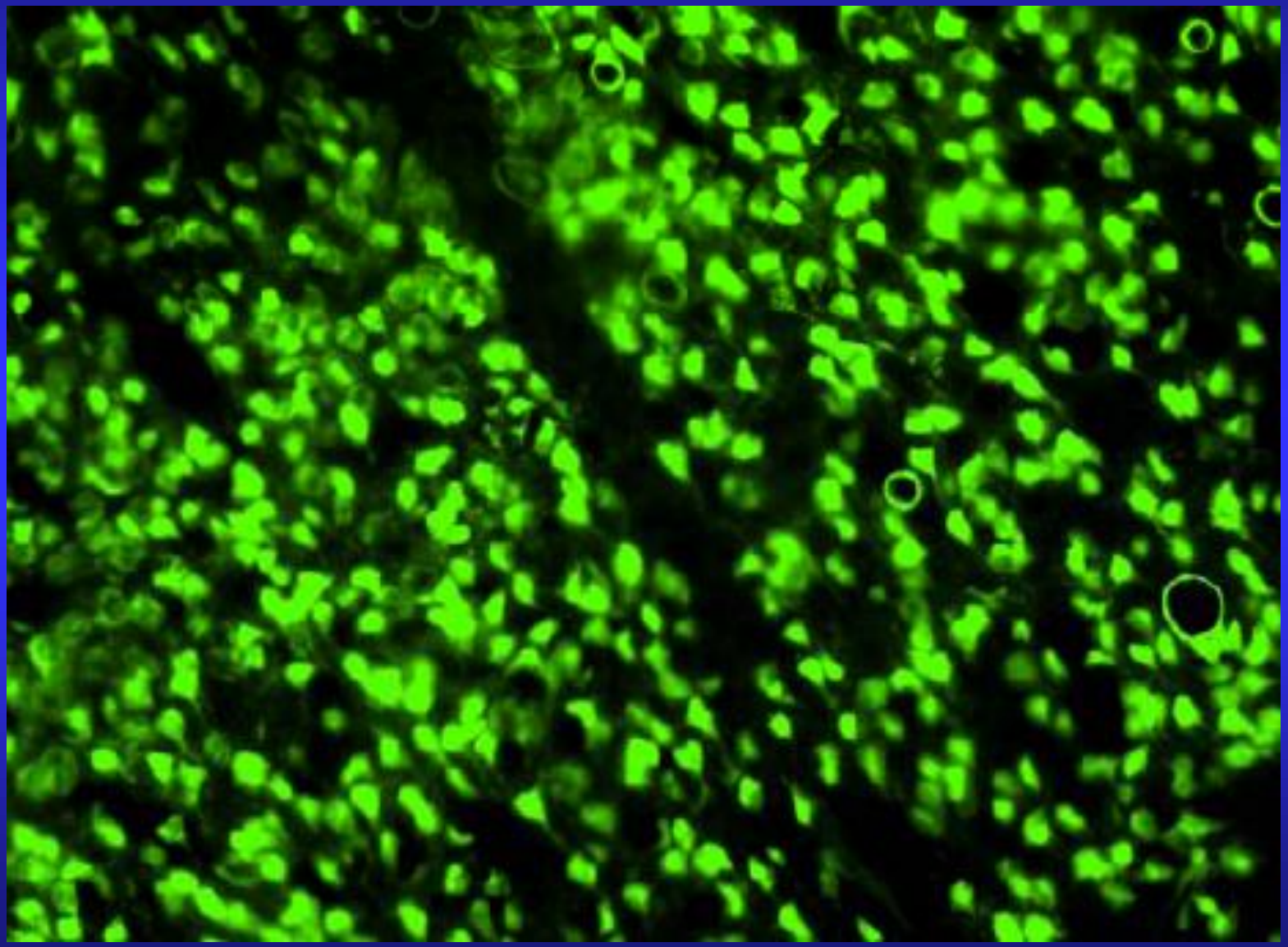


Control T2W coronal MR 6 m after
No sign of edema or radionecrosis

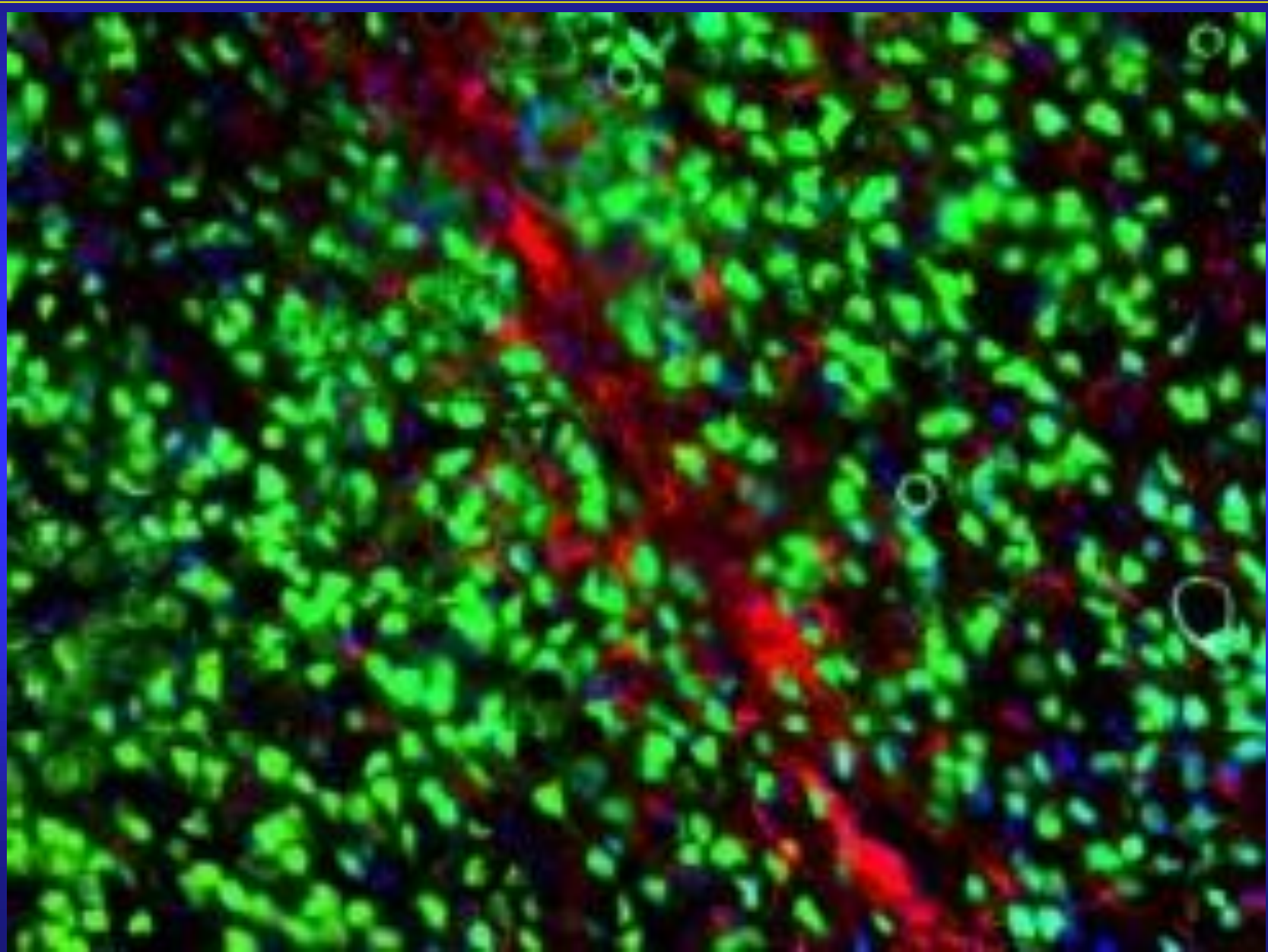
- Microbeam Radiosurgery generates microscopic cortical and hippocampal incisions of the wanted thickness and spacing
- These cortical incisions are equivalent to those obtained by Multiple Subpial Transections(MST), a surgical technique used for the treatment of epileptic foci located over eloquent cortex
- Microbeam Transections replicate the ability of MST to modulate the cortex without functional injury but are non invasive and the size of the incisions and spacings can be predetermined



