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## Synaptic Plasticity in Local Networks of Neocortical Layer 2/3

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To Jonah



## Abstract

The neocortex is a hierachal organ in which information processing takes on place on many levels, from subcellular signalling all the way to neural networks. Neocortical local neuronal networks (microcircuits), composed of interconnected neurons, form elementary information processing units within the cortex. Pyramidal cells, the primary glutamatergic cells in the cortex, receive synaptic input both from within the neocortex and from more distant cortical and sub-cortical regions. The strength of these inputs can be modified on various time scales.

The strength of pyramidal-pyramidal (P-P) cell unitary connections can be modified long-term, depending on the timing of action potentials (APs) in the pre- and post-synaptic cells (spike-timing dependent plasticity, STDP). We reported that the learning rule governing STDP modification is regulated by preceding activity in a postsynaptic neuron. Moreover, we have shown that the difference between STDP observed at layer 2/3 (L2/3) P-P cell connections and STDP studied at other excitatory connections in the neocortex is attributed to a fundamental difference in synaptic properties, suggesting that a L2/3 pyramidal cell is able to recognize its presynaptic partner and form physiologically distinct synapses based on the origin of input.

Additionally, the time-window for the induction phase of spike timing- dependent long-term potentiation (STD-LTP) and depression (LTD) at L2/3 P-P connections and its dependence on post-synaptic cell spine calcium concentrations was further examined using data-based computational modelling. We have shown that the resulting synaptic gain change depends on a 15 ms window following synaptic activation. Our data suggested a theoretical enzyme-like  $\text{Ca}^{2+}$  sensor that could account for the observed synaptic gain changes in L2/3 P-P connections.

Synaptic LTP is thought to be a crucial component underlying learning and memory. Neurodegenerative disorders, such as the Alzheimer's disease (AD) are commonly associated with cognitive impairment and memory loss. We reported that STD-LTP induction at excitatory inputs onto L2/3 pyramidal cells in a mouse model of Alzheimer's disease was impaired as early as at 3.5 months of age, at the very onset of AD-like pathology and prior to amyloid plaque formation. STD-LTP was also abolished at L2/3 P-P connections in wild-type brain slices after soluble non-fibrillar  $\text{A}\beta_{(25-35)}$  application. The underlying mechanism was the selective  $\text{A}\beta$ -induced reduction of AMPAR-mediated currents. Meanwhile, STD-LTP induction could be rescued by application of AMPAR desensitization antagonist, cyclothiazide. Thus, we have demonstrated a novel target for AD pathology as well as a means of rescuing STDP under AD's neurodegenerative conditions.

Synaptic plasticity consists of multiple variations in synaptic gain taking place over different time scales and between different cell types. In another instance, inhibitory connections from FSN interneuron onto the pyramidal cell can undergo short-term changes in synaptic gain following a postsynaptic AP burst. Previous studies suggested that retrograde dendritic release of glutamate regulates such short-term changes. We further clarified the molecular mechanism of retrograde signalling by showing the SAT2-mediated glutamine transport to be is a necessary precursor for retrograde signalling at FSN-pyramidal cell connections, substantiating the role of glutamate as a retrograde messenger at this synapse.

**Keywords:** Neocortical microcircuit, layer 2/3, pyramidal cell, spike timing- dependent plasticity, LTP, LTD, FSN interneuron, retrograde signalling, SAT2.

## List of Publications

This thesis is based on the following papers and manuscripts:

- I. Misha Zilberter\*, Carl Holmgren\*, Isaac Shemer, Gilad Silberberg, Sten Grillner, Tibor Harkany, and Yuri Zilberter (2008) *Input specificity and dependence of STDP on preceding postsynaptic activity at unitary connections between rat neocortical layer 2/3 pyramidal cells.* Cerebral Cortex (in press).
- II. Isaac Shemer\*, Misha Zilberter\*, Carl Holmgren, and Yuri Zilberter (2006) *Calcium dynamics within a short temporal window determines spike timing-dependent plasticity in synapses between layer 2/3 pyramidal cells.* (Manuscript).
- III. Isaac Shemer, Carl Holmgren, Rogier Min, Livia Fulöp, Misha Zilberter, Kyle M. Sousa, Tama's Farkas, Wolfgang Härtig, Botond Penke, Nail Burnashev, Heikki Tanila, Yuri Zilberter, and Tibor Harkany. *Non-fibrillar beta-amyloid abates spike-timing-dependent synaptic potentiation at excitatory synapses in layer 2/3 of the neocortex by targeting postsynaptic AMPA receptors.* Eur J Neurosci, 2006. 23(8): p. 2035-47.
- IV. Monica Jenstad\*, Abrar Z. Quazi\*, Misha Zilberter\*, Camilla Haglerød, Paul Berghuis, Navida Saddique, Michel Goiny, Doungjai Buntup, Svend Davanger, Finn-Mogens S. Haug, Carol A. Barnes, Bruce L. McNaughton, Ole Petter Ottersen, Jon Storm-Mathisen, Tibor Harkany, and Farrukh A. Chaudhry. *System A Transporter SAT2 Mediates Replenishment of Dendritic Glutamate Pools Controlling Retrograde Signaling by Glutamate.* Cerebral Cortex Advance Access published on October 1, 2008.  
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\* denotes equal contribution

