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**Ontogenetic and comparative aspects of cerebellar and motor development**

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# Ontogenetic and comparative aspects of cerebellar and motor development

DOCTORAL DISSERTATION

Bye due permission of the Faculty of Medicine of Lund University to be defended for the degree of Doctor of Philosophy of medical science in Segerfalksalen, Wallenberg Neurocentrum, Sölvegatan 17, Lund, on March 31, 2007, at 10.00 a.m.

by

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Cover layout by Emma Hansson (Microphotographs display the external granular layer in ferret cerebella of different postnatal ages).

# Ontogenetic and comparative aspects of cerebellar and motor development

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# CONTENTS

## ORIGINAL PAPERS

This thesis is based on the following papers, which are referred to by their roman numerals in the text:

I      Christensson M, Garwicz M (2005) Time course of postnatal motor development in ferrets: Ontogenetic and comparative perspectives. *Behav Brain Res;* 158:231-242

II     Christensson M, Garwicz M (2005) Ontogenesis of within-session locomotor habituation in the open field. *Neuroreport;* 16(12):1319-23

III    Christensson M, Broman J, Garwicz M (2007) Time course of cerebellar morphological development in postnatal ferrets: Ontogenetic and comparative perspectives. *J Comp Neurol;* 501:916–930 (2007)

IV     Christensson M, Garwicz M. Functional organization of climbing fibre input to the cerebellum of the postnatal ferret. *Preliminary manuscript*

V      Garwicz M, Christensson M. Can the time course of postnatal motor development in humans be predicted from animal models? *Preliminary manuscript*

# SUMMARY

During the course of development the motor repertoire of animals and humans alike go through dramatic changes. New motor patterns arise; movements become coordinated, improve in precision and are at the same time continuously calibrated to the changing body dimensions. The cerebellum is critical for movement coordination and adaptation in adults. Also, interfering with cerebellar development during early life causes behavioural deficits suggesting an important role of the cerebellum in the formation of motor synergies. Hence, to understand the dramatic change in motor competence that characterizes postnatal development it may be of particular interest to study processes underlying the formation and shaping of the cerebellar neuronal networks. Unfortunately, little is known about *how* the cerebellum actually contributes to motor development. The relationships between structure, function and motor development are likely to be complex, as processes of maturation, adaptation and learning all contribute to the functional development of many neuronal systems.

In order to elucidate the relationships between cerebellar ontogenetic changes and postnatal motor development a suitable animal model for multiple levels of analysis is a prerequisite. In this thesis, we therefore sought to develop and evaluate an experimental model that is suitable for combined behavioural, structural and systems level electrophysiological investigations of cerebellar development. For a number of reasons, the postnatal ferret seemed to be a suitable candidate. Although the ferret is commonly used as an experimental model in developmental studies on sensory systems, the development of its motor systems and motor behaviour had not been previously investigated.

Firstly, we characterized the postnatal motor development in ferret kits in daily sessions from postnatal day (P)2 to P63. A battery of motor tests spanning the entire developmental period was used to assess locomotor activity and ability and the maturation of specific postural dynamic reflexes. Secondly, we characterized the morphological development of the ferret cerebellum. Overall cerebellar size, foliation and thickness of cortical layers were quantified and Purkinje cell morphology was characterized from P1 to P63. Thirdly, we investigated the zonal organization of climbing fibre input to the cerebella of ferret kits; a fundamental and general physiological feature of cerebellar function in the adult animal.

Taken together, these studies provide the first systematic investigation of motor behavioural and cerebellar morphological development in the ferret

and the electrophysiological data obtained may represent a first important step towards the understanding of cerebellar physiological processes in the course of motor development. We further conclude that the ferret in many aspects is a particularly suitable animal model for the study of mechanisms underlying motor development.

In a parallel approach, we assessed how timescales of motor and cerebellar morphological development can be translated between species with differently long developmental time periods, such as the ferret and rat. In practical terms, this was necessary in order to allow translation of data from the numerous studies on rat cerebellar and motor development. Linear regression analysis was used to obtain equations representing the conversion of developmental time between the two species. The results were striking by the congruence in relative timing of cerebellar and motor development in the two species.

Such an analysis also has more general implications for the understanding of comparative aspects of motor development and was therefore extended also to humans. In brief, we formulated a model that takes into consideration comparative time courses of neurogenesis and cerebellar morphogenesis and relative timing of birth. Using behavioural data from rats and ferrets as input, the model predicts corresponding motor developmental dates that fall within 10% of actual mean values for the human population. Such astonishing predictive accuracy indicates that motor development in animals and man is governed by very similar principles and that these principles are successfully captured by our model.

# GENERAL INTRODUCTION

## BACKGROUND

You may have witnessed when a newborn foal rises and takes it first unsteady steps. It is an impressive sight and even more so considering it does it less than an hour after it was born. This familiar yet curious naturally occurring phenomenon points at some fundamental issues of developmental comparative biology in general and motor development in specific. One intriguing issue is that of motor developmental time; whereas the foal can walk and even trot immediately upon birth, the rat, for instance, crawls with its belly off the ground by day ten and walks quite well when it is 16 days of age (Altman and Sudarshan, 1975) and the human baby walks independently on average 357 days after being born (Bayley, 1993). Which factors may contribute to these differences in developmental time between mammalian species?

Biomechanics of the motor apparatus could be one such factor. Plantigrade, digitigrade and unguligrade (hoofed) animals differ with regard to mobility of the extremities. In fact, the ability to walk or even run immediately upon birth is common to all unguligrade animals. Further, the first two mammals in the example above are quadrupedal whereas the latter is bipedal, and thus has to maintain an upright posture. Bipedalism indeed adds a degree of complexity to many motor tasks. Postural constraints impeded by our upright stand are often stressed in the literature as a key factor causing our prolonged motor development (Pearson and Gordon, 2000; Adolph et al., 2003). Apart from differences in motor apparatuses, a fundamental factor when addressing species differences in motor developmental time is the relative level of maturity of the nervous system. For instance, with respect to neurogenesis, the newborn human baby corresponds to a rat that has completed its postnatal development. Yet, while the rat at this age exhibits the motor skills of an adult rat the human baby is relatively helpless (Altman and Sudarshan, 1975; Forssberg, 1985).

Clearly, a key issue in this context is the relationship between maturation and learning in the course of development, which has been the focus for a longstanding debate (Carew et al., 1998). The burning question has been, simply put, what is due to "pure" development (nature) and what is accounted for by learning induced by environmental influences (nurture) that in due course will result in an optimal behaviour for a specific animal.

Numerous studies have demonstrated the necessity of environmental influences in forming functional neural circuits (Wiesel, 1982; Katz and Shatz, 1996; Buonomano and Merzenich, 1998; Knudsen et al., 2000; DeBello and Knudsen, 2004). However, although much knowledge about experience dependent development of sensory neural systems has been gained, little is known about the role of experience dependent activity in shaping developing motor systems. It is clear though, that even relatively simple spinal sensorimotor circuits are functionally shaped by use dependent mechanisms in the course of postnatal development (Petersson et al., 2003).

For animals to accurately move in different surroundings their movements need to be properly calibrated. A major site for mechanisms underlying motor coordination and motor adaptability in vertebrates is the cerebellum (Ito, 1984; Thach et al., 1992; Manni and Petrosini, 1997; Wolpert and Flanagan, 2001; Doyon et al., 2002; Morton and Bastian, 2004; Yamamoto et al., 2004; Ito, 2005; Bastian, 2006). Sensory information of different modalities and from many sources is conveyed to the cerebellum, which integrates these various inputs so that appropriate motor outcome in turn is generated. Relatively simple motor behaviours such as eye movements and head orientation, compound (stereotyped) motor behaviours as locomotion and skilled motor acts like for example complex limb movements, are all under cerebellar control (Flanagan and Wing, 1997; Kawato et al., 2003; Thach and Bastian, 2004). Common to these motor behaviours is the requirement of adaptability in order to meet changes in body properties and environmental settings. The cerebellum is believed exert control over the flexibility/adaptability of these behaviours (Ito, 2006). Indeed, the function of the cerebellum as 'an adaptive control system' is proposed to be fundamental in various kinds of motor learning (Thach, 1998; Hesslow et al., 1999; Medina et al., 2000; Blazquez et al., 2004). Essential for a control system of motor adaptation and motor learning is to have a high degree of modifiability in its operational processes (Ito, 2006). A number of plasticity mechanisms have been described within the connectivity of cerebellar circuits (Hansel et al., 2001; Ito, 2001; Jorntell and Hansel, 2006). Some of these mechanisms are tightly associated with corresponding adjustments in motor behaviour (Boyden et al., 2004).

# FUNCTIONAL ORGANIZATION OF THE ADULT CEREBELLUM

Much of the knowledge regarding cerebellar plasticity mechanisms and cerebellar processing underlying motor adaptation and learning is based on the detailed understanding of the specific connectivity of the relatively few neuronal cell types in the cerebellar cortex. The local neural circuitry is repeated throughout the cerebellar cortex (Eccles, 1967) and it is therefore generally held that information processing performed by its neural circuitry is similar in principle in all parts of the cerebellum (Ito, 1984; Kawato and Gomi, 1992; Boyden et al., 2004). Importantly, however, different regions of the cerebellar cortex receive different kind of input and send projections to different targets. This regional specialization of function (Dichgans and Diener, 1985; Manni and Petrosini, 2004) of the mammalian cerebellum is evident as a compartmentalization of the cerebellar cortex into a number of zones, sagittally oriented across the cerebellar lobules (Oscarsson, 1980; Voogd and Glickstein, 1998). It is well documented, anatomically and electrophysiologically, that each sagittal zone receives climbing fibers from a distinct area in the inferior olive and projects Purkinje cell axons to a specific subdivision of the cerebellar or vestibular nuclei (Voogd and Bigaré, 1980). By way of its afferent and efferent specificity, each zone in turn influences specific motor tracts/systems, and hence is fundamental in the control of specific motor functions (Ito, 1984, 2006). Compartmentalization of the cerebellar cortex is also evident in the biochemical heterogeneity of Purkinje cells. Purkinje cells with similar patterns of gene and protein expression are organized into parasagittal zones, which closely correlate with the longitudinal zones of olivocerebellar projections (Gravel and Hawkes, 1990; Voogd and Ruigrok, 1997, 2004; Larouche and Hawkes, 2006).

