GENERAL INTRODUCTION

1.1 Origins of human cognitive capacities

The human brain is certainly one of the most complex and enigmatic structures in biology. Somehow, a collection of 86 billion neurons (Azevedo et al., 2009), interconnected via hundreds of trillions of synapses (Pakkenberg et al., 2003), supports every thought, memory and experience we have in our lives and endows us with cognitive abilities that surpass those of all other animals (Shettleworth, 2012). Our advanced cognitive abilities are generally considered as defining our humanity, yet what adaptations our brain has undergone to gain these faculties remains one of the central questions in neuroscience. Investigating this may not only help us understand human cognitive function in health and dysfunction in disease, but also help define what it is that makes us human.

Traditionally, answers to this question have been sought in the anatomy of our brain. The human brain is based on a basic structural template shared by all other mammals. Different species have evolved their own variations on this common theme, ensuring each comes with a brain equipped to support the relevant cognitive abilities and behavioural repertoire required for survival (Barton and Harvey, 2000; Harvey and Krebs, 1990). Across the mammalian spectrum, a general trend seems to exist where brain areas supporting the animals most important behaviours and abilities are the most well-developed and enlarged (Barton and Harvey, 2000). Are there unique, defining anatomical features to which we can attribute our cognitive advance?
Chapter 1

Figure 1.1  Evolution of large brains in humans and across the mammalian radiation.

The evolutionary expansion of our brain can be followed through time by measuring the cranial capacity (a proxy for brain size) of fossilised skulls of early hominids. This shows that the current size of our brain is a relatively recent feature, and that most of the dramatic increase has occurred over past 3.5 million years (top panel). ‘The cooking hypothesis’ proposes this increase may have been made possible by the advent of cooking food by our ancestors (Wrangham, 2009; Fonseca-Azevedo and Herculano-Houzel, 2012). Brains are extremely energy demanding (Mink et al., 1981), which puts a limitation on how big a brain an animal can afford (Navarrete et al., 2011). The greater caloric yield from cooked food may therefore have helped to overcome these energetic constraints and allowed our brain to grow to its current size (Fonseca-Azevedo and Herculano-Houzel, 2012). Evidence suggests early hominids started cooking their food about 1.5 million years ago (indicated by orange shading, top panel), and it is in the years since that close to a doubling of the human cranial capacity took place. Our brain is not unique in its size however; as the bottom panel shows, large brains appeared several times in the mammalian radiation. Elephants and many species of dolphins and whales in fact have brains substantially (3-6 times) larger than humans. These species also surpass humans in terms of total number of neurons in the brain and absolute cortical surface area or volume. Top panel formatting from figure in Le Journal du Net (2010), plotted curve is based on double exponential fit published by De Miguel and Henneberg (2001), which was extrapolated to include the more recently found Sahelanthropus (Brunet et al., 2002). Bottom panel is adapted from Herculano-Houzel (2012).
To start, the sheer size of our brain may explain a lot. The past 3.5 million years of human evolution has witnessed an enormous increase in brain size (Figure 1.1, top panel; Pilbeam and Gould, 1974), and since a larger brain can hold more neurons, the basic computational elements of the brain, a larger brain would presumably have a larger computational power (Williams and Herrup, 1988). But there may be more to it than merely overall size; most of the enlargement of the human brain has in fact gone into increasing the volume of the neocortex, the extended six-layered structure that encompasses our brain, to which many higher-order cognitive functions have been accredited. In primates, which also have a large neocortex (Finlay and Darlington, 1995), it is thought to support the many skills associated with high-level cognition, including behavioural innovation, social learning, deception, strategic insight, tool use and planning, which they require to navigate a complex social environment (de Waal, 2007) and face the challenges of foraging for food (Janmaat et al., 2006, 2013). The degree to which different primate species exhibit these hallmarks of intelligence strongly correlates with the volume of their neocortex (Reader et al., 2011). With an area close to 2300 cm² (Elias and Schwartz, 1969), humans have the largest cortical surface area among primates. Such an enlarged neocortex may have allowed humans to further develop the cognitive capacities inherited from their primate ancestors, perhaps giving rise to uniquely human capacities in the process (Shettleworth, 2012).

Increased general brain size, a high number of neurons and a disproportionally large neocortical surface area thus appear to go a long way in explaining our advanced cognition. Yet, gross-anatomical features alone do not explain human intelligence to full satisfaction, since the human brain doesn’t actually stand out as much anatomically as it does in cognitive abilities (Figure 1.1, bottom panel; Premack, 2007; Roth and Dicke, 2005). Humans do not have the largest brain (whether expressed in either absolute size or relative to body mass), the highest number of neurons, or largest neocortex among mammals (Hofman, 1985). Yes, humans have the largest cortex of all mammals in relation to the volume of the entire brain (Hofman, 1988), but only just compared to a number of other primate and non-primate species (Herculano-Houzel, 2012; Hofman, 1985). In fact, the human brain is anatomically in every way a linearly scaled-up primate brain (Azevedo et al., 2009; Herculano-Houzel, 2009), and appears to have little exceptional or extra-ordinary features to which our outstanding cognitive abilities can be straightforwardly attributed (Herculano-Houzel, 2012).