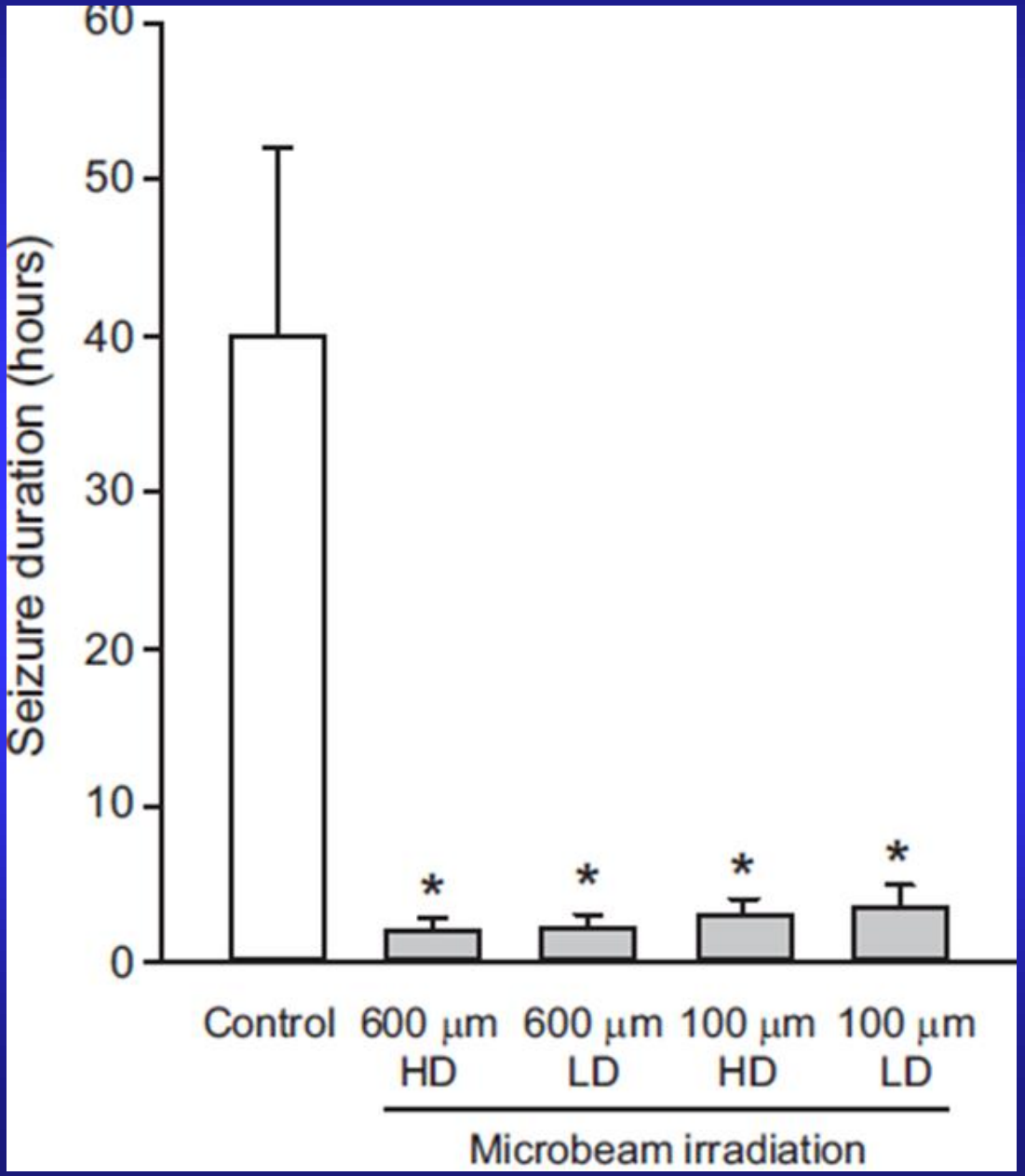
Lack of neuronal nuclei along the microbeam path 7 months after irradiation (NeuN)



Glial cells colonize the microbeam path
GFAP + NeuN



Convulsive seizure duration: controls vs transected



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Synchrotron-generated microbeams induce hippocampal transections in rats

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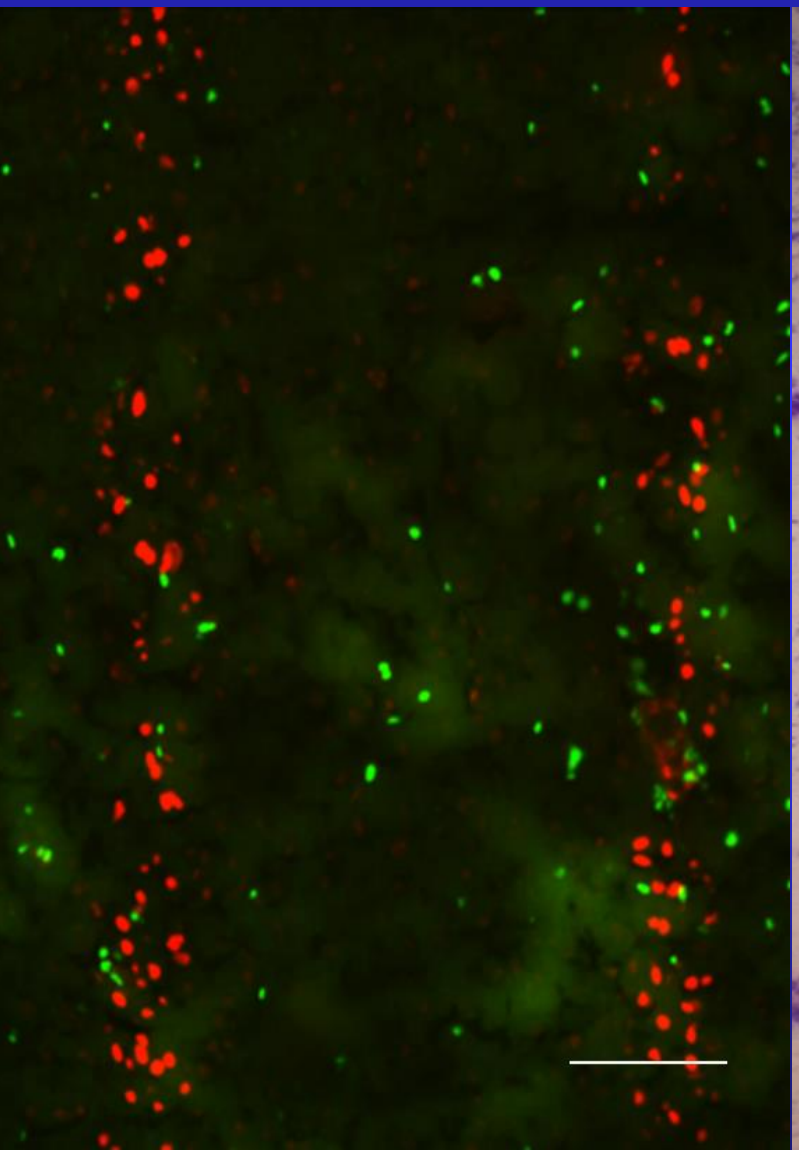
Published online: 09 January 2018

