**GENERAL DISCUSSION**

Our understanding of how the human brain works on the level of neurons and neural circuits is limited and to what extent the advanced cognitive abilities of man can be retraced to its cellular building blocks remains largely unknown. The principle aim of the work presented in this thesis was to examine to what extent the physiological properties of human neurons and synapses resemble those of rodents. Over the course of the past four chapters we have found many basic electrophysiological features that are shared between human and rodent pyramidal neurons, but importantly, we have also come to identify a number of key morphological and physiological differences which may have consequences for how these neurons exchange, integrate and store information.

6.1 **INFORMATION PROCESSING IN HUMAN PYRAMIDAL NEURONS**

6.1.1 **A large volume of information-rich synaptic input**

We started in Chapter 2 by exploring one of the more striking features of human neurons, their extensive dendritic arborisations. Working with acute slices provided an opportunity to overcome some limitations of post-mortem tissue, with the major advantage being the use of substantially thicker brain slices. This enabled us to obtain reconstructions of more complete human neurons and so allow quantitative analysis of full dendritic and axonal arborisations of
human pyramidal neurons. Notably, we were able to reliably capture those more distal, higher-order segments of dendritic branches of human pyramidal neurons, and it was there that we found prominent differences in the length of dendritic branch sections in comparison to mouse. It turns out that human cortical pyramidal neurons have substantially larger dendritic trees, which are almost three times larger than those of mice and macaques. The number of synapses per human L2-3 temporal cortex neuron has been estimated at around 30’000, a number obtained by dividing synaptic density by the corresponding neuronal density in a brain section (DeFelipe, 1999; DeFelipe et al., 2003). Using the same technique, rodent cortical pyramidal neurons were estimated to have around 15’000 synapses (DeFelipe et al., 2003). With human neurons having so much more dendrite as rodents, as we have shown, it would seem they indeed have sufficient dendritic surface area to accommodate twice as many synapses. The expansion of the dendritic arborisation of human neurons therefore allows them to deal with a vastly larger amount of input than rodent neurons and potentially supports a higher degree of connectivity in human cortical circuits (Balter, 2007; Buxhoeveden et al., 2001).

In Chapter 3, we studied the physiology and information transfer capabilities of these synapses using a combination of experimental work and computer modelling. We found that synapses between human L2/3 pyramidal neurons are highly efficient in transferring information from one neuron to the next. These neurons connect with one another via synapses that all show short-term depression, similar to juvenile or adult mouse neurons. However, wherein they markedly differ is their exceptionally fast recovery from this depression. Within 500 ms after a train of activity causing substantial depression, the human synapse had on average recovered to original levels, whereas mouse synapses had only recovered to ~70% of their original amplitude. Comparing the best-fit parameters of the Tsodyks-Markram model showed that the most prominent difference lay in the time constant of recovery, a measure of the speed at which a synapse replenishes its resources. The consequence of having a faster time constant of recovery for human synapses is that during realistic patterns of synaptic activity, synaptic inputs remain more easily resolved. That is, the postsynaptic, receiving neuron is able to more faithfully pick up the message sent by presynaptic, sending neurons. Using an application of information theory to short-term depressing synapses (Fuhrmann et al., 2002), we found that synapses that recover fast from depression, as human synapses do, are capable of transferring substantially more information over a wide range of activity frequencies. Thus, when it comes to receiving information, human cortical pyramidal neurons not only receive an enormous volume of input, made possible by their large dendritic arborisations, but the inputs that they receive are also high in information content, owing to fast recovering synapses.

6.1.2 Strong attenuation of signals from synapse to soma

Besides a larger total dendritic length, human pyramidal neuron dendrites have different structural features as well, as indicated by the isolation of 88% of human L2-3 pyramidal neurons into a unique cluster in the hierarchical agglomerative cluster analysis performed in Chapter 2. There have been studies showing that the dendrites of Golgi-stained neurons in histologically normal human cortical tissue resected during surgery from epilepsy patients have fewer branch points and fewer proximal branches compared to neurons from control post-mortem tissue,
suggesting that neurons in the brain of epilepsy patients have an altered dendritic architecture (Multani et al., 1994). Despite these reports, we do not find any morphological correlates with parameters such as disease duration and severity. It therefore seems more likely that our findings are a reflection of the general theme that occurs throughout the mammalian class, where larger brains house not only more neurons, but also larger, and more complex ones (Elston et al., 2001; Herculano-Houzel et al., 2014; Manger et al., 2013).

