Spontaneous Movements during Sleep Guide Spinal Self-organization: Formation and Expression of a Memory Trace

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Formation and Expression of a Memory Trace

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CONTENTS

Original papers

This thesis is based on the following papers, which will be referred to by their roman numerals:

I. Petersson P., Waldenström A., Fåhraeus C. and Schouenborg J.
”Spontaneous muscle twitches during sleep guide spinal self-organization”
Nature (2003), **424**, 72-75

II. Petersson P., Holmer M., Breslin T., Granmo M. and Schouenborg J.
”An imaging system for monitoring receptive field dynamics”
J Neurosc Meth. (2001), **104/2**, 123-131

III. Petersson P., Granmo M. and Schouenborg J.
”Characterization of connections in a sensorimotor circuit shaped through neonatal experience”
(manuscript)

Introduction

Background

Behaviour is the result of the interaction between the central nervous system (CNS) and the environment. Although certain types of behaviour appear to be innate and presumably genetically determined, behaviour is generally altered by learning and memory - and altered behaviour result in new experiences, etc. During development, the CNS not only has to adjust to a changing environment it also has to adjust to a changing body. Our only way to interact with the world around us is by generating movements; therefore motor learning can in a sense be regarded as the most fundamental form of learning process. To possess this ability something in the CNS needs to be changeable in order to adjust to new situations but at the same time stable enough to guarantee the storage of earlier modifications. - What is the substrate underlying this important plasticity?

In 1894, Ramón y Cajal proposed that memory storage is accounted for by existing nerve cells growing more branches and strengthening connections with other nerve cells (Ramón y Cajal, 1894). Yet, this idea was neglected for almost half a century as other hypotheses attracted a greater interest (Kandel, 2001). But in the 1940s the idea that memory formation and long-term storage were managed by alteration and maintenance of connection strengths in the central nervous system resurfaced and was at this point also advocated on theoretical grounds (McCulloch and Pitts, 1943;Hebb, 1949;Rosenblatt, 1958;Zhang et al., 1998). In investigating the plausibility of these claims reductionistic approaches such as the gill withdrawal reflex in the snail *Aplysia Californica* have proven useful (Kandel, 2001) In *Aplysia* a stronger response to the same input after sensitization can be explained by; a higher excitability in sensory neurons and changes in synaptic strengths between sensory and motoneurones (Marinesco and Carew) and in interneuronal synaptic connections. Thus, the storage of this non-declarative memory is distributed within the reflex pathways (Milner et al., 1998). A closely related withdrawal reflex in mammals is the nociceptive withdrawal reflex; a spinal reflex system that has now been studied for almost a century (Sherrington, 1906;Sherrington, 1910).

Nociceptive withdrawal reflexes

The nociceptive withdrawal reflex is a nocifensive reflex with a primary purpose to minimize tissue damage. When a painful stimulus is applied to a certain body area, a withdrawal reflex is evoked. Sherrington described the withdrawal reflex of the hind limb in decerebrate cats as a ‘flexion reflex’ signifying a reflex simultaneously activating flexor muscles and inhibiting extensors. The receptive field was found to be very large although the pattern of flexor activity depended on which nerve that was stimulated, a phenomenon referred to as the local sign of the flexion reflex. It was not until a detailed and more complete study was carried out, using calibrated and natural noxious stimulation of the skin and electromyographic recordings from most of the hind limb muscles that this reflexes system was more properly characterized (Schouenborg, 2002). It was shown that this reflex system has a modular organization, where each module controls a single or a few synergistic muscles with similar action. Each module has a receptive field that matches the withdrawal function of its muscle. The sensitivity within the receptive field is proportional to withdrawal efficiency in standing position.

Some information on the reflex circuit has also been obtained. In the deep dorsal horn in

the L4 and L5 segments of the spinal cord a group of interneurons having identical receptive fields to that of single hind limb muscles mediating withdrawal reflexes of the hind paw have been found. These cells are assumed to encode the withdrawal reflexes of the hind paw and have thus been termed 'reflex encoders' (Schouenborg et al., 1995).

Are spinal reflex systems innate?

Reflexes are commonly assumed to be innate, controlled by hard-wired genetically determined circuits (Brodal, 1992). However, if the nociceptive withdrawal reflex function were to be directly genetically coded this would require an enormous amount of information to be stored in the genetic sequence in order to build a hard-wired CNS precisely enough to exactly match the body constitution of each individual. On the other hand, if the CNS instead is able to adapt to the biomechanical properties of each individual, the same basic circuits can be used and a wider range of flexibility matching the highly variable needs of different species and individuals can be met. Such a functional adaptation should occur fairly early in life but at a time-point where the systems involved are mature enough to have attained a basic circuit connectivity. Experience dependent adjustments in a sensorimotor system require a repertoire of movements to start off from. This leads to the question, what type of motor behaviour is present early in life?

Spontaneous movements

In studies of newborn human infants (Prechtl, 1974) as well as in rat pups (Blumberg and Lucas, 1996)distinct behavioural states with associated specific motor patterns have been found. As rats are born relatively immature, developmental stages corresponding to human foetal development occur postnatally (Clancy et al., 2001), which makes the study of the possible roles of different kinds of 'foetal' movements more accessible. In rats, motor behaviour during the first postnatal weeks is dominated by brief myoclonic twitches (Blumberg and Lucas, 1996;Blumberg and Lucas, 1994). The detailed origins of these spontaneous twitches have not been established but they have been proposed to occur predominantly during a behavioural state resembling adult REM-sleep termed active sleep (Karlsson and Blumberg, 2002).

Activity dependent organization during development

Many sensory systems need activity during a critical period early in life to develop correctly. For example, it was noted already in the 1930s that removing congenital cataracts between the age of 10 and 20 (which was common practise at the time, but is after the critical period) resulted in a permanent impairment of the ability to perceive shape and form (Kandel et al., 2000). Later studies in animals (Hubel and Wiesel, 1977;Hubel and Wiesel, 1970) confirmed the importance of visual input during postnatal development for adequate function of the primary visual cortex. The development of the visual system in mammals has subsequently been studied in great detail. It has been shown that molecular cues guide the initial formation of the afferent pathways so that axons from the retinal ganglion cells are directed in the optic nerve to the appropriate cells in the later geniculate nucleus and that these cells, in turn, project to the correct cells in the visual cortex. However, this rather crude initial organization is thereafter shaped into its adult pattern first by spontaneous activity in the retina *in utero* and thereafter by visual input during early development (Katz and Shatz, 1996). In the adult visual system input from the different eyes to the brain are separated so that in the lateral geniculate nucleus in the thalamus separate layers receive input primarily from one of the eyes and the projection of these cells to the visual cortex is organized into columns where input from a single eye dominate in each column. This organization is thought to arise because firing patterns of axons from neighbouring ganglion cells in one eye is more synchronized with each other than they are

with inputs from the other eye. The synchronization in the activity patterns is thus regarded as a signal that these cells belong together and should preserve or establish strong connections to each other. Similar grouping mechanisms have been found in other sensory systems such as the auditory system (Sanes and Constantine-Paton, 1983;Zhang et al., 2001) and somatosensory system (Wallace and Fox, 1999;Feldman et al., 1998) in rats and mice. Interestingly, the functional maturation of one of the somatosensory systems in the rat, the vibrissa system, has been reported to be partly dependent on motor output to the whiskers during neonatal active tactile exploration (Nicolelis et al., 1996).

