RESEARCH ACTIVITY OF PROFESSOR RICCARDO BRAMBILLA

SUMMARY

Cognitive processing is central for our ability to gain information from the outside world and create factual knowledge, govern our thoughts and emotions, and being able to socialise with others “sapiens” and “non-sapiens” beings. Specific circuitries in the brain are devoted to distinct forms of cognition and understanding how neural connections may be strengthened or weakened in normal and pathological conditions is not only a hot topic in contemporary Neuroscience but also the overarching goal of my research activity. In particular, I am interested in translating knowledge on the molecular and cellular mechanisms governing cognitive function into effective therapies for neuropsychiatric disorders. Currently, the main focus of my laboratory is on intellectual disability (ID) and autism spectrum disorder (ASD) but, we are also interested in cognitive aspects related to neurodegenerative conditions such as Huntington’s (HD), Parkinson’s (PD), and Alzheimer’s (AD) disease.

RESEARCH TOPICS

I have always been fascinated by molecules and in particular by their function in the nervous system. The whole field of neuropsychopharmacology deals with “drugs” that bind specific target molecules in brain cells and by doing so affect behaviour. In the last few decades, novel powerful genetic technologies have been introduced in the field of neuroscience allowing to modify the expression of specific genes and proteins in a tissue and cell specific manner, thus providing unprecedented novel tools to study the brain. I pioneered the use of “gene knockout” and “viral mediated transgenesis” in the field of learning and memory and cognitive disorders. More recently, my laboratory has designed and validated a number cell penetrating peptides (CPPs) that have the capability to enter the brain and disrupt protein-protein interactions, formidable pharmacological and reversible tools not only to investigate the molecular mechanisms of cognitive deficits but also to set the basis for innovative therapies in patients.

Role of Ras-ERK signalling in memory

Learning and memory processes require that the immediate synaptic information generated by neurotransmitter release is integrated at the cellular and network levels. An essential set of intracellular mechanisms leading to long-term, protein synthesis dependent memories, involved protein kinase cascades. ERK is a major intracellular pathway both controlling cytoplasmic events such as local protein translation and gene expression/chromatin remodelling in the nucleus. The ERK cascade is activated by the Ras family of small GTPases, which before the 1990s were believed to be exclusively involved in cell proliferation and cell survival. In 1992, as a PhD student, I contributed to the cloning of Ras-GRF1, the first ever identified guanine nucleotide exchange factor for Ras, which is essential to activate these small GTPases in response to external stimuli. To our surprise, Ras-GRF1 is exclusively expressed in post-mitotic neurons of the central nervous system, suggesting a specific role of Ras-ERK signalling in adult brain functions. In 1997, as a postdoc at the EMBL in

Heidelberg, I generated the Ras-GRF1 KO mouse strain that was the first published genetic model demonstrating a direct involvement of the Ras-ERK signaling cascade in behavioural plasticity (Brambilla et al, Nature, 1997, cited so far 379 times). Subsequently, as a PI in Milan, I published another important paper demonstrating that ERK1 kinase is a negative regulator of global ERK signaling (Mazzucchelli et al, 2002, Neuron, cited so far 367 times). That paper led us to suggest that the ERK1/ERK2 protein ratio is a major predictive indicator of behavioural and synaptic plasticity changes, particularly in the striatum. The model has been subsequently validated in vitro by my laboratory and has received a number of in vivo confirmations (Vantaggiato et al, 2006, J Biol, cited so far 168 times), supporting the notion that in the absence of ERK1, the remaining ERK2 kinase is facilitated in its nuclear translocation (Marchi et al, PLOSone 2008). Importantly, this model is very essential to explain the phenotypes observed both in 16p11.2 deletion and duplication mouse models, where ERK1 gene is present in one or three copies, respectively. Also, the model has led the development of a novel pharmacological tool, an ERK selective positive modulator.

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-C. Mazzucchelli, et al, and **R. Brambilla.** Knockout of ERK1 MAP kinase enhances synaptic plasticity in the striatum and facilitates striatal-mediated learning and memory. Neuron (2002) **34**, 807-820. *****

-C. Vantaggiato , I. et al, and **R. Brambilla.** ERK1 and ERK2 mitogen-activated protein kinases affect Ras-dependent cell signaling differentially. J. Biol. (now BMC Biology)(2006), 5:14.

-M. Marchi, A. D'Antoni, I. Formentini, R. Parra, **R. Brambilla,** G. M. Ratto and M. Costa. The N-terminal non catalytic domain of ERK1 MAP kinase is responsible for the functional differences with ERK2. PLOS ONE. 2008;3(12):e3873. Epub 2008 Dec 4.