In addition, the longitudinal zones are further partitioned into narrower zones – microzones, which are defined by the specific functional characteristics of their climbing fibre input (Andersson and Oscarsson, 1978; Ekerot et al., 1991a). In specific, all Purkinje cells within a microzone receive climbing fibres with similar receptive field properties (Apps and Garwicz, 2005). The microzonal organization has been studied extensively in the paravermal zones of the cat cerebellar anterior lobe by electrophysiological mapping of receptive fields (Apps and Garwicz, 2005).

The micro-circuitry consisting of a microzone and its associated olivo-cortico-nuclear circuitry is believed to be the operational unit of the cerebellum. The presence of microzones or microcomplexes has been

identified anatomically in the flocculus and nodulus (Ruigrok et al., 1992), and recently, an olivocorticonuclear module in the intermediate part of the cerebellar hemisphere was confirmed by a combination of zebrin immunohistochemistry and tracer techniques (Pijpers et al., 2005).

Although traditionally studies of cerebellar adaptation and learning have been carried out in adult animals, it is clear that normal cerebellar development is required for normal development of motor patterns and abilities (Ferguson, 1996; Chizhikov and Millen, 2003). Indeed, the seminal work by Altman and collaborators in rats has in different ways demonstrated a close parallelism between the development of cerebellar circuitry and the emergence of motor skills (Altman and Bayer, 1997).

## CEREBELLAR DEVELOPMENT

### Early cerebellar development

Cerebellar development occurs in distinct but interconnected steps. Briefly put they include the establishment of the cerebellar territory along the alar plate of the neural tube, the formation of two germinative matrices that together give rise to all neurons of the cerebellum, these cells' further proliferation, migration and differentiation, and finally their assembly into functional neuronal circuits (Hatten and Heintz, 1995; Chizhikov and Millen, 2003; Sotelo, 2004). The origin of the cerebellum has traditionally been thought to be from the anterior most rhombomere of the hindbrain /metencephalon, however by the introduction of experiments with chick/quail chimeras there was strong support for a cerebellar origin also from the caudal mesencephalon in addition to rostral metencephalon (Hallonet et al., 1990; Hallonet and Le Douarin, 1993). Although not crystal clear (Chizhikov and Millen, 2003), there seem to be an agreement of a dual origin today (Sotelo, 2004). Once the cerebellar territory is established cells begin to generate in the ventricular germinal matrix/ventricular zone, and the first to be generated are those that will become neurons of the deep cerebellar nuclei closely followed by cells predestined to become Purkinje cells. In the rat, neurogenesis of cells of cerebellar nuclei and Purkinje cells ceases at E15 and E16, respectively (neurogenesis of lateral hemispheres were not included) (Altman and Bayer, 1978) and in week 8 and 9 in human embryogenesis (Rakic and Sidman, 1970). Later this germinal matrix also gives rise to precursors of the interneurons basket and stellate cells and golgi cells that eventually settle in

the molecular and the granule layers of the cerebellar cortex (Sotelo, 2004). About a decade ago it was shown contrary to what was generally assumed at that time - that stellate, basket and golgi cell precursors divide again after they have left the ventricular zone of the metencephalic alar plates (Zhang and Goldman, 1996a). This division takes place within the cerebellar white matter during their migration to respective layers (Zhang and Goldman, 1996b).

Predestined Purkinje cells settle in specific clusters in the cerebellar anlage. The underlying mechanism whereby the Purkinje cell precursors are separated during their migration so that specific groups of settled Purkinje cells forms is not fully understood (Altman and Bayer, 1997; Hashimoto and Mikoshiba, 2003; Sotelo, 2004). This early, and seemingly 'predetermined', settlement of Purkinje cells into clusters is important in subsequent early stages in cerebellar development (Hatten and Heintz, 1995; Altman and Bayer, 1997). For instance, the early phases of fissuration and lobulation are believed to be regulated by the early clustering of Purkinje cells (Altman and Bayer, 1997). Furthermore, some of the mechanisms underlying early phases of cerebellar compartmentation are suggested to depend on the settling of Purkinje cells into clusters (Oberdick et al., 1998; Larouche and Hawkes, 2006).

Beside intrinsic cell mechanisms and trophic factors, the cerebellar afferents are important already from the beginning of Purkinje cell differentiation (Sotelo, 2004). Both mossy fibers and climbing fibers are present in the cerebellar anlage about the time of Purkinje cell neurogenesis and the olivocerebellar projection is formed during the period from E16 to P2 in rats (Altman and Bayer, 1997; Sotelo, 2004; Sugihara, 2006). This initial projection of olivary axons into the cerebellar anlage is ordered and the topographic pattern of their projection is roughly identical to that in the adult (Sotelo and Chedotal, 2005). By the end of the first postnatal week climbing fibres of single olivary axons terminate in narrow zones in the cerebellar cortex, comparable to microzones of the adult rat (Sugihara et al., 2001; Sugihara, 2005). Thus, suggesting that 'microzonal precision' is established early in development before the final contacts between Purkinje cells and climbing fibres have formed (Sugihara, 2005).

Whereas the formation of the first coarse map of afferent projections and Purkinje cells is believed to be activity-independent (Sotelo, 2004; Sotelo and Chedotal, 2005), the processes underlying the assembly into more or less functional neuronal circuitries are partly dependent on intrinsic and extrinsic neural activity (Wong and Ghosh, 2002; Sotelo, 2004). The probably best studied phenomenon in this respect is the

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regression of the multiple climbing fibre innervation of Purkinje cells (Kakizawa et al., 2000; Hashimoto and Kano, 2003; Scelfo and Strata, 2005). The remodelling and regression processes of multiple transient contacts between climbing fibres and Purkinje cells that result in the typical monoinnervation of Purkinje cells constitute a fundamental feature of the olivocerebellar development (Sugihara, 2006). In electrophysiological recordings functional synapses between climbing fibres and Purkinje cells appear at postnatal day 3 in the rat. The majority of Purkinje cells are multi innervated from P3 to P7 and thereafter there is a rapid decrease to P10 and at P17 all Purkinje cells are single innervated (Crepel et al., 1976; Crepel et al., 1981; Mariani and Changeux, 1981a; Sotelo, 2004). Parallel fibre-Purkinje cell synapses appear first at P7 in rat and the subsequent development of the parallel fibre-Purkinje cell synapses is highly correlated with the time course of the multiple climbing fibre regression. If the parallel fibres are deleted, degenerated or impaired in their function the multiple climbing fibre innervation of Purkinje cells persists (Sotelo, 2004; Lohof et al., 2005).

## EGL and cerebellar development and growth

Although differentiation and further major maturational steps in terms of synaptogenesis and dendritogenesis continue after birth, in principal all proliferation of neuroblasts from the ventricular zone occurs embryonically in mice and rats (Hatten and Heintz, 1995; Goldowitz and Hamre, 1998; Sotelo, 2004). By contrast, the proliferation of granule cell precursors continues postnatally, and in humans this proliferation persists for the first year of life (Rakic and Sidman, 1970).

Precursors of granule cells arise in a region adjacent to the ventricular zone called the rhombic lip. They migrate to the cerebellar anlage and by covering its surface the granule cell precursors form the external granular layer (EGL). What happens next is peculiar for the cerebellum; the EGL constitutes a second germinative matrix and within its outer part granule cell progenitors proliferate extensively and the layer will not only cover the surface of the cerebellar anlage but also increase in thickness during postnatal life (Altman, 1972; Goldowitz and Hamre, 1998; Sotelo, 2004). Initially, the increase in width is due to the outer part of the EGL; the proliferative zone (Altman and Bayer, 1997). Across time however, the postmitotic cells that gather in the inner EGL zone make up an increasingly larger part of the layer (Altman and Bayer, 1997). The inner postmitotic

cells begin to differentiate to granule cells (Sotelo, 2004). As the differentiation process carries on they migrate inward along radial glia/Bergmann glia fiber (additional migration mechanisms are also suggested) to settle under the Purkinje cells, and thus 'building' the granular layer (Altman and Bayer, 1997; Sotelo, 2004).

As mentioned above, this is a lengthy process – in the human cerebellum EGL persists throughout the first year (sometimes longer) although it appears already around day 80 postconception (Rakic and Sidman, 1970; Clancy et al., 2001). Similarly, the proliferation of granule progenitors begins as early as E19.5 in rat whereas EGL disappears by P21 (Altman and Bayer, 1997). This long lasting process of proliferation and subsequent differentiation of granule cells - the largest neuronal population of the CNS - is to a substantial part responsible for the massive postnatal growth of the cerebellum (Altman and Bayer, 1997). The generation of the proper number of granule cells is strictly regulated by predetermined programs for proliferation, cell cycle withdrawal and apoptosis (Goldowitz and Hamre, 1998). Several genes that are important for this regulation have been identified (Goldowitz and Hamre, 1998; Sotelo, 2004). Since the EGL strongly influences overall cerebellar development and growth processes, substantial efforts have been made to gain insight into the mechanisms that regulate the cell division in the superficial EGL zone. It is clear that Purkinje cells are required for the proliferation of granule cell progenitors (Altman and Bayer, 1997; Goldowitz and Hamre, 1998; Chizhikov and Millen, 2003) and mutant analysis (Sonmez and Herrup, 1984; Mullen et al., 1997) and ablation studies (Smeyne et al., 1995) have indicated that Purkinje cells stimulate mitosis in the EGL.

## ROLE OF THE CEREBELLUM IN MOTOR DEVELOPMENT

The knowledge about the role of the cerebellum in motor development comes from investigations using models of impaired cerebellar development (Ferguson, 1996). Studies investigating the effects of cerebellar lesions in the rat report impaired motor performance in various motor test during development (Gramsbergen, 1982; Auvray et al., 1989; Molinari et al., 1990; Petrosini et al., 1990; Zion et al., 1990; Gramsbergen, 1993; Jones et al., 1995). Focal X-ray irradiation of the cerebellum during development constitutes the other commonly used experimental paradigm beside surgical

ablations. X-ray irradiation causes a massive (and selective) reduction of granule cells and results in impaired or delayed acquisition of motor abilities during development (Wallace and Altman, 1970; Altman et al., 1971; Anderson and Altman, 1972; Pellegrino and Altman, 1979; Le Marec et al., 1997; for summary see Ferguson, 1996). Several studies report motor behavioural deficits in mice with natural and induced mutations that result in damaged cerebellar development, however only few assess motor behaviour during development (Thullier et al., 1997; Caston et al., 1998; Krizkova and Vozeh, 2004).