Using computational models of 3D digitally reconstructed neurons, we were able to assess the effect of the distinct dendritic arborisation of human neurons on passive signal propagation. Attenuation turned out to be very severe, with several hundred-fold reductions of signals travelling from distal dendrites to soma. Such strong signal attenuation makes it look difficult for these distal synapses to contribute to somatic integration, so how do neurons cope with this? The somatic depolarisation caused by a synaptic input depends not only on dendritic filtering, but also on its size and time course at the site of origin (Nevian et al., 2007). So, attenuation may be countered by simply starting off with a larger amplitude. Rodent hippocampal pyramidal neurons for example overcome dendritic signal attenuation by expressing more postsynaptic AMPARs at their synapses as the distance to soma increases, resulting in larger local EPSPs for distal synapses. This ensures that after dendritic filtering, each synapse evokes a similar depolarisation at the soma irrespective of dendritic location (Häusser, 2001; Magee and Cook, 2000). In rodent cortical L5 pyramidal neurons, evidence for EPSP scaling has been found in the basal dendrites, where a close to fourfold increase in the mean peak amplitude of local unitary EPSPs was observed over the first 150um from soma (Nevian et al., 2007). This EPSP scaling seems a feature particular to their basal dendrites however, as it was not observed in apical dendrites (Williams and Stuart, 2002).

Alternatively, human neurons may demand a larger volume of coordinated synaptic input. Individual synaptic responses need not be large if many summate together, which may be helped by the phenomenon of supra-linear summation; in rodents, near-synchronous activation of 10-20 synapses on a dendritic branch can trigger a local spike carried by NMDARs which greatly amplifies the postsynaptic response beyond the sum of every individual synaptic potential (Larkum et al., 2009; Major et al., 2013; Schiller et al., 2000). Dendritic NMDA spikes have been shown to occur in vivo, where they facilitate communication between the dendritic tuft and soma, allowing these distal inputs to influence neuronal output (Palmer et al., 2014). Distinguishing between these not necessarily mutually exclusive possibilities should be possible using glutamate uncaging. This technique involves the light-controlled liberation of caged glutamate around the spine head, thereby simulating presynaptic glutamate release (Callaway and Yuste, 2002). The presence of EPSP scaling could then be tested by uncaging glutamate on individual spines and observing somatic response amplitudes to see whether these remain the same as the distance of the activated spine to soma increases. Non-linear summation mechanisms could be detected by near-synchronous glutamate uncaging on multiple spines on a dendritic segment, and comparing the amplitude of the response to the sum of individual responses.
Of course, human neurons may have altered biophysical properties not accounted for in our present model, which may lead to an overestimation of dendritic filtering in human dendrites. Important parameters in this respect are the specific membrane capacitance ($C_m$) and specific membrane resistance ($R_m$), which both impact the low-pass filtering behaviour of the neuronal membrane. The value of $C_m$ is generally assumed to be a biological constant across many types of neurons and lies close to 1 µF/cm$^2$ (Gentet et al., 2000). It is this value that was used in the development of the neuron model presented in Chapter 2. However, during the realisation of this thesis, further modelling efforts carried out by collaborators suggested human neurons may have a substantially lower $C_m$ (Figure 6.1). This prompted us to perform direct measurements of $C_m$ using nucleated patches, which experimentally confirmed this: human neurons have a $C_m$ about half that of rodent neurons (Figure 6.1; Eyal et al. submitted manuscript).

A lower $C_m$ for human neurons would effectively allow a faster propagation velocity and lead to less filtering of signals as they propagate through the dendrites. A first indication on how strongly this will impact dendritic signal propagation in human neurons can be obtained by updating the models presented in Chapter 2 with this new experimentally-derived $C_m$ value. Equally of interest will be to identify what underlies the lowered $C_m$ of human neuronal membranes. Recently, a large-scale analysis of the organisation and evolution of the brain’s lipidome revealed a strikingly rapid evolution of lipids in the primate brain and particularly in the human neocortex (Bozek et al., 2015). Such changes in lipid composition may affect $C_m$, for instance by allowing neuronal membranes to become thicker and thereby hold less charge. Naturally, whether adaptations of the human lipidome explain the low $C_m$ value of human cortical pyramidal neurons requires future exploration, but these preliminary results lead to an intriguing proposition; could the presumed restraint of signal attenuation on neuronal cell size have been overcome in the human brain by evolutionary changes to the composition of neuronal membrane?

### 6.1.3 High frequency tracking ability

When synaptic potentials leave the dendrites and invade the neuronal cell body, the neuron has to translate these inputs into meaningful output. Having established that human pyramidal neurons potentially receive a very large volume of information-rich synaptic input, this is a formidable challenge; in order for human neurons to make use of the information enclosed in the resulting fine temporal voltage fluctuations, they will have to be sensitive to this fine detail and be able to react rapidly. In Chapter 3 of this thesis, we tested the limits of the capacity of human pyramidal neurons to do so using the experimental paradigm of frequency tracking (Köndgen et al., 2008). We found that human neurons were exceptionally adept at encoding fine temporal voltage fluctuations into the timing of their APs, with AP firing remaining phase-locked up to cycles of 1000 Hz with minimal loss of modulation depth. With such a high cut-off frequency, human neurons perform better than rodent neurons, suggesting neuronal information transfer can have a substantially higher bandwidth in human neocortical circuits.