Activity dependent changes in the adult

The observation that synchronous firing strengthens the synapses of all cooperating fibres whereas the non-cooperating fibres decline was in fact assumed by Hebb when he postulated the learning rule governing synaptic plasticity in 1949. Although not explicit in the well-known formulation: 'When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change take place in one or both cells so that A´s efficiency as one of the cells firing B, is increased" (Hebb, 1949), Hebb envisioned that the way the postsynaptic cell was possible to activate in the first place was either through a coactive strong synapse (associativety) or many coactive weaker synapses (cooperativity) (McNaughton, 2003). In 1973 a phenomenon with identical properties was found in the hippocampus of adult animals, a finding that triggered a whole field of research. If a pathway was stimulated briefly but intensely, the strengths of the synapses between neurons in this pathway were potentiated for prolonged periods referred to as long-term potentiation (LTP) (Bliss and Lomo, 1973). The properties of LTP matched the intuitive experience of how memories are laid down and it was subsequently shown that pharmacological interference with an LTP induction mechanism would also interfere with learning (Morris et al., 1986). Although many of the molecular changes underlying long-term potentiation and the reverse type of synaptic modification, long-term depression (LTD), are now known links between these processes and the physiology of learning and memory in the adult still need further corroboration (Bailey et al., 2000). Notably, the fact that the learning rules put forward by Hebb appear to be part of both developmental tuning processes and plastic changes in the adult may indicate that common underlying mechanisms govern both of these important physiological phenomena.

Theoretical approaches and relations to neuronal systems

Since Hebb's early postulate on the physiological bases for learning, the understanding of learning mechanisms has deepened significantly largely because a theoretical framework describing learning principles in artificial neuronal networks has emerged.

A distinction can be made between two types of learning paradigms; learning with a 'teacher' (supervised learning) and learning without a 'teacher' (unsupervised learning). In the first case, the 'teacher' provides the network with an error signal defined as the difference between the desired response and the actual response of the network when exposed to an input from the environment. During a training period gradual adjustments are then made to make the neural network emulate the teacher, whereafter the network can be left to itself.

Two examples of experience dependent tuning in neuronal systems that have been suggested to use this type of learning paradigm are the auditory localization system in barn owls and climbing fibre mediated cerebellar motor learning in cats. In the first system, experimental manipulations of sensory experience have revealed functional, anatomical and pharmacological changes in this system accompanying behavioural training and it is thought that activity from

the visual system is sent into the auditory system as an instructive input to guide the transformation of auditory cues into a topographic map of space (Knudsen, 2002). In the second system, the climbing fibre input to the cerebellar cortex has been taken as an instructive 'error signal' governing adult plasticity (Albus, 1971;Marr, 1969;Ito, 2000).

To learn without a 'teacher' overseeing the learning process an organism can either rely on reinforcement learning which require some external signal from the environment that can be converted to a heuristic reinforcement signal used as an index of performance or, perhaps more surprising, it can learn completely unsupervised without an evaluation of performance – a process referred to as self-organization. In the latter case the network becomes tuned to the statistical regularities of the input data and can thereby, after a learning period, classify input patterns according to certain features. For example the receptive fields of simple cells in primary visual cortex have been suggested to be built-up according to such self-organizing principles (Linsker, 1986).

For the nociceptive withdrawal reflex system it is difficult to see how supra-spinal structures could have a direct instructive role, as the projections down into the spinal cord are very immature during early development. Instead one might think that the adaptation of the nociceptive withdrawal reflex system is likely to be achieved through the first type of unsupervised learning described above using pain as a negative reinforcement signal. However, as the functional adaptation occurs during a period when very little nociceptive input is present (*in utero* or in early postnatal life) this type of learning cue would probably not be very useful. On the other hand it is perplexing how the noisy sensory input reaching the spinal cord more or less continuously, by itself, could guide the organization of circuits performing such a complex sensorimotor transformation.

Imprinting a movement pattern in a sensory pathway

Earlier experiments in rats have revealed that the nociceptive withdrawal reflex system is indeed shaped through experience dependent mechanisms so that an imprint of withdrawal efficiency on the reflex pathways is attained through extensive postnatal adjustments, whereby erroneous connections are eliminated or reduced and the strength of adequate connections becomes proportional to withdrawal efficiency (Holmberg and Schouenborg, 1996a). A number of findings together hint at how the nociceptive withdrawal reflex system is able to functionally adapt during development and have also pointed towards possible learning mechanisms governing the self-organization of this system. The sensorimotor transformation can adapt to neonatal alterations of both peripheral innervation (Holmberg and Schouenborg, 1996b) and movement patterns (Holmberg et al., 1997). The reflex adaptation takes approximately one week and occurs during the first three postnatal weeks depending on body part (Holmberg and Schouenborg, 1996a;Waldenström et al., 2001). The tuning seems to be dependent on tactile input, but independent of nociceptive input (Waldenström et al., 2001).

Taken together these data suggest that a functional tuning takes place during early postnatal development such that the movement pattern resulting from motor output of a given reflex module is used to set the strengths of synaptic connections to its reflex encoder. A comprehensive description and a biologically plausible theoretical model of the adaptation process incorporating all these elements would improve our understanding of this complex system and potentially produce experimentally testable predictions.

The functional importance of foetal movements

Although parents in all times surely have been fascinated by spontaneous activity

displayed by the foetus *in utero*, the importance, if any, of this motility remains unknown. Systematic observation of spontaneous motility has been carried out in a number of studies on foetuses (Sival, 1993), preterm (Prechtl, 1990) and term infants (Cioni et al., 1997). In these studies a clear correlation between quality (but not to the same extent quantity) of the spontaneous movements and the presence of brain lesions and/or the neurological outcome has been found. It has therefore been assumed that they at least reflect some aspects of the maturation of the CNS. It is also clear, that they tend to be displayed in certain behavioural states (Prechtl, 1974). The state of active sleep, involving sudden myoclonic twitches or startles, that is commonly found both in humans and in other mammals is a behaviour displayed predominantly during the perinatal period. A number of suggestions concerning the functional importance of these movements have been put forward. On the one hand the twitches have been regarded just as an epiphenomenon arising due to insufficient inhibition during paradoxical sleep (Chase and Morales, 1983). But on the other hand they have also been suggested to be important for several developmental processes; as a signal preventing neuronal cell death, for muscle fibre differentiation, for synapse elimination in the neuromuscular junction and for basic network arrangement analogously to visual system (Blumberg and Lucas, 1996). It is consequently fair to say that if these spontaneous movements displayed both pre- and postnatally have other more complex functions than the merely 'trophic' role suggested hitherto these are basically unidentified. If these movements are used for spinal self-organization in the nociceptive withdrawal system it is possible to postulate certain effects on the reflex adaptation resulting from manipulations of either the twitches themselves or perhaps the sensory feedback resulting from the twitches.

To selectively alter the feedback on the myoclonic twitches, a very fast automatic system that can differentiate between continuous movements and twitches in a time frame of a few ms and give some type of artificial tactile feedback is required. To construct such a system for hind limb twitches would call for a very high spatial image resolution in order to distinguish between the sometimes very fine movements generated.