Although it is clear that the cerebellum is important for normal motor development, little is known about *how* the cerebellum contributes to the advancement in motor competence in the course of development (see however; (Crepel, 1971; Puro and Woodward, 1977; Mariani and Changeux, 1981b). To gain a deeper understanding of what role the cerebellum plays (and how it does it) in the course of motor development it seems necessary to functionally characterize the physiological changes in cerebellar connectivity at a systems level. The detailed physiological characterization of the paravermal system of the adult cerebellum and its functional connectivity with the spinal cord (Ekerot et al., 1991b; Garwicz et al., 2002; Apps and Garwicz, 2005) provides a unique frame of reference for a systematic exploration of functional organizational modifications in the cerebellum during development. Developing a systems level physiological animal model of the normal development of the cerebellum is a prerequisite for these types of investigations. Recent studies on the nociceptive withdrawal reflex system of the spinal cord have demonstrated the explanatory potential of such an approach (Petersson et al., 2003; Waldenstrom et al., 2003). Knowledge on processes taking place in the developing cerebellum may also further the understanding of information processing in the adult cerebellum (Mauk, 1998).

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# AIMS

The overall aim was to gain a deeper understanding of the relationships between cerebellar ontogenetic changes and postnatal motor development. Two parallel approaches were employed. The first was to develop an experimental model suitable for combined motor behavioural, structural and *in vivo* physiological investigations of the developing cerebellum. In a parallel approach, relationships between motor development and cerebellar morphological development were sought by a cross-species comparative assessment of their dependence on developmental time. The overall aim was broken down into the six specific aims addressed in this thesis.

1. to quantitatively characterize the postnatal motor development of the ferret (I)

2. to quantitatively characterize the ontogenesis of habituation of locomotor activity in the open field (II)

3. to quantitatively characterize the morphological development of the ferret cerebellum (III)

4. to quantitatively compare time courses of motor and cerebellar morphological development in the ferret and rat (I, III)

5. to characterize the functional organization of the climbing fibre input to the developing ferret cerebellum (IV)

6. to formulate and test a model for translation of motor developmental timescales between different mammals (V)

# METHODS

## Animals

Purpose bred ferret kits of mixed sex were used in all experiments. In paper IV two adult female ferrets were used. In all studies, P1 was defined as the day of birth (margin of error 12 h). Ferrets were kept at a 12:12 h (7 a.m.–7 p.m.) light–dark cycle. Room temperature and relative humidity were 18–19 and 55%, respectively. Food and water were provided ad libitum. Kits were kept with their mother until the end of postnatal week 8, but started eating some solid food already at the end of postnatal week 4. Experiments were carried out in accordance with the European Communities Council Directive November 24, 1986. Procedures were reviewed and approved by the Malmö/Lund Ethics Committee for Animal Experimentation and the District Court of Lund.

## Motor behavioural assessments (I, II)

*General test procedures* - Testing was carried out in a room adjacent to the housing facility and limited to one session per day, usually at some point between 1 and 5 p.m. During sessions, pups were away from their mother for a maximum of two hours (animals older than P40 sometimes up to 2.5 hours). Pups were weighed on a more or less daily basis. Their body temperature was measured with an infrared non-contact probe (Thermonitor C-1600 M; Linear Laboratories, Los Altos, CA) three times per session (including at the beginning and end of the open field observation) up to P21 and then once per session up to P42. Thermo-neutrality between animals and environment was strived for to minimize potential thermal stress for young animals during sessions. The floor of the box in which animals were kept between tests was warmed (first two postnatal weeks) as was the floor of the open field (until first indications of crawling in the fourth postnatal week). All animals were id-marked with a felt-tipped pen and test performance was documented individually. Number of tests per daily session varied between 1 and 5 (but usually not more than 3). When assessed, open field behaviour was first in order. In tests with more than one trial, pups were always tested in a sequence to yield a break between subsequent trials for individual pups. Animal behaviour during tests was recorded on digital video (JVC GR-DVL 9800E; 680.000 pixels, 25 images/s or high-speed mode: 100 images/s) for

off-line analysis. Frame-by-frame or slow motion was used when applicable. Some qualitative observations and manual time-measurements were made on-line.

*Open field* - This 'test' was used chiefly for the face value assessment of locomotor activity. Animals were placed individually at the centre of a 75x75 cm plywood surface divided into 5x5 cm squares for scoring and bordered by 1 epoxy (for the sake of filming) and 3 plywood walls (25 cm high up to P45, then 45 cm). Observation time was seven minutes. Illumination consisted of standard fluorescent room lighting supplemented by a lamp placed centrally over the open field. Two video cameras were used, one straight above the open field and one from the side (ca. 30° relative to surface). Locomotor activity was analysed off-line. To quantify length of path covered, the trail of the animal was drawn on a video projection of the open field. The number of squares traversed by all limbs was counted on a minute-by-minute basis. For the analysis of habituation, within-session effects were assessed by a comparison of activity in minutes 1, 3 and 5 of the observation time (paper II).

*Narrow path / beam walking* - The set-up consisted of a start box (22 cm long, 18 cm wide, 15 cm high) connected to a goal box of the same size by a horizontal narrow plywood path with adjustable length (max 90 cm) elevated 60 cm above a cushioned surface. The width of the path was 5 cm. Performance was quantified by counting the number of 5 cm marks that pups crossed. Each animal was given five to seven trials per daily session. Immediately before sessions including the narrow path test food in the animals' pen was withdrawn for ca. three hours.

*Contact righting / righting on a surface* - Animals were held between thumb and index finger at the shoulder and pelvis, belly-up and back in contact with the underlying surface. Upon release they turned to a more or less prone position following a brief latency. Latency to criterion – both forepaw palms on surface – was measured manually with a standard sports stopwatch. For one litter, the corresponding latency was measured also for the hind limb plantae (cut-off time 20 sec; trials in which no hind paw placement was achieved were assigned this value). Each animal was given five trials per session.

*Air righting / righting mid air* - Animals were held between thumb and index finger at the shoulder and pelvis, belly-up, and then dropped from a

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height of 60 cm onto a cushioned surface. Each animal was given five trials per session. The animals' success in turning around in the air to land in the prone position was scored according to the following landing criteria. 0: back; 2: back/side; 3: side/prone; 4: prone.

*Righting on an inclination / geotaxis* - Typically, an animal that is placed belly-down with its head directed down the slope on a high-friction surface rotates to direct its head up the slope. Three inclinations were used: 15°, 30° and 40°. Each animal was tested in three trials per angle per session.
We quantified the number of trials ending in falling off or sliding down the inclined plane and measured the time of rotation from a position with head facing down the plane to a position with head facing up the plane. Cut-off time in all trials was 30 sec.

## Quantitative characterization of cerebellar morphology (III)

*Tissue preparation* - For the histological and morphological assessments of ferret cerebellar development, two sets of cerebellar tissue were prepared; as 40µm and 1.5µm thick sections. Ferrets were given a terminal dose of pentobarbital (60 mg/kg i.p). When deeply anaesthesized, they were fixed by rapid transcardial perfusion fixation. Perfusion fixation was performed by briefly rinsing with PBS preceding administration of fixative (PBS with paraformaldehyde; for the set prepared for semithin sectioning, glutaraldehyde was added) for ca. 20 minutes. Cerebella (for 40µm sections) were cryoprotected in 30% sucrose for 1-2 days and then sectioned (serial parasagittal 40µm sections) on a freezing microtome. Every third section was mounted on slides, stained with thionin and cover-slipped with DPX (BDH, Poole, England). Cerebella prepared for semithin sectioning were cut parasagitally at 200µm on a Vibratome. The wet sections were photographed. The mid-sagittal Vibratome sections were further postfixed in 1% osmium tetroxid, dehydrated in a graded series of ethanol, and embedded in Durcupan (Fluka, Switzerland). The embedded sections were trimmed to include lobulus V. Semithin sections were cut, mounted, and stained with toluidine blue and finally, coverslipped with DPX.

*Measurements and data analysis* - Five parameters of gross morphological development of the cerebellum were assessed. Whole cerebella measurements included the distance across the widest parts of the

hemispheres (laterolateral width) and cerebella weight. The remaining three parameters were measured in midsagittal sections stained with thionin (40µm) or toluidine blue (1.5µm); rostrocaudal length, dorsoventral height and mid-sagittal area. Mid-sagittal area was measuered using NIH Image (http://rsb.info.nih.gov/nih-image/).

The identification of the cardinal lobes and cardinal fissures in the ferret were based on comparisons with the developing rat cerebellum (Altman and Bayer, 1997). The identification of the lobules of Larsell was facilitated by comparisons with the adult and developing dog and cat cerebella (Larsell, 1970) but to some extent also with the developing cerebellum of the rat (Larsell, 1952; Altman and Bayer, 1997). In the quantitative analysis of folia, a folium was defined by its delineating grooves in the cerebellar cortex that were visible in the surface contour limit of the mid-sagittal section.

Thickness measurements of the external granular layer (EGL), the Purkinje cell layer, the molecular layer and the (inner) granular layer were obtained from 40µm and semithin mid-sagittal sections of lobule V. To probe for regional variations in EGL across developmental time, EGL thickness in lobules VI (central lobe; not shown) and IX (posterior lobe) was measured in addition to lobule V (anterodorsal lobe). Thickness of the different cortical layers was consistently assessed at sites with minimal cortical curvature, midway between summit and base of a folium.

Assessments of qualitative features of cortical layers and Purkinje cells were based on observations in semithin mid-sagittal sections of lobule V. The photomicrographs were obtained using a digital microscope camera (Polaroid, DMC 1, USA). The pictures in Figs 1, 3 and 6 in paper III were moderately processed with 'levels', 'brightness-contrast' and 'unsharp mask' functions in Adobe Photoshop to facilitate observations.

### Comparative analyses (I, III, V)

*Ferret rat comparisons* - Comparative analyses were carried out for motor behaviour and cerebellar development. Time points defining the corresponding levels of motor performance in different tests and cerebellar maturation in ferrets and rats were quantitatively compared (rat data from (Altman and Bayer, 1997). Linear regression analyses were performed on all data points. The equations derived that characterize the conversions between time courses of rat and ferret motor development and between time courses of rat and ferret cerebellar development were subsequently

used for the relative scaling of time-axes of the curves in Fig. 5B-G, paper I and Fig. 8B-E, paper III, respectively, to facilitate comparisons between detailed time courses of the specific motor abilities and individual cerebellar structural parameters in the two species.

