POSTERS Friday

POS-FRI-013

NEURAL STEM CELLS RESIDE IN DISTINCT POCKETS IN THE ADULT MAMMALIAN BRAIN

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It is now clear that new neurons continue to be generated in the adult mammalian brain, and that endogenous neural stem cells (NSCs) provide the basis for replacing those neurons lost due to normal processes, such as ageing, or in response to pathological insults, such as spinal cord injury or stroke. Owing to the absence of a positive marker distinguishing NSCs from other precursor populations, we know little about the activity of endogenous stem cells, and even less about their regulation. A defining characteristic that distinguishes endogenous NSCs from more committed progenitors is their relatively quiescent nature. Here we sought to identify infrequently cycling endogenous NSCs by "pulsing" dividing cells with BrdU for a period of 1 month. This was followed by "chasing" these cells for a period of 3 months post-injection, over which rapidly cycling cells will have diluted their label leaving only slow cycling or label retaining cells (LRCs). An analysis of the distribution of LRCs along the adult mouse ventricular neuraxis was accomplished by serially sectioning (14im) the entire brain (n=5 mice), and counting the number of BrdU-immunoreactive cells that did not colocalize with markers of mature cell types (NeuN, GFAP, S-100, O4). This analysis revealed three distinct regions where high concentrations of LRCs were localized. Of interest, these pockets of LRCs correlated exactly with peaks in the number of neurosphere-forming cells that could be harvested. However, as neurosphere formation is indicative of both stem and progenitor cells, we are currently employing the neural-colony forming cell assay (Stem Cell Technologies, Canada), which can distinguish stem from progenitor cells based on colony size, to determine what percentage of LRCs are indeed NSCs.

POS-FRI-014

SOCS3 NEGATIVELY REGULATES LIF SIGNALLING IN NEURAL PRECURSOR CELLS

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Cytokines that signal through the LIFRâ/gp130 receptor complex, including LIF and CNTF, promote the self-renewal of embryonic and adult neural precursor cells (NPCs). In non-CNS tissues, the protein Suppressor of Cytokine Signalling-3 (SOCS3) negatively regulates signalling through gp130. Here, we analyse the role of SOCS3 in inhibiting LIF signalling in NPCs *in vitro*. SOCS3 is rapidly expressed by NPCs in response to LIF stimulation (n=3), with this expression largely dependent on recruitment of STAT proteins to the activated gp130 receptor. Proliferating NPC cultures (n=10 per genotype) can be generated from SOCS3 knockout mice and display prolonged STAT phosphorylation and induction of the GFAP gene in response to LIF. In comparison with wild-type NPCs, SOCS3 deficient cultures display enhanced self-renewal capacity (n=3). However, the clonal potential of wild-type but not SOCS3 deficient NPCs is enhanced by exogenous LIF. Together our data reveal that SOCS3 negatively regulates LIF signalling in NPCs.

POS-FRI-015

NEUREGULIN I MODULATES AGRIN-INDUCED ACETYLCHOLINE RECEPTOR CLUSTERING

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Agrin and neuregulin are two nerve-derived signals that influence the organisation of acetylcholine receptors (AChRs) in the postsynaptic receptor cluster. Agrin and neuregulin have been traditionally believed to act via two distinct and separate signalling pathways. Agrin has been thought to solely drive the clustering of AChRs, while neuregulin is believed to act only on AChR synthesis. Here we report that the signalling pathways of agrin and neuregulin may interact. When added to sub-optimal doses of agrin (100pM), neuregulin potentiated agrin-induced AChR clustering by 2-fold during a short incubation time (4 hours, n=3, $P<0.01$). However, when similar experiments were conducted over a 12 hour incubation period, neuregulin reduced the number of agrin-induced AChR clusters by 2-fold (n=3, $P<0.01$), probably by promoting AChR disassembly. These results support the suggestion that neuregulin signalling may influence agrin-induced assembly of AChR clusters, but also foster a subsequent AChR disassembly process. These complex modulatory actions of neuregulin may help to explain the formation and remodelling of the postsynaptic AChR cluster at the developing synapse.
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POS-FRI-016

RAPSYN, AN EFFECTOR OF POSTSYNAPTIC RECEPTOR CLUSTERING, IS UP-REGULATED BY NEURAL AGRIN VIA A POST-TRANSCRIPTIONAL PATHWAY