Dynamic receptive field mapping

The activity of a sensory neuron in the central nervous system can typically be modulated from an area in the periphery defined as its receptive field. The convergence implies that a large number of synaptic connections collectively determine the sensitivity distribution of the receptive field. To adequately characterize a receptive field a large number of sites activating different afferent inputs have to be stimulated with known intensity several times. This rather slow mapping technique usually requires half an hour for a single map in the case of the nociceptive withdrawal reflex system. This makes it impossible to detect changes of responses over time when the dynamics also occur in the time range of minutes. For example, in order to accurately follow the effect of pharmacological substances in our model system, it is necessary to acquire a map of the receptive field at least every few minutes and ideally each map should contain multiple stimulations of each site to get a more reliable measure and hence a higher sensitivity. It is known both from the visual system and from the recordings in the dorsal horn (Weng et al., 1998;Cook et al., 1987;Woolf and King, 1990) that the input preceding the mapping of a receptive field greatly influence the outcome (Worgotter et al., 1999). This is also the case with the overall excitability. Differences may depend on; the state of the brain, the depth of anaesthesia or as for the decerebrate spinal preparation used in the experiments described here, a progressive excitability increase over time after spinalization. However, an automated system that rapidly

scans receptive fields in a random fashion would allow a high precision at sufficient speed to permit the experimenter to follow the effect of various drugs in real time.

The physical foundation of a long-term memory

In search of the morphological and physiological substrates for long-term memory in mammals, LTP in the hippocampus has been a popular focus (Morris, 2003;Milner et al., 1998). However, long-term storage of most memories is probably not located within the hippocampal structure (Teng and Squire, 1999). Further, little is known about how information is processed in the hippocampus and which are the main sources of input and areas of output communicating with this structure (Brasted et al., 2003). To look for long-term memories in sensory systems may also be problematic. Although activity modulations of sensory cells, in for example the visual system, can be correlated with changes of visual input there is no direct evidence that the cells actually are 'coding for' or representing the 'impression' of the peripheral stimulation (Parker and Newsome, 1998). Instead, to tackle this fundamental question a well characterized system where the functional meaning of both the input and output signals are known is crucial. In addition, to track learning induced changes it must be possible to make repetitive recordings from identified neurones demanding precise anatomical or physiological landmarks in the CNS. Few studied neuronal systems have been characterized in such detail, but the nociceptive withdrawal reflex system may offer an opportunity (c.f. *Aplysia*). In the nociceptive withdrawal reflex system the long-term storage of the neonatal adaptation appears to be managed by maintenance of differential connection strengths, but it is not clear in what respect these strong and weak connections differ. Knowing the nature of the differences in connection strengths in this functionally tuned system, such as number of connections or differences in postsynaptic receptors, would clarify the essence of the end product of the learning mechanism and thus, potentially, shed some light on the long-standing question of how the central nervous system is able to store information during a life-span.

Aims

The Aims of this thesis were:

1) To describe and test the plausibility of a suggested learning principle underlying the functional adaptation of the nociceptive withdrawal reflexes in a computer simulation (I)
2) To validate the theoretical model through behavioural experiments where artificial feedback on spontaneous movements is used to interfere with the learning process during the period when reflex adaptation normally occurs (I)
3) To construct a system that allows for a rapid and reliable mapping of receptive fields to enable characterization of the end product of the learning process (II)
4) To characterize the differences between strong and weak connections in a neural circuit to elucidate the physiological basis of a non-declarative memory (III)

Methods

Wistar rats of both sexes were used.
During the conditioning experiments and in the documentation of sleeping behaviour (I) the rat pups were separated from their mother a maximum of three hours per day. The animals received food and water *ad libitum* and were kept in a 12-h day-night cycle at a constant environmental temperature of 21°C (humidity 65%). The litters were kept under the same conditions as the adult rats. (II-III). Approval for the experiments was obtained in advance from the Malmö/Lund ethical committee on animal experiments.

Computer simulations (I, III)

Simulation of reflex adaptation
The simulations were implemented in MATLAB (Mathwork Inc.). In the computer simulation of the reflex adaptation, experimental data on both the movement patterns of a number of muscles and the receptive fields of these same muscles were needed. Firstly, six movement patterns produced by activation of five different muscles (Peroneus longus (PL), Peroneus brevis (PB), Tibialis anterior (TA), Gastrocnemius (G) and two sub-units of the Extensor digitorum longus with tendon insertions on digit 2 and 3 (EDL23) and digit 4 and 5 (EDL45) respectively were gathered from earlier experiments. These movement patterns were represented in 28x61 matrices (597 of these matrix elements represented sites of the plantar skin and were used in the simulation; >1.5 pixels/mm^2 in an adult) (see (Schouenborg and Weng, 1994) for information on how the matrices were obtained). These patterns were assumed to describe the pressure changes on the plantar skin of the hind paw in standing position and consequently the pattern of afferent input from mechanoreceptors responding to skin pressure on muscle contraction. Secondly, quantified and normalized nociceptive withdrawal receptive fields from intramuscular electromyographic recordings of these muscles were also obtained from previous studies (Schouenborg and Weng, 1994). In the most plausible and successful learning strategy termed MDSI (see results and comments), a learning epoch was initiated by a burst in the reflex encoding interneuron. For each learning epoch, muscles were activated at random with a specified probability. On coactivation of muscles, the withdrawal movement was approximated as the sum of the normalized withdrawal patterns of the individual activated muscles. The cutaneous feedback, which was represented in the same coordinates as the withdrawal efficiency, was conveyed by parallel afferents reaching 597 first order interneurons in substantia gelatinosa (SG_j). These, in turn, act on six reflex encoding deep dorsal horn neurons (RE^k). Hence, 597 connection weights (w_j^k) between substantia gelatinosa neurons and reflex encoders for each of the six modules were updated in each learning epoch. A total of 10,000 epochs was estimated to roughly correspond to the observed number of tail twitches during the adaptation period (Waldenström et al., 2002). Oja's rule $\Delta w_j^k = \eta RE^k \left(SG_j - RE^k w_j^k\right)$ where η is the learning rate, was used for synaptic weight adjustments (w_j^k normalize to $w_j^k \in [0,1]$) (Oja, 1982;Turrigiano et al., 1998;Fregnac and Bienenstock, 1998;Katz and Shatz, 1996).
In the simulation of unsupervised feedforward learning, learning occurred when the postsynaptic activity in the reflex encoding interneurons exceeded ~15% of maximum response. Increasing the activation probability did not improve adult correlations. When activated, weight adjustments were performed exactly like in MDSI learning.

Simulation of pharmacological effects

In paper III, we simulated the effects of NBQX and AP-5 on the transmission in the excitatory pathways. The same basic cellular processes were assumed to be present for all the excitatory cells in the pathway and thus the modulation of the responses in the model can be viewed as taking place at a single cell or being distributed over all the cells in the pathway. It was assumed that the excitatory synaptic transmission is primarily mediated by AMPA and NMDA receptors and that the total charge mediated by the NMDA receptors upon opening was three times as big as that mediated by the AMPA receptors (Umemiya et al., 1999). Synaptic sizes covering a range of 1 to 100 receptors of each type was simulated (with no distinction between whether they are distributed over a number of synapses or all being located in a single synapse). Three different receptor distributions were used: 1) A/N >1 in weak synapses and <1 in strong synapses ($N_{AMPA} \in U[30,55]$; $N_{NMDA} \in U[1,100]$), 2) A/N =1 in all synapses ($N_{AMPA} \in U[1,100]$; $N_{NMDA} \in U[1,100]$) and 3) A/N <1 in weak synapses and >1 in strong synapses ($N_{AMPA} \in U[1,100]$; $N_{NMDA} \in U[30,55]$). The opening of at least 10 AMPA receptors was supposed to be needed for NMDA receptor activation (this threshold was chosen arbitrarily). The amount of depolarization resulting from activation of the receptors was assumed to directly scale with number of action potentials emitted by the neurons (Reyes, 2001). This can be formally stated as:

$$\text{Response} = \left(N_{AMPA} \times 1 + N_{NMDA} \times 3 \right) \times \Theta(N_{AMPA} = 10);$$

(where Θ is the Heaviside step function). The parameter values were chosen to be within a biologically feasible range (Nusser et al., 1998), but altering them did not change the basic outcome of the simulation.