*Time conversion model (paper V)* - Based on the findings from the linear regression analyses of ferret and rat motor and cerebellar development we formulated a general model for predicting developmental events across species. The mathematical model was constructed based on quantitative comparative developmental data. The model equation describes the translation of motor developmental time between two given mammals as a linear equation of the general type $y = kx - p$, where k is the rate constant determined by the relative rate of cerebellar morphological development and p is an offset represented by the difference in the timing of birth. We defined the relative rate of cerebellar morphological development (k) for the given pair of species by calculating the relative duration of the presence of the EGL. A general previously published model of comparative neurogenesis was used to determine time of EGL onset (Clancy et al., 2001) and species specific comparative data for rats (Altman and Bayer, 1997), ferrets (paper III) and humans (Abraham et al., 2001) were used to determine time of EGL termination. Differences in the relative timing of birth in relation to the maturity of the nervous system (p) were derived from the above mentioned comparative model of neurogenesis (Clancy et al., 2001). All figures from which k was calculated are listed in Table 1 together with behavioural data relevant to Fig. 3 in paper V. The full version of the equation is shown under Table 1; paper V.

## Surgical procedures (IV)

In preparation for electrophysiological investigation ferret kits were anaesthetized with sodium pentobarbital, 25-30 mg/kg i.p., and artificially ventilated using a mixture of air 70% and oxygen 30% via a tracheal cannula. The end-expiratory $CO_2$ concentration (4%) was monitored continuously. Cannulae were inserted into the right femoral artery and mean arterial blood pressure (70-120mmHg) was monitored continuously. A continuous infusion of 5% glucose in Ringer acetate was given into the right femoral vein at a rate of 0.6 ml/hr. Blood gases were routinely measured to further check the general condition of the preparation. Rectal temperature was monitored throughout the experiment and kept between

37-38.5 °C using a feedback regulated heating system. The head of the ferret was placed in an adjustable stereotaxic frame and a hole in the dura mater of the caudal brainstem was made in order to provide drainage of cerebrospinal fluid and thereby increase the mechanical stability of the cerebellum. The left cerebellar anterior lobe was subsequently exposed following craniotomy, resection of the occipital lobe and removal of the tentorial dura mater. An agar pool was made to keep the cerebellar surface covered with warm mineral oil. To obtain stable recording conditions, the animals were paralyzed with pancuronium and a bilateral pneumothorax was made. The muscle relaxant was allowed to wear off at regular intervals during the experiment to control for maintained surgical level of anaesthesia, which is characterized by general muscle atonia, completely depressed withdrawal reflexes and a stable blood pressure, also during noxious mechanical stimulation of the skin.

## Electrophysiology (IV)

For the electrophysiological analysis, three pairs of thin stimulation needles were superficially inserted into the left (ipsilateral to the cerebellar exposure) forelimb skin corresponding to the innervation areas of the ulnar and superficial radial nerves, and the hindlimb skin innervated by the sciatic nerve in the ferret. Field potentials evoked on electrical stimulation of these sites were recorded at the cerebellar surface, using a ball-tipped electrode (tip diameter ca. 0.2 mm). Climbing fibre responses were seen as sharply deflecting potentials with a biphasic positive-negative configuration, often preceded by positive potentials reflecting mossy fibre input. At the beginning of each experiment, the cerebellar surface was scanned for input from all three stimulation sites for purposes of identification of sagittal zones, with the recording electrode typically being moved in 0.2 mm steps from medial to lateral along the cerebellar folia of lobule V. The stimulus threshold (T) for evoking a detectable climbing fibre response at the locus with the largest potentials was determined for each stimulation site separately. For data collection, the stimulus intensity was 10xT and field potentials evoked on ten (sometimes 20) consecutive stimuli presented at a rate of 0.5 Hz were sampled for off-line analysis. When lower rates were tested, the results did not differ from those reported here. Recording locations were indicated on a photograph of the cerebellar surface. Input from each peripheral stimulation site was routinely re-assessed at regular intervals by monitoring a few reference recording locations.

At the end of the experiment, the animals were killed by an overdose of sodium pentobarbital and subsequently perfused with 4% depolymerized paraformaldehyde in PBS. The cerebellum was removed for verification of recording locations with respect to the cerebellar lobules.

Data were analysed off-line using tailor-made analysis software (software by Henrik Jörntell). Response latencies were measured from onset of the stimulus artefact to onset of the evoked climbing fibre field potential. Response size was defined as the amplitude from the baseline at onset to the positive peak of the potential. The onset of the climbing fibre potential was usually readily identifiable due to the fast rise time of the potential.

# RESULTS AND COMMENTS

Although with the ultimate goal to understand the potential role of cerebellar processes in postnatal motor learning in the back of our minds, the focus of the first study was to carry out a detailed characterization of *general* motor development in the postnatal ferret. As a parallel aim we wanted to evaluate how timescales of motor development can be translated between species with differently long developmental periods, such as the ferret and the rat (paper I).

The ferret emerged as a suitable candidate for these combined studies since the adult ferret cerebellum is strikingly similar to that of the cat with regard to physiological organization of cerebellar sagittal zones (Garwicz, 1997), and because ferret kits appeared to be born rather immature and develop relatively slowly (Clancy et al., 2001).

The advantages of the ferret's protracted development have been acknowledged in studies on the visual system for some time (Linden et al., 1981; Jackson and Hickey, 1985) and during the last decade it has become increasingly more common as an experimental animal in the field of neuroscience, specifically so in studies focusing on the development of the sensory cortices and subcortical structures (Chapman and Stryker, 1993; King and Carlile, 1993; Chapman et al., 1996; Noctor et al., 1997; Noctor et al., 2001); (King et al., 1998; Issa et al., 1999; Gao et al., 2000; Chiu and Weliky, 2001; Sengpiel and Kind, 2002; Majewska and Sur, 2006); (Li et al., 2006). It has been pointed out previously that the ferret is well suited for behavioural studies (Rabe et al., 1985). Until now, however, the motor development of the ferret had not been investigated nor was anything known about the postnatal development of its motor structures, including the cerebellum (paper I, III).

With regard to the comparative approach, the "ferret-rat pair" was particularly interesting since both species are altricial but phylogenetically distant being a carnivore and rodent, respectively. Importantly, and of course facilitating the comparison between developmental timescales, the two species considered here are born at similar levels of neurogenetic maturity (Clancy et al., 2001).

## Motor development in the ferret

Postnatal motor development has been extensively studied in the rat, and motor behavioural tests in the present study were selected from a battery used in studies by Altman and co-workers of rat development (summarised in (Altman and Bayer, 1997) to facilitate the comparison of motor developmental time between the rat and the ferret. Motor tests were chosen so as to be indicative of the emergence and maturation of motor behaviours occurring at distinct points in, and covering as much as possible of the whole span of, postnatal development. Detailed accounts of the ferret postnatal motor development and novel findings on test specific aspects are given in papers I and II.

The battery of motor tests included assessments of locomotor activities and dynamic postural reflexes. Two litters of ferret kits were followed longitudinally on a more or less daily basis from postnatal day (P) 3 to P55 for Litter 1 and P2 to P63 for Litter 2. In addition, animals that never had been exposed to the experimental set up or handled before were tested at unique occasions only. This group served as control for day-to-day adaptation and learning that might influence behaviour and test performance between sessions in the longitudinal groups. Data from this control group were similar to data from the longitudinal group, suggesting that between-session effects in the latter were negligible.

Locomotor activity was analysed qualitatively, with respect to movement pattern - pivoting, crawling and walking, and quantitatively, with respect to the length of the path covered (or time spent in a certain activity) in the open field. Ferret kits displayed pivoting as newborn and although the time spent pivoting in the open field subsided to close to zero after postnatal week 2, the behaviour persisted in the movement repertoire until the end of postnatal week 5 (Fig. 2A; paper I). In addition to the rotational movement, newborn ferrets displayed 'neonatal crawling', akin to the swimming like behaviour displayed by newborn mammals (Geisler et al., 1993; Clarac et al., 2004). Neonatal crawling was not observed after P4 in ferret. Linear locomotion in the open field was first observed again at the end of postnatal week 4 (Fig. 2B). However, from P22 and onwards, pups systematically displayed crawling in their home cage. During postnatal week 5, there was a transition between crawling and walking and subsequently length of path covered in the open field increased dramatically, reaching a peak during postnatal week 6 (Fig. 2B). In the following weeks, an increase in overall walking speed was clearly observed.

As a direct measure of skilled locomotion we assessed the ferret kits' ability to locomote on an elevated wooden path. Performance was analysed qualitatively, with respect to movement pattern, and quantitatively, with respect to the length of the path covered. Despite a distinct improvement in length covered on the path at the beginning of postnatal week 8, performance was variable and the overall impression was not one of skilled locomotion (Fig. 2D). However, it is possible that the width of the path may have been insufficient, especially with respect to the hind limbs, which are set quite widely apart in the ferret. The rotorod test is likely to be a better indicator of the development of skilled locomotion in ferrets (Gramsbergen, 1993; Chapillon et al., 1998).

In addition to observations in the open field and narrow path walking, development of general postural abilities were investigated in three individual tests. In the contact righting test kits were held belly up on a plywood surface and their ability upon release to turn around to an upright position was assessed by measuring the latencies to criterion, which was placement of forepaws on surface and hindpaws on surface. Kits were able to turn from belly-up to belly-down from P2, although during postnatal weeks 1–2 this did not involve active movement of the hind part of the body (Fig. 3A). Righting gradually became faster, and active positioning of both hind paws occurred around P26 (Fig. 3B). Performance then improved with an exponential decrease in time to criterion, which equalled time to criterion of the forepaws by P43 (Fig. 3A, B). In the air righting test animals were dropped, belly up, from a height of 60 cm above a cushioned surface, and the success in turning around in the air to land upright on the surface was quantified. Kits landed straight on their backs in virtually all trials whenever tested during the first 4 postnatal weeks (Fig. 3C). Systematic righting movements were seen first at ca. P32 and maximum performance score was reached at ca. P42 (Fig. 3C). In the test of geotactic behaviour, i.e. righting on an inclination, ferrets were placed head down on a tilted plane with a high-friction surface. The ability to turn around was assessed on three inclinations; 15°, 30° and 40° relative to the floor and time latency to test criterion – head directed upwards and forelimbs placed horizontally – was measured. After P43 there was a clear improvement in kits' overall capability to righting on an inclination (Fig. 4F). For detailed analysis of performance in this test see paper I pages 236-238.

