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European Journal of Histochemistry
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The *European Journal of Histochemistry* was founded in 1954 by Maffo Vialli and published till 1979 under the title of *Rivista di Istochimica Normale e Patologica*, from 1980 to 1990 as *Basic and Applied Histochemistry* and in 1991 as *European Journal of Basic and Applied Histochemistry*. It is now published under the auspices of the University of Pavia, Italy.
The *European Journal of Histochemistry* is the official organ of the Italian Society of Histochemistry and a member of the journal subcommittee of the International Federation of Societies for Histochemistry and Cytochemistry (IFSHC), and has been an influential cytology journal for over 60 years, publishing research articles on functional cytology and histology in animals and plants.

The Journal publishes Original Papers, Technical Reports, Reviews, Brief Reports, Letters to the Editor, Views and Comments, and Book Reviews concerning investigations by histochemical and immunohistochemical methods, and performed with the aid of light, super-resolution and electron microscopy, cytometry and imaging techniques; attention is also given to articles on newly developed or originally applied histochemical and microscopical techniques.

Coverage extends to:
- functional cell and tissue biology in animals and plants;
- cell differentiation and death;
- cell-cell interaction and molecular trafficking;
- biology of cell development and senescence;
- nerve and muscle cell biology;
- cellular basis of diseases.

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ization protocol that has proven to be efficient for tendons (DN-P1) was compared with a decellularization protocol specifically developed for nerves (DN-P2). The outcomes of both the decellularization protocols were assessed by a series of *in vitro* evaluations, including qualitative and quantitative histological and immunohistochemical analyses, DNA quantification, SEM and TEM ultrastructural analyses, mechanical testing, and viability assay. The overall results showed that DN-P1 could provide promising results if tested *in vivo*, as the *in vitro* characterization demonstrated that DN-P1 conserved a better ultrastructure and ECM components compared to DN-P2. Most importantly, DN-P1 was shown to be highly biocompatible, supporting a greater number of viable metabolically active cells.

PHYLOGENETIC VARIATION OF "IMMATURE" NEURONS IN SUBCORTICAL REGIONS OF MAMMALS: PRELIMINARY RESULTS

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In mammals, brain plasticity can vary depending on animal species. The genesis of new neurons (adult neurogenesis) is spatially restricted to stem cell niches and reduced from mice to humans. A population of prenatally generated (non-dividing) "immature" neurons (INs), which retain expression of typical markers of immaturity, is known to occur in the layer II of rodent paleocortex. We recently demonstrated that these cells are particularly abundant in the neocortex of gyrencephalic brains, also extending in subcortical regions. Here, we analysed claustrum and amygdala of six mammalian species characterized by different brain size, gyrencephaly and cortical IN density. Three young-adult animals/each species were analysed. Whole brain hemisphere, amygdala and claustrum volumes were evaluated on histologically stained serial coronal sections (40 μm thick) scanned with Axioscan. To study INs, doublecortin (DCX) was employed as a marker and quantitative/qualitative analyses were carried out on 480 nm-interval sections covering the whole extension of both subcortical regions. The marker for cell division Ki-67 antigen was used to check the nature of either dividing or "immature" neurons for the DCX+ cells. Direct cell counting was performed to obtain a quadratic/volume cell density (cells/mm^2-mm^3) using Neurolucida Software. Populations of DCX+ cells were found in both claustrum and amygdala of cat, rabbit, marmoset, while no immunoreactive elements were detected in mouse and naked mole rat. Different morphological cell types were identified, spanning from bipolar to multipolar neurons (3 types in amygdala, 2 in claustrum). Quantitative analyses revealed interspecies heterogeneity of IN occurrence, with prevalence in non-rodent mammals. This study confirms that non-rodent mammals, generally characterized by reduction in stem cell-driven adult neurogenesis, can rely on populations of young neurons within brain regions underlying the most important cognitive functions.

EFFECT OF 3D SYNTHETIC MICROSCAFFOLD NICHOID ON THE MORPHOLOGY OF HIPPOCAMPAL NEURONS

Musi C.A.[1,2], Colnaghi L.[2], Giani A.[2], Tomaselli G.[1,2], Marchini G.[2], Conci C.[4], Tironi M.[2], Raimondi M.T.[4], Remuzzi A.[3] Borsello T.[1,2*]

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The human brain is the most complex organ in biology. This complexity is due to the number and the intricate connections of brain cells, and has so far limited the development of *in vitro* models for basic and applied brain research. We decided to create a new, reliable, and cost-effective *in vitro* system of hippocampal neurons and astrocytes co-cultured based on the Nichoid, a 3D microscaffold microfabricated by two photon laser polymerization technology. After 21 days in culture, we morphologically characterized the 3D spatial organization of the hippocampal astrocytes and neurons within the microscaffold and we compared our observations to those made using the classical 2D co-culture system. We found that the co-cultured cells colonized the entire volume of the 3D devices. Using confocal microscopy, we observed that within this time period the different cell types had well differentiated. This was further elaborated with the use of Drebrin and PSD-95 antibodies as markers for mature and differentiated dendritic spines. Drebrin and PDS95 labelled the majority of neurons both in the 2D as well as in the 3D co-cultures. Using scanning electron microscopy, we found that neurons in the 3D co-culture displayed a significantly larger amount of dendritic protrusions compared to neurons in the 2D co-culture. This latter observation indicates that neurons growing in a 3D environment may be more prone to connections than those co-cultured in a 2D condition. Our results show that the Nichoid can act as a 3D device that can be used to investigate structure and morphology of neurons and astrocytes in a 3D volume. In the future, this model can be used as a tool to determine the factors at the basis of different human brain diseases, by plating cells derived directly from patients. This system may potentially further be used for drug screening in various brain diseases.

