Regulation of critical period plasticity in normal development and in Neurofibromatosis type 1

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Summary

During brain development, neuronal networks are still dynamic and being shaped. The brain must be able adapt to sensory stimuli. Especially after birth and in childhood, there are various critical periods in which a neuronal network adapts to external experiences. There are critical periods for correct processing of sensory perceptions such as seeing with both eyes, developing motor skills and learning a first language. These skills must be learned during a specific critical period in order to optimize relevant neuronal networks. Once a critical period is closed, neuronal networks are less dynamic and it becomes more difficult or even impossible to learn certain new skills. The opening and closing of critical periods must therefore be carefully regulated. If the timing of critical periods are altered, developmental disorders can arise with cognitive, behavioral and motor problems. In this dissertation we have investigated various mechanisms that regulate critical periods.

The model we used to investigate critical periods is the visual system of the mouse. During development, the visual system must learn to process information from both eyes to form one image. If one eye is temporarily deprived during a critical period, cells in the visual cortex will react less to this eye and ultimately more to the other eye. This is due to structural and functional changes of neuronal connections and this form of plasticity is called ocular dominance (OD) plasticity. The critical period for OD plasticity occurs in mice between approximately P20 (20 days after birth) and P35 with a peak around P28. After this period, the amount of OD plasticity is greatly reduced.

Critical periods are regulated by various mechanisms. One of the mechanisms that play a role in closing the critical period is the stabilization of axons and synapses. During the critical period for OD plasticity there is a lot of growth and retraction of axons. These axonal changes are greatly reduced after closure of the critical period. In addition, a great deal of synaptic plasticity occurs during the critical period. After critical period closure, there is still local synaptic plasticity, but much less. Another mechanism is inhibition via the neurotransmitter γ-amino butyric acid (GABA), which plays a crucial role in regulation of critical periods. Too much inhibition can lead to early opening and closing of critical periods and a lack of inhibition can cause a delayed or late critical period. This has been extensively studied in animal models where the amount of inhibition is genetically or pharmacologically manipulated. A shift in the excitation / inhibition balance is also the basis for development disorders in which critical periods are disrupted, such as Rett syndrome and Fragile X syndrome.

In this dissertation we looked at both mechanisms involved in the regulation of critical periods. In chapter 2 we looked at structural aspects and found that genes involved in regulation of Wallerian degeneration, a form of axonal degeneration that

normally occurs after damage to axons, are also involved in plasticity during critical periods. In chapters 3 and 4 we have shown that increased inhibition in a mouse model for the developmental disorder Neurofibromatosis type 1 (NF1) can lead to an early closure of the critical period for OD plasticity.

Wallerian degeneration is a form of degeneration in which axons immediately disintegrate after losing contact with the cell body. This process is regulated by the nicotinamide mononucleotide adenylyl transferase (NMNATs) enzymes in the cytoplasm. In a mouse model that produces the fusion protein UBE4b-NMNAT1 due to a mutation (Wld^S mice), Wallerian degeneration is delayed. This type of degeneration is also delayed in mice overexpressing NMNAT into the cytoplasm. In a study analyzing proteins involved in cortical development and OD plasticity, some proteins were found which are also involved in Wallerian degeneration. That is why in **chapter 2** we investigated whether OD plasticity has changed in mice in which Wallerian degeneration is delayed. We found that both Wld^S mice and mice overexpressing NMNAT3 have reduced OD plasticity at the end of the critical period. We also observed that Wld^S mice have an accelerated development of visual acuity, a development process that runs parallel to the critical period for OD plasticity. Despite that we showed that the NMNAT signaling pathway is involved in OD plasticity, we did not find any evidence that Wallerian degeneration actually occurs during this type of plasticity. It is possible that NMNATs not only regulate Wallerian degeneration after damage, but that they also play an important role in the regulation of critical periods during normal cortical development.