The ability of neurons to time APs relative to subthreshold membrane potential changes relies on the AP onset dynamics, with faster AP onsets allowing higher frequency tracking (Fourcaud-trocmé et al., 2003; Ilin et al., 2013). A recent study reported that in order for neurons to track fast varying inputs to the degree we observed in human pyramidal neurons, AP onset rapidity would have to be above 30 mV/ms per mV (Ilin et al., 2013). We found APs fired by human pyramidal neurons had mean onset rapidity values above 32 mV/ms per mV for all firing frequencies tested, and so provide experimental evidence that human neurons indeed have sufficiently fast AP onset dynamics to account for their ability to track fast inputs. But what makes human APs faster? One possibility is that the onset of APs is boosted by the presence of cooperative voltage-gated Na$^+$ channels in the axon initial segment, where the AP is initiated (Huang et al., 2012; Ilin et al., 2013; Naundorf et al., 2006). Voltage-gated Na$^+$ channel activa-
Figure 6.1  Human neurons have an exceptionally low specific membrane capacitance.

The cable parameters of a neuron can be extracted by fitting a neuronal reconstruction-based model to experimentally recorded voltage responses to small injected currents (Rall, 1969). Panel A of this figure displays such a voltage response (black trace) to a brief somatic depolarizing current pulse (2 ms, 200 pA) obtained from the human L2/3 pyramidal neuron on the right. Superimposed coloured traces depict the theoretical transients for an identical input injected to into models of the same cell assuming different cable parameters ($R_m$, $C_m$, and $R_a$). For all 6 human neurons measured, reconstructed and modelled this way, the best fit was invariably obtained with $C_m$ values close to 0.5 μF/cm$^2$ (green trace), which is half the value commonly assumed for neuronal membranes (Gentet et al., 2000). Panel B shows the excellent fit of the model assuming a $C_m$ value of 0.45 μF/cm$^2$ to all voltage transients recorded from this neuron, regardless of stimulus strength and direction (same model as green trace in A). To experimentally validate these predictions, we made nucleated patch recordings from human L2/3 pyramidal neurons using the procedures described by Gentet et al. (2000). After establishing whole-cell configuration (C), the nucleus was carefully retracted from the soma to obtain a nucleated patch (D). Next, hyperpolarising voltage steps (-5 mV) were injected to record capacitive currents (E). The bottom trace in E is a magnification of the area boxed in red in top trace, and displays the mono-exponential fit (red line) to the capacitive current to estimate the membrane time constant. Using the membrane surface area of the nucleated patch, the capacitance of membrane per cm$^2$ could then be calculated. Panel F summarises our results for human (n=6) and mouse (n=4) cortical pyramidal neurons. Human neurons had remarkably low $C_m$ values (0.47±0.06 μF/cm$^2$), which were significantly lower than those of mouse neurons (1.03±0.14 μF/cm$^2$, Student’s t-test, p=0.011). Figure is from Eyal et al. (manuscript in preparation).
tion during the initiation of an AP occurs in an avalanche-like way, with each channel-opening causing further depolarisation and the recruitment of more channels. Modelling studies have predicted that this process of AP initiation can be greatly sped up if even just a small fraction of the Na\(^+\) channels would be spatially clustered in the axonal membrane (Huang et al., 2012; Ilin et al., 2013; Naundorf et al., 2006). This coupling would allow cooperative gating, from which fast-onset APs and high-frequency tracking abilities would follow. This theory is not without dispute however (McCormick et al., 2007), and no direct biological evidence exists for Na\(^+\) channel cooperativity in rodent, let alone human neurons.

Another, more strongly founded possibility is that there may be a role for dendrites here too. In rodents, experimental manipulations of dendritic tree size have directly demonstrated that the speed of AP onset depends on the dendritic load (Bekkers and Häusser, 2007). In a modelling study carried out by collaborators, the effect of human dendritic geometry on action potential kinetics and frequency tracking ability was investigated using a subset of the human neuronal reconstructions presented in Chapter 2 (Eyal et al., 2014). They found that the cable properties of the dendritic tree, which follow from the morphological features, directly affect the shape of the AP onset at the axon initial segment: the larger the dendritic tree, the larger its conductance load relative to the axon initial segment, and the faster the AP onset. Conversely, the smaller rat neurons had a smaller dendritic load, slower AP onset rapidity, and a lower cut-off frequency in these models than human neurons (Eyal et al., 2014). Our results in Chapter 3 are nicely in line with the model-based predictions by Eyal et al. (2014). The larger dendrites of human L2 and L3 pyramidal neurons may therefore explain both the faster AP onset and their ability to encode faster membrane potential changes into AP timing compared to mouse pyramidal neurons.