GENISTEIN: SEXUAL DIMORPHIC EFFECTS ON SEROTONERGIC SYSTEM IN MICE

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Phytoestrogens can act as endocrine disruptors (EDC), producing estrogenic or non-estrogenic effects, and can be dangerous during development, in specific critical periods. Among phytoestrogen, genistein (GEN), an isoflavone naturally present in the plant kingdom with estrogen-like activity, is particularly present in high quantities in the soy plant (*Glycine max*). Several studies have analysed the effects of GEN administration, in the mouse, during development, showing that it can alter brain neural cir-

cuits as well as behaviours, especially anxious behaviour, fertility and energy metabolism. Serotonergic system is deeply involved in almost all the above-mentioned behaviours. Therefore, we hypothesized that GEN exposure may alter the development of this system in sexual dimorphic way. Given that the treatment mimics the effects of raising newborns with soy-based preparations, this study may help to clarify possible caveats in the use of those formulas. Therefore, we analysed the effects of an early postnatal treatment with a dose of GEN comparable to the exposure level in babies fed with soy-based formulas, on serotonin (5-HT) brain circuits and anxious behaviour. We treated male (N=24) and female (N=24) CD1 mice with GEN (50 mg/kg body weight dissolved in sesame oil) or with the vehicle (control, CON). At PND60 six animals per group were tested with Open Field (OF) and Elevated Plus Maze (EPM). At the same time, we collected also feces to measure the fecal corticosterone in order to evaluate the anxious behaviour. The other mice were sacrificed for the immunohistochemical (IHC) analysis of 5-HT. In the OF test, treated mice have a reversal of anxiety-like behaviour: GEN induces an anxiolytic effect in male and an anxiogenic effect in female. The OF results are confirmed by the corticosterone levels, higher in treated male than in untreated male. Rather, the EPM showed only a sexual dimorphism in CON mice, with male more anxious than female, but, unlike OF, not an inversion of behaviour in treated mice. These findings are confirmed by the results of the IHC study (male, N=8; female, N= 8), indicating that GEN induced a reversal of serotonin neuron content in raphe nuclei, dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN). Specifically, GEN influenced 5-HT neuronal populations in a sexually dimorphic manner, notably in DRN: treated female mice showed a decrease of 5-HT neurons, whereas treated male mice showed an increase, compared with control.

SESSION II
NEURODEGENERATION AND NEUROPROTECTION

NEURODYNAMIC EFFECTS ON NERVE REGENERATION AND PAIN

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Nerve injuries and diseases affecting nerves are a rising problem leading to disability due to sensory and motor impairments often associated with neuropathic pain and in particular mechanical allodynia and hyperalgesia. The neurodynamic treatment (NDT) consisting of selective uniaxial nerve repeated tension protocols has been described to effectively reduce mechanical allodynia and hyperalgesia in neuropathic pain patients. Nevertheless, even if some studies on *in vivo* and *in vitro* models reported the ability of NDT to promote nerve regeneration and pain modulation the most of the biological effects involved are still unknown. Moreover, no standardized protocols are available. Since mechanical allodynia and hyperalgesia are linked to processes detected in the dorsal root ganglia (DRG), we aimed to define *in vivo* and *ex vivo* whether NDT protocols could induce selective biological effects promoting nerve regeneration and mechanical allodynia suppression. A model of rat median and ulnar nerve crush injury was adopted to define the effects of NDT on motor and sensory nerve recovery and pain modulation. A DRG ex vivo model was adopted to confirm the NDT effects on sensory recovery and in pain modulation to be selective on sensory neurons and not to be dependent on other non-neural tissue mediated responses. The obtained results show that NDT induced significant sensory and motor recovery preventing intraneural fibrosis. The regulation of TACAN and PIEZO1 gene expression in the DRG ipsilateral to the nerve injury was observed. No protective effect of NDT on nerve injury-related pain and loss of functions were detected. Ex vivo results confirmed that NDT promoted a dose-dependent neurite outgrowth and significantly downregulates the expression of TACAN and PIEZO1. Also, NDT does not promote pro-apoptotic effects in the DRG. Notably, the analyzed genes involved in mechanical allodynia are expressed in murine and human DRGs and coding for a high threshold mechanosensitive channel induced by inflammation (TACAN) and a low threshold mechanosensitive ion channel and are related to mechanical pain modulation. No significant effects are induced on other genes involved in neuroinflammation and myelin-activated mechanical allodynia. These results show that NDT activates selective anti-allodynic mechanisms related to the regulation of the mechanosensitive channel helping to understand its efficacy in pain reduction and nerve regeneration promotion.