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## List of Abbreviations

A $\beta$ <sub>(25-35)</sub> :	Amyloid beta 25-35 peptide fragment
AMPA(R):	$\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (receptor)
AP:	action potential
bAP:	back-propagating action potential
EPSP:	excitatory post-synaptic potential
FSN:	fast-spiking nonaccomodating interneuron
GABA(R):	gamma-aminobutyric acid (receptor)
IPSP:	inhibitory post-synaptic potential
L:	neocortical layer #
LTD:	Long Term Depression
LTP:	Long Term Potentiation
MeAIB:	$\alpha$ -methylamino-iso-butyric acid, a prototypic SAT inhibitor
mGluR	metabotropic glutamate receptor
NMDA(R):	N-methyl-D-aspartate (receptor)
P-P:	Pyramidal cell-Pyramidal cell (unitary connection)
SAT2:	system A transporter
STDP:	spike timing-dependent plasticity
VGCC:	voltage-gated calcium channel

# Introduction

Organisms inhabit environments in which they are presented with diverse stimuli. Throughout their development they must respond to changes in both their internal and external environments and this leads to a requirement for a flexible information processing system. Within the mammalian neocortex this system is based around pyramidal cells, the main cortical cell type thought to correspond to elementary integrative information processing units. These neurons receive information from a variety of cortical and sub-cortical regions, both local and distant. Once processed the pyramidal cell output is communicated in the form of action potential trains. However, the flow of information through the cortex is not unidirectional and feedback occurs at many different hierarchical (from synapses to systems) and temporal (from short-term negative feedback to long-term synaptic potentiation or depression) scales.

This thesis focuses on a few of the mechanisms *via* which the strength of synaptic inputs to a L2/3 pyramidal cell can be changed depending on neuronal activity in the microcircuits. Occurring on different time scales, from short-term changes in synaptic strength dependent purely on postsynaptic cell bursting, to long-term modifications in synaptic gain determined by both pre- and postsynaptic firing, these synaptic modifications ultimately influence information processing by neuronal networks.

The first three papers in thesis examine changes in the strength of excitatory unitary synaptic input onto pyramidal cells and how these changes are affected when specific synaptic inputs become functionally impaired in a transgenic model of Alzheimer's disease model. The final paper studies the involvement of retrograde signaling, through transporter-dependent dendritic glutamine release, in short-term inhibition of local GABAergic inputs onto pyramidal cells.

## The Neocortex

A striking feature of the neocortex is the presence of distinct cellular layers ranging from the sparsely populated layer 1 to the more densely populated layers 2-6. Meynert (1867-1868, 1869-1872 cited in E. Jones Ch1, Vol1) provided the first systematic descriptions of this neocortical layering, describing 5 cortical layers. A number of different schemes were provided by subsequent researchers, prior to the adoption of the modern 6-layer scheme by Brodmann and Vogt.

In this thesis neurons within L2/3 of the somatosensory and visual cortices are studied. L2/3 serves as the first major information processing layer for input arriving at a cortical region. Known as the association layer and populated by a high density of small pyramidal cells and a diverse population of interneurons, L2/3 handles a wide range of incoming information. L2/3 neurons receive strong synaptic

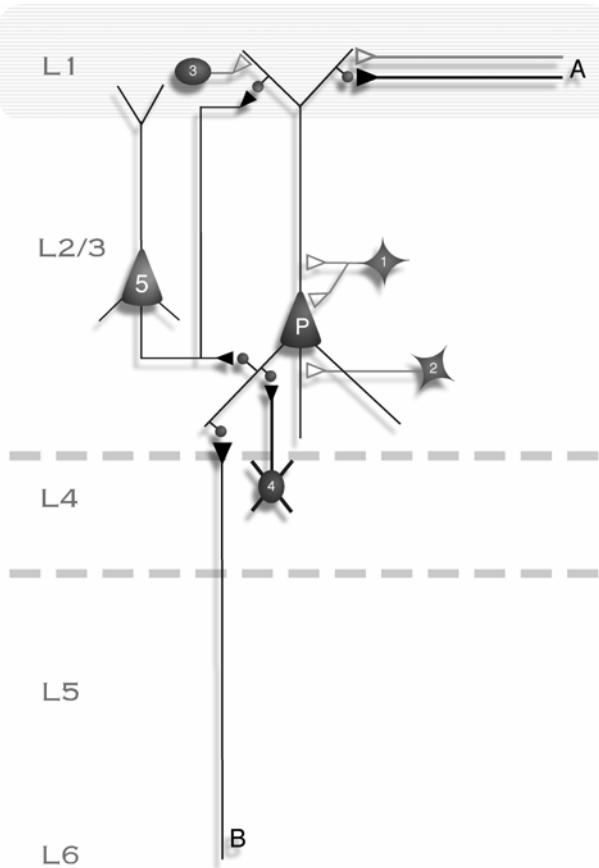
input from afferent fibers in L1 and from spiny stellate cells within L4. L1 contains afferent thalamic and cortico-cortical afferents together with projections from the basal forebrain and brainstem [1] [2].