Ras-ERK signalling in striatal-dependent plasticity disorders

In the last decade my lab has contributed to expand our knowledge of the role of Ras-ERK signalling in behavioural plasticity. More specifically, I have published a number of important papers involving this pathway in striatal dependent synaptic plasticity and in the responses to drugs of abuse (Fasano et al, 2009; Papale et al, 2016, E-Life). In addition, I have shown that ERK signalling is an important mediator the abnormal involuntary movements caused by L-DOPA, the gold standard therapy for Parkinson's Disease (Fasano et al, 2010, PNAS; Cerovic et al, 2015 Biol Psych).

-S. Fasano, et al, and **R. Brambilla.** Ras-Guanine Nucleotide-Releasing Factor 1 (Ras-GRF1) Controls Activation of Extracellular Signal-Regulated Kinase (ERK) Signaling in the Striatum and Long-Term Behavioral Responses to Cocaine. Biol Psychiatry, 66:758 –768 (2009). Doi:10.1016/j.biopsych.2009.03.014

-Fasano, S., et al, and **Brambilla R**. Inhibition of Ras-GRF1 in the striatum reverts motor symptoms associated to L-DOPA induced Dyskinesia. PNAS 107, 21824–21829 (2010). Doi:10.1073/pnas.1012071107

-Cerovic M, et al, **Brambilla R**. Derangement of Ras-Guanine Nucleotide-Releasing Factor 1 (Ras-GRF1) and Extracellular Signal-Regulated Kinase (ERK) Dependent Striatal Plasticity in L-DOPA-Induced Dyskinesia. Biol Psych (2015). 10.1016/j.biopsych.2014.04.002.

-Papale A[*], et al, **Brambilla R[#],** and Fasano S. Impairment of cocaine-mediated

behaviors by clinically relevant Ras-ERK inhibitors. eLife 2016;5:e17111. DOI: 10.7554/eLife.17111. **[#] co-corresponding-author**

Cell signalling in Intellectual Disability and neurodegenerative disorders: search for effective treatments

In recent year my laboratory has been involved in a number of projects with the aim of testing novel therapeutic approaches for cognitive disorders in which ERK signaling is deregulated. In a work published in 2017, we demonstrated that in a mouse model of a severe form of Rasopathies, the K-Ras G12V model, aberrant GABAergic synaptogenesis mediated by ERK signalling can be rescued by administration during postnatal development of cell penetrating peptides we devised to attenuate ERK signalling (Papale et al, 2016; Papale et al, 2017). With a similar approach, we also showed in a mouse model of 16p11.2 deletion, that both brain anatomical and behavioural deficits can be rescued (Pucilowska et al, 2018). This last publication support part of the work in the present grant application. More recently, our work using the novel ERK positive modulator indicated that both neurodegeneration and cognitive impairments in mouse models of Alzheimer's and Huntington's Disease can be prevented by stimulating this signalling pathway. In the recently awarded MRC Programme Grant, we are planning to use a similar approach to treat a mouse model of 16p11.2 duplication, in which our preliminary data indicate a significant reduction of ERK activity in the brain (Indrigo et al, 2017; Indrigo et al, 2018).

-Papale A, et al, and **Brambilla R.** Severe intellectual disability and enhanced GABAergic synaptogenesis in a novel model of rare RASopathies. Biol Psych (2017). doi: 10.1016/j.biopsych.2016.06.016.

-J. Pucilowska, et al, **R. Brambilla** and G.E. Landreth. Pharmacological inhibition of the ERK Signaling Pathway Rescues Cellular and Behavioural impairments associated with 16p11.2 Chromosomal Deletion in Mice. J Neurosci. 2018 Jun 22. pii: 0515-17. doi: 10.1523/JNEUROSCI.0515-17.2018.

-Indrigo I, et al, and **Riccardo Brambilla[#]** and Stefania Fasano. Modulation of ERK1/MAPK3 potentiates ERK nuclear signalling, facilitates neuronal cell survival and improves memory in mouse models of neurodegenerative disorders. *bioRxiv* (2018). doi: https://doi.org/10.1101/496141. **[#] co-corresponding-author**

- M. Indrigo, A. Papale, S. Fasano and **R. Brambilla**. Neuroprotective peptide (GB1719520.7, November 27, 2017)(PCT/GB2018/053384, November 23, 2018)