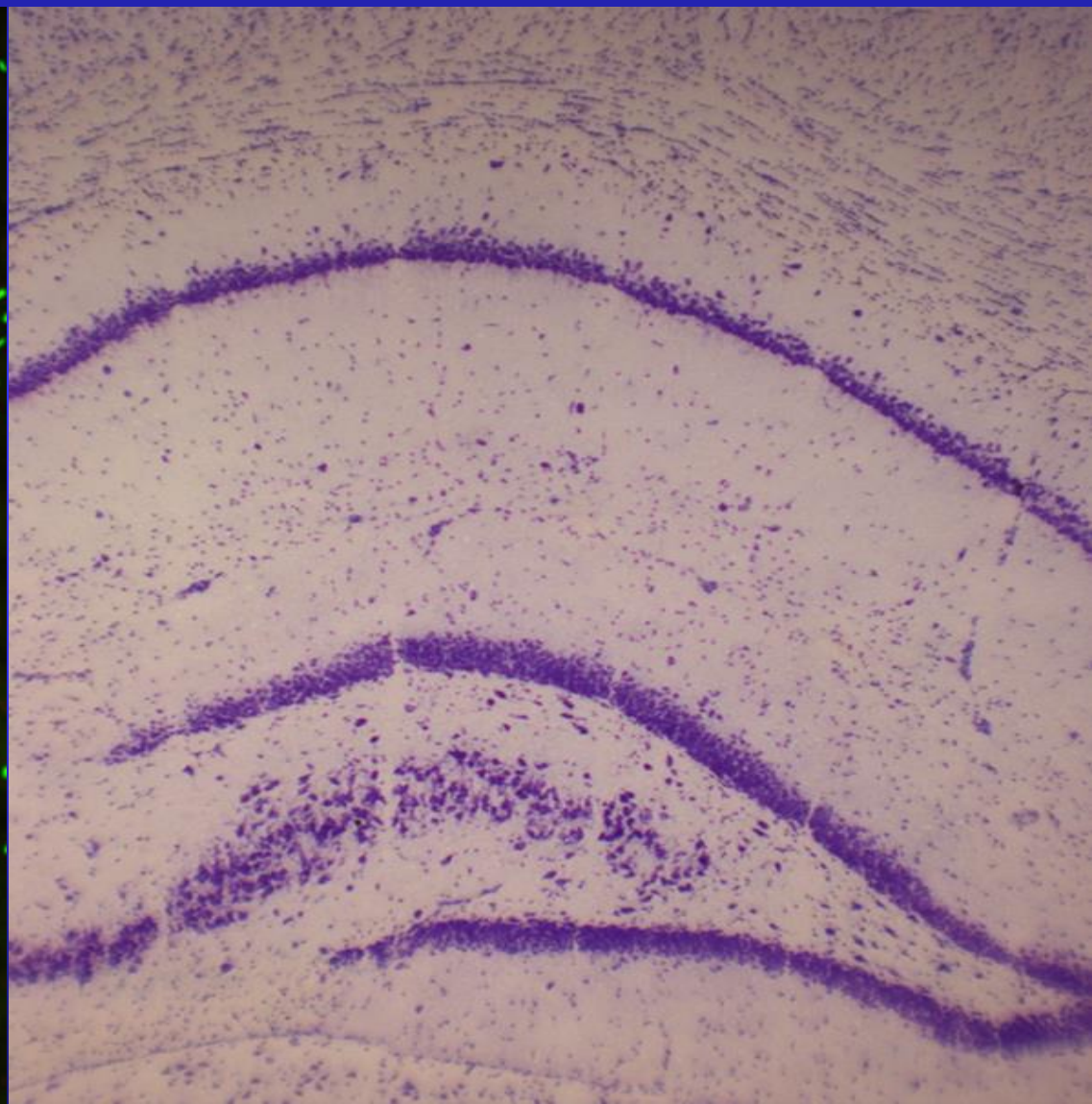
Erminia Fardone[1,9], Benoît Pouyatos[2], Elke Bräuer-Krisch[1], Stefan Bartzsch[3,4], Hervè Mathieu[2], Herwig Requardt[1], Domenico Bucci[5], Giacomo Barbone[6], Paola Coan[6,7], Giuseppe Battaglia[5], Geraldine Le Duc[1], Alberto Bravin[1] & Pantaleo Romanelli[8]

Synchrotron-generated microplanar beams (microbeams) provide the most stereo-selective irradiation modality known today. This novel irradiation modality has been shown to control seizures originating from eloquent cortex causing no neurological deficit in experimental animals. To test the hypothesis that application of microbeams in the hippocampus, the most common source of refractory seizures, is safe and does not induce severe side effects, we used microbeams to induce transections to the hippocampus of healthy rats. An array of parallel microbeams carrying an incident dose of 600 Gy was delivered to the rat hippocampus. Immunohistochemistry of phosphorylated γ-H2AX showed cell death along the microbeam irradiation paths in rats 48 hours after irradiation. No evident behavioral or neurological deficits were observed during the 3-month period of observation. MR imaging showed no signs of radio-induced edema or radionecrosis 3 months after irradiation. Histological analysis showed a very well preserved hippocampal cytoarchitecture and confirmed the presence of clear-cut microscopic transections across the hippocampus. These data support the use of synchrotron-generated microbeams as a novel tool to slice the hippocampus of living rats in a minimally invasive way, providing (i) a novel experimental model to study hippocampal function and (ii) a new treatment tool for patients affected by refractory epilepsy induced by mesial temporal sclerosis.

HIPPOCAMPAL TRANSECTIONS

Immediate cell death and adjacent replication assessed respectively by pH2AX(red dots) and Ki67 (green) and late histology(Nissl) after synchrotron-generated microbeams (75 µm) delivering an incident dose of 600Gy





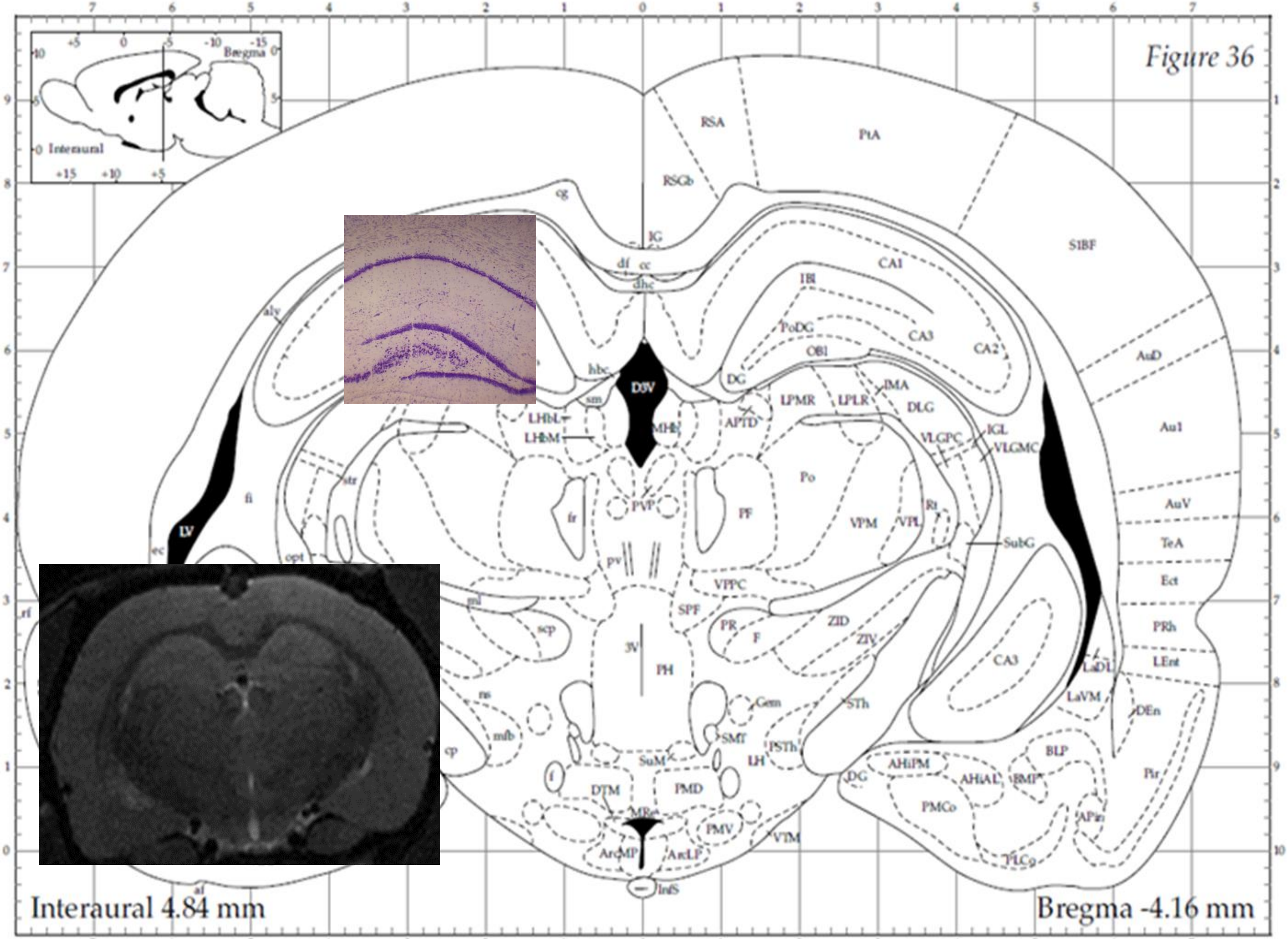
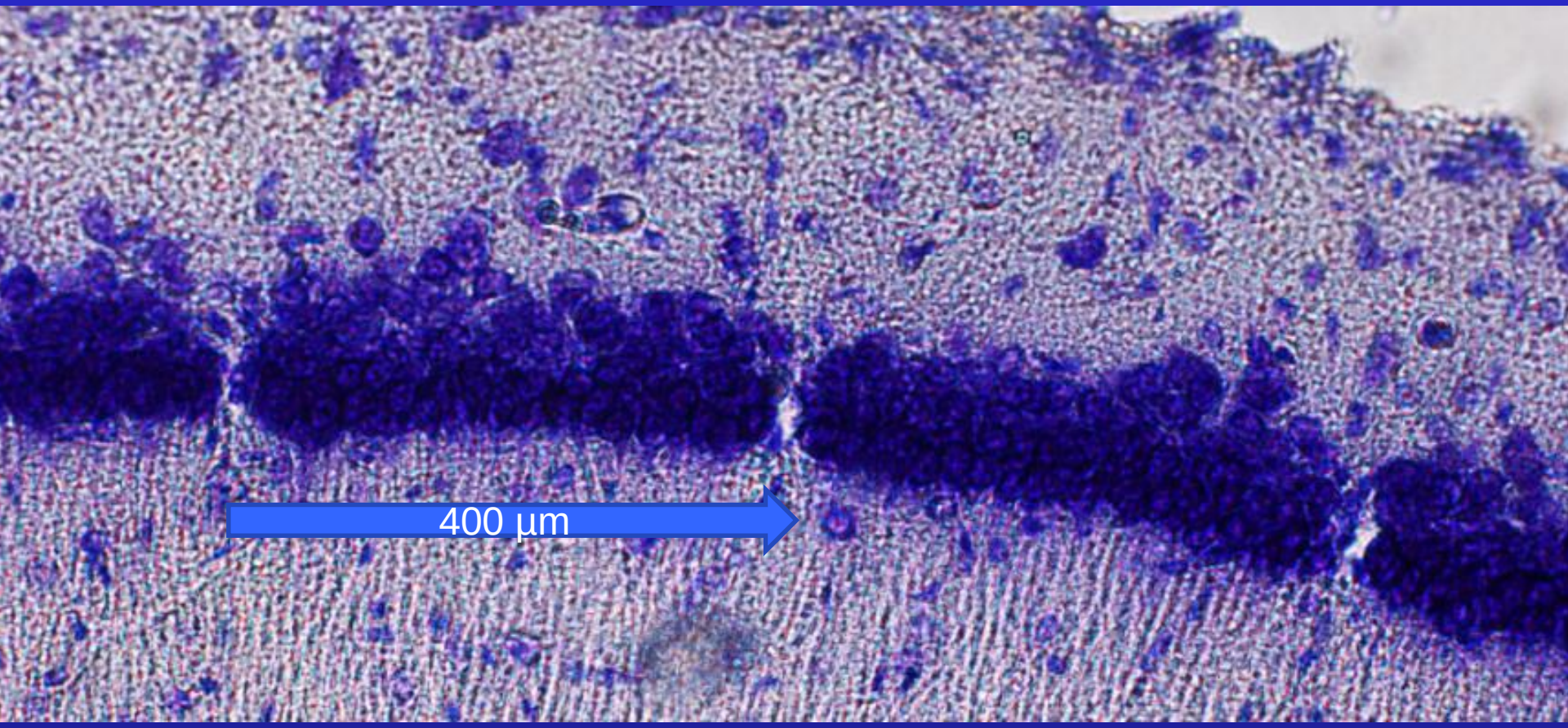