In addition to structural changes, inhibition also plays a major role in regulation of critical periods. In a mouse model for the developmental disorder NF1 (*nf1*$^{+/-}$ mice), GABAergic inhibition is increased in distinct parts of the brain. NF1 is a developmental disorder that is associated with cognitive, motor and behavioral problems. In addition, patients often suffer from skin problems, disfiguration and an increased risk of tumors. NF1 is caused by a mutation in the *nf1* gene leading to reduced expression of the protein neurofibromin. There is no therapy other than controlling symptoms. Elevated GABAergic inhibition seems to underlie the cognitive problems in NF1. Since increased inhibition may affect the opening and closing of critical periods, we examined the development of inhibition in the visual cortex and whether the timing of the critical period for OD plasticity has changed.

In **chapter 3** we studied the development of the cerebral cortex in *nf1*$^{+/-}$ mice. We first investigated how GABAergic inhibition and the activity of pyramidal cells in the visual cortex develop. After eye opening, the activity of pyramidal cells increased rapidly. The excitability of these neurons also slightly increased in *nf1*$^{+/-}$ mice. After eye opening, the amount of GABAergic inhibition should slowly increase, but it increases rapidly in *nf1*$^{+/-}$ mice. The disturbed balance between excitation and inhibition can influence spontaneous activity during the early development of the

cerebral cortex. This spontaneous activity is generated in the retina or in the cerebral cortex and spreads throughout cortical areas of the brain. This is important for the establishment of proper neuronal connections. In the visual system, for example, this is important for establishment of optimal connections between the retina, thalamus and the visual cortex. Therefore, we investigated the development of spontaneous cortical activity. However, we detected no difference in spontaneous activity in the visual cortex between *nf1*$^{+/-}$ mice and their wild-type controls. Therefore, it appears that the cognitive problems in NF1 develop at a later stage.

Chapter 4 is a continuation of the study in chapter 3 and investigates cortical development of one month old *nf1*$^{+/-}$ mice. We studied the activity of GABAergic interneurons and pyramidal cells in the visual cortex of *nf1*$^{+/-}$ mice during the critical period for OD plasticity. We show that cortical inhibition remains elevated and that the excitability of pyramidal cells is normal at that age. Importantly, we showed that the critical period for OD plasticity closes early in *nf1*$^{+/-}$ mice. During the normal peak of the critical period (P28), *nf1*$^{+/-}$ mice exhibit much less OD plasticity compared to wild-type mice, whereas one week earlier in development the amount of plasticity is normal. It is also interesting that the critical period for OD plasticity starts normally and that the total duration of the critical period for OD plasticity is therefore shortened in these animals. This can have major consequences later in life, because there is less time to optimize the neuronal networks. Proper regulation of critical periods is also important because these critical periods succeed each other. Plasticity often occurs in lower cortical areas first, followed by critical periods in higher, more specialized cortical areas. If this is not properly coordinated, this can result in cognitive, motor and behavioral problems, such as in patients with NF1.

Because cognitive problems in NF1 are mainly caused by increased GABAergic inhibition, most clinical studies focus on (pharmacologically) reducing inhibition. So far, these clinical studies have not been successful. It is possible that the timing of the treatments are not correct and that they start after the closure of critical periods. Additionally, the time at which inhibition should be reduced and the required dose could differ depending on brain areas. It will be challenging to reduce the amount of inhibition at different moments in different brain areas. Another approach is extending the critical period and rescuing the early closure. In mice, critical periods can be extended by environmental enrichment, which reduces the amount of inhibition. We showed that by raising *nf1*$^{+/-}$ mice in an enriched environment, the differences in GABAergic inhibition and OD plasticity disappeared. This means that enriching the environment can prevent developmental problems.

In this dissertation we have shown that timing and regulation of critical periods are very important. We have unraveled a structural aspect of the closure of critical

periods and looked at the role of inhibition in the regulation of critical periods. We have found that cytoplasmic NMNATs play a role in OD plasticity. Previously it was not known that these proteins play a role in normal development, and were considered to be only involved after neuronal injury. In addition, we have demonstrated that increased inhibition results in early closure of critical periods in *nf1*$^{+/-}$ mice and that this can be saved by rearing these mice in an enriched environment. Because of these findings, we now know more about the mechanisms that regulate critical periods. This creates new opportunities for treatment strategies or for development of new therapeutic approaches.