



**Karolinska
Institutet**

Institutionen för Cell- och Molekylärbiologi

Regenerating the Brain: Lessons from the Red Spotted Newt

AKADEMISK AVHANDLING

som för avläggande av medicine doktorexamen vid Karolinska
Institutet offentligen försvaras i Föreläsningssal Farmakologi, Nanna
Svartz väg 2

Fredagen den 28 September, 2012, kl 10.00

av

Daniel Berg

Huvudhandledare:

Dr. András Simon
Karolinska Institutet
Institutionen för Cell och Molekylär biologi

Fakultetsopponent:

Professor Fred Gage
Salk Institute
Laboratory of Genetics

Bihandledare:

Professor Ernest Arenas
Karolinska Institutet
Institutionen för Medicinsk Biokemi och
Biofysik

Betygsnämnd:

Professor Michael Thorndyke
Göteborgs Universitet
Institutionen för Biologi och Miljövetenskap

Professor Karin Forsberg-Nilsson

Uppsala Universitet
Institutionen för Immunologi, Genetik och
Patologi

Docent Elisabet Andersson

Karolinska Institutet
Institutionen för Cell och Molekylärbiologi

Stockholm 2012

ABSTRACT

Unlike mammals, adult salamanders can regenerate their brain after injury in a process fuelled by neurogenesis. The aim of this thesis is to identify the cells that give rise to new neurons after injury and examine the mechanisms controlling the initiation and termination of brain regeneration in the newt. The reasons why newts can regenerate their brain while mammals cannot are unknown, and it has been argued that the presence of constantly proliferating cells is a prerequisite for regeneration to occur.

In **Paper I** of this thesis we mapped the distribution of proliferating cells in the adult newt brain and identified the areas of the brain where neurons are added under normal conditions. We observed that similarly to mammals, neurons are continuously added to the newt forebrain, while no neurogenesis was detected in the midbrain. After injury to the newt midbrain, radial glia-like cells (RGLs) start to proliferate and progeny of these cells differentiate into neurons. These results show that regeneration is not dependent on constitutive neurogenesis.

In **Paper II** we wanted to test if the neurotransmitter dopamine is involved in controlling regeneration of dopaminergic (DA) neurons. We found that synthetically increasing the concentration of dopamine in the regenerating newt brain by administration of the dopamine precursor L-dopa, inhibits regeneration by blocking the proliferation of the progenitor cells. We also found that antagonizing dopamine signalling in the uninjured brain is sufficient to evoke proliferation of the otherwise quiescent RGLs and we found that progeny of these cells give rise to increased numbers of neurons in the midbrain.

The newt is not the only vertebrate animal able to regenerate its brain; both fish and reptile species can repair injured brain. Interestingly, fish and reptiles are also able to survive hypoxia and are exposed to these conditions in their natural habitat. In **Paper III** of my thesis we wanted to examine if the ability to regenerate brain tissue is linked to an animal's ability to survive varying levels of oxygen. We observed that the red spotted newt is able to survive hypoxia, but this treatment leads to increased cell death in the brain. Reoxygenation of the brain leads to an elevated production of reactive oxygen species, which is concomitant with an increase in proliferation of the ventricular progenitor cells, suggesting that a regenerative process has been initiated. Inhibiting production of reactive oxygen species during reoxygenation results in reduced progenitor cell proliferation.

The major findings of this thesis are summarized as follows: the red spotted newt is able to regenerate areas of the brain that are normally devoid of proliferating cells. In this process, ventricular RGLs act as progenitor cells in the adult newt brain and give rise to new neurons after injury. We have also shown that the neurotransmitter dopamine inhibits the proliferation of DA progenitor cells in a feedback-like manner and thus identified a mechanism for how the brain senses the degree of neuronal loss. These results pinpoint several features of naturally occurring brain regeneration and could aid the development of techniques to evoke brain regeneration in humans. Finally we have found that the newt, similarly to other animals capable of brain regeneration, is able to survive hypoxia. Hypoxia leads to increased cell death in the brain, but molecules that are activated by reoxygenation induce regenerative mechanisms such as increased proliferation of neural progenitor cells. These discoveries suggest that the trait of brain regeneration has evolved together with the capacity to survive variation in oxygen levels.