CAREER DEVELOPMENT AWARDEES

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Research Interests	A basic question in neurobiology is how neuronal activity causes long lasting changes in synaptic structure and function that may contribute to learning and memory. Synapses are the sites of most information processing in the brain, and changes in the molecular composition of the postsynaptic compartment are likely critical for the modulation of synaptic efficacy in normal and pathological conditions. My laboratory is interested in understanding the functions of proteins which regulate synapses formation and plasticity. **1) The Shank family of protein.** The postsynaptic density (PSD) consists of a network of interacting proteins that form an electron-dense organelle right beneath the postsynaptic membrane. Most PSD proteins function as scaffolds that anchor and link glutamate receptors and other postsynaptic membrane proteins to cytoskeletal elements and signaling pathways. Shank families of proteins are among some of the major scaffold proteins that organize the PSD. Shank1-3 proteins are large scaffold proteins that contain ankyrin repeats, an SH3 domain, a PDZ domain, a proline-rich domain and a SAM domain. They are associated with the NMDA receptor-PSD-95 complex by their binding to C-terminal GKAP, and with type I mGluRs via an interaction with Homer in the proline-rich domain. A number of actin regulatory molecules bind to Shank in the proline-rich domain (cortactin, IRSp53, and AbP1), or in the PDZ

domains, (beta-PIX).

It is also known that the overexpression of Shank1 in hippocampal cultures induces synapse maturation associated with the enlargement of dendritic spines (Sala et al., 2001). These data suggest that Shank acts as a major scaffold for postsynaptic proteins and as a molecular bridge linking multiple glutamate receptor subtypes to the postsynaptic cytoskeleton. The important role of Shank in synapse function is also supported by the finding that in humans mutations or haploinsufficiency of Shank3, found in the 22q13 deletion syndrome, causes mental retardation and autism.

In the last few years we have contributed in showing that 1. stability and targeting to synapses depend on the interaction with GKAP/SAPAP and PSD-95, also because the dissociation of PSD-95 from this complex induces the degradation of Shank and GKAP (Romorini et al., 2004); 2. Shank1 promotes the accumulation of postsynaptic density proteins, such as GKAP and NR1, in dendritic spines, it increases the F-actin content and recruits functional sER compartments in spines (Sala et al., 2001; Sala et al., 2003; Sala et al., 2005).

For Shank3 we have shown that its overexpression in cerebellum granule cells induces dendritic spine and synapse formation by recruiting different subtypes of glutamate receptors, whereas the inhibition of Shank3 expression in hippocampal neurons reduces the number of dendritic spines (Roussignol et al., 2005).

We also studied the regulation of Shank3 expression by tissue specific methylation. We showed, by DNA methylation analysis in lymphocytes, brain cortex, cerebellum and heart, that the three *SHANK* genes possess several methylated CpG boxes, but only *SHANK3* CpG islands are highly methylated in tissues where protein expression is low or absent and unmethylated where expression is present. These findings suggest the possibility to regulate the expression of Shank3 pharmacologically and open the possibility of a therapy for the 22q13 deletion syndrome.

The role of Shank3 mutation in causing mental retardation has stimulated the study of other synaptic genes whose mutations have been demonstrated to be causing mental retardation, even if the molecular function of these proteins has not been demonstrated.

2) Identification of molecular mechanisms regulating synaptic plasticity

We are developing new proteomic approaches to reveal changes in protein synthesis, degradation and post-transalational modifications in rat hyppocampal neurons during development and synaptic activity. Using these approaches we have studied the differential expression of neuronal proteins in hippocampal neurons treated with synaptic activity regulators. Neurons at DIV20-21, when synapses are mature, were treated with synaptic activity stimulators (bicuculline, a GABA receptor inhibitor) or repressors (TTX, a voltage sodium channel blocker), and analyzed by means of Western blot and 2D-gel. The proteins spots in each condition, that statistically changed the level of expression, were identified by means of MALDI-TOF mass spectrometry. About 60 proteins that change expression have been identified. We are currently studying the functional significance of these changes and the role on synaptic plasticity of some of the identified proteins.