Ultimately, it is the behaviour of the neurons in our brain, communicating with one another via electrical discharges in an intricately organised circuitry, that deal with the encoding, processing, and storage of information on which brain functions rely. The question arises then, to what extent our advanced cognitive capacities may also be down to adaptations on this much smaller level, that of neurons and circuits (Balter, 2007; Preuss, 2010). Unfortunately, we currently know very little about the organisation and physiology of the human brain on this scale and so the existence and nature of uniquely human neural and circuit specialisations are largely unknown. The reason for this is that in order to investigate how our neurons behave, communicate, and wire up into circuits, one requires living human brain tissue. This is obviously hard to come by, so as a result virtually our entire understanding of these processes is based on studies of the brains of laboratory animals, which are typically rats and mice of a few weeks old.
If we are ever going to fully understand the human brain and the origins of its cognitive abilities, this will have to change. While studies of the rodent brain can tell us how a brain may work, the degree to which they explain how our brain works is questionable in the face of an extensive body of literature showing a remarkable variation in the cellular organisation of brains across species (reviewed in: DeFelipe et al., 2003; Elston, 2007; Preuss, 2000, 2010). At some point, we need to study the function and physiology of neurons in the human brain directly and determine to what extent the fundamental principles of neuronal information processing and storage as they have been described in rodents also apply to humans (Altemus et al., 2005; Logan, 1999; Preuss, 2000). Initiation of grand projects such as the BRAIN\(^1\) initiative (Insel et al., 2013) and the Human Brain Project (Markram, 2012), with their ambitious goals of finding new methods to study human neuronal networks and integrating currently available data into a computational model of the human brain only adds urgency to this; such models need accurate estimates of human neurophysiological parameters in order to realise their full potential.

A few of laboratories have turned to using brain tissue derived from human neurosurgery to fulfil these aims. On occasion, neurosurgeons need to remove a piece of brain tissue in order to approach deeper regions in need of treatment. Say, for instance, an epileptic focus or tumour needs to be removed from the hippocampus, a piece of temporal cortex may have to be removed simply to gain access. It is this piece of neocortical tissue, removed not as part of the treatment but by practical necessity, which can be sliced into thin sections and kept alive for many hours in the lab. Using this preparation, direct electrophysiological measurements can be obtained from living human neurons still embedded within their native circuits, allowing detailed study of human neuronal and synaptic physiology.

The procedures to maintain slices of human cortex alive and in good condition were already developed in the early seventies (Kato et al., 1973), and the first intracellular recordings from human neurons were made shortly after (Schwartzkroin and Prince, 1976). Since then however, only about 30 papers have appeared using living human brain tissue for this purpose. Factors playing a role in this likely include the limited availability of tissue (operations yielding resected brain tissue may occur only a few times a year) and the fact that laboratories have to be in the immediate vicinity of the operating room to minimise the transition time between resection from the brain and slicing, which is important to maintain viable slices.

This thesis presents the work on human brain tissue that was carried out in our laboratory, which is privileged in being only a few hundred meters from the neurosurgery department of the VU Medical Centre, where brain surgeries are regularly performed. In the chapters to come, we will use human brain slices to obtain a detailed morphological and physiological characterisation of the main neuronal constituent of the neocortex, the pyramidal neuron. The principle aim is to determine to what extent physiological properties of human neurons and synapses resemble those of neurons and synapses in the rodent brain. What follows in this introduction is a review of some of the basic concepts of neuron-level information processing and storage that will be investigated.

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\(^1\) The Brain Research through Advancing Innovative Neurotechnologies
1.2 INFORMATION PROCESSING, FROM INPUT TO OUTPUT

1.2.1 Receiving information – dendrites

The neuron is a processor that receives, integrates and relays information. Information enters a neuron via its dendrites, the long protrusions that serve as its receiving antennae. Neuronal dendrites of the typical neocortical pyramidal neuron can be subdivided into two basic domains; the basal dendrites that extend more or less horizontally from soma and spread within the layer of the soma, and the apical dendrite that extends vertically across layers towards pia. Apical dendrites can be further subdivided into the main apical trunk, from which oblique dendrites branch off horizontally, and which usually ends with a characteristic tuft in the most superficial layers. Different pyramidal neuron types can diverge from this basic blueprint of dendritic morphology, which has direct consequences for the connectivity and electrophysiology of these neurons and how they process information.