Behavioural tests (I)

Testing nociceptive withdrawal reflexes

Every test included 10 stimulations (25 ms CO_2-laser pulses, 5W, beam width 3 mm) to each side of the distal tail, which reliably evoked C-fibre mediated reflexes as judged by the onset latencies. The evoked reflexes were stored using the optical system for twitch detection (see below) and analyses of movement directions were made from these film sequences off-line. The person performing the analyses did not know which type of conditioning stimulation the animal had received. Only tail-tip movements > 0.02 rad (~1 mm) was considered as reflexes.

Conditioning stimulation

To be able to detect spontaneous twitches and give feedback fast enough to match the natural situation, a very fast imaging system that automatically could give tactile feedback had to be constructed. The fastest camera available at the time was a CMOS sensor camera (CCi4, C-Cam Technologies, acquisition rate ~180 images/s). The images from the camera were collected and air-puffs were triggered through software written in LabVIEW 5.2 (National Instruments Corp.) on a PC.

The criteria for emitting feedback were selected after a careful qualitative evaluation of the systems classification of different movement patterns. Twitches with a lateral deviation of the mid-section of the tail >9.2 radians/s (~0.28 m/s) after >300 ms immobility triggered an air-puff directed to the lateral side of the tail. The air-puffs, generated by loudspeakers, had a peak pressure of ~20 kPa and stimulation latency from twitch initiation of ~40 ms at normal tail position. Conditioning stimulation was given once per day during 2h per animal.

Documentation of sleeping behaviour

For the analysis of the position of the hind paws during sleep and the quantification of twitches dominated by single muscle contractions, video recordings, using a JVC GR-DVL9800E digital video camera (25

images/s and 680.000 pixel for hind paw position and 100 images/s and 170.000 pixels for twitches) were made in the afternoon (the entire litter 30 min/day for hind paw position and each rat pup for at least 5 min for twitch quantification, in a surrounding temperature of 35º C). Analyses were performed by visual inspection in slow motion on a TV-monitor.

Surgery (II, III)

The animals were anaesthetized with halothane (0.9 - 2.0%), in a mixture of 65% nitrous oxide and 35% oxygen, and were ventilated artificially via a tracheal cannula. The expiratory CO_2 (3.0 - 4.0%) was monitored continuously. An infusion of 5% glucose in Ringer acetate (pH = 7.0) at a rate of 40 - 80 µl/min, was administered via the right jugular vein. Mean arterial blood pressure (65 - 140 mm Hg) was monitored continuously in the right carotid artery. Core temperature was maintained between 36.5 and 38.5°C using a thermostatically controlled, feedback-regulated heating system. Local infiltration of 3.5 mg/ml lidocaine *(Xylocaine)* with 2.2 µg/ml adrenaline was used to reduce nociceptive input during surgery. A craniotomy was performed, and the brain rostral to the inferior colliculus was removed. The anaesthesia was then discontinued. A laminectomy of the tenth thoracic vertebrae was carried out and the rat was spinalized using a pair of fine scissors. In experiments using pharmacological interventions, a laminectomy of the thirteenth thoracic and first and second lumbar vertebrae was made and the underlying meninges were removed. An agar pool with artificial cerebrospinal fluid (aCFS) (Edwards et al., 1989) over the lumbar cord was then created. Experiments were terminated upon signs of deterioration such as a precipitous drop in blood pressure or in expiratory CO_2-level. After termination of the experiments, the animals were given a lethal dose of halothane.

Electrophysiology (II, III)

Cutaneous stimulation

Nociceptive stimulation was performed either with CO_2-laser (beam diameter 3 mm, intensity 5W pulse duration 12-28 ms resulting in an estimated T_{max}~43-64 °C at a depth of 100 µm (Haimi-Cohen et al., 1983) or by intracutaneous cathodal electrical stimulation using fine steel needle electrodes (n = 16, see below). No visible damage of the skin or marked changes in response properties at the stimulation sites was detectable at these intensities.

Electromyographic recordings

The needles used for skin stimulation and electromyographic recordings were electrolytically pointed and insulated with a varnish coating except for about 40 µm and 80 µm, respectively, at the tip. A small metal plate, used as anode, was placed subcutaneously well outside the stimulation area. For electromyographic recordings, a small opening was made in the skin overlying the muscle, and the same type of electrode that was used for intracutaneous stimulation (isolated except for about 80 µm at the tip) was inserted into the mid-region of each muscle belly. A reference electrode was inserted in an adjacent skin flap. Generally, the electromyographic activity in two to four hindlimb muscles was recorded simultaneously in each experiment.

Signal analysis

A computerized system for the rapid mapping of receptive fields was developed (see paper II). The method, termed receptive field imaging (RFI), is highly flexible and can consequently be useful in a wide range of applications that usually require multi-channel stimulation/registration and data processing. RFI uses random stimulation of multiple sites, in combination with an averaging procedure, to extract the relative contribution from each of the stimulated sites. The ability to quickly scan, in parallel, the receptive fields of several reflex pathways involved in the withdrawal reflex

enables the user to follow experimentally induced changes with a temporal resolution of minutes. The same 16 stimulation sites were used in all experiments. Automated stimulation and recording, with spike detection and counting, were performed on-line by the RFI programme. Parameters used for spike detection and counting based on the raw data sweeps presented in the *Sweeps* window were set manually. The mean number of classified spikes generated from each stimulation site was displayed simultaneously, superimposed on the imported map of the stimulated region to enable on-line analysis.

Topographical representation of receptive fields

From the measured response map, 36x76-matrices were computed (intermediate sites were interpolated by spatial low-pass filtering). From these matrices, in turn, contour maps were constructed and plotted on the paw surface (using Surfer software from Golden Inc.).

Pharmacological interventions (II, III)

The drugs were applied topically on the spinal cord. Before and after drug administration, the exposed lumbar spinal cord was kept in a bath of aCSF. The aCSF was then removed by gentle suction with a syringe and the drugs were administered. (II) Morphine (Kabi Pharmacia) was diluted to 1 mg/ml, and naloxone (Du Pont Pharmaceuticals Ltd) to 0.2 mg/ml in artificial cerebrospinal fluid at pH = 7.3 and (III) *6-nitro-7-sulfamoylbenzo(f)quinoxaline-2,3-dione (*NBQX) (1-7 µg) or *D-2-amino-5-phosphonovalerate* (AP-5) (0.1-1 µg) were diluted in 100µl aCSF and titrated to pH 7.4 before administration into the bath. The effect of the drug was monitored through receptive field mapping, using RFI, every third minute for approximately ½ h. After this period the drug was gradually washed out with aCSF.

Results and comments

Simulation of motor-directed somatosensory imprinting (MDSI)

To test the plausibility and clarify the implications of a self-organizing learning principle based on movement patterns of individual modules we set out to construct a computer model of the reflex adaptation (I). If this model 'behaved' according to known experimental data with respect to timing and character of reflex patterns during the adaptation process this would considerably strengthen the hypothesis. Further, certain testable predictions, such as the sensitivity to perturbations during reflex adaptations may be possible to make.