## Pyramidal Cells

As their name implies, pyramidal cells generally have a distinctive somatic morphology. Their most distinctive feature is an apical dendrite projecting vertically towards layer 1, where it arborises extensively. This provides contact sites for afferent projections from intrinsic neurons within layer 1, in addition to the numerous afferent thalamic, cortico-cortical, basal forebrain, and brainstem projections which course through this layer. Around the base of the soma a spray of dendrites provides contact sites for synaptic inputs from local neurons, in addition to projections from spiny stellate cells within L4, L6 and thalamus. Additionally, local interneurons form synaptic connections on specific regions of the pyramidal cell, ranging from the axon to the apical tuft (Fig.1).

Locally, pyramidal cells form microcircuits with neighbouring pyramidal cells, with which they form 3-8 synaptic contacts (Paper I). These synapses invariably map onto the basal dendritic arbor, with some additional connections at oblique and apical dendrite segments provided by axon collaterals coursing towards L1 [3]. Pyramidal cells display regular spiking behaviour [4] in response to stepped depolarization from their resting potential. After an initial high frequency two-spike burst they show accommodation of firing (mediated by both voltage and calcium dependent K<sup>+</sup> channels [5] with increased interspike intervals, in response to steady state depolarizing current.

Our understanding of the pyramidal cell function has evolved considerably. Starting from a purely “integrate-and-fire” model, where the neuron plays the simple role of an integrator and the computation thus occurs on the larger network level, the focus has shifted back to the single neuron and its active computational capabilities. Neuronal dendrites are now known to exhibit a range of linear and non-linear properties that allows them to perform computations all of their own [6]. Interestingly, recent studies have reported a wide variation in the extent of dendritic arborization (complexity of dendritic architecture) of L2/3 pyramidal cells across different cortical areas and across different mammalian species [7, 8]. While it is not known what selects for such a diversity, a pyramidal cell with more extended dendritic architecture is presumably able to integrate and process a larger population of inputs, raising its computational capacity. A continuous gradient of increased dendritic complexity was shown to be in the direction from more vegetative areas to the more executive areas. For example, pyramidal cells in the prefrontal cortex have on average 23 times more dendritic spines (putative sites for excitatory input) than those located in primary visual cortex. (for review, see [9]).



**Fig.1 Synaptic inputs onto layer 2/3 pyramidal cells.**

Synaptic contacts form on (P) L2/3 pyramidal cell's (1) proximal dendrites and soma (eg. basket cells), (2) axon initial segment (chandelier cells), (3) distal dendrites (eg. intrinsic L1 cells). Excitatory synaptic input is provided by (4) L4 spiny stellate cells and (5) neighbouring L2/3 pyramidal cells. Afferent excitatory and inhibitory fibres from cortical and subcortical regions project via (A) L1 or (B) white matter.

## Neocortical local networks

Information processing within the brain occurs on a variety of levels, ranging from the molecular interactions to complex neuronal networks. Even though the cellular morphology has been shown to vary across cortical areas, the neocortex is considered by many to be primarily uniform in structure and composed of a repeated circuit [10-12]. Neocortical local networks (also called microcircuits), comprised of connected neuronal pairs, form the elementary processing units at the cellular level.

Examination of the neocortex shows neuronal populations in which dendrites and axons from different neurons are intertwined. This suggests that connectivity between any two neurons is likely to be random. However, studies suggest that despite first appearances, connectivity between neocortical neurons is in fact stereotyped [13, 14] [15, 16]. Presynaptic cell axons seem to target specific cell types, and even specific regions of those postsynaptic cells (Fig.1). Additionally, the

pyramidal cell population displays stereotypical connectivity with other pyramidal cells. Neighbouring L5 pyramidal cells form synaptic connections on basal or apical oblique dendrites, while distant pyramidal cells contact the distal dendrites [15]. Intralaminar connectivity repeats this pattern. Individual neuronal classes within L2/3 are targeted by specific laminar input [13]. L6 pyramidal cells project axons vertically, and select particular neurons in specific cortical layers, ignoring a host of post-synaptic elements on the way [17]. This laminar specificity can be maintained even in the face of disruption of normal laminar organisation, as in the case of reeler mutant mice [17]. Moreover, L2/3 pyramidal cells frequently contact L5 large tufted, bursting firing pyramidal cells, but ignore their smaller regular firing pyramidal cell neighbours [18] [19]. Connections within neocortical networks thus appear to be selective and frequently stereotyped. However, to what extent input selectivity is pre- or post-synaptically determined and what molecular mechanisms govern the information transfer is as yet unclear.

Exactly how larger networks are formed from their constituent microcircuits remains unclear. The supposed next organizational level from microcircuits is the microcolumn, where the local networks would assemble, via vertical connections, to form a columnar computational unit spanning all layers. While cortical microcolumn existence has been suggested by morphological studies [20], no electrophysiological substrate for their formation has yet been reported [21, 22]. Among other factors, such architecture would require directional connectivity preference, i.e. a neuron within a microcolumn should display a higher connectivity to its columnar partners and less connectivity to those neurons in the vicinity but outside the microcolumn. As a result, the neurons within a microcolumn should exhibit a higher correlation in firing. It has been suggested that synaptic connectivity in microcircuits is non-random and that stronger connections occur in clusters in L5 [23]. Nevertheless, the L5 thick-tufted pyramids within clusters that assemble the dendritic bundles have so far been shown to receive input that is no more correlated than that sent to non-clustered cells [22]. Furthermore, these cells exhibit no connectivity preference and no increased correlation in output [22]. In L2/3, connections between pyramidal cells were also shown to be weak and with no clear clusters [3]. Thus, the principle underlying the assembly of microcircuits into larger functional networks remains elusive.