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Neural agrin is a key signal from the presynaptic nerve terminal that regulates postsynaptic acetylcholine receptor (AChR) clustering during neuromuscular synapse formation. The AChR-associated protein, rapsyn, acts downstream of signaling pathways initiated by neural agrin and its receptor tyrosine kinase, MuSK. The signaling pathway/s downstream of MuSK activation remain only partly understood but a number of different protein kinases and other second messenger proteins including Alb1/2 kinase and Src/Fyn kinases have been implicated. Transcriptional and post-translational changes are both thought to be involved in forming and stabilizing postsynaptic AChR clusters, but precisely how they work together remains uncertain. We have recently shown that increasing the level of rapsyn at adult synapses slows the rate of metabolic turnover of postsynaptic AChR's. Here we report that in cultured C2 muscle cells, neural agrin rapidly increases the level of rapsyn protein in a dose- and time-dependent manner (n=4 experiments). Our results also indicate that the up-regulation of rapsyn occurs downstream of Abl and Src kinase activation via a post-transcriptional mechanism (n=4). Together, these results lead us to the hypothesis that neural agrin fosters the formation and/or stabilization of postsynaptic AChR clusters by increasing the level of expression of rapsyn beneath the postsynaptic membrane.

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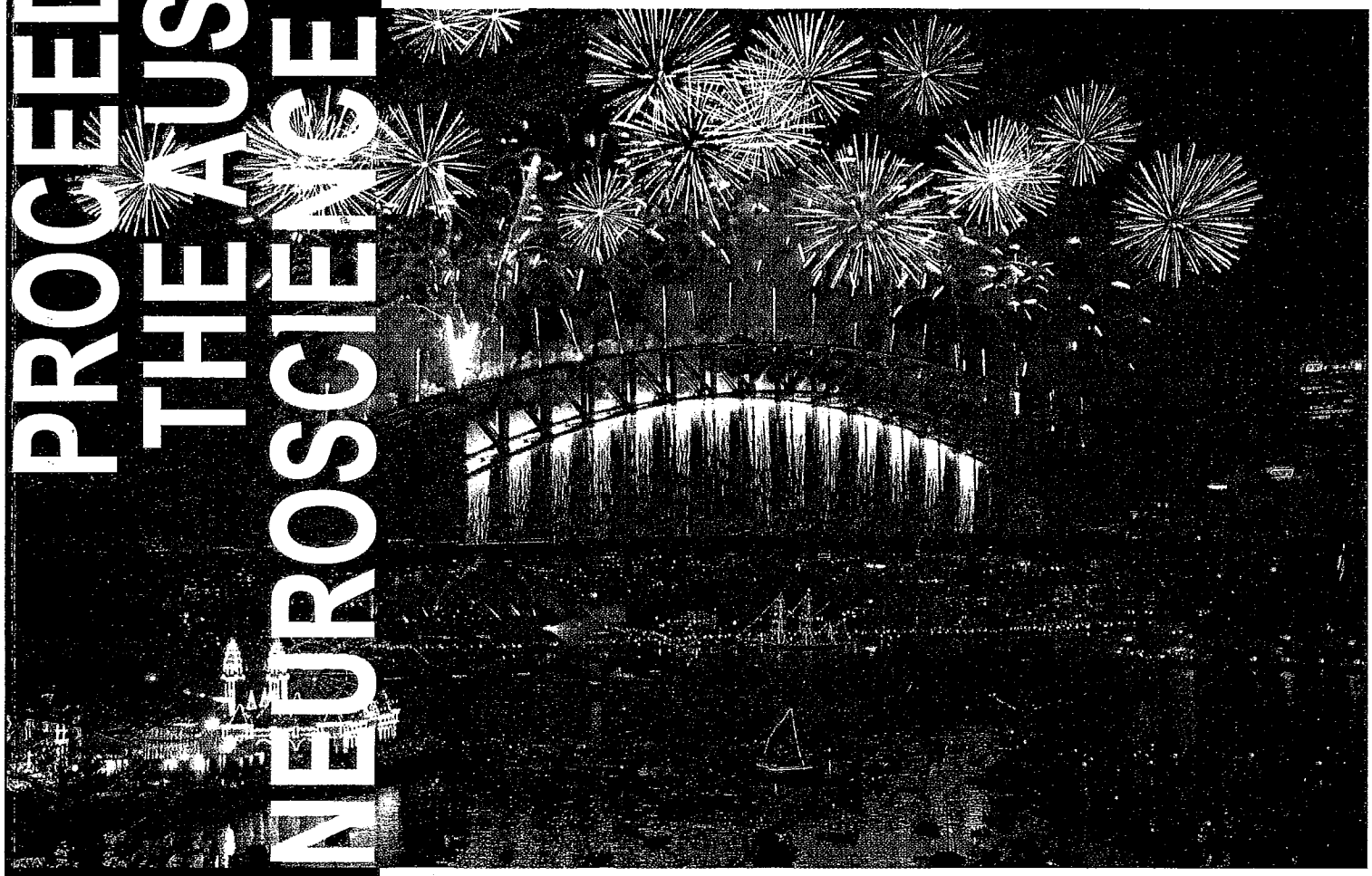

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