Figure 36
Interaural 4.84 mm
Bregma -4.16 mm
RSA
PtA
RSGb
S1BF
CA1
CA3
CA2
AuD
Au1
AuV
TeA
Ect
PRh
LEnt
DG
LV
D3V
MHb
LHbL
LHbM
APTD
LPMR
LPLR
DLG
Po
PF
VPM
VPL
Rt
SubG
VPPC
SPF
ZID
ZIV
3V
PH
STh
SuM
PMD
PMV
DTM
ArcMP
ArcLP
InfS
VTM
AHiPM
AHiAL
BMP
BLP
PMCo
APir
PLCo
Pir
DEn
LaDL
LaVM

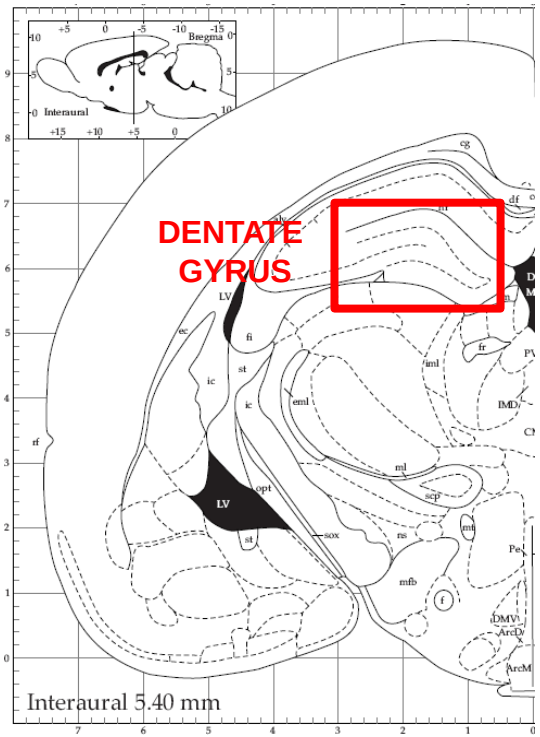
Cellular-scale Radiosurgery

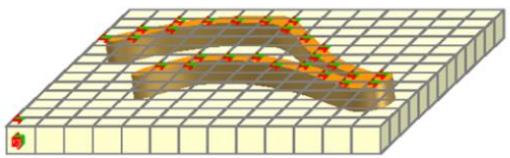
Nissl staining 3 months after delivery of an array of 9 parallel 75 μm beams with an incident dose of 600 Gy

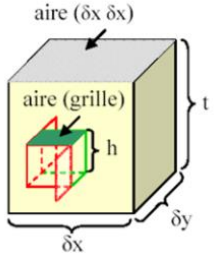


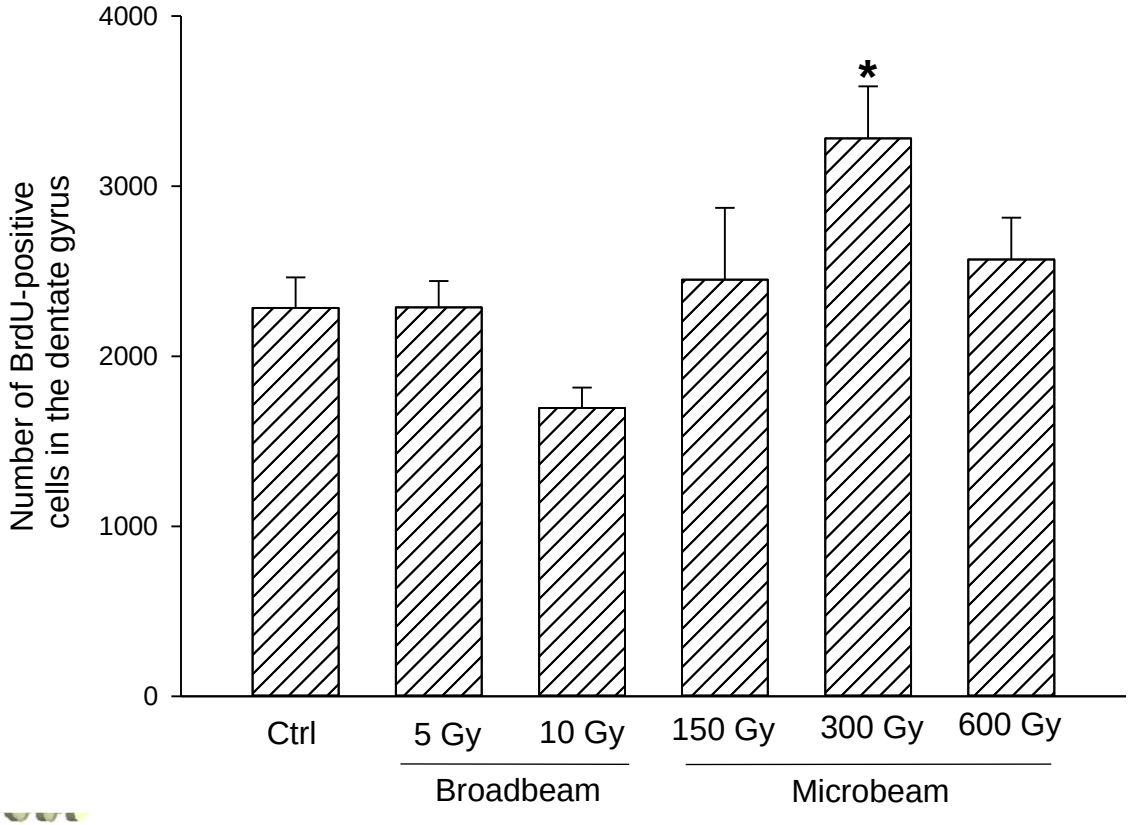
Dentate gyrus unicellular transections

Microbeam irradiation (300 Gy) increases the number of BrdU-positive cells in the rat hippocampal dentate gyrus