As microcircuits process synaptic input both from their connected partners and from a diverse array of cortical and sub-cortical afferent synaptic inputs, pyramidal cells within these circuits face the challenging task of trying to discern small individual synaptic input drops on the background waterfall of hundreds and thousands of other active synapses. To enable them to do this a variety of mechanisms exist within the pre- and postsynaptic elements to modify synaptic strength. This allows pyramidal cells to attend or ignore signals dependent on the source of the signal and the context in which it is received.

Using the technique of dual-whole cell recording in cortical brain slices, this thesis focuses on a selected number of ways in which the strength of synaptic input to L2/3 pyramidal cells can be modified. The first papers in the thesis examine the learning rules and mechanisms underlying long-term changes in the strength of excitatory unitary synaptic input and how those mechanisms are affected when synaptic integrity is compromised. The final paper studies the role of glutamine and its dendritic transporter SAT2 as the substrate for the short-term synaptic depression at inhibitory connections onto L2/3 pyramidal cells.

## Synaptic Plasticity

### Long Term Synaptic Potentiation

It is hard to talk about memory and learning without mentioning the phenomenon called synaptic LTP, or Long Term Potentiation. Long-term potentiation (LTP) and depression (LTD) of synaptic strength have been the subject of intensive study for decades (reviewed in [24]). Long-term changes in synaptic strength are widely believed to at least partially underlie learning and memory [25, 26] as well as neural circuitry formation [27, 28]. Although first discovered in 1966 by Terje Lømo [29], the idea that such a mechanism must exist has been around long before. It has been accepted that the adult brain does not produce new neurons in a significant way, yet an adult is able to learn and form new memories; therefore the system must be able to encode long-lasting information using the available neuronal population. Cajal first proposed that memories must be formed as a result of strengthening connections between neurons [30]. Hebb built on that concept, stating in 1949 that

“...the persistence or repetition of a reverberatory activity (or “trace”) tends to induce lasting cellular changes that add to its stability.... 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 takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.”[31]

This hypothesis is now known as the Hebb's postulate, and the rule where the input that causes a post-synaptic cell to fire is potentiated is known as the Hebbian learning rule.

However, if the synapses could only undergo potentiation, the network would soon saturate. Therefore, a mechanism must exist for the opposing effect on synaptic change. Such a mechanism is known as LTD, or Long Term Depression. LTD in, e.g.

hippocampus is thought to be involved in clearing memory traces, just as LTP is thought to be required for their formation. In cerebellum, LTD is suggested to be involved in motor learning [32]. Often, LTP and LTD at the same synapse occur through different molecular pathways. However, one underlying deterministic signal that seems to be common to most types of LTP and LTD is synaptic  $\text{Ca}^{2+}$ . The direction of the change in synaptic strength depends on the timing and frequency of the input [33]. According to the Bienenstock, Cooper and Munro model (BCM model), low  $\text{Ca}^{2+}$  levels lead to LTD, whereas higher  $\text{Ca}^{2+}$  levels that cross a certain threshold lead to LTP [34].

LTP is divided into two phases: early (E-LTP) and late (L-LTP). The early phase, underlain by synaptic changes and on the scale of minutes and hours, is followed by the late phase that involves morphological changes that can become permanent, such as the formation of perforated synapses and the addition of new ones. However, such a mechanism is potentially important not only for memory, but for functional network formation – two cells whose connection is strengthened *via* LTP will exhibit more correlated firing, as the input from one cell to another is stronger and more likely to cause postsynaptic firing. Likewise, if LTD is induced, the connection becomes “less important” and the correlation will decrease. By “pruning” the connections through LTP and LTD, a particular network can be formed and become more efficient, increasing the input-to-noise ratio in information processing.

### **Spike timing- dependent plasticity**

Towards the end of the last decade, the means by which the strength of synaptic connections can be modified depending on the precise timing of pre- and post-synaptic cell spikes has been identified [35]. This mechanism, termed spike-timing dependent plasticity (STDP), has generated interest due to its possible implications *in vivo* [36-39]. STDP appears to be a widespread mechanism for the induction of long-term plasticity, operating at both excitatory and inhibitory connections. STDP at specific subsets of synapses has been observed in the neocortex, hippocampus, basal ganglia, and cerebellum [40].

Action potentials, generated at the axon initial segment [41] (or first node [42]), backpropagate into the neuronal dendrites [41]. These bAPs can evoke a transient increase in spine and dendritic  $\text{Ca}^{2+}$  concentration through activation of dendritic VGCCs and subsequent relief of NMDA channels from magnesium block [43-45]. If coincident with synaptic activation this can result in supra-linear summation of calcium, while activation of the synapse after bAP action results in sublinear calcium summation in the post-synaptic cell dendrites or spines[43-45]. Consequently LTP or LTD induction, both of which rely on post-synaptic  $\text{Ca}^{2+}$

elevation, can be induced depending on the precise order of pre- and post-synaptic cell firing.

