An increasing number of studies indicate that neurons generated in the post-natal hippocampus participate to mechanisms of learning and memory, but also mood control. Indeed, new neurons display increased plastic properties and the role of new neurons in the formation of memory traces was confirmed by optogenetic silencing in mice undergoing a Morris water maze test, which resulted in impaired hippocampal memory retrieval. Similarly, inhibiting post-natal neurogenesis reduces the behavioral effects of antidepressant treatments. It is thought that the functional role of neurons born post-natally critically depends on the mechanisms by which they integrate into the mature hippocampal network, which are still poorly-known.

By integrating into the mature network, new neurons preferentially contact pre-existing pre- and postsynaptic partners, which are already involved in synaptic contacts with other neurons. Furthermore, previous work suggests that, upon maturation, new neurons eventually displace or eliminate the pre-existing partners from the synapses they contact, suggesting that a competition at the synaptic level may occur between new and pre-existing neurons. Further substantiating the possibility of competition, the elimination of NMDA receptors from neurons born post-natally decreases their survival, which can be partially restored upon the pharmacological silencing of NMDA receptors of all hippocampal neurons. Together, these results indicate that neurons born post-natally may compete with pre-existing neurons, eventually resulting in the integration or elimination of new neurons. This competition mechanism may underlie the elimination of a great proportion of post-natal-born neurons during the course of their maturation and the mitigation of this elimination process by neuronal activity.

A question arising from these observations is whether improving the synaptic integration of new neurons may result in their increased survival and, eventually, enhance hippocampal function, a hypothesis we tested in a recent study. To this aim, we used a retroviral approach to overexpress synaptic adhesion molecules in a cohort of birth-dated post-natal-born neurons. Synaptic adhesion molecules are transmembrane proteins that mediate the physical connection between pre- and post-synaptic neurons at the synapse. Their expression in non-neuronal cells induces

**ABSTRACT**
Post-natal hippocampal neurogenesis plays a role in hippocampal function, and neurons born post-natally participate to spatial memory and mood control. However, a great proportion of granule neurons generated in the post-natal hippocampus are eliminated during the first 3 weeks of their maturation, a mechanism that depends on their synaptic integration. In a recent study, we examined the possibility of enhancing the synaptic integration of neurons born post-natally, by specifically overexpressing synaptic cell adhesion molecules in these cells. Synaptic cell adhesion molecules are transmembrane proteins mediating the physical connection between pre- and post-synaptic neurons at the synapse, and their overexpression enhances synapse formation. Accordingly, we found that overexpressing synaptic adhesion molecules increased the synaptic integration and survival of newborn neurons. Surprisingly, the synaptic adhesion molecule with the strongest effect on new neurons’ survival, Neuroligin-2A, decreased memory performances in a water maze task. We present here hypotheses explaining these surprising results, in the light of the current knowledge of the mechanisms of synaptic integration of new neurons in the post-natal hippocampus.
the formation of synaptic contacts with axon terminals of co-cultured neurons. The best described synaptic adhesion molecules include SynCAM, Neuroligins and their isoforms, which play distinct roles on synapses. Indeed, the cell-autonomous overexpression of SynCAM1 but not Neuroligin1 (NL1) increases synaptic efficacy in cultured hippocampal neurons, whereas only overexpression of NL1 increases synapse number. In cultured rat hippocampal neurons, Neuroligin 1B (NL1B) overexpression increases glutamatergic puncta whereas Neuroligin-2A (NL2A) overexpression increases the number of both glutamatergic and GABAergic puncta. We therefore reasoned that overexpressing these synaptic adhesion molecules in post-natal-born neurons may enhance their synaptic integration in the pre-existing hippocampal network.

