CHAPTER 6

General discussion and summary
GENERAL DISCUSSION AND SUMMARY

In order to establish functional and mature circuits, neurons must extend their axons and dendrites on the way to reach their potential synaptic target. They also must select the neurons with which to make synapses, and finally they must eliminate those connections that are inappropriate or irrelevant. The changes in synaptic receptor repertoire are important for the establishment of the synaptic specificity of the neurons. The localization of receptors at the pre- and postsynaptic membrane determines to a large extent the efficacy of synaptic transmission. However, the molecular basis and the mechanisms responsible for all these processes are not well understood.

The corticotropin-releasing factor (CRF) and related peptide urocortin (UCN) are widely expressed in the brain (Palkovitz et al., 1987; Swinny et al., 2002). Both peptides are involved in stress-related illnesses such as major depression, anxiety-related disorders (Gold, 1996; Muller et al., 1998; Arborelius et al., 1999; Dautzenberg and Hauger, 2002) and Alzheimer's, Parkinson's, Huntington's disease (De Souza, 1995; Behan et al., 1995). In the cerebellum they are modulators of dendritic development (Swinny et al., 2004; Chen et al., 2004). The trophic factors and neural activity as well as the interaction of the receptors may play a role in regulating pre-/postsynaptic differentiation and in the patterning of cells. The exact role and the way in which CRF and UCN are involved in the development of Purkinje cells and subsequent stabilization of the cerebellar circuitry are still not clear. Recently, neuropeptides like CRF and urocortin have been hypothesized to exhibit a dual role in the cerebellum by acting during developmental at early stages and later changing to a neuromodulatory role (by inducing LTD) in the adult. The research in this thesis has provided data about the roles and involvement of CRF-related peptides and their receptors in the development of Purkinje cells and their afferents.

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In chapter 2, we demonstrate the cellular distribution of full-length isoform CRF-R2. The full-length form of CRF-R2 is characterized by marked changes during postnatal development in the rat cerebellum. The full-length form CRF-R2 has a clearly different distribution than the truncated isoform of CRF-R2. Our data demonstrate that the expression and the localization of the full-length form CRF-R2 correlate with development of the cerebellum. First, the higher expression of full-length of CRF-R2 in Purkinje cells demonstrated that UCN and CRF might play a role in initial stage of Purkinje cells growth and synaptogenesis encoded by the full-length form of CRF-R2. In the next stage of cerebellar development, we observed labelling of full-length CRF-R2 in glutamatergic systems of the cerebellum: parallel fibers, climbing fibers as well as granule cells. The expression of receptors in this system takes place when the process of the reduction of supernumerary Purkinje cell – climbing fiber synapses is ongoing. And finally, in the late stage of cerebellar development, the functional significance of the localization of full-length of CRF-R2 in presynaptic terminals of climbing fibers as well as of parallel fibers could be reflected by the role of CRF-R2 receptor in the autoregulation pathway (Bishop et al., 2000) and modulation of the synaptic transmission. In summary, the investigation of CRF receptors types has not only increase our understanding of CRF physiology and the role in CNS but the results also provide a basis in rational drug design for the treatment of diseases that are associated with abnormal CRF levels.

In chapter 3, we describe the effects of CRF and UCN on Purkinje cells in organotypic slices. We demonstrate that both CRF and UCN are capable of activating gene expression of glutamate receptor delta2 (GluRδ2), although only UCN treatment leads to the increase in GluRδ2 protein level. CRF appears to affect the pattern of GluRδ2 distribution on the PSD (gives clustering of receptor on PSD), while UCN upregulates GluRδ2, but does not induce clustering of GluRδ2.
GluRδ2 involved in the process of elimination of supernumerary climbing fibers in cerebellum (Hashimoto et al., 2001; Ichikawa et al., 2002). The degradation of the climbing fibers terminals leads to the arrangement of the synapses with parallel fibers at identical positions (Morando et al., 2001) and such Purkinje cell – parallel fiber synapses are characteristic GluRδ2-positive structures. We observed that the number of GluRδ2-positive structures in Purkinje cells decreases upon CRF application. Thus, CRF might lead to the preservation of some or all climbing fiber – Purkinje cell synapses, together with a consequent decrease of the number of parallel fiber – Purkinje cell synapses and therefore most likely it is involved in the axonal competition process. On the other hand, since upregulation of the GluRδ2 gene by CRF does not lead to any statistically significant increase of δ2 receptor protein at all, it is possible that the posttranscriptional mechanism allowing superproduction of GluRδ2 protein is only governed by UCN, but not by CRF. The presence of few receptor forms (or isoforms) in the same cell may provide a molecular basis for distinct developmental processes. CRF and UCN mediate their effects via two receptors, CRF-R1 and CRF-R2 (full-length and truncated isoforms) (Chalmers et al., 1996; Bishop et al., 2000). CRF and UCN bind with equal affinity to CRF-R1; however, UCN has a 40-fold greater affinity for CRF-R2 (Latchman, 2002), suggesting that UCN is the natural ligand for CRF-R2. We propose, in this chapter, a model suggesting how CRF and UCN, via different receptors regulate distinct cellular functions in Purkinje cells (particularly affect on GluRδ2) since they predominantly act through different receptors, resulting in different fine tuning of the same signalling pathways.