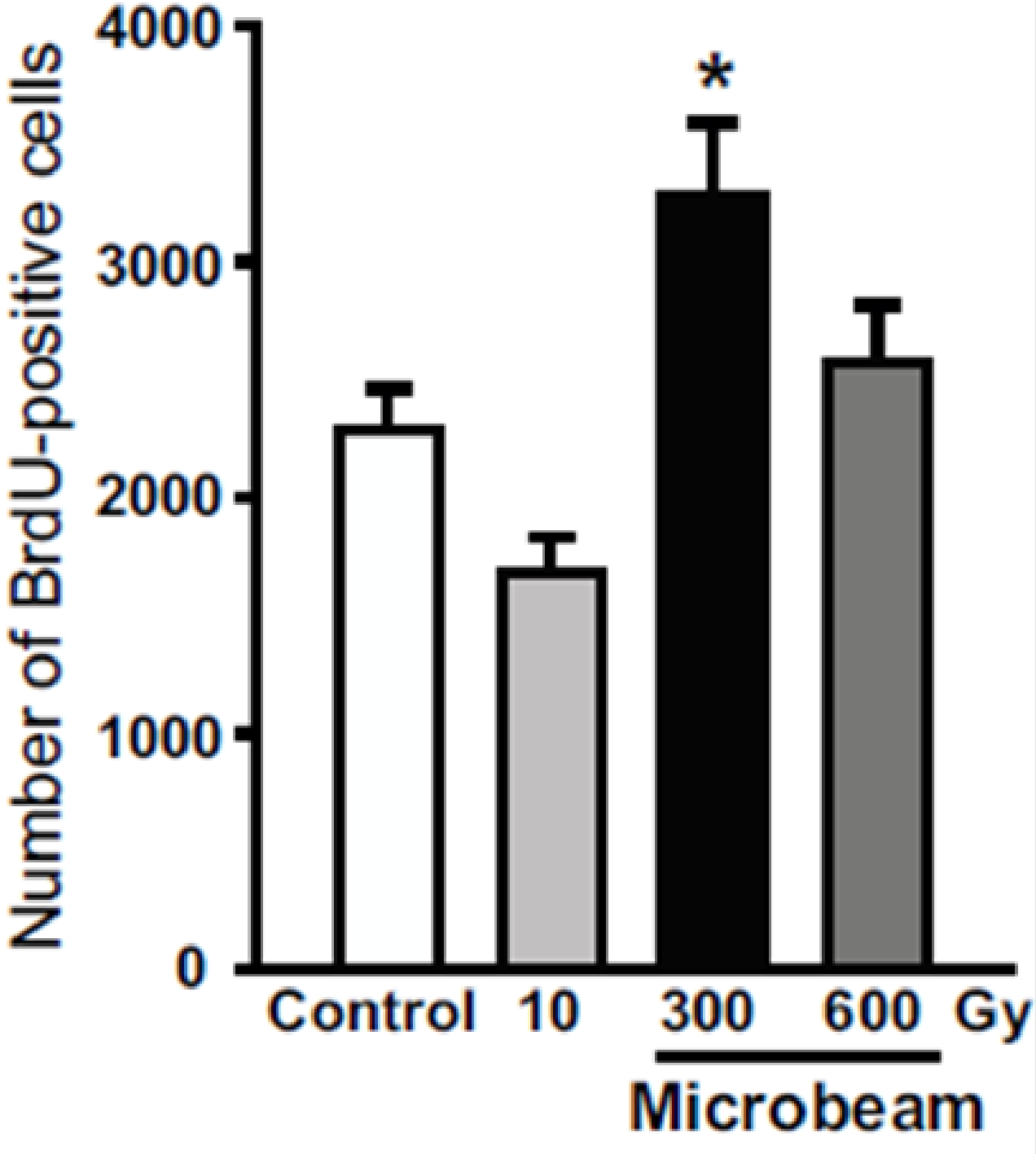


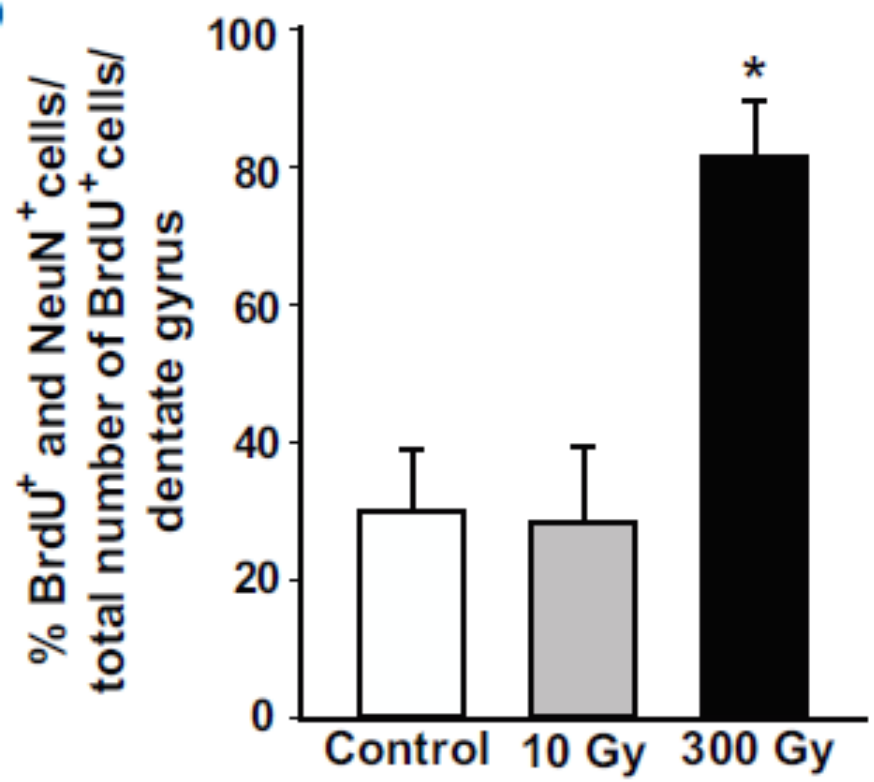


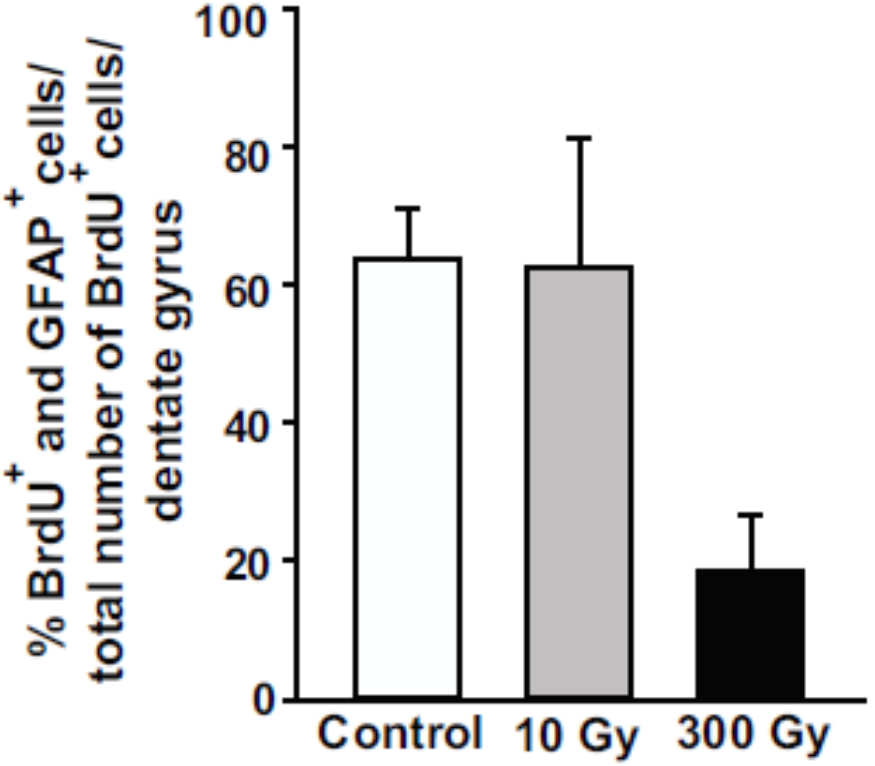




* p<0.05 (One-Way ANOVA+ Fisher's LSD test) vs. control (ctrl) non irradiated rats