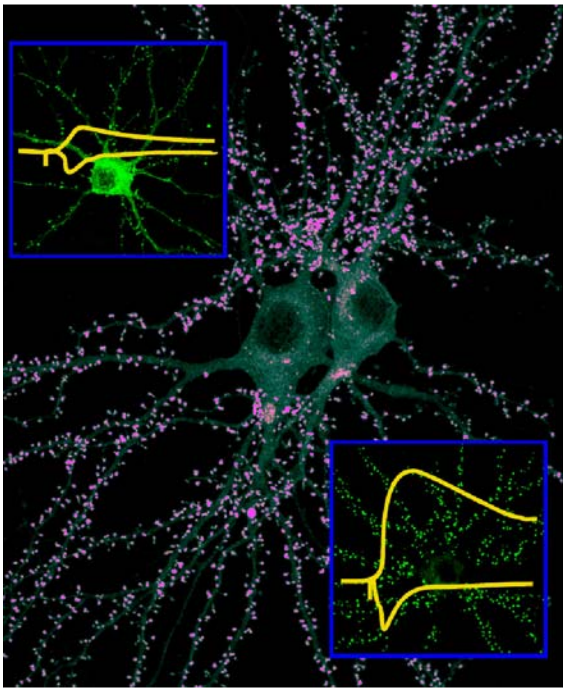

Figure 1Expression in neurons of the activity-inducible gene Homer1a causes loss of dendritic spines and depression of postsynaptic glutamate receptor currents (top left panel), compared with

	control (bottom right). In the background, hippocampal cultured neurons with dendritic spines are highlighted in pink.
Selected Publications	Hayashi, M. K., Tang, C., Verpelli, C., Narayanan, R., Stearns, M. H., Xu, R. M., Li, H., **Sala, C**., and Hayashi, Y. (2009). The postsynaptic density proteins Homer and Shank form a polymeric network structure. Cell 137, 159-171. Arstikaitis, P., Gauthier-Campbell, C., Carolina Gutierrez Herrera, R., Huang, K., Levinson, J. N., Murphy, T. H., Kilimann, M. W., **Sala, C.**, Colicos, M. A., and El-Husseini, A. (2008). Paralemmin-1, a modulator of filopodia induction is required for spine maturation. Mol Biol Cell 19, 2026-2038. Candiani, G., Pezzoli, D., Cabras, M., Ristori, S., Pellegrini, C., Kajaste-Rudnitski, A., Vicenzi, E., **Sala, C.**, and Zanda, M. (2008). A dimerizable cationic lipid with potential for gene delivery. J Gene Med 10, 637-645. Hung, A. Y., Futai, K., **Sala, C**., Valtschanoff, J. G., Ryu, J., Woodworth, M. A., Kidd, F. L., Sung, C. C., Miyakawa, T., Bear, M. F., et al. (2008). Smaller dendritic spines, weaker synaptic transmission, but enhanced spatial learning in mice lacking Shank1. J Neurosci 28, 1697-1708. **Sala, C.**, Cambianica, I., and Rossi, F. (2008). Molecular mechanisms of dendritic spine development and maintenance. Acta Neurobiol Exp (Wars) 68, 289-304. Gardoni F, Mauceri D, Marcello E, **Sala C**, Di Luca M, Jeromin A. 2007. SAP97 directs the localization of KV4.2 to spines in hippocampal neurons: Regulation by camkII. J Biol Chem. Piccoli G, Verpelli C, Tonna N, Romorini S, Alessio M, Nairn AC, Bachi A, **Sala C**. 2007. Proteomic analysis of activity-dependent synaptic plasticity in hippocampal neurons. J Proteome Res 6:3203-3215. Saglietti L, Dequidt C, Kamieniarz K, Rousset MC, Valnegri P, Thoumine O, Beretta F, Fagni L, Choquet D, **Sala C**, Sheng M, Passafaro M. (2007) Extracellular interactions between GluR2 and N-cadherin in spine regulation. Neuron 54: 461-77 Beri S, Tonna N, Menozzi G, Bonaglia MC, **Sala C**, Giorda R. (2007) DNA methylation regulates tissue-specific expression of Shank3. J Neurochem 101: 1380-91. Marcello E, Gardoni F, Mauceri D, Romorini S, Jeromin A, Epis R, Boron B, Cattabeni F, **Sala C**, Padovani A, Di Luca M (2007) Synapse-associated protein-97 mediates alpha-secretase ADAM10 trafficking and promotes its activity. J Neurosci 27: 1682-91. Ferrari F, Mercaldo V, Piccoli G, **Sala C**, Cannata S, Achsel T, Bagni C. (2007) The fragile X mental retardation protein-RNP granules show an mGluR-dependent localization in the post-synaptic spines. Mol Cell Neurosci 34:343-54. Gerrow G, Romorini R, Nabi SM, Colicos MA, **Sala C**, El-Husseini A. (2006) A preformed complex of postsynaptic proteins is involved in excitatory synapse development. Neuron 49:547-62. Ko J, Yoon C, Piccoli G, Chung HS, Kim K, Lee JR, Lee JW, Kim J, **Sala C**, Kim E (2006) Organization of the presynaptic active zone by ERC2/CAST1-dependent clustering of the tandem PDZ protein syntenin-1. J Neurosci 26:963-70. **Sala C**, Roussignol G, Meldolesi J, Fagni L (2005) Key role of the PSD scaffold proteins, Shank and Homer, in the functional architecture of Ca^{2+} homeostasis at dendritic spines in hippocampal neurons. J Neurosci 25:4587-92

