european journal of histochemistry
a journal of functional cytology

ISSN 1121-760X
volume 62/ supplement 1
2018

64° CONVEGNO GEI
SOCIETÀ ITALIANA DI BIOLOGIA DELLO SVILUPPO E DELLA CELLULA

L'Aquila, 11-14 June 2018

Rettorato GSSI Gran Sasso Science Institute
Via Michele Iacobucci, 2 - 67100 L'Aquila

Guest Editors
Prof. Mario Pestarino
Prof.ssa Annamaria Cimini

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the University of Pavia, Italy

Published by PAGEPress, Pavia, Italy

Editorial Office:
Via A. Cavagna Sangiuliani 5, 27100 Pavia, Italy
Phone: +39.0382.464340 - Fax: +39.0382.34872
E-mail: info@pagepress.org

Printed quarterly by:
Press Up s.r.l.
via La Spezia, 118/C 00055 - Ladispoli (RM)
Phone and Fax: +39.076.15.27.351

Annual Subscriptions
Europe: Euro 200
All other Countries: $ 250

Subscriptions, cancellations, business correspondence and any enquiries must be sent to PAGEPress Publications, Pavia, Italy.
Cancellations must be received before the end of September to take effect at the end of the same year.

Reg. Tribunale di Pavia n. 289/23.2.1984.

Recognized by the Ministero per i Beni e le Attività Culturali, Italy as a publication of high cultural value.

Associato all'USPI
Unione Stampa Periodica Italiana

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NEURONAL DEVELOPMENT: ORDER AND DISORDER

CRISPR/CAS9-INDUCED INACTIVATION OF THE AUTISM RISK GENE SETD5 LEADS TO SOCIAL IMPAIRMENTS IN ZEBRAFISH

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The SETD5 gene encodes for a putative histone H3 methyltransferase whose loss-of-function (LoF) mutations in humans have been recently associated to intellectual disability (ID) and autistic spectrum disorders (ASD). The aim of this study is to generate and characterize zebrafish models in which *setd5* has been knocked down or knocked out. *setd5* is expressed at early developmental stages while at later stages its expression is localized to the developing central nervous system (CNS) of zebrafish larvae. *setd5* morphant embryos are characterized by microcephaly, cardiac edema and reduced locomotor behavior. *setd5* knockdown determined a reduction of the expression domain of CNS specification markers paralleled by a reduced brain size compared to control embryos, associated to increased apoptosis. Furthermore, we generated stable setd5 mutant zebrafish lines through Crispr/Cas9 strategy: *setd5* LoF causes microcephaly, a significant reduction of body length and locomotor activity. Moreover, we characterized the behaviorial features of heterozygous setd5 LoF adults, focusing on social interaction. In particular, in a social preference test, setd5 heterozygous adults showed reduced sociality when compared to wild type siblings and these altered behavioral traits triggered by setd5 LoF are ameliorated by risperidone, an antipsychotic drug commonly used to treat behavioral traits in ASD patients.
These zebrafish models will be extremely useful to identify the molecular mechanisms underlying SETD5 LoF phenotype. The future perspective is to screen for targeted compounds able to rescue the developmental and behavioral defects, to identify novel promising therapeutic compounds for individuals affected by ASD and ID due to SETD5 haploinsufficiency.

NEURITIN IN MOUSE NEURONAL DEVELOPMENT: A NEW THERAPEUTIC TARGET FOR RETT SYNDROME?

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Rett syndrome (RTT) is a genetic X-linked, progressive neurodevelopmental disorder mainly caused by sporadic mutations in the MECP2 gene. In RTT, neuronal development and synaptic coupling are incomplete. In mice, MeCP2 ablation causes neuronal dysfunctions and morphological aberrations, including brain atrophy. The current hypothesis is that neuronal atrophy in RTT is caused by reduced synaptic activity leading to poor trophic support of the neuronal dendritic arborization and spines. Among the potential neurotrophic factors candidates to rescue neuronal atrophy, Neuritin has recently emerged for its ability to promote growth and stabilization of axonal and dendritic arbors, synapse formation and maturation during development. Aim of this study is to characterize the expression of Neuritin in WT and MeCP2 KO or heterozygous mice brains in order to ascertain a possible disregualtion in the animal model of the pathology and investigate its effects on RTT neuronal atrophy.
During development, neuronal processes explore their environment to identify appropriate partners before establishing presynaptic and postsynaptic contacts leading to formation of stable synapses. We previously showed that this process can be reproduced *in vitro*, in primary mouse hippocampal neuronal cultures that achieve a mature state through 6 developmental stages. Using this *in vitro* model, we previously identified the developmental stages at which neuronal atrophy (days *in vitro*, DIV, 9-12) and synaptic uncoupling (DIV 15) occur in RTT neurons. In this study, we are investigating the expression of Neuritin in WT and KO brains and testing its ability to counteract neuronal atrophy in MecP2 KO neurons *in vitro*. Preliminary results show that levels of Neuritin in mouse whole brains at post natal day 42 are unchanged while data from primary hippocampal cultures at 12 DIV show an mRNA reduction in these neurons. These preliminary results, suggest that Neuritin is disregulated in RTT during development but not in the adult brains.

DEVELOPMENT OF SEROTONERGIC FIBERS IN THE POST-NATAL MOUSE BRAIN

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Raphe nuclei serotonergic neurons provide a diffuse innervation of the central nervous system and are involved in the modulation of several functions in both developing and adult brain.[1,2] Classical studies have described the post-natal development of serotonergic axons as a linear process of terminal field innervation.[3,4] However, technical limitations have hampered a fine morphological characterization. We used the Tph2GFP knock-in mouse line, in which GFP expression allows a specific labelling of serotonergic neurons and axons,[5] for confocal microscope imaging, and we performed 3-dimensional reconstruction to morphologically describe the development of serotonergic fibers in specified brain targets from birth to adulthood. Our analysis highlighted region-specific developmental patterns of serotonergic fiber density ranging from a linear and progressive colonization of the target to a transient increase in fiber density occurring with a region-specific timing. Despite a common pattern of early post-natal morphological maturation in which a progressive rearrangement from a dot-shaped to a regular and smooth fiber morphology was observed, starting from post-natal day 28 serotonergic fibers acquire the regional morphological features observed in the adult. In conclusion, we provided novel, target-specific insights on the morphology and temporal dynamics of the developing serotonergic fibers.

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