Firstly, the total extent of a neuron’s dendritic arborisation will impact the amount of information received, since the total dendritic surface area provides a physical limit to the maximum amount of synapses a neuron can accommodate. Further, the distribution of dendritic branches across layers will impact the nature of information received; axons from a particular source are often guided in bundles to approximate locations along the pia-white matter axis (Meyer et al., 2010), but precisely how synaptic contacts are organised on a dendrite is determined more or less randomly (Morales et al., 2014). This is referred to as “Peter’s rule”; synaptic contacts occur where dendrites and axons are in close apposition² (Peters and Feldman, 1976). The amount and distribution of branches will subsequently determine the chance of connecting to specific axon bundles and thereby influence the relative contribution of inputs from that source to its activity. As a consequence, different dendritic domains often receive inputs from distinct sources (Meyer et al., 2010; Petreanu et al., 2007, 2009; Spruston, 2008). An added computational benefit of branched dendrites is that it allows inputs on the same branch section to interact more than with those from a different branch. Modelling studies have predicted that such compartmentalisation of input greatly increases the information processing capacity of neurons (Poirazi and Mel, 2001).

Another important way by which dendrites impact information processing is in how they shape input signals as they travel to the soma. EPSPs progressively decline as they propagate through stretches of dendrite because current is lost along the way over the membrane resistance ($R_m$). This attenuation can be severe up to the point that distal inputs hardly change somatic membrane potential (Nevian et al., 2007; Spruston, 2008; Williams and Stuart, 2002). Cable theory describes how morphological properties including dendritic length, diameter and branching patterns, and physiological properties such as membrane resistance, axial resistance and membrane capacitance, together determine in what form a synaptic potential arrives at soma after passively propagating through the dendrites (Rall, 1964, 1967). To counteract signal loss, dendrites express a diversity of voltage sensitive channels including voltage-gated calcium channels (VGCCs) and N-Methyl-D-Aspartate (NMDA) receptors, which can boost depolarisations and can even support regenerative events such as dendritic calcium spikes and NMDA spikes, respectively (Kampa et al., 2006; Larkum et al., 2009; Nevian et al., 2007). This way, signals can be greatly amplified and actively transported over longer distances.

² It must be noted that the extent to which Peter’s rule holds in the neocortex is debated (e.g. Shepherd et al., 2005), and was most recently challenged by Kasthuri et al. (2015), who demonstrated with a detailed electron microscopy reconstruction of a volume of cortex that physical proximity between axon and dendrite alone is insufficient to predict synaptic connectivity.
In short, dendritic morphology and physiology have a profound influence over the what and how of receiving information. In this light, it is interesting to see that a diversity of dendritic morphologies exists not only between different neuron types within a species, but also for the same kind of neuron across species. There appears to be a close to linear relationship between overall brain size and the average size of neurons it contains (Herculano-Houzel et al., 2014), with the largest brains holding the largest neurons. Besides growing, pyramidal neuron dendrites also gain in complexity as brain size increases; an index of neuronal complexity scales predictably with brain mass among primates, including humans (Figure 1.2; Manger et al., 2013). Could such differences in neuronal morphological complexity underlie species differences in cognitive performance? Indeed, the increase in neuronal complexity is particularly prominent for cortical brain areas involved in more complex, higher-order cognitive functions such as the temporal and prefrontal cortex (Manger et al., 2013). This has led to the proposition that it is the higher neuronal complexity that endows neurons with the enhanced computational power required to support the sophistication of large-brained primate behavioural repertoires (Manger et al., 2013).

The study of neuronal morphology and complexity may represent a rewarding avenue towards understanding differences in cognitive abilities between species, but for the human brain—and many other large-brained mammals—progress is unfortunately hampered by a number of limitations related to the currently available techniques used for neuronal visualisation. Traditionally, human dendrites are studied in fixed post-mortem tissue and visualised using staining techniques such as Golgi stains or by injecting neurons with fluorescent dyes such as Lucifer Yellow (Benavides-Piccione et al., 2012). The issue with post-mortem tissue is that delays to brain tissue fixation may affect morphology of fine cellular structures and delays have to be very short (less than 24hrs) to leave these unaffected (Oberheim et al., 2009; de Ruiter and Uylings, 1987; Swaab and Uylings, 1988). Concerning neuronal visualisation, a drawback of the commonly used Golgi stain is that the set of labelled neurons in the tissue sample is more or less random, allowing no control over which neurons are labelled. Finally, and perhaps most importantly, the sections used in Golgi or Lucifer yellow studies are typically required to be very thin, much thinner than the horizontal or vertical span of the dendritic tree (Elston et al., 2001), which means that only partial cellular morphologies can be resolved and quantitative analysis can only be performed on sub-compartments of the apical/basal dendritic tree that are relatively proximal to the cell body (Braak, 1980; Elston et al., 2001; Jacobs et al., 2001; Ong and Garey, 1990; Petanjek et al., 2011; Rosoklija et al., 2014).