The function of the nervous system critically relies on the establishment of precise and specific synaptic connections between neurons and specific target cells (Cohen-Cory, 2002). The simple mechanism underling synaptic heterogeneity is the cell-specific expression of a protein, and
targeting of that protein to all presynaptic or postsynaptic sites made by a cell (Craig & Boudin, 2001). Sperry (Sperry, 1963) proposed that differences in the concentrations of cell-surface molecules can convey positional information. However, far less is known about the molecules and signalling involved in target recognition. Since, the cerebellar expression of CRF starts at early embryonic stages, sooner than any functional connections have been formed (Bishop & King, 1999; Chang et al., 1993), CRF may play a role in target recognition as well as in synaptic organization (Cummings et al., 1994), and consequently in the establishment of connections in the cerebellum.

In chapter 4, we evaluate the effects of an overabundance of CRF on afferent systems of cerebellar Purkinje cells using mice, over-expressing CRF. The reason using mice, over-expressing CRF is that the higher CRF level is continuously present throughout the development and in adult cerebellum. First, we found that the overexpression of CRF does not lead to changes in Purkinje cell number and/or density. Second, the overexpression of CRF to marked changes in the pattern of distribution of climbing fibers is due to increasing the number of synapses in the cerebellum. In the normal cerebellum, CRF is concentrated in climbing fibers (Palkovits et al., 1987). Furthermore, we did not observe any effects of the overabundance of CRF in parallel fiber system, where CRF is not present. Thus, we conclude that CRF could be involved in the developmental control of the climbing fiber (establishment of the specificity of axonal projections) and stabilization of their contacts with Purkinje cells.

The formation and stabilization of synapses is a crucial step in the development of cerebellar circuitry. In the chapter 5, we focused on the cellular development of spines of Purkinje cells and the topological matching of presynaptic and postsynaptic specialization of synapses Purkinje cells. Using application by CRF and UCN cerebellar organotypic slices we demonstrate
increasing the length of the active zone as well as the PSD in synapses between Purkinje cells and parallel fibers and subsequently, morphologically matching pre- and post- terminals of synapses. Since, the potential synaptic efficacy directly correlates with synaptic size (Pierce & Lewin, 1994; Schikorski & Stevens, 1997), increasing the length of active zones and PSDs in parallel fiber – Purkinje cell synapses after application slices with CRF and UCN, this probably leads to changes in strengthening of synapses and stabilization of synapses (topological matching).

On the other hand, CRF and UCN increase the density spines of Purkinje cells. Nevertheless, CRF application leads to the appearance of higher number of spines of Purkinje cells than UCN treatment. These observations are in accordance with earlies data shown (Swinny et al., 2004), showing that CRF and UCN affect dendritic outgrowth and elongation. In vitro, Purkinje cells treated with CRF and UCN had longer dendrites and increased dendritic branching per cell compared to control cells. However, CRF leads to increased the number of dendritic branching compared to UCN (Swinny et al., 2004) as well it provides higher numbers of tiny dendritic protrusions – spines. Moreover, CRF and UCN stimulate a different population of spines. They increase the mushroom spines (CRF) and the thin spines (UCN). Increasing the population of the thin spines in UCN treated cells, could be correlated with the capability of the thin spines to isolate Ca\(^{2+}\) transients from parent dendrites (Segal et al., 2000; Tyler & Pozzo-Miller, 2003). On the other hand, mushroom spines (induced by CRF) could represent a state of a spine in which membrane dynamics and receptor turnover are occurring (Lee et al., 2004). The spines of Purkinje cell might be regulated by their local afferents (Bravin et al., 1999). CRF can selectively promote spines for climbing fibers, since CRF is selectively localized in climbing and mossy fibers (Palkovits et al., 1987), but not in parallel fibers. Whilst, UCN that is localized in the
varicose terminals of parallel fibers, where CRF is not present (Swinny et al., 2002) might promote spines for parallel fibers.

In conclusion, our results indicate that CRF and UCN participate in the development of Purkinje cells as well as in their connections and subsequent stabilization of synapses of Purkinje cells. Also, we suggest that CRF and UCN induce local dendritic instability, allowing activity-dependent morphological changes in spines of Purkinje dendrites and as a result has wide effects on synaptic transmission and plasticity.

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