Dendritic tree size alone did not explain the entire phenomenon of fast-onset APs and high frequency tracking (Eyal et al., 2014). Other mechanisms may contribute as well, such as the abovementioned lower \(C_m\) of human neurons. For a given value of \(R_m\), a reduced \(C_m\) value implies that (1) the membrane is more readily charged by depolarising currents to reach threshold for AP initiation and that (2) active signals, such as axonal spikes, propagate at higher velocity (Eyal et al. submitted manuscript; Jack et al., 1975). It will be interesting to see how a lower \(C_m\) will impact the speed of AP onset and frequency tracking abilities of the neurons modelled in the study by Eyal et al. (2014). One would expect that the spread of the AP from the axon initial segment to the soma would be sped up drastically.

### 6.1.4 The role of dendrites

Taking together what is discussed in the three previous sections, it is becoming increasingly apparent that the distinct, extended dendritic architecture of human cortical pyramidal neurons affects many levels of information processing. First, increased dendritic surface area allows for the accommodation of more synapses, supporting a higher degree of connectivity and the potential to receive inputs from many more sources. Second, the extensive dendritic structure also impacts the way inputs are processed, through strong signal attenuation of distal inputs, suggesting a larger role for dendritic integration for human neurons. Third, an increased number of branch points provides more compartments for local dendritic integration which is suspected to increase computational power (Poirazi and Mel, 2001). Fourth, the larger dendritic load imposed on the axon initial segment by the large human dendritic arborisations are predicted to increase the speed of AP onset, which in turn enhances their frequency tracking capabilities and allows them to be reliably encode fast changes in synaptic inputs into the timing of their AP (Eyal et al., 2014). Thus, it seems the evolutionary extension of the dendritic arborisation may have provided human neurons with greater encoding capabilities and information processing capacity.
For such a central feature shaping neuronal function, it is unfortunate that next to nothing is known about the processes taking place in human dendrites. The distinct morphology of human cortical pyramidal neurons calls into question to what extent our knowledge of dendritic processes in rodents may apply in humans and underscores the need for direct measurements of human dendritic electrophysiology. Our present model on dendritic signal propagation has to be experimentally validated, to elucidate the passive and active propagation mechanisms involved in transferring synaptic information to soma. Dual recordings from the human pyramidal neuron soma and dendrite would allow direct measurement of the cable properties of human dendrites and how these affect steady-state voltage attenuation and the propagation of synaptic potentials. Furthermore, such experiments would provide a closer, more detailed look at synaptic properties as well; the distance-dependent scaling of local EPSPs and any non-linearities in their summation could investigated this way, and also to what extent the use-dependent depression—and recovery thereof—depends on synapse location (Williams and Stuart, 2002). Results from these challenging, but not impossible experiments should lead us to more refined models of human pyramidal neurons and to an improved understanding of the implications of such large dendritic trees on dendritic signal propagation and integration.

### 6.2 Information Storage in Human Pyramidal Neurons

The neocortex in our brain stores long-term memories by changing the strength of connections between neurons. To date, the rules and mechanisms that govern activity-induced synaptic changes at human cortical synapses are poorly understood and have not been studied directly at a cellular level. In Chapter 4 and 5 of this thesis, we investigated spike timing-dependent synaptic plasticity at pyramidal neuron synapses in the adult human neocortex, to elucidate the rules and mechanisms of STDP and its regulation by the neuromodulator acetylcholine.