To better understand how an increase in brain size has affected human dendritic morphology, to explore human dendritic signal propagation and integration, and to examine the effects of increased neuronal complexity in neural models, a better picture of what the complete human cortical pyramidal neuron looks like is required. The live human brain slice preparation allows the use of techniques for neuronal visualisation that exist for living rather than dead, fixed tissue and provides a straightforward and efficient way of dealing to a satisfactory degree with most of the aforementioned problems facing the study of neuronal morphology: (1) fixation delays are not applicable, since neurons are labelled in living tissue fresh from the brain, (2) neurons can be selectively targeted for loading, and (3) the use of substantially thicker brain sections preserves more complete neurons for visualisation. In Chapter 2 of this thesis, we will use this technique to examine the dendritic morphology of pyramidal neurons across layers of the adult human temporal cortex and compare these to the dendritic morphology of adult mouse and macaque temporal cortex pyramidal neurons.
1.2.2 Receiving information – synaptic input

The somatic depolarisation caused by a synaptic input does not only depend on the extent to which it has been filtered by propagating through the dendrites, but also on its size and time course at the site of origin. Postsynaptic potentials following synaptic activation are not fixed, but are dynamic in nature and dependent on recent activity. Upon repeated activation, many synapses display transient changes in strength, which can either be facilitating, where the synaptic response progressively grows, or depressing, where the synaptic response progressively decreases. Rodent neocortical pyramidal-to-pyramidal neuron synapses most commonly display the latter form, named short-term depression. The mechanistic basis of short-term depression is that every time a synapse is active it uses up some of its resources, that is, it uses up a portion of the readily releasable pool of vesicles that contain its neurotransmitter (Zucker and Regehr, 2002). If the synapse is then reactivated, the diminished number of neurotransmitter vesicles available for release results in a reduced response, and so with repeated activity, responses become smaller and smaller. What makes these changes ‘short-term’ is that synaptic resources are continuously restocked to allow responses to eventually recover to original levels, a process that can take tens to hundreds of milliseconds, depending on the degree of depression and type of synapse.

Figure 1.2  Pyramidal neuron morphology; variations in complexity across brain areas and species.
Cortical pyramidal neuron morphology can vary substantially across different cortical layers, brain areas and species. To illustrate this, two pyramidal neurons from different neocortical areas in the macaque brain are shown on the left; in orange is a L3 pyramidal neuron from the inferotemporal cortical area (TE), and in blue a L3 pyramidal neuron from the primary visual area (V1). These neurons clearly exhibit distinct growth patterns, differing greatly in the total length of the dendritic tree and degree of branching. Morphological complexity can be captured in an index that is constructed using the area of the dendritic field, the number of dendritic branches and the number of spines (Manger et al., 2013). On the right, morphological complexity of neurons is compared across different brain areas in different primate species. It is evident that as brain size increases, so too does neuronal complexity. Of note is that this increase in complexity is most prominent in areas associated with higher forms of cognition, such as the temporal (TE) and prefrontal cortex (PFC). The bars on the primate brain schematics represent total number of spines reported for neurons in different brain areas. Figure adapted/merged from Elston et al. (2014) and Manger et al. (2013). PFC = prefrontal cortex; TE = temporal cortex; V1,2,4 = primary, secondary and quaternary visual areas, respectively.
Various parameters determine short-term plasticity at a synapse, among which is the pattern and frequency of activity. Also important are a set of synaptic parameters among which the maximum strength of the synapse (i.e. how strong would the response be if all resources would be used in one go), the amount of resources used with every activation, and the time constant of recovery that describes the rate at which an activated synapse replenishes its resources to restore synaptic strength to its original level. The Tsodyks-Markram model incorporates these synaptic parameters and others into a mathematical description of short-term plasticity at a synapse (Tsodyks and Markram, 1997). Different kinds of synapses may have different synaptic parameters, which results in a variety of short-term plasticity rules at different synapses in the brain. In fact, even synapses from the same axon can have different modes of short-term plasticity depending on the postsynaptic target (Markram et al., 1998a), and synapses on the same neuron can have different degrees of short-term plasticity depending on synapse location (Williams and Stuart, 2002).

What the different forms of short-term plasticity have in common is that changes in strength are frequency dependent, such that synapses show more pronounced facilitation or depression in response to higher frequencies of activation. Thus, the magnitude of the postsynaptic response is dependent on the recent history of synaptic activation; for depressing synapses, this means that the shorter the time interval since the last presynaptic action potential, the smaller the postsynaptic response, and vice versa. This way, short-term plasticity allows synaptic response amplitude to contain information about the temporal structure of preceding presynaptic activity. The transfer of this information can be quantified by combining the Tsodyks-Markram model with information theory. This is the mathematical theory of communication formulated by Shannon in the 1940s to describe a system where a sender and a receiver communicate with each other via a channel with limited capacity (Shannon, 1948). The application of this theory to neurons communicating via synapses that show short-term plasticity allows the amount and rate of information transfer to be quantified for a given pair of connected neurons in terms of bits and bits/sec, respectively (Fuhrmann et al., 2002). Thus, using this model, one can explore how specific synaptic parameters will impact the efficacy of information transfer between neurons.