By itself, this fulfils Hebb's criteria for the modification of synaptic strength as potentiation of an excitatory postsynaptic potential (EPSP) occurs if a presynaptic AP is accompanied by an increase in the probability of a postsynaptic AP during the period of association [40]. It also fulfils the additional criteria that synapses that did not participate in output generation and were only active after the post-synaptic cell had fired APs should be depressed [46]. However, to consider STDP induction as dependent purely on the order of spike timing might lead to oversimplification of the phenomenon actually taking place. Interestingly, LTP and LTD at different cortical excitatory synapses show different dependencies on the temporal order of pre- and post-synaptic cell firing, resulting in different learning rules at these sites (for review, see [47]). Additionally, anti-Hebbian learning rule, where pre-before-post timings induce LTD while post-before-pre timings induce LTP, has been found at the inhibitory inputs onto L2/3 pyramidal cells [3] as well as in the dorsal cochlear nucleus [48]. Some of the factors suggested to influence the plasticity outcome include the synapse's distance from the soma and dendritic bAP filtering.

bAPs, the associative signal for STDP [49, 50], show decremental propagation along pyramidal cell dendrites [41]. This could result in a situation in which distal synaptic contacts are in effect isolated from the soma [51]. The degree to which bAPs can travel through the dendritic tree depends on a number of active and passive dendritic properties. bAP amplitude decreases while their AP half-width increases as they propagate distally [41]. Neuronal morphology plays a key role in determining the decremental propagation of bAPs [52] but see [53].

It is no surprise, therefore, that excitatory connections in neocortex studied to date are reported to exhibit distance-dependent modification of learning rules. Excitatory inputs located distal to the cell soma are weakly influenced by bAPs, leading to a lack of supralinear  $\text{Ca}^{2+}$  summation through NMDA and VGCC channels. Such inputs, therefore, would require further conditions to be met for LTP to occur under standard spike coincidence timing. Synaptic cooperativity, where multiple excitatory inputs are activated simultaneously, leads to additional dendritic depolarization, helping remove the NMDAR's  $\text{Mg}^{2+}$  block and restoring LTP induction in L5 pyramidal cell synapses [54]. Another reported mechanism for overcoming the distance dependence for LTP is postsynaptic cell bursting. In this case, multiple bAPs at high frequency overcome the dendritic attenuation and are more effective in depolarizing distal synaptic sites. If the bAP burst frequency reaches a critical frequency,  $\text{Ca}^{2+}$  spikes are generated, in which the  $\text{Ca}^{2+}$  current becomes self-regenerating and can back-propagate into distal dendritic locations, again leading to membrane depolarization and restoration of LTP [55].

The ability of a cell to recognize its synaptic partner is paramount to efficient network formation and information processing. Altogether, the distance-dependent modification of STDP learning rules has been suggested to be a mechanism for input

specificity in cortical pyramidal cells. Activation of other signalling pathways, for instance metabotropic glutamate receptor (mGluR) [56, 57] and endocannabinoid signalling through CB1 cannabinoid receptors [58, 59] can also contribute to STDP induction, resulting in input-specific STDP (for review, see [47]).

Recent studies have shown that the cortex is a dynamic entity; previously potentiated synapses can be “de-potentiated” (weakened), depressed ones can be “de-depressed” (restored or re-potentiated) and synaptic connections continuously form and dissolve over a period of hours [60, 61]. It has been reported that STDP can play a role in long-term connectivity dynamics of the microcircuits, with newly formed and potentiated synapses being morphologically reinforced and weak ones dissolved altogether [60].

### Retrograde signalling and negative feedback at inhibitory synapses

Classically, synaptic information transfer at synapses has been viewed as a unidirectional process, from pre- to postsynaptic neuron. However, feedback is used to regulate the activity of numerous systems within the body, ranging from cardiac to endocrine. Consequently, it is perhaps not so surprising that communication at synaptic connections should turn out to be a dialogue between pre- and post-synaptic cells, rather than a pre-synaptic cell monologue. In fact retrograde signalling has been shown to occur at a number of sites throughout the brain [62].

This section will specifically look at one form of retrograde signalling, short-term negative feedback. Rapid negative feedback at inhibitory synaptic connections was shown to regulate the degree of inhibition of Purkinje cells [63] and CA1 pyramidal cells [64]. In both regions the action of retrograde signalling was expressed pre-synaptically. Induction required depolarisation of the post-synaptic membrane and elevation of post-synaptic  $\text{Ca}^{2+}$  levels. As IPSP amplitude could also be decreased by mGluR inhibition [65, 66], glutamate was suggested to be a candidate retrograde messenger. Other studies have however called into question the identity of the retrograde messenger at these sites. Endocannabinoids have been shown to act as retrograde messengers at hippocampal [67], cerebellar [68, 69] and neocortical [70, 71] synapses.