## Habituation of locomotor activity

When an animal is placed in an open field, it will engage in activities that together constitute exploratory behaviour (Cerbone and Sadile, 1994; Wright et al., 2004). The level of activity, which is determined by a number of factors, will normally subside with time during the same exposure if the environment does not change. This decrease in activity is commonly defined as within-session habituation (Lat, 1973; Altman and Bayer, 1997).

   Habituation means not to respond to biologically non-significant stimuli and constitutes the simplest form of nonassociative learning. Although habituation is a type of learning considered to be fundamental to a wide range of abilities and potentially defective in certain neurodevelopmental psychiatric disorders (Lipska and Weinberger, 2000) little is known about its ontogenesis, and findings thereof have been somewhat contradictionary (Einon et al., 1975; Pellegrino and Altman, 1979; Shaywitz et al., 1979; Laviola et al., 1988; Ba and Seri, 1995). We therefore systematically characterized developmental changes in activity profiles within individual open field sessions (paper II). Developing ferrets' ability to habituate to the open field environment was assessed by comparing activity in minutes 1, 3 and 5 of within-session observation time from P1 to P62. Our findings showed that a systematic shift between increment and decrement of within-session activity occurred in the course of development (Figs. 1B and 2; paper II). The increment-to-decrement turning point was around postnatal day 48 (Figs. 2 and 3A). The changes from increment to decrement of within session activity profiles across developmental time were robust both within and between the litters and control animals (control animals were tested at unique time points; Figs. 1-3; paper II).

*In sum,* postnatal weeks 6, 7 and 8 seem to be especially dynamic with regard to development of key motor abilities in the postnatal ferret (paper I). Extending previous knowledge on open field habituation during ontogenesis, time profiles of locomotor activity changed systematically across developmental time: within-session increment switched to decrement around P48 (paper II). The high reproducibility of data despite the relatively small number of animals used was notable (papers I, II). One factor that may contribute to the striking similarities between individual animals is that the gestation period of ferrets is less variable than that of many other species, such as cats and rats.

## Conversion of motor developmental time between ferret and rat

Whereas ferrets crawled at P22, walked at P35 and managed air righting at P42 (paper I) rats display the corresponding level of motor ability at P10, P16 and P18, respectively (Altman and Bayer, 1997). Hence, the differences in postnatal motor development time were obvious. In order to quantitatively compare time courses of motor development between the ferret and the rat we performed a linear regression analysis on motor developmental time points representing corresponding levels of performance in the two species The equation derived, characterizing the conversion between time courses of rat and ferret motor development, was $y = 2.46x - 4.18$ (y and x: postnatal days of ferret and rat). Thus, the sequence and relative timing of various milestones of motor development in ferrets and rats were remarkably congruent. It was interesting to compare our findings to a recently published model for cross-species developmental time based on neurogenesis and axonal growth (Clancy et al., 2001). Although the bulk of experimental data for the ferret was on visual and cortical systems, there are strong similarities between the two studies with regard to relative developmental rates. The model suggests that the postnatal ferret develops 2.33 times slower than the rat, as compared to our coefficient of 2.46.

*To conclude*, the use of similar experimental set-up and analysis made it possible for us to assess and quantitatively express for the first time the relation between motor developmental time of the rat and that of another animal (Altman and Bayer, 1997). The results were striking by the congruence in relative timing of motor development in the two species with differently long postnatal development. In addition, a calibration between the ferret and rat developmental time scales makes it possible to use the vast reference material on motor development in the rat.

## Morphological development of the ferret cerebellum

As pointed out earlier, the use of the ferret as an experimental model of developing sensory systems has increased. However, the development of its motor structures has not been characterized before. Following our characterization of the ferret's motor development we continued the work of establishing a model for combined behavioural, structural and physiological investigations of the developing cerebellum by characterizing the morphological development of the ferret cerebellum (paper III).

Additionally, we wanted to extend the comparative analysis of how developmental time translates between species to also encompass time courses of structural development. The cerebellum, which is highly conserved in phylogenesis and develops late in neuro-ontogeny and has a simple architecture (Crosby and Lauer, 1967; Eccles, 1967; Hatten and Heintz, 1995; Altman and Bayer, 1997; Voogd and Glickstein, 1998; Sillitoe et al., 2005) was considered an appropriate model motor structure.

We quantified various measures of cerebellar morphogenesis in a total of 43 ferrets ranging in age from postnatal day (P) 1 to P63 and together covering 24 time-points in postnatal development (paper III). For a complete account of results see paper III. To assess gross morphological development we measured cerebellar laterolateral width and weight in whole specimens and dorsoventral height and rostrocaudal length as well as midsagittal area in vermal midsagittal sections. In essence, length and height increased at constant rates from birth on to ca P35, when growth rates decreased noticeably for both parameters (Fig. 2C, D; paper III). Cerebellar width (Fig. 2B), by contrast, was rather constant during the first two postnatal weeks, followed (at ca. P12) by a shift to high growth rate, which was then noticeably reduced around P35. Similarly timed shifts were seen also with regard to cerebellar weight (Fig. 2A), but with quadrupling of growth rate at the transition from the initial low rate to the high rate phase. By the end of the developmental period included in our study i.e. postnatal week 9, these measures of cerebellar size had by and large reached adult values.

We further quantified foliation in midsagittal vermal sections. The foliated surface is a distinct feature of the avian and mammalian cerebellum. Deeper fissures group the cerebellar folia into lobules. During the first three postnatal weeks the number of folia increased with about ca. 2 folia per day and the transformation of the ferret cerebellum into a foliated structure was more or less complete by the end of week three (Fig. 5A; paper III).

The anatomical pattern of cerebella folia has been characterized in various species (Larsell, 1970; Voogd and Glickstein, 1998). Lobules of the adult ferret cerebellum have been outlined (Voogd, 1969) according to Larsell's general classification of cerebellar lobules. In principle, the definition by Larsell means that the hemispheric lobules are extensions of the ten (I-X) vermal lobules (Voogd and Glickstein, 1998). The resulting configuration of lobules I-X in the present study was compatible with that previously presented schematically for the adult ferret cerebellum (Voogd, 1969). In our characterization of lobules in developing ferret cerebella we

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were aided by Larsell's description of the adult and developing cat and dog cerebella (Larsell, 1970). Altman and collaborators have made a comprehensive description of the developing rat cerebellum's division into lobes and lobules (Altman and Bayer, 1997), and for the identification of cardinal lobes and cardinal fissures in the ferret we were guided by comparisons with the developing rat cerebellum (Larsell, 1952; Altman and Bayer, 1997). The rat cerebellum, however has fewer folia, and thus made direct comparison of lobules' foliation pattern across developmental time less straightforward. In addition to comparison with developing dog and cat cerebella we defined the boundaries between lobules in terms of the branching of medullary fibre rays in combination with the formation of fissures evident at the cortical surface. We used the cardinal fissures and lobes evident at P1 in mid-sagittal sections of the ferret cerebellum as a starting point, and followed the lobes' subsequent partitioning into lobules (and their further foliation) between consecutive ages throughout the postnatal period (Figs. 3 and 4; paper III). Conversely, beginning with adult cerebella we tracked the formation of lobules backwards from the mature to the early stages. In the newborn ferret the four cardinal fissures - f1 (preculminata), f2 (prima), f3 (secunda) and f4 (posterolateralis)- were present and defined the boundaries between the five cardinal lobes (Fig. 3A). Segregation of these lobes into lobules had begun before birth, and at P1 all ten Larsell lobules were present, and subdivision of lobules I, IV and V had begun. Compared to the degree of variation between for instance individual cat cerebella (Larsell, 1970), there appeared to be rather small variations between individual ferret cerebella with regard to the configuration of lobules and sublobules. Nonetheless, when present, variants could be identified early on during development (cf Fig. 4 and page 919; paper III).

As yet it is not known how the foliation of the mammalian cerebellum is regulated in development. Different processes have been suggested to have important roles in cerebellar foliation yet although the mechanisms as such is debated, it is in general agreement that the genesis of cerebellar granule cells influences the formation of lobules and fissures (Altman and Bayer, 1997; Doughty et al., 1998; Goldowitz and Hamre, 1998). In the developing cerebellum of the rat, regional variations in the timing of foliation parallel regional variations in the postnatal time course of external granular layer (EGL) formation (Altman and Bayer, 1997; Goldowitz and Hamre, 1998). We wanted to know if similar relationship between time courses of foliation and EGL formation were evident in the developing ferret cerebellum. When pooling data into weekly bins the relative time

courses for foliation of lobules V, VI and IX (Fig. 5C-E; paper III) were compatible with the corresponding differences between these lobules with regard to time courses of EGL thickness (Fig. 7A; paper III). Accordingly, lobules VI and IX reached their final number of folia earlier (postnatal week 3) than lobule V (postnatal week 4), which indicate that an earlier relative increase in EGL thickness causes an earlier and faster foliation in lobules VI and IX of the central and posterior lobes, respectively.

We quantified widths of the cortical layers in midsagittal vermal sections. 'Qualitative milestones' of cerebellar cortical and Purkinje cell development were assessed in semi-thin sections stained with toluidine blue. Changes in laminar organization of the cerebellar cortex in lobule V are summarized below.

At P1 the cerebellar cortex of the ferret was essentially made up of the *external granular layer,* EGL that consisted of the outer zone of proliferating granule cells. During the first postnatal week, the EGL (in lobule V) thickened steadily. By P9, the inner zone of differentiating granule cells was clearly identifiable. EGL reached its maximal thickness between P18 and P25. (Note that in paper III, Fig. 6A, B lobule VI is depicted. In Fig. 7A, EGL thickness curve for lobule IX and V are shown). By P28, the depth of the outer proliferative zone had declined and at P35, the inner differentiating zone made a relatively larger contribution to the total width of the layer. By P43, the layer had more or less disappeared, except for some small cells visible at the very surface. At P56 no EGL could be seen (Figs. 6A and 7A).