The complexity of the withdrawal task has led several researches to assume that only a distributed multilayer network could accomplish this type of sensorimotor transformation (Pouget and Snyder, 2000;Lockery and Sejnowski, 1993;Lockery and Sejnowski, 1992). However, as the nociceptive withdrawal reflex system has a modular organization, in which each withdrawal reflex module acts primarily on one muscle a more feasible learning principle would be that each module is self-organizing and learns about its withdrawal efficiency by probing the tactile feedback that arises from spontaneous activity in its reflex interneurons, activating the associated principal muscle. The temporal correlation between the initial activity in the reflex interneurons triggering the movement and the ensuing tactile feedback would allow a reversed type of Hebbian learning (with the postsynaptic activity in reflex interneurons preceding the afferent input). We have termed this unsupervised learning principle motor-directed somatosensory imprinting (MDSI). In the simulation, the network organization consisted of an input layer of interneurones in substantia gelatinosa that receives nociceptive and tactile input from the same skin area, and an output layer of deep dorsal-horn neurons encoding the strength of the withdrawal reflex, termed reflex encoders, that project to the motor neurons (Fig. 1). This very simple network architecture is similar to a classical Rosenblatt perceptron (which linearly combines synaptic inputs and fires if the sum is above threshold) (Rosenblatt, 1958). A learning rule deduced from Hebb's postulate but with the addition of a synaptic scaling factor that readjusts synaptic weights gradually when uncorrelated postsynaptic activity is present was used - Oja's rule (Oja, 1982) (see Methods). Thus, Oja's rule is self-normalizing, a property that intuitively seems reasonable; a redistribution of material due to limited resources (Miller, 1996), but which recently also has gained experimental support (Turrigiano et al., 1998). In view of this, it is worth noting that, in MDSI, it is the relative amplitude rather than the absolute amplitude of the tactile feedback that determines the change in connection strengths. That is, connections from skin areas from which stronger tactile input is received after a twitch will weaken compared with those from skin areas with less tactile input.

However, the type of central activity modulation caused by the tactile feedback specifies the type of learning (strengthening or depression of connections) that will generate adequate weight distributions. For the data shown in Fig. 3, inhibitory feedback connections of tactile input to the substantia gelatinosa-cells (as shown in Fig. 1) were modelled (Cervero and Iggo, 1980). If tactile input instead exerts excitatory effects on the substantia gelatinosa-cells (Nakatsuka et al., 2000) then anti-Ojan learning (substituting η with $-\eta$ in Oja's rule) yields similar relative adult weight distributions and comparable learning curves.

In the simulation, correlations between synaptic weight-maps of the developing receptive fields and the movement pattern of each muscle were calculated at different time points during the adaptation process (Fig. 3a). The learning curves for the six modules

that were obtained from the simulation were similar to previous experimental data (Fig. 2). Simulated (MDSI) and experimental (receptive fields and movement matrices) adult correlations, were respectively: r_{EDL23}=0.94/0.91, r_{EDL45}=0.93/0.95, r_G=0.85/0.95, r_{PB}=0.94/0.88, r_{PL}=0.93/0.91, r_{TA}=0.93/0.90. By contrast, unsupervised feedforward learning based on the same input patterns and Oja's rule did not result in an adequate reflex adaptation (correlations <0.4) (Fig. 3a). The model was relatively insensitive to the choice of parameter values (such as twitching probability, learning rate, sensory noise level and initial synaptic weights). As learning only occurs in conjunction with twitches, activity in other motor systems has a minor effect. For the same reason a fairly high learning rate and a noise level with an input many times stronger than that caused by the twitches still results in adequate tuning in the simulation. Also the problem of co-activation was small, as the modules were co-active with different muscles allowing the module to rather quickly sort out the pattern of its own muscle.

An en passant observation in the simulations was that if the pattern of tactile feedback was reversed for a period, the effect of this aberrant feedback had a stronger impact on reflex adaptation (a deterioration) than normal feedback under the same period. From this observation we therefore predict a stronger effect of aberrant input than of normal during reflex adaptation.

Summing up the outcomes of the simulation, we conclude that the agreement between the simulation of MDSI and earlier experiments indicate that our model has a high validity. All aspects of adaptation found in the simulation have also been established experimentally, and besides that, we were able to make an experimentally testable prediction regarding a differential effect of aberrant and normal feedback on the adaptation process.

The role of spontaneous movements – behavioural studies

The theory of motor-directed somatosensory imprinting ascribes a critical function to spontaneous movements in the developmental organization of reflex systems. We therefore firstly, analysed twitching behaviour during sleep to expand on certain aspects that had not been described previously in the literature and secondly, evaluated the role of spontaneous muscle twitches during sleep for spinal self-organization experimentally (I). In the examination of sleeping behaviour two features of the spontaneous motility were analysed in particular: 1) the position of the hind paws during sleep and 2) the percentage of total number of twitches that are dominated by single muscle contractions. Firstly, the reason the position during sleep had to be evaluated is that the receptive fields of nociceptive withdrawal reflexes of the hind limb are clearly adapted to the unloading pattern of the hind paw in a low tonus standing position (the movement patterns used in the simulation (Schouenborg and Weng, 1994) were obtained in anaesthetized rats). Hence, the typical position during sleep should be standing-like for the twitches to generate a similar type of unloading. It was confirmed that the plantar side of the hind paw in sleeping rat pups is usually (~92% of the time) in contact with, or in proximity to, the ground during early postnatal development (P5-P17). Secondly, a potential problem to the model would be if muscle twitches only occur in synergies, in that case the single modules would not be able to sort out the movement patterns arising due to the muscle activity in their own muscle (unless many different muscle combinations were present in the synergies). For this reason the quantification of single muscle twitches was conducted. It was shown that about 20 % of the spontaneous hindlimb movements during the time of peak twitching are dominated by single muscle contractions. The remaining spontaneous movements consist of other and

more complex movement patterns such as quivering and startles (SI(I)) .
Hence, during sleep the hind paw twitches are usually performed in a standing-like position and muscle twitches dominated by single muscle contractions are present. These findings strongly suggest that the MDSI during sleep is a plausible phenomenon.

To directly prove the role of spontaneous muscle twitches during sleep for nociceptive withdrawal reflex adaptation, behavioural experiments in which artificial sensory feedback was given shortly after spontaneous muscle twitches were performed. An automatic imaging system that triggered air-puff stimulation was developed. This stimulation was directed to either side of the tail upon detection of isolated, rapid tail movements. Before training began, the animals were randomized to either air-puff stimulation from the side of the set-up that the tail was moving towards or from the side it was moving away from. Because an adequate nociceptive withdrawal reflex can cause an increased input from the skin area moving towards external objects but should not cause an increased input from the skin area withdrawn, these two air-puff stimulations are referred to as 'normal' and 'aberrant', respectively (Fig. 4a). Rats were trained in the system for 2h a day during the period when adequate tail nociceptive withdrawal reflexes are normally learned (P12–P17) (Waldenström et al., 2001). The effect of training was examined by comparing the error rate of nociceptive withdrawal reflex responses before and after each training session. Reflexes were elicited by cutaneous nociceptive CO_2-laser stimulation of either side of the tail in the horizontal plane. Reflex movements towards the laser source were classified as erroneous. After aberrant air-puff conditioning ($n=895 \pm 493$) the mean nociceptive withdrawal reflex error rate was increased by 9.2% per training session ($P<0.01$) whereas normal air-puff stimulation ($n=1032 \pm 593$) had no significant effect (Fig. 4b) (however if the change in error rate was adjusted to account for the amount of feedback the animals had received there was a tendency for normal air-puff stimulation to reduce the error rate, as predicted by the findings in the simulation). Uncorrelated air-puff stimulation ($n=1159 \pm 833$) had no effect on reflex adaptation. These results demonstrate that this rather short training period was enough to induce a clear difference in reflex adaptation in young animals, given that they received the feedback in direct connection to the spontaneous twitches.
This is the first time that a direct functional role of spontaneous movements, corresponding to human foetal movements, for the maturation of the CNS has been demonstrated.