We examined the effect of the cell-autonomous overexpression of these proteins on the maturation and survival of post-natal-born neurons in the dentate gyrus of 7- to 9-week-old mice. We found that SynCAM1 increased the morphological maturation of dendritic spines and mossy fiber terminals while NL1B increased dendritic spine density. However, the effects of SynCAM1 and NL1B overexpression were slight and did not induce modifications in newborn neuron survival. In contrast, NL2A increased both spine density and size as well as GABAergic innervation and resulted in a drastic increase of neuronal survival. These results are consistent with the notion that, in vivo, synaptic adhesion molecules play a role in synaptogenesis in the post-natal brain and contribute to the regulation of synaptic integration of new neurons. Furthermore, the effect of these manipulations on the survival of post-natal-born neurons supports the competition hypothesis. Several studies showed that increased post-natal neurogenesis results in improved performances in spatial memory tasks. In view of these results, we expected that mice with NL2A overexpressing post-natal-born neurons would display enhanced memory performances. It therefore came as a surprise that we observed decreased memory performances of these mice in a Morris water maze task.

The apparent discrepancy between our expectations and observations may be viewed in the light of the mechanisms of synaptic integration of newborn neurons. In particular, we consider 4 possibilities:

The first possibility is that synaptic adhesion molecule overexpression interfered with the synaptic plasticity of newborn neurons. Indeed, immature neurons display increased long-term potentiation (LTP) at around 4 weeks after division, a developmental stage at which their involvement in mechanisms of learning is the greatest. LTP requires structural plasticity of synapses, a mechanism that may be hindered by inter-cellular adhesion. Indeed, the overexpression of proteases increases LTP expression and memory performances, while the overexpression of NL1 and SynCAM1 reduce synaptic plasticity in CA1 neurons in vivo. Hence, it is possible that NL2A overexpression in post-natal-born neurons inhibited the synaptic remodeling necessary for the expression of LTP or LTD and therefore reduced their synaptic plasticity. However, when we assessed the expression of the immediate-early gene activity-regulated cytoskeleton-associated protein (Arc) in these neurons in the context of environmental enrichment, we found that NL2A overexpressing neurons had increased Arc expression compared with their wild-type counterparts. The increased Arc expression in neurons born post-natally argues against impaired synaptic plasticity in these cells.

The second possibility is that NL2A overexpression altered the excitation/inhibition balance of newborn neurons by differentially increasing the density of excitatory and inhibitory synapses. If the increase in the density of inhibitory synapses is more prominent than the increase in excitatory synapses, NL2A-overexpressing newborn neurons might sustain increased inhibition. This might lead to decreased newborn neuron activity, which could underlie the observed memory impairment. However, this possibility is not supported by the increased Arc expression in these neurons. Alternatively, if the increase in the density of excitatory synapses is more important than the increase in inhibitory synapses, this might lower their excitation threshold for a given input from the entorhinal cortex. As a results, the sparse nature of neuronal activity in the dentate gyrus, thought to be critical for information processing, might be disrupted. In line with this hypothesis, Dieni et al. showed that low excitatory innervation balanced high excitability of immature neurons in the dentate gyrus and prevented broad responsiveness of these neurons. Although this hypothesis cannot be ruled out, according to our data, the number of GABAergic synapses increased as much as the number of dendritic spines in NL2A-overexpressing post-natal-born neurons, suggesting that the inhibitory/excitatory balance is maintained in these neurons. As NL1B overexpression in post-natal-born neurons selectively increased excitatory synapse density, assessing the
effect of NL1B overexpression in post-natal-born neurons on learning and memory performances would give insight on whether keeping a proper excitation/inhibition balance is crucial for maintaining the proper function of the neuronal network.

The third possibility is that the overexpression of synaptic adhesion molecules in newborn neurons might lead to the sequestration of pre-synaptic molecules such as neurexins at synapses formed with adult-born neurons, leading to an overall decrease of available synaptic adhesion molecules in pre-synaptic neurons. This, in turn, may impair their ability to form synapses with other post-synaptic partners, thus impairing their function, and leading to decreased memory performances. In line with this hypothesis, perforant path synaptic loss correlates with cognitive impairment in subjects aged 90 and older.25