At present, the nature of these synaptic parameters at human synapses is unknown and their use-dependency has not been investigated directly. Humans have been found to have an increased complexity of synaptic protein networks, which has been proposed to underlie our more advanced cognitive abilities (Bayés et al., 2012; Nithianantharajah et al., 2013; Ryan and Grant, 2009). In rodents, pre- and postsynaptic proteins are known to have a prominent role in shaping short-term plasticity (Caillard et al., 2000; von Engelhardt et al., 2010). Altered synaptic protein networks in human synapses thus may affect the efficacy of these to transfer information which, given our neocortex alone has in the order of 150 trillion synapses (Pakkenberg et al., 2003), would have profound consequences for the computational capacity of the brain in its entirety. To better understand the nature of synaptic communication between human neurons, we explore short-term plasticity at human pyramidal-to-pyramidal neuron synapses in the initial part of Chapter 3.

1.2.3 Integrating information – encoding input into timed output
The major site of neuronal information integration is the soma and the axon initial segment, which includes the spike-initiation zone. Here, the synaptic inputs that the neuron is bombarded with throughout its dendritic tree funnel together, causing rapid voltage fluctuations of the somatic membrane potential. It is the neuron’s challenging task to constantly judge these inputs for whether or not it should respond with the firing of an action potential (AP). There is an upper limit to the ability of a neuron to react to rapid changes in synaptic inputs. At
some point, the corresponding voltage fluctuations may occur at a time-scale faster than it can respond to. Thus, a neuron has a limited frequency bandwidth wherein fluctuations in synaptic inputs can be meaningfully encoded into timed AP output. This bandwidth stands as a measure of the processing capacity of a neuron and can be explored using an experimental paradigm called frequency tracking (Köndgen et al., 2008). In short, this involves injecting noisy sinusoid currents into a neuron close to AP threshold, such that the neuron fires at 10-15 Hz. At low oscillation frequencies, the neuron will time its APs at a specific phase of the sinus cycle, thus encoding the temporal variations in its input into the timing of its output. By increasing the frequency of the oscillation to ever higher frequencies, one can test at what point the neuron can no longer fire its APs phase-locked to the oscillation. The point where this occurs is called the cut-off frequency; voltage fluctuations occurring at a rate faster than cut off frequency cannot be reliably encoded in AP timing and the input-output relationship is lost. In juvenile mouse neurons, this was found to occur at 200-300Hz (Köndgen et al., 2008).

Experimental and computational work has shown that the ability of neurons to track input frequencies is determined by their AP kinetics; the faster the onset of an AP, the faster the input frequencies it can track (Fourcaud-trocme et al., 2003; Ilin et al., 2013). The reason for this can be intuitively understood: a steep, rapid action potential onset enables the axon to respond fast to rapid membrane potential changes, whereas a less steep action potential onset will not be fast enough and, in the extreme case, will “ignore” the underlying voltage modulations. Then, the neuron will fire action potentials at arbitrary time points with respect to fast synaptic inputs. The encoding capabilities of human neurons have never been investigated and so how fast human APs are and how high frequencies they can track is not known. This aspect of information integration by human pyramidal neurons will therefore be investigated in the second part of Chapter 3.

1.3 INFORMATION STORAGE

1.3.1 Memory

A central feature of the brain, from the simplest nematodes to humans, is its ability to use past experience to shape and adapt current behaviour. This means that besides processing information about the current state, neurons and circuits in the brain also have to have some form of recollection of previous activity. Experiences must be stored in order for an animal to learn, remember, and refine behaviours. The human brain is outstanding in these capacities. We can effortlessly remember things from a distant past, rapidly encode memories (Ison et al., 2015) and learn new skills throughout life. Surely, knowing how memories are formed, stored and recalled in our brain is key to its understanding.

Memories are believed to be encoded by relatively small populations of neurons (Josselyn, 2010; Silva et al., 2009), the activation of which is both necessary (Han, Jin-hee, Josselyn, 2009) and sufficient (Liu et al., 2012) for memory retrieval. This sparse neuronal ensemble is called the memory engram, a term coined by Richard Semon (1921) for the permanent record stored in an organism after exposure to a stimulus. Precisely what mechanisms regulate where information is stored within a neural circuit and how memories are allocated to specific subpopulations of neurons is an area of intense research (Silva et al., 2009). It has been found that connections between neurons part of an engram are more common and, importantly, stronger than connections with neurons not part of the engram (Liu et al., 2012). This synaptic strengthening has been found to contribute to both the formation of engrams (Nonaka et al., 2014) and their reactivation during memory recall (Ryan et al., 2015).
1.3.2 Synaptic plasticity