#### 6.2.1 Unconventional STDP rules

In Chapter 4, we found that adult human neocortical synapses can bi-directionally change strength in response to STDP induction protocols at least up to 65 years of age. It is commonly held that our ability to remember declines as we grow older, but we found no concurrent loss in STDP efficacy. Significant changes in synapse strength were induced regardless of age, indicating that the human brain retains its ability to adapt neural circuits and learn throughout life. The temporal window for associative changes in synapse strength for human synapses is rather at odds to the Hebbian STDP present at many synapses in rodents. Firstly, with a range of ±100 ms, the STDP window for human cortical synapses is wider compared to rodents, where the conventional STDP window has a range of ~40 ms (Bi and Poo, 1998). Secondly, the STDP window is apparently reversed compared to what is generally found in rodents, with LTP at negative pairing intervals and a window for LTD at positive intervals. This reversed and wide STDP window for human cortical synapses is unconventional, but bears a striking resemblance to some of the human STDP-like plasticity windows of motor evoked potentials shown in vivo (Conde et al., 2013; Koch et al., 2013; Thabit et al., 2010).
Interestingly, human cortical STDP rules were similar, but not the same as human hippocampal STDP rules we obtained earlier (see Figure 6.2; Testa-Silva et al., 2010). At adult human hippocampal synapses, LTP was also observed at negative timing intervals, where EPSPs following a postsynaptic burst of APs within 80ms potentiated. Hippocampal synapses could also undergo LTD, but in contrast to cortical synapses, this was only observed when EPSPs followed the burst by more than 80ms (Testa-Silva et al., 2010). Human STDP rules therefore appear to differ between brain areas, perhaps tailored to the specific functions of each brain region. To see how STDP rules at human cortical synapses compare to those in rodents we collected a parallel dataset in adult rat temporal cortex. We found that rat and human synapses share a number of core principles for STDP, including a maximum of timing dependent changes close to 0 ms and an overall wide temporal window that includes LTP at negative intervals. A notable difference however is that adult rat temporal cortex synapses did not show LTD within our tested range of timing intervals. Thus, synapses in adult human cortex, adult human hippocampus and adult rat cortex essentially have similar STDP rules, but differ in the presence and location of an LTD window.
6.2.2 Mechanisms of STDP

What are the underlying mechanisms of human cortical plasticity and how do these shape the characteristic STDP window? A number of possible mechanisms have been discussed in Chapter 4, including a role for inhibition in setting the LTD window at positive intervals, and the concurrent activation of synapses with the dendritic calcium dynamics induced by AP back-propagation in setting an LTP window at negative intervals. In light of the clearly established role of bAPs in STDP (i.e. how their kinetics affect STDP rules), and the relation between dendritic morphology and bAP kinetics, the putative effects of the extensive dendritic trees of human neurons described in Chapter 2 warrants further discussion.

In the general introduction of this thesis, the distance-dependent shift in STDP rules at rodent cortical synapses was introduced: changes in the bAP waveform as it propagates into the dendrites lead to changes in the rules for STDP at synapses along the way. In Chapter 4, we noted the similarity between the reversed STDP rules observed at distal rodent synapses and those of human synapses, but concluded that a contribution of this synapse location-dependent form of anti-Hebbian STDP was unlikely, because the synapses we studied in humans were likely not distal, but proximal. However, in light of the work presented in Chapter 2, perhaps we should reconsider this possibility. We know now that human neurons have dendritic trees very different from rodents, having not only longer dendrites, but also distinct branching patterns and structural properties. Such morphological differences can translate into substantial differences in AP back-propagation (Kim and Connors, 1993; Vetter et al., 2001), and so the distance-dependent shift in STDP rules may take a different form in human cortical neurons.

Neuronal reconstruction-based models also predict that dendritic morphology impacts the degree to which bAPs are sensitive to changes in dendritic voltage-gated channel distributions (Vetter et al., 2001). Investigating a wide variety of dendritic trees of different cell-types, Vetter et al. (2001) found that bAP kinetics in pyramidal-like arborisations display the greatest sensitivity to such changes. Although VGCCs were not included in these models, the authors suggested it was likely that their findings would extend to these. In Chapter 4, we found that dendritic L-type VGCCs were recruited during back-propagation of single APs, and had their activity reflected in the area of the somatic ADP. Between neurons, ADP area varied considerably, which suggests different levels of expression or recruitment of these channels. It will be interesting to see to what extent these differences affect the waveform of bAPs in human dendrites.

To further improve our understanding of human cortical STDP mechanisms, it is clear that here too dendritic recordings will be essential. AP back-propagation needs to be directly measured in the dendrites, preferably at different distances from soma, to measure their kinetics and how these change with distance travelled. Only then can we begin to appreciate how the postsynaptic signal of activity required for STDP takes form at the synapses involved. The role of L-type VGCCs in bAP propagation and shaping its waveform can then be probed as well, for example by simulating different levels of expression with pharmacological tools. Simultaneous somatic recordings may also shed light on the relationship between bAP kinetics and somatic ADPs, and elucidate why larger ADP areas are associated with stronger LTP.
6.2.3 Implications of reversed STDP rules