Receptive field imaging
A crucial step towards an understanding of the effects of different drugs on pain pathways, as well as how these neuronal circuits participate in signal transmission of pain, is to develop techniques allowing observation of interactions in real time. In the nociceptive withdrawal system a less time consuming mapping procedure would enable such a rapid analysis of receptive field changes during various treatments. With this in mind we decided to develop a computerized method with the aim of achieving rapid mapping of multiple receptive fields and their respective sensitivity distributions (II). This method was termed receptive field imaging (RFI). RFI uses random stimulation of multiple sites, in combination with an averaging procedure, to extract the relative contribution from each of the stimulated sites. Automated multi-electrode stimulation and recording, with spike detection and counting, are performed on-line by the RFI programme. A series of imaging experiments was carried out to evaluate the functional capacity of the system in the nociceptive withdrawal reflex system of adult rats.
The trail-to-trail variance proved to be small enough to yield a stable representation of the receptive field, thereby achieving a high

sensitivity in dynamic imaging experiments. The large number of stimulation (n=32) and registration (n=60) sites that can be monitored in parallel permits detailed network analysis of up to 32x60 different connections of the reflex pathway. RFI replicated the results obtained with conventional methods and allowed the display of receptive field dynamics induced by topical spinal cord application of morphine and naloxone on a minute-to-minute time scale (Fig. 5). In this perspective, it is worth pointing out that this technique could be a useful complement in the development of pharmaceutical compounds aimed at pain relief. Perhaps even more so, if transmission to cortex and other supra-spinal structures are measured instead of reflex responses.

Differences between strong and weak connections

The preserved differences between strong and weak connections after the establishment of the memory trace should be possible to determine in electrophysiological terms and perhaps even with respect to certain receptors involved using pharmacological techniques in the adult animal. We carried out a large study involving many thousand receptive field mappings in order to properly characterize the electrophysiological properties of the mature and functionally adapted nociceptive withdrawal reflex system (III) using the RFI system (II). To study the input-output characteristics of the circuit, the reflex gain for individual skin sites within the receptive fields was mapped by applying CO_2-laser stimulation with different pulse duration. The electromyographic responses from three hind limb muscles were mapped.

Responses for sites within the receptive field increased proportionally with increasing stimulation intensity until a plateau was reached. For every site within the receptive field, a specific and constant factor of amplification was found for the entire stimulation range, referred to as the *gain* of the connection (Fig. 6). No distinct differences were found between response thresholds of strong and weak connections. The main trait defining the connection strength is consequently the gain of the connection. This finding adheres closely to the concept of synaptic weight in artificial neuronal networks (Haykin, 2002).

It was also shown that the onset latency differences for different sites within the receptive fields have a distinct spatial distribution very much akin to the response amplitude distribution of the same muscle (Fig. 7). Hence, a temporal code is present where the latency to the first action potential in principle can inform about which skin site that has been stimulated, although this does not imply that this information is actually used. When performing multiple mappings of the same receptive field a measure of the trail–to-trail variability can be obtained. The variance increased almost linearly with the mean response amplitude of the different sites and not in a squared manner as would be expected if just taking the gain difference into consideration. This could be taken as an indication of a greater number of input pathways participating in the stronger pathways (Shadlen and Newsome, 1998).

Alternatively, there could be qualitative differences of connections in different pathways leading to different post-synaptic responses in response to pre-synaptic activation. One such difference could be the type of receptors that are present in the different synapses.

During the last years, a number of researchers (Hayashi et al., 2000;Shi et al., 1999;Carroll et al., 1999;Heynen et al., 2000;Luthi et al., 1999) have advocated a molecular explanation for LTP/LTD based on the insertion and withdrawal of AMPA-channels into/out of the postsynaptic plasma membrane. Perhaps synapses in the stronger connections of the nociceptive withdrawal reflex circuitry have been boosted by LTP during developmental tuning and have kept a higher number of AMPA-receptors in the membrane ever since, mediating the more efficient transmission (or analogously, a

lower number of AMPA-receptors in weaker connections after LTD).

Not many reports have shown a similar mechanism for NMDA-receptors (Grosshans et al., 2002). However, in studies of spontaneous excitatory postsynaptic potentials in single cells some findings indicate that AMPA- and NMDA-receptors are in the long run somehow co-regulated so that the ratio of these receptor types is more or less constant in all synapses (Umemiya et al., 1999;Watt et al., 2000). To test weather differences in receptor populations could explain the differences between strong and weak synapses in the nociceptive withdrawal reflex system we tried applying selective AMPA/NMDA antagonists while mapping the receptive field every third minute (Fig. 5).

The NMDA-antagonist (AP-5) caused a response decrease almost exactly proportional to the strength of the connections for all sites, whereas AMPA-antagonist (NBQX) application resulted in an absolute decrease of responses regardless of site (weaker sites stopped responding) and only a moderate proportional decrease (Fig. 8D-G). We also ran a computer simulation in a greatly simplified model (see Methods), comparing the effect of the antagonists for different proportions of the two receptor types in weak and strong synapses (Fig. 8A-C).

Comparing the experimental with the theoretical results, there appears to be no major difference with regard to receptor populations (only a slightly higher percentage of NMDA-receptors in stronger connections would fit the experimental data). The main difference between connections instead may be the number and/or size of their synapses, while the AMPA to NMDA-receptor ratio is essentially the same in all synapses.

Clearly, the strength of a connection is directly related to the number of NMDA-receptors, so the role of NMDA-receptors is in a sense to set the gain of the connection, whether this is done in single synapses or just reflecting a greater number of connections remain to be sorted out.

General discussion

A key issue in neuroscience is how the CNS is adapted to the constitution of the body during development. This problem is formidable in view of the complex biomechanics and body shape of mammals. Moreover, body constitution changes during normal life and after injury. In this thesis, it is shown that one highly effective strategy whereby this adaptation occurs is by learning from the sensory input that arises on spontaneous movements generated by endogenous activity in motor circuits. This learning principle is termed motor-directed somatosensory imprinting, MDSI, to emphasize that motor activity guides the somatosensory imprint on the CNS. Importantly, MDSI is demonstrated in one of the most basic neuronal circuits. Since a variety of different types of spontaneous movements are elicited during early development (see SI(I) and (Prechtl, 1974)) concomitant with the functional adaptation of motor systems, it is conceivable that also sensorimotor transformation in other more complex motor systems are functionally adapted by similar mechanisms. Understanding the formation of this relatively simple reflex system may thus be an important step towards a more complete description of how behaviour is changed by learning and memory. In the second part of the thesis, an attempt was made to clarify the nature of the resulting imprint. Through the development of a new technical system (RFI) it became possible to study the expression of the imprinted memory trace. Some of the physiological differences between connections of different strength, constituting the memory, are described. The implications of these findings will be discussed below.

What MDSI can tell us about neuronal representations

Given that MDSI proves to be a general mechanism for functional adaptation of sensorimotor transformation, it would follow that sensory representations in motor systems are based on movement patterns. This would be highly advantageous as it immediately solves the problem of translating sensory to motor coordinates (Pouget and Snyder, 2000), the transformation is already made by the weighted connections. This hypothesis is strengthened by the recent finding that olivo-cerebellar climbing fibres projecting to the C1, C3 and Y zones in the cerebellum have receptive fields with weight distributions identical to those in the spinal nociceptive withdrawal reflex system (Garwicz et al., 2002).