The ability of synapses to undergo lasting changes in strength is called long-term synaptic plasticity and differs from the short-term plasticity discussed earlier in that changes last not in the order of milliseconds, but in the order of days, weeks or months (Abraham, 2003). The theory is that when connected neurons are synchronously active together, the synapses between them become stronger. As a result, future activity of just a subset of neurons in the ensemble will more likely recruit the others, thus retrieving the memory. These activity-dependent changes in synapse strength are a wide-spread phenomenon, expressed throughout the central nervous system at both excitatory and inhibitory synapses, contributing to experience-dependent modifications of brain function, learning, and memory (Malenka and Bear, 2004). Although the hippocampal formation seems to play a role in the initial consolidation of memories, the principal site for long-term storage of information appears to be in the cerebral cortex, as shown with in vivo studies in rodents (Hasan et al., 2013) and with fMRI in humans (Yamashita et al., 2009).

So how are these changes in synaptic strength brought about? Synaptic strength is principally determined by the amount of neurotransmitter released and amount of postsynaptic receptors for that neurotransmitter, both of which can undergo lasting changes. The canonical form of synaptic plasticity at glutamatergic synapses is expressed at the postsynaptic site, and is brought about by changes in the number of postsynaptic AMPARs and/or their state of phosphorylation. Incorporation of more AMPARs or increasing their phosphorylation leaves a stronger, potentiated synapse (long-term potentiation (LTP)), and reducing the number of AMPARs or decreasing their phosphorylation results in a weaker, depressed synapse (long-term depression (LTD)). The molecular machinery involved in modifying synaptic strength cascades into action in response to changes in intracellular calcium levels ([Ca$^{2+}$]) in local nanodomains in and around the synapse. Small increases in Ca$^{2+}$ result in LTD, whereas higher increases lead to LTP and so the timing and time course of Ca$^{2+}$ signals determine the sign of change (Rubin et al., 2005; Zhou et al., 2005). Multiple postsynaptic sources of Ca$^{2+}$ exist, including internal Ca$^{2+}$ stores andVGCCs, a family of channels that permit influx of Ca$^{2+}$ in response to depolarisation (Catterall, 2011). A channel deserving special consideration here is the NMDA receptor, who’s involvement in synaptic plasticity has been long established (Malenka and Nicoll, 1993). These are ligand-gated ion channels that upon activation permit the flow of multiple ionic species across the channel, including Na$^+$, K$^+$ and a substantial amount of Ca$^{2+}$. So critical is the influx of Ca$^{2+}$ though these receptor channels for synaptic plasticity that in fact at many synapses, synaptic plasticity cannot occur without them (Malenka and Nicoll, 1993).

NMDARs require glutamate binding for the channel to open. However, at the neuronal resting membrane potential, the channel pore is still blocked by a Mg$^{2+}$ ion. This Mg$^{2+}$ block is only removed when the cell becomes more depolarised (Mayer et al., 1984; Nowak et al., 1984). This dual requirement for complete activation allows NMDARs to take on the physiological role of coincidence detectors of correlated pre- and postsynaptic activity; NMDARs will be triggered only when glutamate is released by presynaptic activity and binds to the receptor while the postsynaptic neuron is depolarised. Thus, the biophysical properties of NMDARs allow them to gate synaptic plasticity by timing the influx of Ca$^{2+}$ to when both the pre- and postsynaptic neuron are coincidently active. The temporal pattern of pre- and postsynaptic activity will shape the time course of glutamate availability and time course of postsynaptic depolarisations, respectively, and will thereby determine the degree and duration of NMDAR and VGCC activation (Shouval et al., 2002). This, in turn, will affect the time course of intracellular Ca$^{2+}$ levels and thereby determine the sign and magnitude of the change in synaptic strength. Thus, a direct relationship exists between the precise temporal pattern at which a pre- and postsynaptic neuron are firing and the change in strength of connections between them (Bi and Poo, 1998; Dan and Poo, 2004; Markram et al., 1997).
1.3.3 Spike timing-dependent plasticity

The temporal relationships of pre- and postsynaptic activity and resulting change in synapse strength have been explored in great detail in the mouse brain. There, it was found that the precise millisecond timing difference between pre- and postsynaptic AP firing determines the magnitude of change, and that their firing order determines the sign of change, so whether the synapses are strengthened or weakened (Bi and Poo, 1998). Typically, when the presynaptic neuron fires before the postsynaptic neuron (‘pre-before-post’) the synapse is potentiated, whereas if the order is reversed (‘post-before-pre’) the synapse is depressed. The time window for synaptic modification is restricted to about 40ms and a sharp switch in the direction of synaptic change exists at the 0 millisecond timing interval (Figure 1.3; Bi and Poo, 1998). STDP can be investigated experimentally by either recording from two connected neurons simultaneously, or by stimulating presynaptic axons via extracellular stimulation, as shown in panel B. The STDP window shown in E is a schematic example of the classic Hebbian STDP window first described by Bi and Poo (1998).

