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# *European Journal of Histochemistry*

## *a journal of functional cytology*

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:

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## WHOLE BRAIN REPRESENTATION OF IMPRINTED CUES

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Establishing sensory-related memories during infancy is a shared process among several animal species and it is fundamental in a wide spectrum of vital animal behaviours. One among many is sexual imprinting, a process of instinctive learning that happens early in life, when individuals acquire memories of odours, vocalizations, and other characteristics of their relatives, and then utilize this information for mate choice as adults<sup>1-2</sup>. Despite sexual imprinting has evolved in many taxa, almost nothing is known about the underlying neuronal mechanisms. Past works in inbred mice showed females display an innate preference for novel unfamiliar males of a different strain, but only if reared with their own father<sup>3</sup>. By using an interdisciplinary approach, we investigate how imprinted (familiar) or novel (unfamiliar) sensory cues are represented in the adult female brain. We perform whole brain mapping of immediate early genes (IEGs) after acute exposure of adult females to familiar or unfamiliar odours (urine). We combine iDISCO<sup>4</sup> tissue clearing with light-sheet fluorescence microscopy to image IEGs stained neurons across the whole brain. We use ClearMap<sup>5</sup>, an open-source software for single cell segmentation and atlas alignment, to evaluate the number of IEGs positive cells for all brain areas. Our data show that both familiar and unfamiliar olfactory cues recruit brain areas known to guide mating and social interaction such as hypothalamic and amygdalar areas. Despite these areas were activated in both conditions, unfamiliar odours tend to elicit stronger activation. Importantly, we identified a small subset of hypothalamic regions which were recruited by unfamiliar, but not familiar, odours. These preliminary results indicate that hypothalamic areas might play a prominent role in discriminating imprinted odours, contributing to male preference during mate selection.

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## EXTRACELLULAR VESICLES FROM MESOANGIOBLASTS MODULATE MACROPHAGE BEHAVIOUR

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It is now well known that stem cells release large amount of extracellular vesicles (EV) that are involved in tissue regeneration. Inflammation also plays an important role in tissue repair and regeneration.

The aim of our work was to determine the effect of EV released by mesoangioblast stem cells (C57) on macrophages (Raw264.7), as they play a central role in all stages of the inflammatory response. In damaged tissue macrophages migrate to carry out their function of identifying and removing dead cells, debris, and foreign particles *via* phagocytosis.

To this aim we have proved that EV treatment influenced negatively Raw264.7 cells cell proliferation index, and positively their migratory phenotype, when compared to untreated cells. We also proved, that this enhanced migration is due to an elevated expression and activity of MMP2/9. Moreover, *in vitro* phagocytosis index calculation highlighted that EV treatment is able to improve Raw264.7 phagocytic ability, which is important at early stages of tissue repair. As C57-EV contain Hsp70 as a transmembrane protein to elucidate whether it is involved in phagocytosis modulation, we performed phagocytosis assays also in the presence of neutralizing antibodies (*i.e.*, anti-Hsp70, anti-TLR2, anti-TLR4 antibodies), or in the presence of exogenous Hsp70. The obtained results demonstrated that C57-EVs are able to increase Raw264.7 phagocytosis through Hsp70 and its surface receptors.

The healing process consists of three overlapping phases: inflammation, tissue regeneration, and tissue remodelling. It is known that "classically activated" M1 macrophages dominate at early times after injury, while M2 macrophages dominate at later stages, although they are also present at initial time points. To point out whether EV modulate macrophage phenotype we explored the presence of M1/M2 markers (*i.e.*, iNOS and arginase), and NO and cytokine synthesis. At the initial stage after EV treatment Raw264.7 synthesized both iNOS and NO, whereas no arginase mRNA was detected. These data suggest an M1 phenotype. After a recovery following EV treatment, was observed an increased release of anti-inflammatory cytokines, typical of M2 macrophages.

These data suggest that C57-EV could positively influence tissue repair through macrophage modulation.