At P14 the *molecular layer* was distinct. Horizontally oriented cells, probably early differentiating basket cells, were relatively common in the layer (Fig. 6A, B). The molecular layer expanded with a relatively constant growth rate of ca 5µm per day from the mid-third week (Fig. 7B). The most striking change in the laminar organization after the fourth week was the expansion of the molecular layer that measured ca. 110µm in thickness at P28 (Fig. 7B). At P56 the layer had reached a width of about 200µm.

By P1 the *Purkinje cells* typically had hypertrophied apical cytoplasm and formed a multilayer (Fig. 6). The multilayer decreased in thickness until the Purkinje cells were completely aligned into a monolayer by P14. At this age the onset of dendritic development was more obvious with delineated threadlike processes radiating in various directions from the apical cap of each Purkinje cell. Major changes in the shape of the Purkinje cells and in dendritic growth had taken place by P22. The thin processes that spanned from various sites at the apical swelling at P14 had disappeared and the apical cap had become transformed into one stem

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dendrite that gave off extensive secondary branches that in turn gave off dendrite branchlets extending primarily in the lateral dimension. At P35, the dendrites of the Purkinje cells reached far in the vertical direction towards the EGL and at P56 the extensive dendritic trees of the Purkinje cells traversed the whole width of the molecular layer (Fig. 6A).

At P14, migrating granule cells were seen both above and beneath the Purkinje cells. At P22 the *granular layer* was a distinct entity measuring ca 100µm in thickness between the Purkinje cell layer and the newly formed medullary layer (Figs. 6A and 7D). The layer had increased a lot during the fourth week measuring to ca. 140µm at P28 (Fig. 7D). Thereafter there was a slower increase in thickness, but the packing density of granule cells became notably higher.

*To conclude*, the external granular layer (EGL), which in addition to generating cerebellar granule cells also influences the morphological development of the cerebellum as a whole, was present during seven to eight weeks in ferrets. The thickness of the cerebellar cortical molecular layer developed in strict proportion to the thickness of the EGL (see paper III, Figs. 7A, B, E, F and in paper V, Fig. 1B).

## Comparative timescales of cerebellar morphological development

From the literature it is clear in general terms that the cerebellum in mammals develops through a largely stereotyped sequence of events. However, as yet there has been no study quantitatively addressing the time course of cerebellar morphogenesis between species or any explicit analysis of it either. We compared the time course of the ferret cerebellar morphological development (paper III) with that of the rat (Altman and Bayer, 1997). The cerebellar structural development was markedly slower in the ferret than in the rat. For instance, in the rat, the EGL reaches its maximal thickness by P9 and disappears by P21; the Purkinje cells become aligned into a monolayer by P5 and the rate of foliation of the cerebellar cortex declines abruptly by P10 (Hatten and Heintz, 1995; Altman and Bayer, 1997). In ferrets, the EGL was thickest around P22 and disappeared by P56; Purkinje cells had formed an incomplete monolayer by P9, and with respect to formation of new folia there was an abrupt decline around P20. Similarly to the analysis of relative time courses of motor behavioural development in ferrets and rats, we determined time-points of corresponding relative developmental maturity of morphological features

and analysed their relationship by linear regression. The derived equation characterizing the conversion between time courses of rat and ferret cerebellar development, was $y = 2.34x - 2.28$ (y and x: postnatal days of ferret and rat, respectively). The determination coefficient $r^2$ was 0.95. The parameters included in the analysis were foliation, midsagittal area and EGL and molecular layer thickness.

We wanted to see whether this methodology of time translation between the ferret and rat applied also for other mammals. Since all parameters of cerebellar development that we measured were highly congruent, any one of them should be possible to use to derive the relative time course of cerebellar development for a pair of species. Thus, by using time points of corresponding levels of relative EGL thickness, before and after its developmental peak, we acquired a first approximation of the conversion equation between timescales of the mouse and ferret cerebellar development (Fig. 8A, paper III). The determination coefficient ($r^2$) was 0.99, indicating that the ferret-rat pair was not unique with regard to constancy of cerebellar development. Data on mouse EGL development was adapted from the literature (Yamasaki et al., 2001).

*In sum*, a comparative timetable of cerebellar morphology may be useful not only for translation of data between studies of ferrets and rats but also for comparisons between motor and sensory structures and functions in the developing ferret. In addition, by using linear regression analysis on quantitative measures of cerebellar gross morphology and cerebellar cortical laminar structure, the present study extends previous work on comparative cerebellar ontogeny (Laxson and King, 1983), and the precisely defined relationship of time between the cerebellum of two rather distantly related species underscores how highly conserved this structure is in the evolution of mammals.


## Functional organization of climbing fibre input during development

To understand how the regional specialization of cerebellar function is formed in the course of development constitutes an important step towards a fuller understanding of the interplay between developmental changes in cerebellar processing and motor development. The climbing fibre system is a determinant of the functional zonal organization and thus, a natural first step was therefore to investigate the organization of climbing fibre input to the cerebella of ferret kits (paper IV).

In this preliminary report, we focused on latencies of climbing fibre responses evoked on electrical cutaneous stimulation of the forelimb (and hindlimb). Recordings were made in lobulus V in cerebella of five ferret kits and in two adult ferrets (paper IV). The three weeks time period following the onset of walking at P35 was a particularly dynamic phase with regard to both quantitative and qualitative observations of motor achievements in the ferret kit (paper I). The postnatal ages in focus for this study were therefore chosen to be within that time span.

In the previous study characterizing the adult sagittal zonal organization in ferret, climbing fibre responses were evoked on direct stimulation of the ulnar and superficial radial nerves (Garwicz, 1997). Since we were interested in investigating climbing fibre responses evoked on cutaneous stimulation, we first pursued two experiments in the adult cerebellum. We found that absolute latencies of climbing fibre responses evoked on electrical cutaneous stimulation for most zones were overall similar to those using direct nerve stimulation despite the difference in stimulation type and peripheral conduction distance (Table 1; paper IV).

In the ferret kits we defined putative sagittal zones based on location on the folial surface with regard to the distance from the midline, the medial to lateral sequence and location within the rostrocaudal range. In addition to the location we compared the latencies with the relative differences in response latencies between zones demonstrated in the adult previously (Garwicz, 1997). With regard to these premises a sagittal organization of climbing fibre input were evident from P38 through P53 (Fig. 1a; paper IV).

Latencies of climbing fibre responses for all the identified zones were overall longer than those of the adult. Latencies were longest in the youngest animal and decreased progressively with age (Figs. 1B and 3A, B). In the investigated time span, the changes in latencies of climbing fibre responses appeared to be gradual rather than stepwise and amounted to a total of around five milliseconds in the two week period starting at P38. For instance, at this age, mean latencies for the Cx and D2 zones were 16.5 and 22.7, milliseconds, respectively (Figs. 1B). At P53, the mean latencies of 12.6 and 16.8 had still not reached adult levels (Fig. 1B). Similar gross patterns of latency changes were seen in other zones (Fig. 2A, B).

Amplitudes of climbing fibre responses were relatively constant across the whole period investigated but differed distinctly from the larger amplitudes in the adult ferrets. Mean amplitudes in both the Cx and D2 zones overall ranged from ca. 115µV to 130µV (Fig. 3A, B). These response amplitudes were much smaller than the mean amplitudes in the

adult ferrets, which amounted to 415µV and 245µV for the Cx and D2 zones, respectively (Fig. 3A, B). We did not find any proportionality between latencies and amplitudes across developmental time, which indicated that the changes in latency not were due to a corresponding change in the strength of peripheral input (Fig. 3C).

*In conclusion*, this is the first systems level physiological analysis of climbing fibre input to sagittal zones of the developing cerebellum. The developmental time period characterized by dramatic advances in motor competence has not been investigated before in any mammal, and in this regard the present study extends previous studies of the physiological development of the climbing fibre system (Crepel, 1971; Puro and Woodward, 1977). The gradual change in latency with postnatal age observed here may be due to a combination of changes at any of the elements involved in transmitting the signal from the periphery to the cerebellar cortex, i.e. principally the peripheral nerve, the spino-olivary pathway, the olivo-cerebellar projection and all the intervening synapses. Another factor adding to the complexity of this issue is the fact that the number of synaptic relays included in the spino-olivary pathway varies depending on the specific zone (Oscarsson, 1973). Further investigations are needed to elucidate what the likely mechanism underlying the change in latency is.

## Comparative aspects of climbing fibre input during development

Although latencies of peripherally evoked climbing fibre input to the developing rat cerebellum have been investigated (Puro and Woodward, 1977), the zonal organization has not been addressed. Furthermore, the time period characterized by advances in motor capabilities in the rat (Altman and Sudarshan, 1975) that corresponds to the time span investigated here in the ferret, were not included in the study by (Puro and Woodward, 1977). In addition, mean latencies of climbing fibre responses in the rat of the previous study were much longer than those recorded here in the ferret. This is to some degree likely due to the use of different anaesthetics. All in all, these differences preclude any direct or detailed comparisons between rats and ferrets with regard to changes in climbing fibre latencies during this time period. However, the cerebellar area recorded from in the previous study is largely similar as is the body part stimulated (Puro and Woodward, 1977). So to make an approximate

comparison we used the equation for ferret-rat time conversion of cerebellar development. In relative terms then, the period of rather constant latencies P12-P17 in the rat (predicted ferret age: P26-P38) and the substantial decrease in latencies in the rat between P17 and P22 (predicted ferret age: P38-P49) is compatible with the present findings in the ferret.