Figure 1.3  Spike timing-dependent plasticity.

Memories are thought to be encoded by small neuronal populations, called memory engrams. These ensembles are characterised by having higher connectivity and stronger synaptic contacts with members of the engram than with other neurons, as illustrated in the schematic of panel A. Synaptic strengthening between engram neurons may be established through long-term synaptic plasticity processes such as spike-timing-dependent plasticity (STDP). Panels B-D show how in two connected neurons (B), the order of presynaptic vs. postsynaptic activity (C) can lead to either long-term synaptic potentiation or depression (D). The timing interval determines the magnitude of change (E), with the largest change in synapse strength occurring when pre- and postsynaptic neuron firing is most tightly correlated (i.e. with the smallest delay). STDP can be investigated experimentally by either recording from two connected neurons simultaneously, or by stimulating presynaptic axons via extracellular stimulation, as shown in panel B. The STDP window shown in E is a schematic example of the classic Hebbian STDP window first described by Bi and Poo (1998).
A cardinal feature of STDP is the strong dependence on the back-propagating action potential (bAP) (Magee and Johnston, 1997). APs fired by a neuron propagate not only down the axon but also back ‘upstream’ into the dendrites, informing the synapse that the postsynaptic cell was active and providing a major source of depolarisation for NMDARs and dendritic VGCCs (Magee and Johnston, 1997). The importance of bAP signalling to STDP is nicely demonstrated by the progressive distance-dependent shift in the timing requirements for the induction of LTP and LTD that exists from proximal to distal synapses of rodent cortical pyramidal neurons (Letzkus et al., 2006; Sjöström and Häusser, 2006). This shift is due to a broadening of the bAP waveform as it propagates into the dendrites, which means that synapses at proximal dendritic locations experience sharper dendritic Ca\(^{2+}\) signals than distal synapses (Froemke et al., 2010; Letzkus et al., 2006; Sjöström and Häusser, 2006). The result is a shift from classic Hebbian STDP rules at proximal synapses, towards reversed, anti-Hebbian STDP rules at distal synapses.

STDP has now been observed at synapses in many brain areas and animal species in vitro (Abbott and Nelson, 2000), and in vivo in the macaque brain (Nishimura et al., 2013), but the temporal rules for STDP appear to not always be the same for every synapse; different synaptic learning rules can be observed between brain areas, neuron types and different species. This means that STDP rules and mechanisms identified at rodent synapses may not necessarily translate to those of humans, where STDP has remained unexplored. In fact, currently only indirect evidence exists for STDP in the human brain (Figure 1.4; Cooke and Bliss, 2006). A timing-dependent form of plasticity of motor-evoked potentials (MEPs) can be induced in human subjects by pairing peripheral nerve stimulation (PNS, analogous to presynaptic stimulation) with a transcranial magnetic stimulation (TMS, analogous to postsynaptic
stimulation) of the motor cortex (Stefan et al., 2000, 2002). Such paired associative stimulation (PAS) can induce both LTP-like increases and LTD-like decreases in MEP amplitude, with the sign of change depending on the relative timing of associated stimuli (De Beaumont et al., 2012; Conde et al., 2013; Koch et al., 2013; Thabit et al., 2010; Wolters et al., 2003, 2005). These changes have been suggested to be of cortical origin and related to STDP at synapses in the upper cortical layers (Stefan et al., 2000; Wolters et al., 2003) and the sensitivity of this STDP-like plasticity to NMDAR and VGCC blockers (Wankerl et al., 2010) is strongly suggestive of an underlying synaptic plasticity process. However, the reported time windows for STDP-like plasticity observed with this method are notably wider, and in some cases even reversed compared to the Hebbian STDP discussed above. To what extent PAS-induced changes in MEP amplitude and their corresponding timing windows reflect STDP at cortical synapses is unclear. In Chapter 4 of this thesis, we therefore explore human cortical STDP rules and mechanisms to understand how information is stored by pyramidal neurons of the human cortex.

### 1.3.4 STDP modulation

Synaptic plasticity rules are not fixed, but subject to change by the actions of neuromodulators. In rodents, neuromodulators have been reported to alter (Couey et al., 2007; Pawlak and Kerr, 2008; Zhang et al., 2009) or even completely reverse STDP rules (Pawlak et al., 2010; Seol et al., 2007). The presence or absence of particular neuromodulators can this way create windows during which specific timing of neuronal activity will lead to synaptic changes or not. Neuromodulators thereby provide the brain with temporal and spatial control over the synaptic modifications occurring within its circuitries. Acetylcholine (ACh) is one such neuromodulator which is involved in regulating neuronal network activity and synaptic plasticity and which is thought to have an important role in learning and the encoding of new memories (Hasselmo, 2006). The effects of ACh are mediated by two types of receptors; metabotropic muscarinic receptors (mAChRs) and ionotropic nicotinic receptors (nAChRs), the latter of which we shall focus on here. Upon binding ACh, these channels permit influx of multiple ionic species, most notably Na\(^+\) and Ca\(^{2+}\), resulting in membrane depolarisation. Brain nAChRs are composed of multiple subunits, either heteromeric combinations of \(\alpha(2-10)\) and \(\beta(2-4)\) subunits or homopentamers consisting of \(\alpha7\) subunits, and their precise subunit composition affects their biophysical (Ca\(^{2+}\) permeability, kinetics) and pharmacological properties (affinity, desensitisation) (Gotti et al., 2006; Hogg et al., 2003).