Finally, an intriguing possibility is that NL2A overexpression altered the specificity of partner choice at the dendritic level, and therefore altered input selectivity. The rules of presynaptic partner choice are unclear, however several lines of evidence suggest that adhesion molecules may be a key mechanism in matching specific presynaptic and postsynaptic partners: In the vertebrate retina, immunoglobulin superfamily (IgSF) adhesion molecules Sidekick-1 and-2 and Dscam/DscamL were found to be important for correct targeting of axons to different sublamina of inner plexiform layer.26,27 In the chick, retinal ganglion cell axons target the correct retino-recipient laminae in vitro, and this targeting is partially dependent on the expression of the cell adhesion molecule N-cadherin. In a similar way, N-cadherin has been implicated in the targeting of thalamocortical projections.28 Some cell adhesion molecules are also thought to serve as a guide for synapse formation at the subcellular level. In the cerebellum, specialized inhibitory basket neurons form synapses, called pinceau synapses, specifically with the axon initial segment (AIS) of Purkinje neurons. The intracellular membrane associated adaptor protein Ankyrin-G is required for basket axon targeting and pinceau synapse formation at the Purkinje AIS. In Ankyrin-G knockout mice, basket axons are no longer restricted to the AIS but form synapses on slightly more distal Purkinje axon segments.28 Thus, interfering with synaptic adhesion molecules may impair the connectivity of new granule neurons.

Filopodia are widely thought to be the precursors of dendritic spines.29 In our recent work, we observed that any given filopodium from a granule neuron born post-natally is surrounded by an average of 5 presynaptic axon terminals, each of which being a potential presynaptic partner (Fig. 1).30 Given that individual perforant path axons contact hippocampal granule cells by very few synapses, changes in the connectivity of individual spines may have drastic repercussions on the hippocampal network. Therefore, it is possible that by interfering with presynaptic partner choice of nascent filopodia, NL2A overexpression led to connections with

Figure 1. Left panel: Dendritic filopodia from neurons born post-natally (gray) grow in the direction of pre-existing axon terminals. Upper right panel: In normal conditions, upon maturation, some filopodia will retract while others will establish mature synaptic connections with appropriate presynaptic partners (green) but not with inappropriate synaptic partners (red). Lower right panel: Overexpressing Neuroligin-2A (red lines) in neurons born post-natally may lead to the formation of aberrant synaptic connections with axon terminals that would otherwise not have been connected (red). These aberrant connections may lead to malfunction of the hippocampal network.
presynaptic partners that should otherwise not have been connected, which impaired the connectivity of post-natal-born neurons and resulted in their aberrant integration (Fig. 1). The wrong targets may include natural pre-synaptic partners that would otherwise not have been chosen, such as axon terminals of the perforant path or of pyramidal basket cells, but also aberrant targets such as axon terminals of neuronal types that do not connect to the post-synaptic specializations of granule neurons in physiologic conditions but are located in the molecular layer of the dentate gyrus. In turn, although the aberrant connectivity of new neurons may have enhanced their survival, it resulted in impaired function and thus impaired memory performances.

The synaptic integration of new neurons occurs simultaneously to the activity-dependent elimination of the majority of these cells. Although the mechanisms involved in this selective elimination remain unknown, synaptic connectivity seems to be an important factor for newborn neuron survival, and the specificity of this connectivity is required for their proper function. Thus, beyond the number of connections, the identity of their synaptic partners may be a critical factor for the functional role of new hippocampal neurons. It is clear that further research is required to determine how new neurons select specific synaptic partners from the crowd offered by the mature brain and it will be interesting to assess the role of specific adhesion molecules or relative activity between adjacent fibers in this process.

Beyond post-natal neurogenesis, a proper level of expression of cell adhesion molecules may also be required for proper synaptic integration of neurons during embryonic neurogenesis. Since mutations in cell adhesion molecules such as neurexins and neuroligins are linked to neuropsychiatric disorders such as schizophrenia and bipolar disorder, it is tempting to think that the aberrant functional integration of neurons during post-natal neurogenesis but also during embryogenesis may contribute to the impaired cognitive function associated with these pathologies.

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