## How may timescales of motor development be translated from different experimental animal models to man?

In papers I and III we investigated how time scales of motor development and cerebellar morphological development depend on the duration of postnatal developmental time in ferret and rat. We found that the relative rate of postnatal motor development in these two species appears to be proportional to the relative rate of cerebellar morphological development.
As a means to test the general validity of this finding we extended the comparison to humans (paper V). As a measure of motor developmental rate we used the relative crawling-to-walking latencies in the two species to be compared. In humans, the onset of crawling and the onset of independent walking occur, on average, at the age of 7 and 11.7 months, respectively (Bayley, 1993). This amounts to a crawling-to-walking latency of 4.7 months, or 143 days. In the rat, the corresponding latency in open field observations is 6 days, from postnatal day (P) 10 to P16 (Altman and Sudarshan, 1975). Hence, the relative man-to-rat rate is 143/6, i.e. 23.8 for motor behavioural development. Noticeably, the relative man-to-rat rate for the duration of cerebellar morphogenesis, defining $k$ in our model equation, is 22.6, as the EGL in man and rat cerebellum is present for 519 and 23 days, respectively (Table 1; paper V) (Altman, 1972; Abraham et al., 2001; Clancy et al., 2001). By the corresponding criteria, relative ferret-to-rat rates for motor behavioural development and cerebellar morphogenesis are 2.33 (paper I) and 2.43 (paper III), respectively. These data indicated, in accordance with our working hypothesis, that there is a close correspondence between the two rates for a wide range of cross-species comparisons (Fig. 2A; paper V). Although the comparison between rat and ferret suggested that the rate of cerebellar morphological development as such determines the rate of motor development, this relationship does not hold for humans. If the level of motor competence were in a strict one-to-one fashion determined by the developmental stage of the cerebellum analogy with the rat would suggest that humans should start walking at the age of six and a half months (Fig. 2B; paper V). This is more than five

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months earlier than the actual mean for the human population. According to our working hypothesis, this discrepancy is due to the difference in the relative timing of birth, $p$ in the model equation. Indeed, when $p$ (cf. Table 1; paper V) is taken into consideration, the model predicts the timing of specific discrete events such as the onset of crawling or of independent walking in humans with astonishing accuracy, whether using input from rats (Fig. 3A; paper V) or ferrets (Fig. 3B; paper V).

*In conclusion,* the model represents a first step towards a quantitative comparative approach to motor development at multiple levels of analysis. Its predictive capacity is promising, although a number of factors need to be addressed before a true generalization is possible.

# RESULTS AT A GLANCE

- The suitability of the ferret as an animal model for combined behavioural, morphological and systems level *in vivo* investigations of cerebellar development was underscored by the protracted time course with respect to motor and cerebellar development and the high reproducibility of data.

- Analysis of habituation of locomotor activity in the open field during development revealed striking changes in within-session activity profiles across developmental time, with a systematic shift between increment and decrement of activity.

- Relative time courses of emergence of motor skills in ferrets and rats were highly similar despite substantially different duration of postnatal periods and could be represented by a simple linear equation ($r^2$ = 0.81).

- Relative time courses of cerebellar development in the ferret and rat were highly congruent and again translatable by a linear equation ($r^2$ = 0.95), indicating in quantitative terms how remarkably conserved the cerebellum is in phylogenesis.

- Latencies of climbing fibre responses in cerebellar sagittal zones decreased progressively during postnatal weeks 6 trough 8. Amplitudes of climbing fibre responses were by contrast relatively constant across the period investigated yet distinctly lower than response amplitudes in adult ferrets.

- The similarity between the time-conversion equations describing cerebellar morphological development and motor development in ferret and rat indicate that in two species born at the same level of neurogenetic maturity, cerebellar morphological developmental events can predict the timing of key events in motor development. In humans, a similar prediction seems possible if the relative timing of birth is taken into consideration.

# GENERAL DISCUSSION

The cerebellum is one of the first nervous system structures to begin to differentiate; yet it is one of the last to reach maturity. In humans for instance, neurons predestined to become granule cells are born already in embryonic week eleven while further proliferation and their subsequent maturation will continue throughout the first year (Rakic and Sidman, 1970). The prolonged development of the cerebellum has made it an attractive nervous system structure for study of developmental biology. Adding to its lengthy development, its uniform architecture with relatively well-defined anatomy and afferent and efferent projections has contributed to the extensive knowledge of processes that govern its development. Analyses of spontaneous and gene-targeted mutant mice have been one important source of information and have given much insight into the molecular and cellular mechanisms directing the regulation of cerebellar formation (Chizhikov and Millen, 2003). However, the large number of these studies has concerned the 'early' stages in cerebellar development including neurogenesis, cell fate determination, proliferation, migration and target recognition, which mainly are genetically predetermined. The shaping of cerebellar connectivity that follows the beginning of synaptogenesis and layout of coarse cerebellar maps is poorly understood (Altman and Bayer, 1997; Sotelo, 2004).

In fact, very little is known about functional development of cerebellar micro-circuitry in general. However, its complexity in the adult animal (Apps and Garwicz, 2005; Ito, 2006) indicates that adaptive mechanism almost certainly are involved in the fine tuning of both intrinsic and extrinsic cerebellar connections. In this perspective, our electrophysiological study, although preliminary, represents a first step towards a functional analysis at the level of neuronal networks (paper IV). The fine tuning of cerebellar neuronal networks most likely is closely correlated to the development of fundamental motor skills. Hence, a full understanding of motor development must include a functional analysis of central nervous micro-circuitry, in particularly in the cerebellum (cf. Ito, 2006; Jorntell and Hansel, 2006). In common experimental animals, such as the rat and the ferret, these processes occur in parallel to the morphological development of the cerebellum (Altman and Bayer, 1997; paper I and III).

Since the cerebellum is a structure that is highly conserved in evolution a valid question to ask is whether the same relationships between structure, functional adaptation and motor behaviour hold true also for the human

species. The prolonged motor development in humans as compared to other mammals has traditionally been thought to reflect qualitative differences rather than quantitative and hence quantifiable ones (Pick, 2003). In fact, it has sometimes been argued that there are only weak relationships between structure and function with regard to motor development in humans (Touwen, 1998). However, it might be in fact quantitative differences that cause the striking differences in motor developmental time between, for instance, the rat and the human baby (Finlay and Darlington, 1995).

This touches upon an intriguing general issue in developmental biology – how developmental time translates between species (Bayer et al., 1993; Finlay et al., 1998). The issue is striking when considering the huge differences in motor developmental time that exist between different mammalian species – humans probably being the most extreme with a very prolonged motor development. Studies addressing possible causes for these differences in developmental time usually put forward the often rather large differences that exist in mammalians' body constitutions and hence motor apparatuses as the prime factors of developmental time (Adolph et al., 2003). As yet there has been no quantification of the relationship between motor developmental time courses in species with differently long development. And it has not been expressed in quantitative terms how the species specific features that are considered fundamental to a species developmental time length relate to the timing of motor developmental events.

In this thesis, it is shown that the time courses for motor development in the rat and ferret – two species with different duration of their developmental time period - are highly congruent and that the relationship between them can be expressed by a linear equation derived from quantitative data (paper I). Conspicuously, relative time courses for cerebellar morphological development in the two species were highly similar to the relative time courses of their motor development (paper III). This suggests that cerebellar developmental time could be an indicative measure of motor developmental time, and thus of the timing of specific milestones in motor development, perhaps also in other species.

A plausible explanation for the high level of morphological congruence in the evolutionary perspective is to be found in the duration of EGL a key determinant of cerebellar development. In phylogenesis, the regulation of a single key factor, such as the number of mitotic cycles or the total duration of mitotic activity in the EGL, could be responsible for differences in cerebellar morphological development between species with different

lengths of developmental time (Finlay and Darlington, 1995; Finlay et al., 1998). This regulation could depend on, for instance, thyroid hormones, which have been shown to play a key role in the control of brain development (Koibuchi and Chin, 2000), by acting on nuclear receptors and their linked target genes (Yousefi et al., 2005), and in the cerebellum influence the timing of the disappearance of the EGL (Nicholson and Altman, 1972). Matching in developmental time between parameters that are set by EGL and structural changes in individual Purkinje cells is perhaps not unexpected. In ontogenesis, interactions between differentiating granule cells and Purkinje cells are important for the maturation of both cell types (Goldowitz and Hamre, 1998), which have different origins in the rhombic lip and ventricular zone, respectively (Sotelo, 2004). Indeed, time and rate of Purkinje cells' maturation coincide with the genesis of granule cells in the EGL (Altman and Bayer, 1997). For instance, synaptic contacts between parallel fibres and Purkinje cells are required for normal development of the Purkinje cell dendritic tree (Hatten and Heintz, 1995). Conversely, it is clear that Purkinje cells are required for the proliferation of granule cell progenitors (Goldowitz and Hamre, 1998). This intricate and tightly linked interplay between architectural and cellular development is evident also in our own study. The ferret-to-rat time conversion equation based on cerebellar architectural data (i.e. thickness of cortical layers, number of folia and midsagittal area) predicted with high accuracy the time points of actual morphological changes in individual Purkinje cells (paper III). Given that the signalling pathways involved in the interaction between Purkinje cells and granule cells (Dahmane and Ruiz-i-Altaba, 1999; Wallace, 1999; Wechsler-Reya and Scott, 1999; Solecki et al., 2001; Corrales et al., 2006) are highly conserved in evolution (Louvi and Artavanis-Tsakonas, 2006) and that there seems to be a straightforward relationship between cerebellar and motor development, both ontogenetically and across species, why is this relationship not evident in humans?

In our concluding paper, we extended the comparative analysis of the relationship between cerebellar developmental rate and motor developmental rate to also include the human species (paper V). We hypothesized that motor development depends on sensorimotor activity dependent adaptive mechanisms at work during the postnatal period and that these mechanisms are implemented to a significant degree by the cerebellum. To test the implications of our hypothesis we constructed a simple mathematical model based on quantitative comparative developmental data. The relative rate of cerebellar morphological

development for the given pair of species (man, ferret or rat) was set by calculating the relative duration of the presence of the EGL. As a measure of motor developmental rate we used the relative crawling-to-walking latencies in the two species to be compared. Both the relative man-to-rat rate for motor behavioural development and the relative man-to-rat rate for the duration of cerebellar morphological development were highly similar to those of the ferret-rat pair. Whereas the comparison between rats and ferrets seemed to suggest that the rate of cerebellar development as such determines the rate of motor development this was certainly not the case in humans. When the difference in the relative timing of birth was taken into consideration however, the model predicted the timing of specific discrete events such as the onset of crawling or of independent walking in humans with astonishing accuracy, whether using input from rats or ferrets.

The temporal dissociation between cerebellar structural development and motor development, with a preserved proportionality of developmental rate, indicates that the neuronal correlates of motor development are not to be sought simply in the emergence of structures as such, but rather in the adaptive processes that occur within those structures. Birth appears to act as a trigger of the adaptive processes underlying the developmental sequence of events, whether it occurs relatively early (rat and ferret) or late (human) in relation to the maturation of the nervous system (Romijn et al., 1991; Bayer et al., 1993; Clancy et al., 2001). This indicates that the adaptive processes are dependent on sensorimotor activity, since this activity changes in many respects at birth (Johnson, 2001). Being free to move implies changes in biomechanics, which affect both the movements as such and the resulting sensory feedback (Schouenborg and Kalliomaki, 1990; Holmberg et al., 1997; Salinas, 2006).

