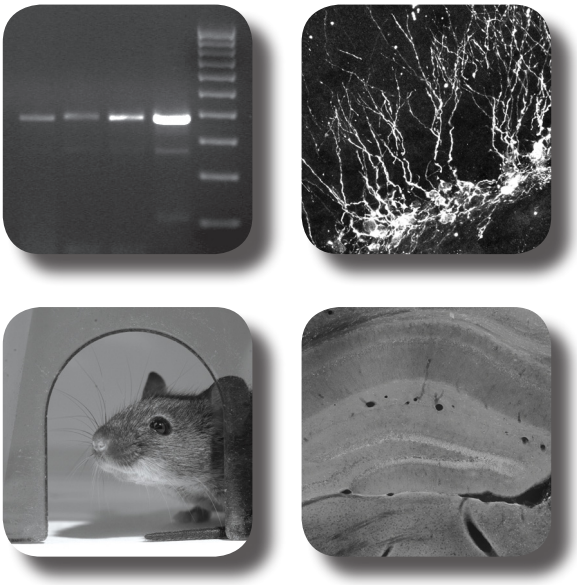
Chapter 7

Summary/Samenvatting



Summary

Adult neurogenesis is a process in which neuronal stem cells in the adult brain give birth to new neurons. Two locations within the brain do show adult neurogenesis; the hippocampal dentate gyrus (DG) and the subventricular zone (SVZ) of the lateral ventricles. Within the DG neuronal progenitor cells (NPC's) develop into mature neurons within the same DG. They migrate over a short distance within the granule cell layer. NPC's born in the SVZ migrate over a longer distance along the rostral migratory stream (RMS) towards the olfactory bulb (OB) were they develop into mature neurons. Doublecortin (DCX) and the highly homologous Doublecortin-like (DCL) play crucial roles during embryonic development. However, for DCL it is unknown whether it is also involved in adult neurogenesis. To study the role of DCL in the adult brain and specifically in adult neurogenesis we developed a DCL specific antibody and an inducible siRNA expressing mouse targeting DCL.

In **chapter 2** we described a DCL specific antibody and mapped the expression of DCL in the adult brain. DCL is encoded by the complex DCLK1 gene generating several splice variants like DCLK-long, DCLK-short and DCL. Since conventional DCLK1 antibodies do not distinguish between all splice variants, a novel, DCL specific antibody was designed. As expected, we identified DCL in neurogenic areas like the SVZ and SGZ of the dentate gyrus where it co localized with DCX. These findings indicate that DCL plays an important role in adult neurogenesis. Interestingly, DCL protein was also found in several non-neurogenic areas like the Islands of Calleja (ICj), Suprachiasmatic nucleus (SCN) and hypothalamic tanycytes. These novel findings raised questions about the function of DCL in these areas.

In **chapter 3**, the DCL-KD mouse model was validated by showing specific DCL knockdown after doxycycline administration. Doxycycline in food pellets resulted in a strong up-regulation of shRNA targeting DCL. Western blot analysis showed a strong reduction in DCL protein expression whereas DCLK-long and DCLK-short were not affected. Furthermore, the effect of DCL knockdown was studied on adult hippocampal neurogenesis using stereological techniques. Bromodeoxyuridine (BrdU) labelling studies showed an increase in proliferation and a reduction in cell survival. Also the number of DCX positive immature neurons was strongly reduced. In addition, we studied the effect of DCL knockdown on hippocampus-dependent behaviour. In a circular hole board paradigm the spatial memory performance of normal and DCL-KD mice was tested. Impaired neurogenesis does not affect spatial memory formation, DCL-KD animals performed equally compared to non-induced littermates. Interestingly, although DCL-KD mice reached the exit hole in the same time as their non-induced littermates, they showed a much longer escape latency.

In **chapter 4**, the findings described in chapter 3 were confirmed. The effect of DCL knockdown on hippocampus-dependent learning was measured using a contextual fear conditioning paradigm. During 6 training sessions, mice were put in a conditioning chamber

were they received a mild electric shock during the presence of a light and tone cue. In 6 memory retrieval sessions, freezing behaviour was recorded in the same conditioning box with the presence of the cue but without the shock. Hippocampus-dependent learning was not affected after DCL knockdown since DCL-KD animals (compared to wildtype littermates) showed equal amounts of freezing behaviour during the periods in the context. Also during the presence of the cue, freezing behaviour of DCL-KD animals was similar as the behaviour of their littermate controls. However, subtle differences were found. During memory retrieval, the first out of six presented cue's induced s significant stronger freezing response in DCL-KD animals. Also the amount of tail rattling behaviour was strongly reduced after DCL knockdown.

A rather unexpected finding was the presence of DCL in hypothalamic tanycytes as reported in chapter 2. Hypothalamic tanycytes are part of the Hypothalamic-Pituitary-Thyroid axis (HPT-axis), which is involved in energy metabolism. Therefore, we address the question whether or not DCL is involved in thyroid hormone signalling in **chapter 5**. We measured bodyweight, serum T3 and T4 concentrations and D2 activity in hypothalamic tissue of DCL-knockdown (KD) mice and their littermate controls. Furthermore, we measured mRNA expression of TRH, NPY, D2 and D3 in hypothalamic punches containing the ARC-ME or the PVN. We observed a strong reduction in DCL expression in hypothalamic tanycytes, which was associated with reduced body weight growth and a significant increase in D2 activity, the enzyme metabolizing inactive T4 into active T3. However, serum levels of T4 and T3 did not differ between wildtype and DCL-KD animals and also the expression of TRH, NPY, D2 and D3 mRNA in the hypothalamus was not affected by DCL knockdown. Together, our data indicate a role for DCL in the regulation of D2 activity in hypothalamic tanycytes and a possible subtle role in thyroid signalling.

In **chapter 6**, we discussed our DCL findings in the adult mouse brain. First of all, for the first time, we show immunohistochemically the presence of DCL in the adult brain. As expected, DCL is expressed in the neurogenic regions of the hippocampus and forebrain. The presence of DCL in other brain areas raises the question to what extend these brain areas have a neurogenic or neuroplastic nature. The generation of a doxycycline-inducible DCL-KD mouse model was validated and DCL knockdown resulted in impaired adult hippocampal neurogenesis. However, hippocampus-dependent learning is not affected by DCL knockdown. DCL may play a role in hypothalamus-regulated energy metabolism since D2 activity is increased after DCL knockdown. In conclusion, The DCL-KD mouse model seems a suitable model to study DCL in the adult brain also by circumventing possible developmental compensation by DCX. This model may be instrumental to elucidate the role of DCL in neurogenesis and neuronal plasticity in brain areas like hippocampus, hypothalamus and forebrain.