We proposed that when endocannabinoid signalling is irrelevant due to the lack of CB1 cannabinoid receptors at presynaptic terminals, glutamate may function as a retrograde messenger at fast-spiking interneuron-pyramidal cell inhibitory connections within the neocortex [72]. Glutamate is released via vesicular dendritic exocytosis in response to pyramidal cell depolarisation. It depresses IPSPs via pre-synaptic mGluR activation, and the consequent decreased calcium influx into the presynaptic terminal. What may be the functions of this short-term retrograde signalling? Retrograde signalling provides a means of regulating the moment-by-moment activity of the pre-synaptic cell [73]. This allows the post-synaptic cell to

rapidly decrease EPSP and IPSP amplitude at excitatory and inhibitory synaptic connections respectively. Pyramidal cells are frequently reciprocally connected to local interneurons [72, 74-77]. Therefore any activation of the pyramidal cell will increase excitability of the connected interneuron. Consequently, this could result in increased inhibition of pyramidal cell excitability. Retrograde signalling however allows the pyramidal cell to activate the reciprocally connected interneuron, without being subsequently “punished” by the increased interneuron activity. Decreased IPSP amplitude, induced by retrograde signalling, could also result in a change in back-propagating action potential flow through the dendritic tree [78, 79], thus affecting the STDP induction kinetics at synapses along the same dendritic branch.

Therefore, rapid retrograde signalling provides a means to control synaptic input, regulate synaptic plasticity, affect PSP summation and possibly regulate the oscillatory behaviour of the network.

## Summary

Neocortical local networks play a vital role the processing of information within the cortex. Their ability to regulate synaptic strength, by short-term feedback and STDP, allows them to selectively attend certain synaptic inputs, thus increasing the signal-to-noise ratio. Input specificity of synaptic connections further allows for increased flexibility of the larger network. Synaptic and dendritic processing by cortical pyramidal cells is thought to underlie information processing in the cortex. Therefore, the modifications of synapses even at their basic unitary level play an important role in cortical function – and studying the rules underlying such changes can help advance our understanding of how cortical networks form and operate.

## Aims

In this thesis my aims were:

- To study spike timing dependent plasticity at L2/3 pyramidal-pyramidal cell connections. In particular, the apparent difference with STDP at other cortical synapses as well as the mechanisms underlying such a variation; such as
  - Change of the synaptic learning rule dependant on the preceding postsynaptic activity and  $\text{Ca}^{2+}$  levels
  - Expression of LTP and LTD loci and their receptor dependence (Paper I)
- To elucidate the more complex role of synaptic/spine  $\text{Ca}^{2+}$  dynamics on determining the direction of synaptic gain change in STDP at L2/3 P-P connections (Paper II)
- To see how STDP at L2/3 P-P connections is affected by progressive synaptic impairment in an Alzheimer's disease model, at what stage, and the possible mechanisms of effect (Paper III)
- To investigate the role of glutamine and its transporter SAT2 in negative feedback plasticity at FSN-pyramidal cell synapses. Specifically, whether glutamine acts as a precursor to glutamate, a retrograde messenger at these inhibitory connections; as well as the role of dendritic glutamine transporter SAT2 in this process (Paper IV).

## Methods

The main technique used in this study was dual whole-cell recording of neocortical L2/3 pyramidal cells and FSN interneurons.

Specific details of methodologies can be found in the individual papers:

1. Neocortical slice preparation – Papers 1 and 3
2. Electrophysiological methods: Papers 1,3,4
3. STDP conditioning protocols: Papers 1 and 3
4. Morphometric analysis of pyramidal cell connections: Paper 1
5.  $\text{Ca}^{2+}$  imaging: Paper 1
6. Synaptic  $\text{Ca}^{2+}$  modelling and simulation: Paper 2

## Results and Discussion

### **Paper 1: Input specificity and STDP dependence on preceding postsynaptic activity at unitary connections between rat neocortical layer 2/3 pyramidal cells**

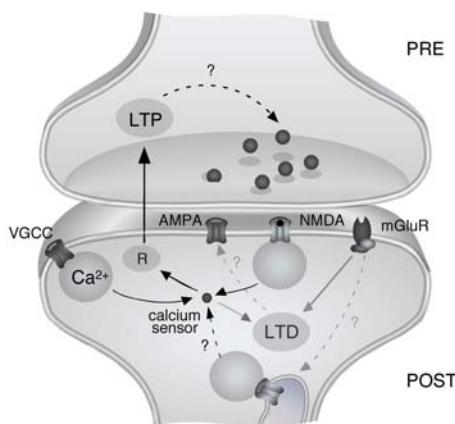
In this paper STDP at P-P cell connections in L2/3 was studied. We showed that L2/3 pyramidal cell unitary connections exhibit properties distinct from those at other cortical excitatory synapses. This variation is not due to distance-dependant modification of STDP learning rules, as suggested for other connections, where the determining factor is the dendritic filtering of bAPs. Rather, the synapses operating at these connections are proximal to the soma and functionally distinct, suggesting a mechanism for a single L2/3 pyramidal cell to identify its presynaptic partner and form corresponding synapses accordingly, even when the synaptic locations overlap between different input sources.