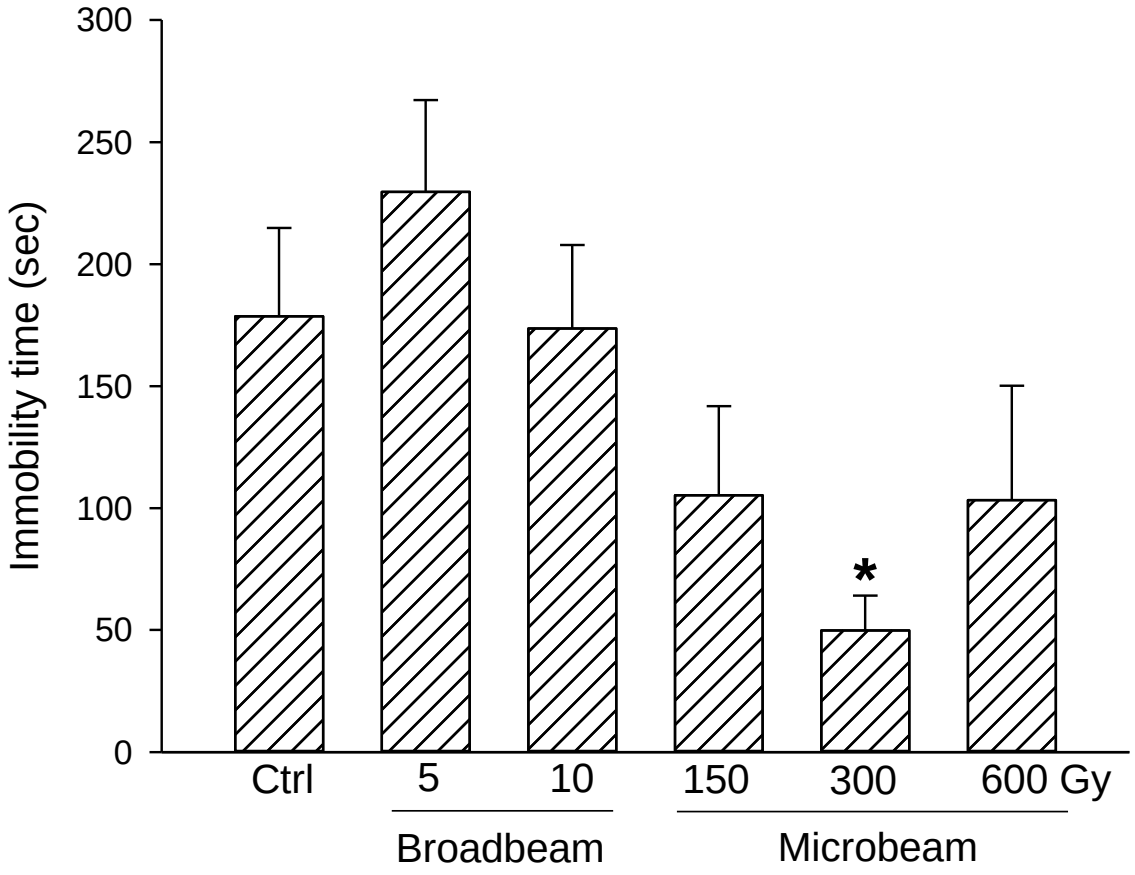

Number of BrdU-positive cells
4000
3000
2000
1000
0
*
Control
10
300
600
Gy
Microbeam


% BrdU+ and NeuN+ cells/
total number of BrdU+ cells/
dentate gyrus
100
80
60
40
20
0
*
Control
10 Gy
300 Gy

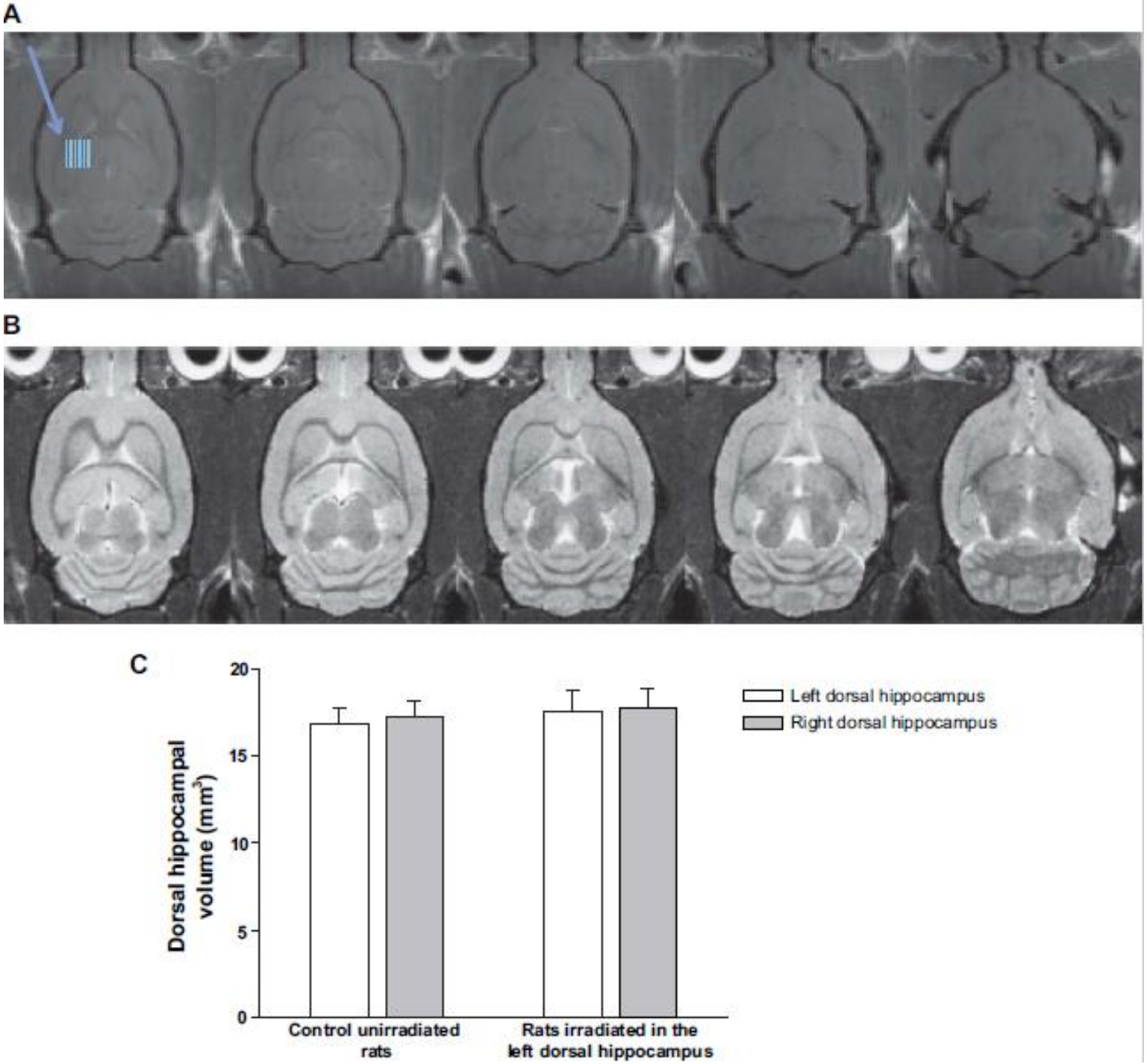

% BrdU+ and GFAP+ cells/
total number of BrdU+ cells/
dentate gyrus
100
80
60
40
20
0
Control
10 Gy
300 Gy