The observed human cortical STDP rules are unconventional and reversed compared to what is generally found in rodents, which leads to the question of what their functional role may be. As discussed in the general introduction of this thesis, a reason why the classic, Hebbian STDP rules may have become such a popular concept is that the synaptic learning rules it imposes on the synapse are rather intuitive: synapses which contribute to AP generation are rewarded, uncorrelated, presumably meaningless input is depressed. Causality, in a way, captured and stored by the synapse. Reversing this relationship seems at first glance to make little sense. It is important to note in this respect however that although broadly reversed, human cortical STDP rules are not precisely anti-Hebbian in the strict sense; the switch from LTP to LTD occurs not at 0 ms, but somewhere between +5 and +10 ms. This means that the typical pre-before-post activity patterns with small EPSP-AP delays (<5 ms) will still give rise to LTP, ensuring that AP-contributing synapses, provided they were active in the few milliseconds preceding the AP, still get their ‘reward’. Only if synapses are active 10ms or more before the AP, will they be subject to depression. If we consider synaptic depression as a ‘punishment’ for not contributing to AP generation, an LTD window at these larger positive intervals (>10ms) may actually be useful. Synaptic EPSPs peak within 10 ms or so, and therefore EPSPs that precede the AP by more than that will already be in the decaying phase, or at longer delays may have even returned back to baseline. Hence, the contribution of such synaptic activity to AP generation may in fact be minimal and would thereby justify their depression.

Still in the context of rewarding causality, the finding that human cortical synapses –and, for that matter, human hippocampal and adult rat cortical synapses- are also strengthened in a broad time window after AP generation is difficult to reconcile. Unless, that is, these synaptic inputs are somehow related to the incoming signal triggering the AP but arrive later in time. This could occur for instance if the AP-triggering inputs and the input arriving later are part of the same related volley of synaptic activity. Potentiation of synapses active after the AP may lead to the neuron switching its firing response to this volley of input from single APs to bursts. An alternative scenario where a synapse would be consistently active after a postsynaptic AP is if its activity would somehow be a direct result of the AP. The firing of one neuron may trigger activity of downstream neurons with projections back to the first. An intriguing consequence of LTD at negative intervals would then be the strengthening of feed-back synapses in the circuit. How the reversed human cortical STDP rules impact the behaviour and organisation of human cortical circuits will have to be studied using computational models of neural networks with plastic synapses (e.g. Zenke et al., 2013).

6.2.4 Layer-specific modulation of STDP by acetylcholine

Neuromodulators provide the brain with strong temporal and spatial control over neuronal excitability and synaptic plasticity. To investigate how STDP rules can be altered by neuromodulators, we started in Chapter 5 with a digression from adult human temporal cortex to the juvenile mouse mPFC. The neuromodulator of interest was acetylcholine, which is known to affect STDP in the mPFC by acting on nicotinic receptors (Couey et al., 2007). The finding that cholinergic neurons at different locations in the BF innervate distinct prefrontal cortical layers (Bloem et al., 2014) and that nAChRs are expressed with layer-specificity (Poorthuis et al., 2013a), put forward the question whether cholinergic control of synaptic plasticity is layer-dependent in mPFC. We found that nAChR stimulation indeed has different effects on synaptic plasticity in L5 and L6 of mouse mPFC; nicotine blocks LTP in L5 pyramidal neuron synapses (Couey et al., 2007), but facilitates LTP in those of L6. Importantly, these effects were not only seen upon application of exogenous bath- or puff-applied nicotine, but also in response to light-evoked endogenous ACh release at naturalistic stimulation patterns. Layer-specific expression of functional nAChRs and a corresponding layer-specific modulation of plasticity
was also found in human cortex. The topographically organised BF cholinergic system therefore appears to have layer-specific control of cortical processing and plasticity that is enforced by layer-specific nAChR expression (Figure 6.3), which is also in place in the human neocortex.

Opposite cholinergic regulation of STDP in mouse L5 and 6 mPFC results from the opposite effects of nAChR activity on bAPs in L5 and L6 pyramidal neurons: in L5, nAChR activation triggers inhibition onto L5 neurons, resulting in reduced bAP signalling and a corresponding increased threshold for LTP. Conversely, in L6 pyramidal neurons, nAChR activation leads to enhancement of bAPs through activation of dendritic α5*nAChRs, which results in a facilitation of LTP. It is particularly interesting to see that L6 pyramidal neurons express the LTP-facilitating nAChRs along their dendrites; L6 pyramidal neurons have very thin dendrites which, in contrast to L5 neurons, are hyperpolarised in respect to soma (Ledergerber and Larkum, 2010). These factors make efficient AP back-propagation into the dendrites challenging, because in the resting state the dendrite will be further away from threshold of voltage-gated channels that may otherwise boost bAPs, such as dendritic Na⁺ channels and VGCCs. Perhaps this explains the limited amount of tLTP observed in control conditions in both human and mouse L6 pyramidal neurons. Interestingly, it was found in rat L6 pyramidal neurons that small depolarisations of the dendrites could lead to a substantial amplification of the bAP amplitude, made possible by dendritic voltage-gated Na⁺ channels (Ledergerber and Larkum, 2010). Dendritic nAChRs in L6 pyramidal neurons may well act as a source for such dendritic depolarisations, functioning as an on/off switch for bAP enhancement and the induction of tLTP. These findings demonstrate how the brain’s cholinergic system can exploit the dependence of STDP on bAP-signalling to transiently modify synaptic learning rules with layer-specificity.
Is the layer-specific, cholinergic control over dendritic bAP signalling also present in human cortex? A likely candidate for a source of inhibition that dampens AP back-propagation in mouse mPFC L5 pyramidal neurons are Martinotti cells. These are β2*nAChR-bearing interneurons that preferentially target pyramidal neuron apical dendrites (Couey et al., 2007; Kawaguchi and Kubota, 1997). In human cortex, we did on a few occasions record from interneurons with the characteristic low-threshold spiking behaviour of Martinotti cells, which in 2 out of 3 cases exhibited potent nAChR-mediated currents (Figure 6.4). Future experiments will have to establish whether these neurons actually target L2/3 pyramidal neurons and are responsible for the cholinergic blockade of LTP in human superficial cortical layers. As for the facilitation of LTP in human L6 pyramidal neurons, the question is whether the bAP-amplifying, LTP-facilitating nAChRs are similarly expressed along their dendrites, as in mouse. The optogenetic techniques used to demonstrate dendritic localisation of nAChRs in mouse L6 pyramidal neurons in Chapter 5 are obviously unavailable for human brain slices, but the recent development of RuBi-nicotine, a caged nicotine compound releasable by light (Filevich et al., 2010), may offer the control and spatial precision of nAChR activation required to probe the distribution of nAChRs on human L6 pyramidal neurons.

