Hypothalamus as a pharmacologically relevant neurogenic area

R. Salem[1,2], V. Bortolotto[1,2], P.L. Canonico[2], M. Grilli[1,2]

[1]Laboratory of Neuroplasticity, [2]Dept. of Pharmaceutical Sciences, University of Piemonte Orientale, Via Bovio 6, 28100 Novara, Italy

Adult neurogenesis, the generation of functionally integrated neurons throughout life, has been described in the subventricular zone and the subgranular zone of hippocampal dentate gyrus. Recently, accumulating evidence suggested that also hypothalamus, a brain region involved in functions including metabolism and energy regulation, exhibits a considerable neurogenic capacity which can be influenced by nutritional and metabolic cues. The NF-kB family of transcription factors is expressed in neurogenic areas and its role in the modulation of adult neurogenesis is well established, especially in the hippocampus. As an example, homozygous deletion of NF-kB p50 in mice was shown to impair adult hippocampal neurogenesis (Denis-Donini et al., 2008). More recently, *in vivo* studies emphasized a possible role of the NF-kB pathway in the regulation of adult hypothalamic neurogenesis in obese and diabetic mice (Li et al., 2012).

At present identity and characteristics of hypothalamic adult neural stem/progenitor cells are not clarified and very few studies use *in vitro* models of these cells. For this reason we have set up primary cultures from the adult murine hypothalamus of C57BL/6 WT and p50KO mice utilizing an established protocol for adult hippocampal neural progenitor cells (NPC) (Meneghini et al., 2013). Within 10 days *in vitro* floating primary neurospheres appeared in culture, and, after dissociation and replating, cells could be grown as neurospheres for up to 40 passages. We phenotypically characterized cells under proliferative conditions by single immunocytochemistry for Sox-2 and nestin (markers of multipotential progenitors), NG2 (an oligodendrocyte precursor cell marker) and GFAP (a marker of both neural stem cells and astrocytes). Our results showed that both WT and p50KO derived cells stably expressed nestin and Sox-2 (about 85% nestin$^+$ and 95% Sox-2$^+$ cells). Conversely, with passaging, NG2$^+$ cells increased from 15% to about 35% in both WT and p50KO cultures. A few GFAP$^+$ cells (about 5%) were present in culture at early passages (P0/P1) in both WT and p50KO-derived cultures and disappeared before P8-10. When hypothalamic cells were cultured under differentiating conditions (in absence of EGF and FGF-2), they gave rise to both MAP2$^+$ (marker for neurons) and GFAP$^+$ cells. Interestingly, both WT and p50KO cells differentiated similarly toward the neuronal lineage, while p50KO cells generated significantly more GFAP$^+$ compared to their WT counterpart. Interestingly, no such difference could be reported between WT and p50KO hippocampal NPC when grown under differentiating conditions. Altogether these results suggest that we are able to generate from adult hypothalamus neural progenitors which can self-renew, express markers of undifferentiated cells and can differentiate toward both the neuronal and astroglial lineages. Moreover they point to a different contribution of NF-kB p50-mediated transcription in adult hypothalamic and hippocampal neural progenitors.

Our future goal is to further investigate the modulation of hypothalamic NPC in response to stimuli such as neurotransmitters, adipokines, as well as metabolic and nutritional cues, and dissect the contribution of NF-kB factors to their physiology. Additionally we would like to reveal distinctive properties of hypothalamic and hippocampal adult neural progenitors, which may help us understanding more about their functional significance under physiological and pathophysiological conditions.