At the outset of the Introduction, differences between a few species in developmental time, or more specifically, in motor capabilities in early life, were exemplified. The foal manages to locomote more or less immediately upon birth in contrast to the rat and at the other end of the spectrum the human baby. One may assume, in accordance with the assumptions underlying the model equation and its emphasis on adaptive mechanisms (paper V) that the reason why newborn unguligrade animals can walk virtually without practice, while digitigrade and plantigrade animals cannot, is largely due to the difference in complexity of the motor apparatus (Bernstein, 1967). A challenge in future models will be to express in quantitative terms how these important features of relative complexity influence the time course required for postnatal development.

## Clinical significance

The well-conserved sequences of developmental events previously demonstrated at the levels of neurogenesis (Bayer et al., 1993); (Clancy et al., 2001) and morphological development (paper III) appear to result in a fundamentally stereotypic sequence of events also at the level of behaviour (paper I, III). Furthermore, it appears that once triggered, behavioural motor developmental in animals and humans evolves with the same relative timing between these events (paper V). As a result, the onsets of different motor developmental events have a precise timing that is highly predictable across species, including humans. Potentially, these findings may have important clinical implications.

Cerebellar pathology has been associated with autism and other complex neurodevelopmental disorders that typically appear during the first three years of life (Eigsti and Shapiro, 2003; Bauman and Kemper, 2005). There is evidence that "cognitive dysmetria", which has been launched as an integrative theory of schizophrenia, depends on dysfunction in cortical-subcortical-cerebellar circuitry (Crespo-Facorro et al., 1999; Toulopoulou et al., 2004; see also Schmahmann, 1998). The cerebellum is also one of the most consistent locations for structural differences between dyslexic and control subjects in imaging studies (Eckert et al., 2003). Deficiencies in social cognition in the fragile X premutation, a milder form of the neurodevelopmental disorder fragile X syndrome, have been suggested to be attributable to impairment of neural pathways modulated by the cerebellum (Cornish et al., 2005). Highly relevant to recent progress in neonatology, it has been suggested that the long-term neurodevelopmental disabilities seen in survivors of premature birth may be attributable in part to impaired cerebellar development (Limperopoulos et al., 2005). In view of its combined capacity for coordination and plasticity it is perhaps not surprising that the cerebellum has been implicated as a key site of pathology underlying such a wide range of neurodevelopmental disorders. Conspicuously, a salient feature of these disorders appears to be a derangement of the interplay between different central nervous functions, progressively worsened in the process of development (Karmiloff-Smith et al., 2002). Finally, one may speculate that maladaptive changes in cerebellar neuronal networks contribute to the movement disability that is an important problem for many children with cerebral palsy (Shapiro, 2004).

A well-characterized animal model of cerebellar physiological development should be of immediate use for analytically testing the influence on the cerebellum of pathogenic circumstances or agents thought

to play a role in the etiology of neurodevelopmental disorders (for references see Lipska and Weinberger, 2000). More specifically, the issues of cerebellar physiological development and its role in the learning of fundamental movement synergies in the postnatal period constitute uncharted scientific territory. A clarification of the extent to which the cerebellum contributes to this learning and what the underlying mechanism are would, however, not only signify a breakthrough in the understanding of normal cerebellar function. In addition, we hope that it will provide insights into the function of sensory feedback for motor learning in general and open possibilities to interpret motor pathological conditions in terms of erroneous, maladaptive learning. Such knowledge could then be used for rational design of protocols for relearning of postures and movement patterns in the context of motor rehabilitation, in particular for young individuals. The acute changes in motor control caused by injuries to the motor apparatus or the central nervous system are often followed by maladaptive changes in muscle tone and/or reflex excitability. Although these changes to some extent most likely are due to neuronal post-injury degeneration it is likely that plastic learning mechanism play a role in contributing to changes in motor output that are not functional but instead often make the condition worse. The possibilities to alleviate such conditions would greatly benefit from an analysis of the organization of the systems housing the neuronal plastic mechanisms and a better understanding of how the mechanisms are influenced by sensory input from the motor apparatus.

# POPULÄRVETENSKAPLIG SAMMANFATTNING

Traditionellt liknas lillhjärnan ofta vid en autopilot, med vars hjälp våra rörelsekomponenter sätts samman till en harmonisk helhet. Men under senare år har det visat sig att lillhjärnan inte bara samordnar våra rörelser, utan även komponenter av vårt språk, våra tankar, kanske till och med olika aspekter av vårt sociala beteende. I grunden för alla dessa samordnande funktioner ligger lillhjärnans plasticitet, d.v.s. dess förmåga att förändra kopplingarna mellan sina nervceller som ett resultat av de erfarenheter som individen gör. Med tanke på att de mest dramatiska förändringarna i dessa kopplingar sannolikt sker tidigt under vår uppväxt då kontrollen av t.ex. vår kroppsmotorik grundläggs är det anmärkningsvärt hur lite som är känt om fysiologiska aspekter på lillhjärnans utveckling.

Det övergripande målet för denna avhandling har varit att närma sig en sådan förståelse genom studier av hur lillhjärnan bidrar till vår förfinade förmåga att kontrollera våra rörelser tidigt i livet. Det komplexa förhållande mellan struktur, funktion och beteende som ligger till grund för kroppsmotorikens utveckling beror på ett samspel mellan mognadsprocesser, gradvisa anpassningar och inlärning baserat på erfarenheter. För att kunna klarlägga eventuella samband mellan lillhjärnans utveckling och den motoriska utvecklingen tidigt i livet är det nödvändigt att ha en experimentell modell som lämpar sig för analyser på olika nivåer. Ett viktigt mål med arbetet i denna avhandling har varit att utveckla och utvärdera en experimentell modell för kombinerade strukturella och elektrofysiologiska studier av lillhjärnans utveckling samt beteendeanalys under den tidiga utvecklingen. Vi visste sedan tidigare att illern föds omogen (jämfört med t.ex. katt) och utvecklas relativt långsamt (jämfört med råtta) varför den skulle kunna utgöra en lämplig djurmodell. Även om illern har blivit allt vanligare som djurmodell, i synnerhet i utvecklingsstudier av sensoriska system, har dess motoriska utveckling inte studerats tidigare, vare sig vad det gäller beteende eller nervsystemets anatomi.

I delarbete (I) karaktäriserades illerns motoriska utveckling. Djuren studerades dagligen under de första tio levnadsveckorna i ett antal motoriska tester. Testerna tog främst fasta på djurens förmåga att krypa eller gå och olika rättningsreflexer, och valdes så att motoriska beteenden under hela utvecklingstiden kunde utvärderas. Genom en vidareutveckling av beteendeanalysen i delarbete (II) kunde en fundamental

inlärningsmekanism studeras. Det visade sig att djurens aktivitet i en given situation förändrades på ett mycket systematiskt sätt med åldern. I delarbete (III) studerades hur illerns lillhjärna utvecklas strukturellt. Olika mått på lillhjärnans tillväxt, lillhjärnbarkens foliering och förändringar i olika lager i lillhjärnbarken kvantifierades samt karaktäriserades förändringar i Purkinje-cellers morfologi från postnatal dag 1 till dag 63. I delarbete (IV) studerades utvecklingen av lillhjärnans funktionella organisation elektrofysiologiskt. Inflödet från de s.k. klätterträdarna, som utgör ett av de huvudsakliga inåtgående signalsystemen till lillhjärnan, analyserades.

I avhandlingen presenteras den första systematiska studien av motorisk beteendeutveckling och lillhjärnans morfologiska utveckling hos iller. Utöver specifika fynd inom de enskilda delarbetena, drar vi slutsatsen att illern utgör en särskilt lämplig modell för att direkt studera samspelet mellan lillhjärnans fysiologiska utveckling och den samtidiga utvecklingen av kroppsmotoriken.

En parallell strategi i avhandlingen har varit att studera hur tidsförloppen för lillhjärnans utveckling och motorisk beteendeutveckling förhåller sig för arter som utvecklas under olika lång tid. Genom att göra kvantitativa jämförelser mellan olika arters utveckling med avseende på lillhjärnans struktur samt vissa motoriska beteenden fann vi mycket tydliga samband som beskriver hur olika utvecklingssteg följer på varandra. Det visade sig till och med möjligt att med en enkel matematisk modell förutsäga när och i vilken följd olika utvecklingssteg följer hos en given art baserade på data från en annan. Genom att studera samband mellan illrars och råttors motoriska utveckling kunde vi förutsäga när vissa utvecklingssteg sker hos människan med en felmarginal på endast 10 % i förhållande till de faktiska medelvärdena för människan. Denna häpnadsväckande precision i förutsägelserna rörande vitt skilda arter antyder att motorutvecklingen i människor likväl som i flera andra djur följer likartade principer och att vår matematiska modell tar fasta på dessa mekanismer på ett rimligt sätt.

Mot bakgrund av lillhjärnans roll som samordnare och dess plasticitet under en människas tidiga utveckling är det kanske föga förvånande att avvikelser i lillhjärnan verkar vara den gemensamma nämnaren för en rad så kallade utvecklingsjukdomar som drabbar nervsystemet. Exempel på utvecklingsjukdomar där avvikelser i lillhjärnan observerats är autism, schizofreni, dyslexi, långsiktiga handikapp som drabbar för tidigt födda barn och sannolikt cerebral pares. För närvarande förstår man ytterst lite om vilka orsakssamband som råder mellan avvikelserna i lillhjärnan och de

symptom som den drabbade individen har. Ännu mindre vet man hur de förändringar i kopplingarna mellan lillhjärnans nervceller som äger rum under utvecklingen skiljer sig i en frisk och en sjuk individ. Innan en sådan jämförelse kan göras och förhoppningsvis kasta ljus över dessa sjukdomar måste man förstå den normala utvecklingen av lillhjärnans nervceller och kopplingarna dem emellan. Mer specifikt torde ett klargörande av lillhjärnans roll i inlärning av grundläggande rörelsemönster hos unga individer öppna helt nya möjligheter för att korrigera felaktiga anpassningar i ett rehabiliteringssammanhang.

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