If, as has been suggested before (Schouenborg and Weng, 1994), the reflex modules are used as building bricks by higher motor systems, deviations from the expected sensory input is weighted in relation to the expected function of the modules. Sensory feedback would thus only have a corrective effect when it deviates from normal.

It should be pointed out in this context, however, that the coding of motor actions is a complex problem; as a matter of fact even when faced with the same experimental data different researchers sometimes come to different conclusions regarding the internal code of motor actions. For primary motor cortex, for example, a classification of neurones as being related to the direction, position, velocity or acceleration of a limb has been used (based on how much of the variance in the firing frequency that can explain the variance in one of the kinematic entities). The interpretation of such measures is problematic when also taking forces into account (Todorov, 2000a;Todorov, 2000b). If MDSI turns out to be a common principle for the organization of sensorimotor circuits one should, with knowledge of the movement patterns displayed by a given motor system, fairly easily be able to read out the neuronal

code of sensory and motor representations in this system, written in the 'alphabet' of movements.

Sleep and dreaming – a self-testing procedure?

Almost a third of our lives we are engaged in a behaviour about which we know nearly nothing. The functional meaning of sleep and dreams have always been a source of fascination but in spite of their clear necessity for our well-being and survival no direct evidence pinpointing the physiological reason for this has been presented. Different hypotheses have suggested roles in brain thermoregulation, brain detoxification and tissue restoration (Maquet, 2001). More recently the importance of sleep for learning and memory has been a subject of particular interest. The working-hypothesis being that a memory trace remains weak until sleep processes consolidate it during a following sleep period. Different studies have shown that sleep does indeed play a role in learning and memory processing in for example procedural learning of a visual discrimination task (subjects do significantly better after a sleeping period) (Gais et al., 2000), but also during development of the visual cortex (sleep enhances plasticity) (Frank et al., 2001) and likely in sensorimotor vocal learning in songbirds (internal rehearsal of motor output and predicted sensory feedback during sleep has been suggested) (Dave and Margoliash, 2000). In these examples of both developmental and adult plasticity, the effect of previous learning seems to be manifested during the following sleeping period. Hence, there may be a relation between learning and memory in the adult and experience dependent tuning during development also with regard to the functional importance of sleep.

It is not clear how the brain's state influences the learning in the nociceptive withdrawal system, but as neonatal spinalization interferes with reflex adaptation, notwithstanding that the connections from the brain and brain stem are not yet appropriately established, it is probable that supra-spinal structures act permissively, rather than instructively. As the twitches are predominantly displayed during REM-like sleep (Karlsson and Blumberg, 2002), it is possible that disinhibition or excitatory input from brain stem nuclei (Blumberg and Lucas, 1994) may be partly responsible for generating the twitches and perhaps thereby also putting the spinal cord into a 'learning mode'. It is tempting to speculate that processes where test pulses are used to probe responses of various neuronal systems are ongoing ubiquitously and are instrumental in memory consolidation in the brain during sleep. The spontaneous activity, triggering MDSI, is in that perspective only one reflection of this self-testing process (Blumberg and Lucas, 1996).

Cellular mechanisms underlying MDSI

A central part of MDSI is that modules switch between "learning" and "transmission" mode. Hence, learning would only occur in conjunction with activity in the module itself, limiting contamination from activity in other modules. By switching to "learning mode" concomitant with initiating a movement, a simple perceptron based network architecture is able to self-organize despite being trained with a complex repertoire of rather similar movement patterns in a noisy environment. In, for example, feedforward learning this would not be possible - only linearly separable input patterns can be classified under such circumstances (Haykin, 2002). Nevertheless, although MDSI is distinctly different from previously described forms of learning mechanisms, such as habituation, sensitization, error-correction, unsupervised feedforward and associative learning, it may well share cellular or molecular mechanisms with these other forms of learning.

Depending on organization of the reflex circuitry (which is not fully known at present), the suggested relationship of the pre- and post-synaptic activity in the substantia gelatinosa to reflex encoder

synaptic connections is likely to provide clues as to which type of synaptic modifications that follows (see also(Abbott and Nelson, 2000)). If tactile input onto substantia gelatinosa neurons is forwarded by inhibitory or excitatory pathways (see Results), LTP- or LTD-like mechanisms, respectively, would generate similar relative weight distributions. However, the somatosensory imprint on the withdrawal circuits appears to be more stable (total sensory deprivation in an adult for a week does not change the reflex patterns, Waldenström et al, *in press*) than what has usually been described for the changes induced by LTP and LTD induction. It is thus conceivable that the product of MDSI - the imprint - is further consolidated. Such consolidation could result in altered number of synaptic connections (III) or other structural changes at the synaptic level. As mentioned in the introduction such changes are known to underly the changes in connection strengths during sensitization and habituation in the withdrawal reflex system in *Aplysia* (Milner et al., 1998). The alternative possibility that differences in the composition of glutamate receptors (for example the ratio of AMPA to NMDA receptors) underly different connection strengths appears less likely in view of the present result which suggest a relatively constant AMPA to NMDA receptor ratio in strong and weak connections.

Clinical implications

1) Spontaneous movements as diagnostic tools. Even though spontaneous movements previously have been used to assess the status of the foetus, the possible functional role of these movements in the maturation of the central nervous system has not been understood. The importance of these movements in MDSI indicates that more attention should be paid to the movement repertoire in neonatal care of pre-term infants and that further diagnostic tools in foetal medicine may be at hands (Cioni et al., 1997).

2) Can MDSI be used to mend the injured spinal cord? Mending an injured spinal cord is a daunting task in view of the number of systems present, each performing detailed sensorimotor transformations, and the numerous ascending and descending pathways that need to connect precisely with the circuitries. Recently, progress has been made in molecular biology rising hope that regeneration in the spinal cord after injury may in fact be possible within a few years. Unfortunately, it is presumably not enough to trick nerve fibres into growing across an injury zone; they also have to find appropriate targets. This problem has not yet been appropriately focused on (Nicholls and Saunders, 1996). The potential problems due to chaotic or imprecise regeneration or sprouting from adjacent intact fibres into the deafferented zone under growth promoting treatment (Raineteau et al., 2002) are also yet to be studied. Therefore, even if a combination of tissue bridging, stem-cell implants and pharmacology will be in clinical use within a reasonably near future this will most likely not mean that the functions of every day life for spinal cord injured patients will be restored satisfactorily. By studying the normally occurring functional adaptation during development (MDSI), the required gene expression and physiological mechanisms that guide the construction of motor circuits can be revealed. It may then be possible to repeat this scenario in the adult to functionally adjust the connection weights and eliminate incorrect synapses from an initially chaotic mesh of connections. It is thus conceivable that the discovered principles for functional tuning during development will reveal new therapeutic possibilities to train regenerated connections in injured patients thereby restoring function.

3) Neural prostheses and artificial limbs
In cases where regeneration is not possible, neural prostheses – devices used to stimulate

nerves to restore functions lost after neural damage – could help bridging the signal gap after a spinal transection (Prochazka et al., 2001) but this requires an understanding of the basic code of the descending motor commands (see above). This is also true for amputees that are in need of functional limb prostheses, for example upper-limb prostheses with a controllable hand. Ideally, signals from the nerve stump should be used to directly control hand movements (Dahlin et al., 2000). In this latter group, a limited number of neuronal connections must be used to control the prosthesis, and to convey the sensory feedback to the CNS. This potential problem might be partially overcome by introducing a computational unit responsible for the fine-tuning of movements. By registering the outgoing motor commands via motor nerves and the sensory feedback from the prosthesis while executing movements the unit could be trained to make the prosthesis follow the intentions of the patient better (see e.g. www.lucs.lu.se/ArtHand/). In any case, a sensorimotor transformation has to be made by the computational unit following all movements to assure a proper response of the prosthesis to sensory feedback when interacting with objects in the outer world. A natural approach to this problem is of course to try to mimic the calculations normally made by the spinal cord. In view of this, it is interesting to note that MDSI probably could prove very efficient as an adaptive paradigm also for artificial neural networks and the challenge of constructing an artificial hand could also have enormous importance for the field of robotics where the task of making machines able to use sensory information to manipulate objects is a key issue.