Cholinergic layer-specific control over STDP rules, seen here in both mouse and human cortex, may represent an evolutionarily conserved mechanism for switching synaptic learning rules during different behavioural contexts. Our findings provide insight into the cellular processes that underlie nicotinic modulation of non-invasive brain stimulation-induced neuroplasticity via β2*nAChRs in human subjects (Batsikadze et al., 2014; Grundey et al., 2012; Thirugnanasambandam et al., 2011). These processes may contribute to the impact of nicotine on various cognitive functions in humans, including attention, working memory and episodic memory (Heishman et al., 2010; Kumari et al., 2003).
6.3 Placing human neurophysiology in context

6.3.1 Healthy or epileptic human brain?

Human brain tissue used in our studies derived from brains of epilepsy patients. Although resected tissue always originated from cortical regions away from the epileptic focus, tumour, or other primary cause for surgery and showed no functional abnormalities on pre-operative MRIs, the question remains to what extent the pathological processes that occurred in the brains of these patients may have affected our tissue samples and the measurements we obtained from them. These interpretation issues have perhaps contributed to the relatively limited use of neurosurgery-derived human brain tissue for electrophysiology and need to be resolved if we are to make full use of its potential.

The approach taken in this thesis was to check whether there were any relations between disease history and our results, the rationale being that if a certain morphological or physiological feature is due to the fact that the patient has epilepsy, one would expect to find some degree of correlation of these features with disease duration or severity. We found no such correlations; seizure frequency and duration of epilepsy explained little of the variance in dendritic morphology, short-term plasticity and recovery from depression were similar between patients with different disease backgrounds (MTS epilepsy vs tumour or other), and so too were the results on long-term synaptic plasticity. This suggests little involvement of epilepsy in the differences we observed and that these features may therefore generalise to the non-epileptic, healthy human brain. Yet, with a highly heterogeneous patient population differing in age, gender, medical and psychological history, comes an inherently large variability. This could easily mask subtle, but important relationships of electrophysiological parameters with disease history, so a dose of caution remains necessary. The best possible control to test the degree to which results may be influenced by patient-status would be to obtain tissue from subjects completely free of epilepsy, as for instance would sometimes come available in treatment of brain trauma. This is being done in a few laboratories, but the rare incidence of such cases and the wide variety of brain areas from which tissue may be obtained would make the collection of datasets comparable to ours a very long process and would probably require the collaboration of many laboratories to become feasible.

6.3.2 Uniquely human specialisations?

When comparing human to rodent neuronal electrophysiology, we are comparing two species on opposite ends of the mammalian spectrum in terms of brain size, neuron size and cognitive complexity. This means that even if we have good cause to believe our results are telling us something about neurons in the healthy human brain, the question remains how differences in physiology between human and rodent neurons should be interpreted. What features are due to us having a much larger brain, what do we share with other primates, and what may be uniquely human specialisations? With present literature dominated by work on rodents and little neuronal electrophysiology data available on species more similar to us, answering these questions is difficult; interpretation of human electrophysiology is troubled by the lack of an appropriate electrophysiological context.