Microbeam irradiation (300 Gy) significantly reduces depressive-like behaviour

PORSOLT FORCED SWIM TEST



*p<0.05 (One-Way ANOVA+Fisher's LSD test) vs. control (ctrl) non irradiated rats (n=6-8)


A
B
C
Dorsal hippocampal volume (mm3)
20
15
10
5
0
Control unirradiated rats
Rats irradiated in the left dorsal hippocampus
Left dorsal hippocampus
Right dorsal hippocampus

Microbeam Hippocampal Transections and Neurogenesis

- Hippocampal transections are well tolerated
- Transected rats showed improved behavioral scores

NeuN (but not GFAP) cells increase strongly after microbeam transections

If these data are confirmed, a novel unexpected avenue to study and induce neurogenesis will open

ELSEVIER

International Journal of Radiation Oncology*Biology*Physics

Volume 101, Issue 4, 15 July 2018, Pages 965-984

Physics Contribution

Micro-imaging of Brain Cancer Radiation Therapy Using Phase-contrast Computed Tomography

Giacomo E. Barbone MSc [*], Alberto Bravin PhD [†], Pantaleo Romanelli MD [‡], Alberto Mittone PhD [†], Domenico Bucci PhD [§], Thomas Gaaβ PhD [‖], Géraldine Le Duc PhD [†], Sigrid Auweter PhD [‖], Maximilian F. Reiser MD, PhD [‖], Markus J. Kraiger PhD [¶], Martin Hrabě de Angelis PhD [¶, #, **], Giuseppe Battaglia MD, PhD [§], Paola Coan PhD [*, ‖]

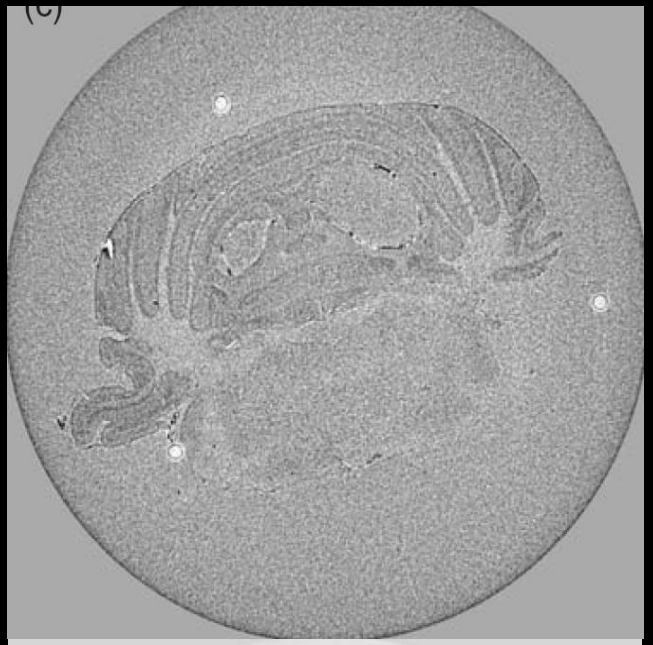
PCI-CT visualizes the brain anatomy and microvasculature in 3 dimensions and distinguishes cancerous tissue morphology, necrosis, and intratumor accumulation of iron and calcium deposits. Moreover, PCI-CT detects the effects of MRT throughout the treatment target areas (eg, the formation of micrometer-thick radiation-induced tissue ablation). The observed neurostructures were confirmed by histologic and immunohistochemistry.

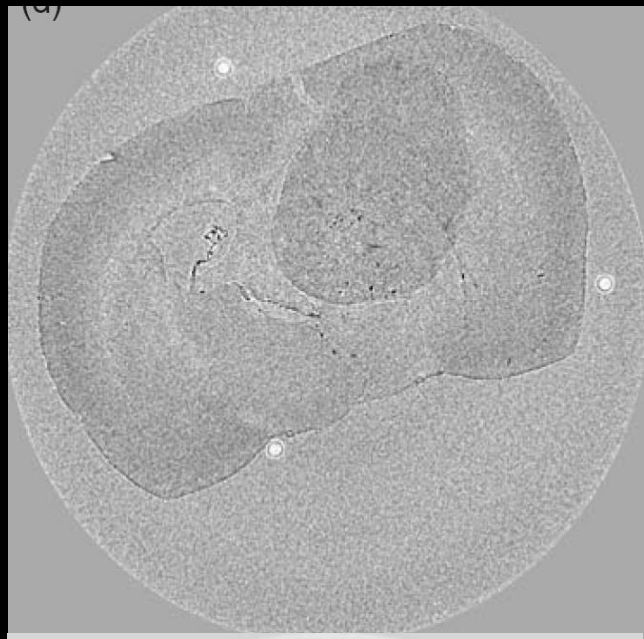
Brain tumor visualization in rat: absorption vs phase contrast

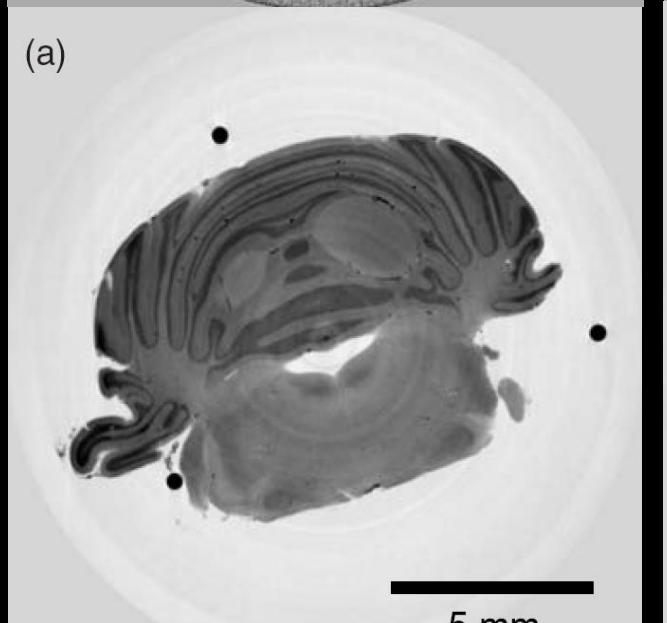
same radiation dose and experimental parameters