Summary

During development, information about the three-dimensional shape and mechanical properties of the body is laid down in the synaptic connectivity of sensorimotor systems through adaptive mechanisms. In spinal reflex systems, this enables the fast transformation of complex sensory information into adequate correction of movements. In this thesis, it is shown, in a computer simulation, that an unsupervised correlation-based learning mechanism, guided by spontaneous muscle twitches, can account for the functional adaptation of the nociceptive withdrawal reflex system (NWR). This new learning principle was termed Motor-Directed Somatosensory Imprinting (MDSI). MDSI proved to be a highly efficient mechanism, far better than conventional Hebbian feedforward learning, and to be relatively independent on such parameters as learning rate and noise level. By developing and using a new fast optical analysis system for detection and classification of spontaneous movements, it was demonstrated that tactile feedback resulting from spontaneous muscle twitches indeed modifies sensorimotor transformation in young rats in a predictable manner. This learning occurs during “active sleep” which is similar to REM sleep in adults, indicating a novel role for sleep in learning and memory.

A new analysis system for rapid imaging of receptive fields, termed RFI, was developed to characterize the differences between strong and weak connections in the NWR in adult rats. Connections of different strengths differed with regard to gain, onset latency and relative variability. These differences represent the preserved end product of MDSI. Neither the inhibitory input to NWR nor differences in glutamate receptors could explain the differences in strengths between strong and weak connections, although it became clear that NMDA receptors are important in setting the overall gain in nociceptive transmission.

In conclusion, this thesis demonstrates for the first time, that spontaneous twitches during sleep, corresponding to human foetal movements, play a key role in spinal self-organization and tentatively suggests that this learning results in structural changes, such as elimination or pruning of erroneous connections, in the spinal circuits. Since a variety of spontaneous movements are present concomitant with the maturation of motor systems, it is conceivable that spontaneous movements reflect a general mechanism whereby motor systems are functionally adapted during development.

Populärvetenskaplig sammanfattning

Till ryggmärgen kommer känselinformation om vad som händer med kroppen. Denna information använder vi sedan för att kunna korrigera våra rörelser. Många av dessa korrektioner utförs mer eller mindre självständigt av nervkretsar belägna i ryggmärgen. Att snabbt omvandla dessa känselsignaler till muskelaktivitet är oerhört komplicerat och beräkningskrävande eftersom kroppens anatomi och biomekanik är mycket komplex. Det verkar som nervsystemets lösning på detta problem är att gradvis justera kopplingarna i ryggmärgen under utvecklingen så att information om de olika motoriska systemens funktion lagras in som mönster i förbindelserna mellan nervcellerna.

I studierna i denna avhandling har vi undersökt hur inpräglingen går till och hur ett minnesspår i ryggmärgens kretsar sedan gestaltar sig hos den vuxne.

Vi visar för första gången att en självtestande inlärningsprocedur förkommer tidigt i livet då ryggmärgen förmår att själv organisera sig i ett antal funktionella enheter - moduler - som var och en styr aktiveringen av en enskild eller ett fåtal muskler. Inlärningsprocessen bygger på spontanaktivitet i de olika nätverken och yttrar sig i fosterrörelser. På så vis får de olika motoriska kretsarna sensorisk information om sin egen funktion och kan med hjälp av denna information finstämma sina egna kopplingar. För bortdragningsreflexerna, vårt modellsystem, leder detta till allt mer precisa rörelsemönster, och att varje muskel aktiveras just från det hudområde som den mest effektivt drar bort. Under adaptationsprocessen försvagas eller elimineras inkorrekta förbindelser medan korrekta blir gradvis starkare. Hela denna inlärningsprocess äger rum hos fostret/barnet medan det sover. Det verkar som den här typen av sömn liknar det som ibland kallas drömsömn (REM-sömn) hos vuxna. Kanske tyder denna upptäckt på att en viktig funktion med sömn och drömmar är just att etablera minnesspår och därigenom befästa de minnen som vi samlat på oss under dagen.

Vi har vidare studerat vilka egenskaper det etablerade minnet har hos den vuxne. För att förstå skillnaderna mellan starka och svaga förbindelser i bortdragningsreflexsystemet krävdes det att vi skapade ett system som snabbt och effektivt kan karaktärisera hur smärtsignalerna från huden omvandlas till rörelsemönster. Med hjälp av detta system har vi sedan kunnat studera kopplingarnas olika egenskaper i detalj. Det visade sig att betydande skillnader fanns med avseende på hur lång tid det tog för smärtsignalen att passera genom olika delar av smärtkretsen liksom hur mycket en signal förstärktes på vägen. Dessutom visade sig starka och svaga förbindelser ha olika grad av variabilitet i sin signaltransmission. Alla dessa faktorer utgör tillsammans de skillnader som karaktäriserar det minnesspår som lagrats för att möjliggöra adekvata motoriska svar på olika typer av smärtinflöde. I de farmakologiska experimenten gjordes ett fynd som kan tyda på att den gängse uppfattningen om vilka typer av jonkanaler som är viktiga för att ställa styrkan i de excitatoriska synapserna inte är korrekt. Två typer av jonkanaler är speciellt betydelsefulla för överföringen av aktiverande nervsignaler i dessa kopplingar, de benämns oftast med sina respektive förkortningar: AMPA och NMDA. Tvärtemot det vanliga synsättet så tycks det inte vara antalet AMPA-kanaler som avgör styrkan i en förbindelse utan antalet NMDA-kanaler. NMDA-kanaler har tidigare setts som viktiga för smärttransmissionen vid mycket kraftiga och upprepade smärtstimuleringar eller vid inflammatoriska tillstånd men inte vid normal fortledning av en smärtsignal. Våra fynd tyder på att NMDA-kanalerna i själva verket ställer förstärkningen i kretsen av en inkommande smärtsignal och därför är avgörande för hur kraftig den fortsatta responsen skall bli. Det

är inte möjligt att avgöra om dessa NMDA-kanaler är fördelade på ett större antal förbindelser, så att det i realiteten blir antalet synapser som faktiskt skiljer mellan starka och svaga förbindelser eller om de är samlade i större synapser. Däremot verkar andelen AMPA- och NMDA-kanaler i alla synapser vara ungefär den samma, ett fynd som också det skiljer sig ifrån vad man tidigare funnit då man studerat odlade celler eller uttagna skivor av hjärnvävnad där man på konstgjord väg inducerat förstärkningar eller försvagningar av enskilda synapser.

Betydelse: Genom att studera de underliggande cellulära mekanismerna för dessa förändringar är det möjligt att få viktig information om hur ryggmärgens kretsar formas. Denna forskning ger också viktig insikt i hur minnen bildas och lagras i centrala nervsystemet. Med kunskap om processerna bakom den funktionella adaptationen av ryggmärgen som sker naturligt under utvecklingen finns också, potentiellt, möjlighet att utnyttja liknande strategier både i försök att återställa funktioner i den skadade ryggmärgen men också för att reglera ner smärtsignaleringen i vissa nervkretsar vid kroniska smärttillstånd.

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