More specifically, we showed that STDP at L2/3 unitary connections operates in a symmetric and anti-Hebbian learning mode – i.e., in response to all “classic” LTP induction protocols, LTD is induced independent of spike timing. However, if the postsynaptic cell produces a burst of bAPs prior to synaptic activation, LTP can be induced as well as LTD, dependent on the EPSP’s timing in relation to a neighbouring post-synaptic AP in the burst. A preceding post-synaptic bAP burst, therefore, recovers the standard asymmetric Hebbian STDP mode at these synapses. The role of the burst turns out to be VGCC (more specifically, L-Type  $\text{Ca}^{2+}$  channel)-mediated  $\text{Ca}^{2+}$  influx in the period immediately prior to synaptic activation.

Another interesting detail of STDP induction at these particular synapses is if the single presynaptic spike is temporally embedded within the postsynaptic AP burst, the asymmetric Hebbian rule still holds, and with seemingly identical timing dependence in relation to the nearest bAP in the burst as in the case of a purely preceding burst paradigm. This could be physiologically relevant, as one cannot expect a clear “preceding burst – bAP” pairing to take place *in vivo*, where the adjacent neurons fire in temporally intertwined patterns.

The difference in L2/3 P-P connections’ STDP is underlain by loci of plasticity expression as well as by receptor dependence that differ from those at other excitatory synapses on L5 as well as L2/3 pyramidal cells as reported by other studies. In contrast to layer 5 pyramidal cells and non-specific excitatory inputs onto L2/3 pyramidal cells, LTD is NMDAR and CB1 cannabinoid receptor independent, expressed post-synaptically, and is mediated via activation of metabotropic glutamate receptors (mGluRs); LTP is mGluR-independent, NMDAR-dependent. The expression site of LTP is presynaptic, suggesting the release of a retrograde messenger from the postsynaptic dendrite. However, LTP proved to also be CB1 cannabinoid receptor -independent.

Overall, we reported a new form of STDP rule modulation at L2/3 P-P connections, one that is independent of dendritic filtering. The synapses at these connections are also physiologically distinct from neighbouring synaptic contacts, suggesting a novel input specificity mechanism not based on spatial specificity. Finally, the apparent requirement for postsynaptic activity before LTP can occur and symmetric LTD induction otherwise suggest a possible mechanism for dynamic, task-specific functional column formation in the neocortex.



**Fig. 2. Induction of LTP and LTD at L2/3 pyramidal-pyramidal cell unitary connections.**

Grey and black arrows indicate pathways involved in LTD, or LTP, respectively. Calcium from VGCCs is involved in both LTD and LTP induction. The  $\text{Ca}^{2+}$  sensor is indicated as a black sphere in the post-synaptic cell, while pre-synaptic spheres indicate vesicles.

## Future studies

While we have described the apparent STDP induction requirements at L2/3 P-P connections, the underlying molecular mechanisms remain unclear. The presynaptic locus of LTP expression suggests retrograde messenger release, one that is not endocannabinoid-related. What is the retrograde messenger that causes presynaptic potentiation? At the same time, LTD at these synapses is post-synaptic and mGluR-dependent. What is the mechanism of LTD induction downstream of mGluR activation? From the experimental data, it seems that LTP and LTD are mutually exclusive, i.e. if one is induced, the other is blocked. Does this suggest two different competing processes, or just one? How do the two processes coexist? What serves as the  $\text{Ca}^{2+}$  sensor (or sensors)? Answering these questions will require detailed future experiments involving and combination of electrophysiology, live cell imaging and molecular genetics.

## **Paper 2: Calcium Dynamics within a Short Temporal Window Determines Spike-timing-dependent Plasticity in Synapses between L2/3 Pyramidal Cells**

Using NEURON simulation environment and with published experimental data as the basis, we have constructed a computational model of a L2/3 pyramidal cell, including the dendrite as well as a dendritic spine. We then modelled spine  $\text{Ca}^{2+}$  dynamics that take place under different conditioning protocols used for induction of STDP. Using the experimental data reported in Paper 1, we have investigated how  $\text{Ca}^{2+}$  dynamics affect the direction of plasticity at L2/3 P-P synapses. From the moment of synaptic activation the level and kinetics of spine calcium determine the sign of synaptic plasticity, within a narrow time window of about 15 ms. Following this period further  $\text{Ca}^{2+}$  elevation does not influence the development of plasticity. In this study we show that STDP in unitary connections between L2/3 pyramidal cells displays several unique properties not addressed by Paper 1. In particular, that there is a lack of the stringent time window between LTD and LTP induction, previously observed in a variety of preparations. Modulation of synaptic gain correlates with spine  $\text{Ca}^{2+}$  dynamics within a narrow time window following synaptic activation. In addition, STDP in synapses constituting the same excitatory input may significantly differ depending on dendritic spine morphology.