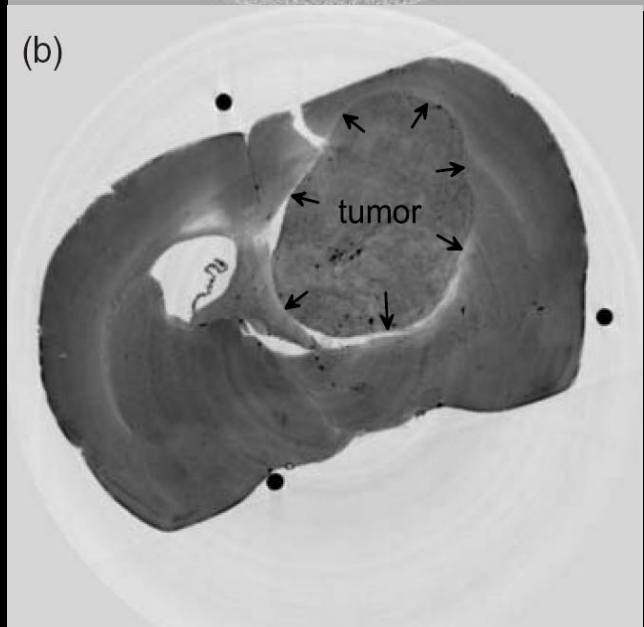
Absorption CT

Phase-contrast CT





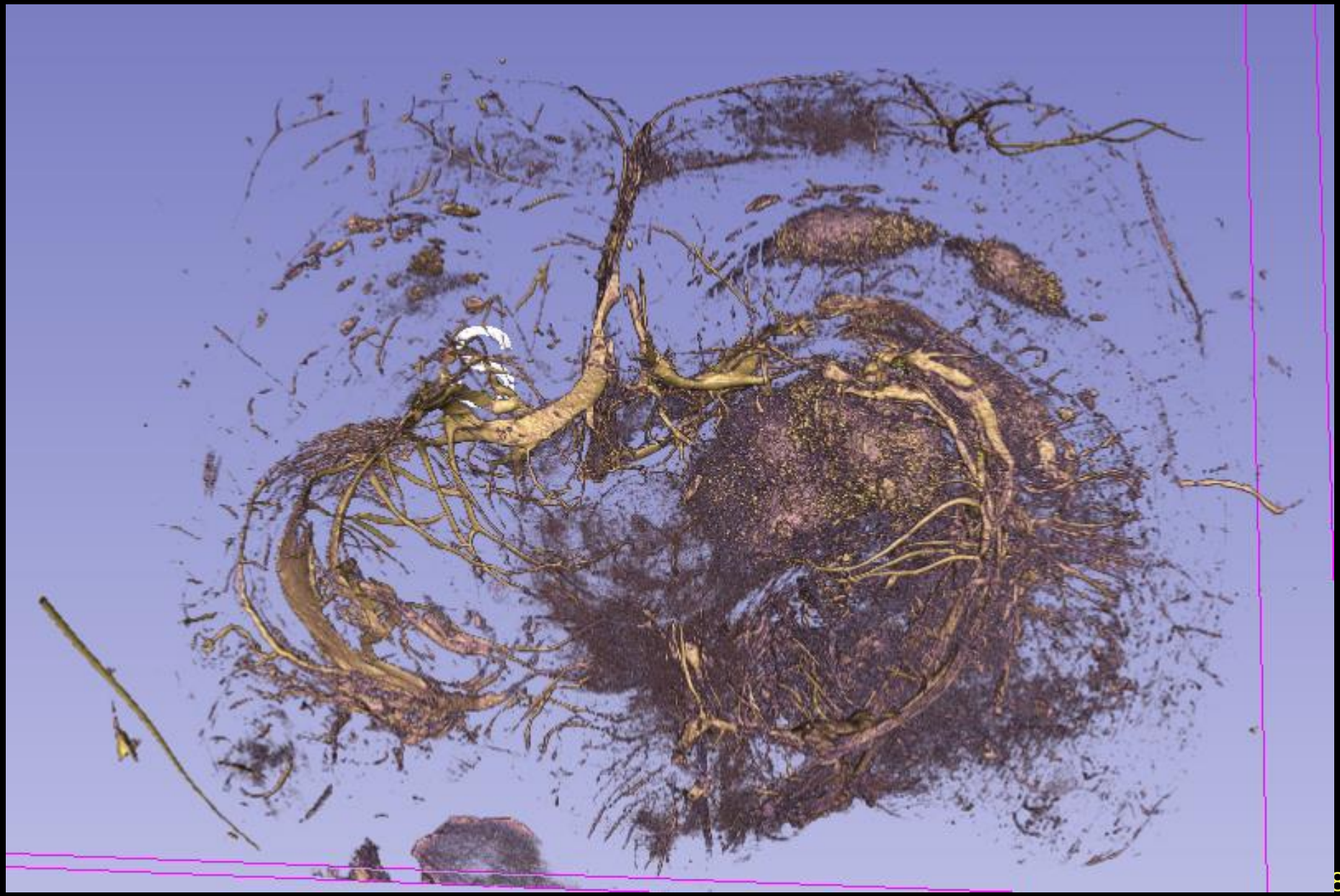


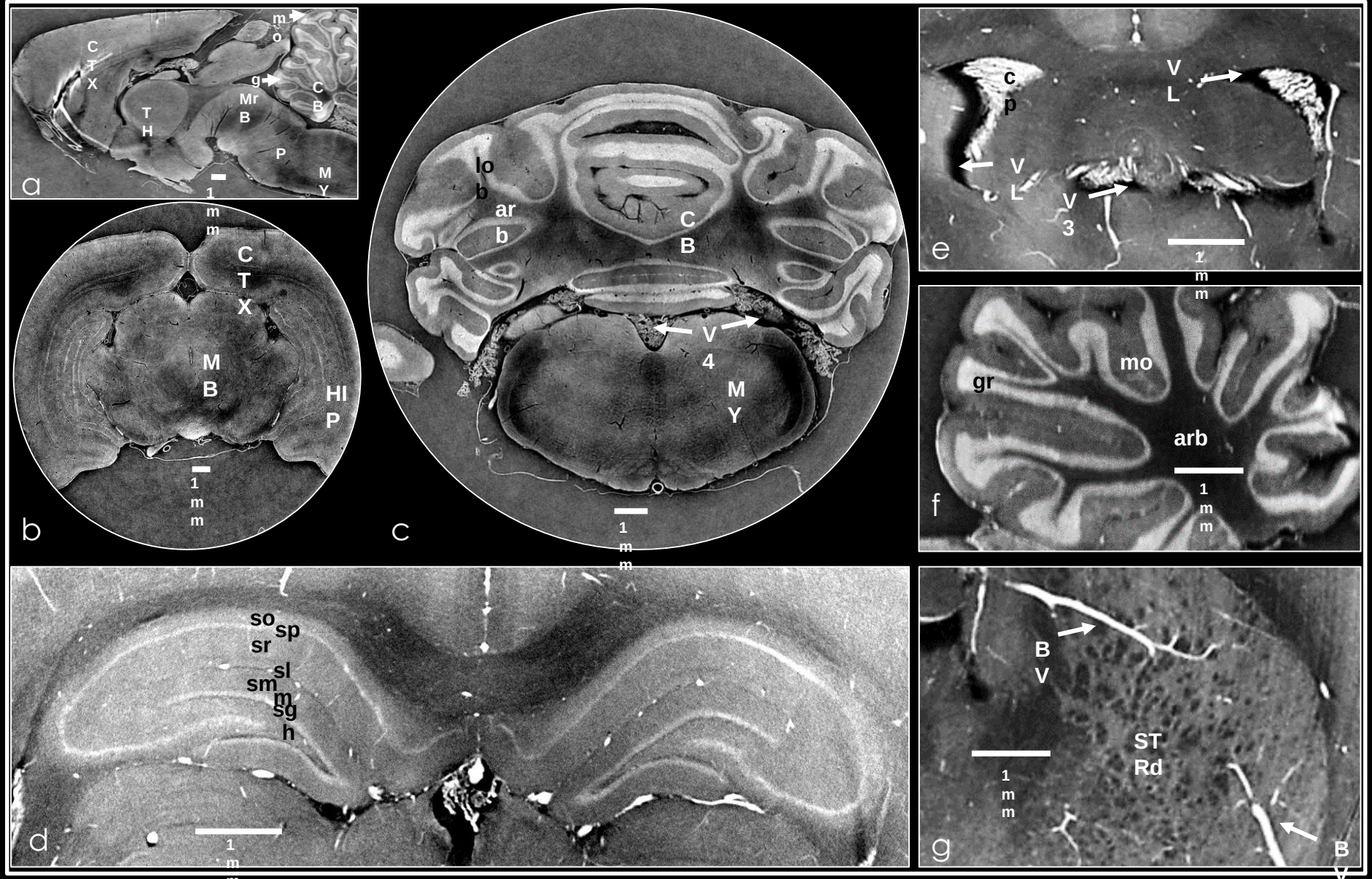


- X-ray energy = 25 keV
- Pixel size = 16 μm

Phase contrast micro-CT: tumor-bearing rat brain MRT

Segmentation of the vascular network and micro-calcifications




a
CTX
TH
mo
g
MrB
CB
P
MY
1 mm
b
CTX
MB
HIP
1 mm
c
lob
arb
CB
V4
MY
1 mm
d
so
sp
sr
sl
sm
m
sg
h
1 mm
e
c
p
VL
VL
V3
1 mm
f
gr
mo
arb
1 mm
g
BV
ST
Rd
1 mm
BV

Conclusions

- Microbeam Radiosurgery provides a method to deliver overwhelmingly high x-ray doses to selected brain volumes without radio-induced edema or radionecrosis
- This unique stereo-selective irradiation modality allows to replicate microsurgical brain transection providing a novel avenue to treat refractory epilepsy and other functional brain disorders
- No need to mention brain tumors/cancer….
- Hippocampal transections appear to induce neurogenesis
- Microbeam-based imaging provide cellular-scale details
- And of course…….much more research it's needed

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