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	myotubes. Neurosci Lett 256:1-4. **Sala C**, O'Malley J, Xu R, Fumagalli G, Salpeter MM (1997) Epsilon subunit-containing acetylcholine receptors in myotubes belong to the slowly degrading population. J Neurosci 17:8937-8944. Passafaro M, Rosa P, **Sala C**, Clementi F, Sher E (1996) N-type Ca2+ channels are present in secretory granules and are transiently translocated to the plasma membrane during regulated exocytosis. J Biol Chem 271:30096-30104. **Sala C**, Kimura I, Santoro G, Kimura M, Fumagalli G (1996) Expression of two neuronal nicotinic receptor subunits in innervated and denervated adult rat muscle. Neurosci Lett 215:71-74. **Sala C**, Andreose JS, Fumagalli G, Lomo T (1995) Calcitonin gene-related peptide: possible role in formation and maintenance of neuromuscular junctions. J Neurosci 15:520-528. Trabucchi G, Brancato R, Verdi M, Carones F, **Sala C** (1994) Corneal nerve damage and regeneration after excimer laser photokeratectomy in rabbit eyes. Invest Ophthalmol Vis Sci 35:229-235. Rubboli F, Court JA, **Sala C**, Morris C, Perry E, Clementi F (1994) Distribution of neuronal nicotinic receptor subunits in human brain. Neurochem Int 25:69-71. Rubboli F, Court JA, **Sala C**, Morris C, Chini B, Perry E, Clementi F (1994) Distribution of nicotinic receptors in the human hippocampus and thalamus. Eur J Neurosci 6:1596-1604. Tomei G, Spagnoli D, Ducati A, Landi A, Villani R, Fumagalli G, **Sala C**, Gennarelli T (1990) Morphology and neurophysiology of focal axonal injury experimentally induced in the guinea pig optic nerve. Acta Neuropathol (Berl) 80:506-513. Matteoli M, Balbi S, **Sala C**, Chini B, Cimino M, Vitadello M, Fumagalli G (1990) Developmentally regulated expression of calcitonin gene-related peptide at mammalian neuromuscular junction. J Mol Neurosci 2:175-184.
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