## **Paper 3: Non-fibrillar $\beta$ -amyloid abates spike-timing-dependent synaptic potentiation at excitatory synapses in layer 2/3 of the neocortex by targeting postsynaptic AMPA receptors**

Alzheimer's disease, a progressive neurodegenerative disorder affecting 26.6 million people worldwide, is commonly associated with memory loss and cognitive deficits. According to the amyloid hypothesis, extracellular amyloid beta peptide (A $\beta$ ) deposits and abnormalities due to A $\beta$ -induced synaptic impairment are a fundamental cause of the disease. Two of the mechanisms that have been shown to be impaired under disease pathology are synaptic plasticity as well as neuronal communication in complex cortical neuronal networks. However, synaptic plasticity studied to date under neurodegenerative conditions involved only "conventional" LTP studies and not STDP [80-82]. We therefore set out to investigate whether STDP in L2/3 excitatory connections is impaired under experimental conditions recapitulating some major hallmarks of Alzheimer's disease. We found that STDP impairment already occurs at the onset of A $\beta$  plaque formation and advances with age in a mouse model of Alzheimer's disease. Pathogenic changes underlying STDP induction in this model are such that STDP cannot be evoked at the age of 7 months, coincident with robust plaque pathology. Our findings suggest that STDP is particularly sensitive in experimental conditions resembling those seen in Alzheimer's disease.

To achieve this, we used both a mouse model of Alzheimer's disease (APPswe/PS1dE9 mice that recapitulate critical features of the molecular pathogenesis of Alzheimer's disease) and acute application of soluble, non-fibrillar A $\beta$ <sub>(25–35)</sub> to neurons in rodent brain slice preparations. Pharmacological experiments were performed in acute rat and mouse brain slices. First, we showed that while STD-LTP at excitatory inputs to L2/3 pyramidal cells could be reliably induced using accepted induction protocols [36, 83] in wild-type mice, STD-LTP is impaired in APPswe/PS1dE9 mutants. The severity of this impairment increased with age but was already present at 3.5 months of age, prior to gross extracellular signs of A $\beta$  deposition (plaque formation). At 7 months of age, STDP-LTP was almost completely abolished. This LTP abatement was underlain by a reduced AMPA/NMDA ratio as measured by nucleated patch method in both wild-type and mutant mice L2/3 pyramidal cells. In addition, we found that acute application of soluble, non-fibrillar A $\beta$ <sub>(25–35)</sub> eliminated STD-LTP induction at L2/3 P-P cell connections when using an optimal induction protocol (Paper I). Again, the apparently reduced AMPA/NMDA ratio underpinned STDP failure. Quantitative experiments using nucleated patch-clamp recordings revealed that it is the AMPAR-mediated currents which are selectively reduced by acute, soluble A $\beta$  application.

Finally, we reported that application of cyclothiazide, an AMPAR desensitization inhibitor, rescues STD-LTP induction at non-specific excitatory inputs onto L2/3 pyramidal neurons.

## Future studies

While we have shown that non-fibrillar A $\beta$  targets postsynaptic AMPARs, the exact mechanism through which A $\beta$  pathology impairs STDP in L2/3 P-P connections remains unclear. Firstly, it is not known whether cyclothiazide rescues STD-LTP specifically in L2/3 P-P connections. Would fibrillar A $\beta$  species have similar effects? It is known that Ca<sup>2+</sup> homeostasis and kinetics are disturbed in Alzheimer's disease. As both slow and fast [Ca<sup>2+</sup>] changes were shown to play a role in STDP induction, the effect of A $\beta$  could be exerted through modifying these kinetics.

## Paper 4: System A Transporter SAT2 Mediates Replenishment of Dendritic Glutamate Pools Controlling Retrograde Signaling by Glutamate

Glutamate is an amino acid that is a prime excitatory neurotransmitter in the brain. Additionally, glutamate has been suggested to function as a retrograde messenger at inhibitory connections from FSNs to pyramidal cells, providing negative feedback to GABAergic transmission at these synapses [72, 84, 85]. However, unequivocal proof of such signalling has been missing.

Glutamine, a glutamate precursor, is recycled and regenerated by perisynaptic astroglia, where it is converted from glutamate. It is then recycled into the presynaptic terminals to be regenerated back into glutamate for synaptic transmission. To date, it remained unclear of the exact nature of the glutamate cycle involved in retrograde dendritic signalling.

In this paper, we reported that glutamine transporter SAT2 is primarily located in somatodendritic compartments of glutamatergic cells in many brain regions, including the neocortex. We also showed that the compartments containing SAT2 accumulate high levels of glutamine, which is readily converted to glutamate upon neuronal activity (induced by electrical stimulation *in vivo* and depolarization *in vitro*). Next we showed that the prototypic system A transporter inhibitor MeAIB ( $\alpha$ -methylamino-iso-butyric acid), which also inhibits SAT2 action, significantly reduces intracellular glutamate and glutamine levels, further supporting the role of SAT2 in glutamate formation.

Finally, using standard electrophysiological methods at synapses studied previously [72] we reported that SAT2 blockade by MeAIB, via inhibition of dendritic glutamine uptake and subsequent impairment of retrograde signalling by glutamate, blocks negative feedback at FSN-pyramidal cells inhibitory synapses in L2/3.

Overall, our study has further elucidated the nature of a metabolic glutamine/glutamate cycle in synaptic signalling, the role of glutamine and its transporter SAT2 and significantly strengthened the case for glutamate as the retrograde messenger at L2/3 FSN-pyramidal cell connections' negative feedback signalling.

## Future studies

To provide unequivocal proof that this mechanism indeed occurs under physiological conditions, experiments using CB1R and VGLUT (Vesicular Glutamate Transporter) transgenic mice should be performed.

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