From comparative neuroanatomy it is well established that many aspects of the brain scale across mammals in predictable manners, some of which already mentioned in the general introduction: encephalisation scales nicely with cognitive complexity in primates (Reader et al., 2011), neocortical surface area scales with a host of features including animal sociability (Sawaguchi and Kudo, 1990), and numbers of neurons and their size scale predictably with brain mass (Herculano-Houzel et al., 2014), to name a few. Cross-species comparative electrophysiology (e.g. Altemus et al., 2005; John et al., 1997; Petanjek et al., 2008) is a field...
far less developed, but may reveal similar cross-species scaling rules and provide the much needed comparison material. Several features of human neurons that impact information processing (many more synapses per neuron, dendritic signal propagation, fast-onset APs and high-frequency tracking) are directly or indirectly related to neuronal size and morphology. If neuronal morphology is indeed a good predictor of these electrophysiological features, one would expect them to scale in step with the scaling of neuronal morphology (e.g. size, complexity) across species with different brain sizes. Will species with comparably sized dendritic trees have equally fast AP onset kinetics and frequency tracking abilities as human neurons? Only cross-species comparative electrophysiology across the mammalian radiation will inform us to what extent the differences found between rodents and humans are due to differently sized brains and neurons.

Identification of uniquely human specialisations must at some point involve a direct comparison with our closest relatives. To what extent do we share our neuronal physiology with other primates? Is the human brain electrophysiologically a similarly ‘scaled-up primate brain’ as it seems to be anatomically (Herculano-Houzel, 2012)? Primate neuroscience usually has a focus on behaviour and studying the electrophysiology of their neurons and synapses is seldom the main research objective. Nevertheless, a number of studies exist where the neuronal physiology of macaque neurons was directly compared to that of rodents, which revealed both similarities and differences between these species. Basic electrophysiological properties such as resting membrane potential, input resistance, rheobase and more were found to be very similar in mossy cells, hippocampal CA1 pyramidal neurons and proximal CA3 pyramidal neurons of macaques and rodents (Buckmaster and Amaral, 2001; Altemus et al., 2005). Dentate granule cells were also found to have a virtually identical morphology and physiology, with the exception of having significantly different AP kinetics; APs had significantly slower rise and fall times in macaques (John et al., 1997). In the macaque dorso-lateral PFC (dLPFC), fast-spiking interneurons have very similar firing behaviour and properties of synaptic inhibition as those in the rodent PFC (Rotaru et al., 2015), yet other interneuron types show different firing patterns and levels of excitability (Povysheva et al., 2007, 2008). The population of L2/3 pyramidal neurons in macaque dLPFC also appears to be much more electrophysiologically diverse than that in rodents (Zaitsev et al., 2012). The few studies that investigated synaptic physiology investigated short-term plasticity of excitatory synapses in macaque dLPFC (Gonzalez-Burgos et al., 2005), and long term plasticity in visual cortex (Huang et al., 2014) and hippocampus (Urban et al., 1996). The results obtained in these studies were mostly similar for macaque and rodent synapses.

Unfortunately, differences in brain regions, neuron types investigated and experimental design make that direct comparisons cannot be made between the neurophysiology data obtained from non-human primates in the literature and that from humans presented in this thesis. Future investigations should therefore be aimed at acquiring more comparable, parallel datasets on human and non-human primate neuronal and synaptic physiology. The studies on macaques mentioned above were mostly performed using tissue from hippocampal regions or from frontal cortical regions of the brain. These regions are sometimes resected during epilepsy surgery as well, which opens the possibility to repeat these experiments in human tissue in the future. Alternatively, perhaps labs with access to macaque brain tissue may turn to the relatively understudied temporal cortex, to obtain datasets on short-term plasticity and information transfer, frequency tracking, AP onset rapidity, and STDP from the pyramidal neurons in this area. Will the electrophysiology of macaque pyramidal neurons and synapses be closer to that of humans than rodents were? Such studies will be an important step forward in placing human neuronal, synaptic and circuit physiology into an evolutionary and large-brained primate context.
6.4 Conclusions

The study of human neuronal and circuit physiology is still in its infancy and much remains to be explored. Human brain slice electrophysiology has allowed us to directly probe the brain’s elementary building blocks, which have emerged as true powerhouses of information processing; the huge number of synapses they receive, made possible by extensive dendritic arborisations, make that large quantities of information converge on a single human neuron. Moreover, each presynaptic source of input is able to convey its messages very efficiently owing to the rapid recovery of synapses from depression. To integrate the resulting large volume of high information content synaptic input, human neurons come equipped with action potentials with fast onset dynamics, ensuring they can make the most of the finest, most rapid voltage fluctuations and encode these into the timing of their APs. Finally, their synapses retain their plasticity throughout life, capable of undergoing bidirectional changes in strength with high efficacy and providing neurons with a large capacity for storing information. In sum, when considering the origins of the cognitive capacities of the human brain, one cannot merely point to its size and numbers of neurons, but should also consider an array of neuron-level adaptations with large implications for the computational power of the system as a whole.