nAChRs are known to modulate cortical STDP rules. In L5 pyramidal neurons of mouse medial prefrontal cortex (mPFC), Couey et al. (2007) showed that spike timing-induced LTP (tLTP) was eliminated in the presence of nicotine and a depression of the excitatory inputs was observed instead. This nicotinic modulation of plasticity was abolished by inhibitors of GABA type A receptors, indicating the effects of nicotine where due to its actions on presynaptic interneurons. Different types of GABAergic interneurons found in the PFC express nAChRs on their soma that activate these neurons when nicotine is present. Thereby, nAChR stimulation enhanced GABAergic inputs to L5 pyramidal neuron dendrites, resulting in reduced dendritic Ca\(^{2+}\) entry during AP back-propagation (Couey et al., 2007, McGehee, 2007). Increasing dendritic bAPs by burst-like stimulation of the pyramidal neuron in the presence of nicotine could restore dendritic Ca\(^{2+}\) signals to levels comparable to those seen in the absence of nicotine, and restored LTP as well, indicating that strong post-synaptic stimulation could overcome the nicotinic modulation. Thus, activation of nAChRs expressed by mPFC interneurons that target pyramidal neuron dendrites can alter the rules for STDP and increase the threshold for the induction of tLTP.
NAChR expression has been shown to be cell-type and layer-specific across many cortical areas in rodents (Hedrick and Waters, 2015; Poorthuis et al., 2013a; Tian et al., 2014; Tu et al., 2009). The result is a layer-specific control of pyramidal neuron excitability by nAChRs (Poorthuis et al., 2013a, 2013b); superficial L2/3 pyramidal neurons are inhibited by nAChR activation on presynaptic interneurons, L5 pyramidal neurons receive a mix of excitatory and inhibitory input, while L6 pyramidal neurons are mostly excited by postsynaptic nAChRs and experience little change in inhibition (Bailey et al., 2012; Kassam et al., 2008; Poorthuis et al., 2013a; Tian et al., 2014). These highly specific expression patterns of nAChRs may provide neuronal networks with the option to locally modulate synaptic plasticity, allowing a particular layer, neuron or even synapse to respond differently than the average of the surrounding circuitry (Ji et al., 2001). Since the cellular and sub-cellular location of nAChRs determines how synaptic plasticity is altered by cholinergic signals, layer differences in expression may translate into layer differences in STDP modulation. Certainly, the mechanisms by which nAChRs alter synaptic plasticity of glutamatergic synapses in L5 pyramidal neurons of mouse mPFC seem not to be in place in L6. In Chapter 5 of this thesis, we therefore start with a study of the modulation of STDP rules by nAChRs in L6 pyramidal neurons of mouse mPFC. Since autoradiography studies have reported a laminar distribution of nAChRs in human cortex as well (Sihver et al., 1998), the second part of this chapter is devoted to investigating whether layer-specific cholinergic modulation of STDP rules occurs in human cortex.

1.4 SYNOPSIS

In this thesis, we will explore the physiological properties of human neurons and synapses in an effort to identify which features our neurons share with rodents, and in which they differ. A related aim is to see whether we can identify human neuronal morphological and/or physiological features that may impact information processing and storage in these neurons in a way that may explain our enhanced cognitive abilities. To achieve this, we use whole-cell recordings from living human pyramidal neurons in brain tissue resected during neurosurgery. The results of this work will be presented in the following 4 chapters, of which the first two will focus on aspects of concerning information processing (transfer, receiving and integration), and the final two will deal with information storage by human cortical pyramidal neurons.

Chapter 2

**Research question:** How do the dendritic arborisations of human cortical pyramidal neurons compare to those of other species and how do they affect dendritic signal propagation?

Dendrites are the antennae of a neuron, the place where information is received. Quantitative datasets on full dendritic trees of human pyramidal neurons are currently not available. We therefore start in Chapter 2 with an examination of neuronal morphology using 3D reconstructions of human cortical pyramidal neurons. Comparison of dendritic morphologies of human cortical pyramidal neurons with those of mice and macaques shows that human layer 2/3 pyramidal neurons have about twice as much dendrite and a distinct branching architecture. The effect of the distinct human dendritic arborisation on passive signal propagation is explored using computational modelling, which reveals strong attenuation of signals in human dendrites.
Chapter 3

Research question: What are the properties of short-term plasticity and information transfer at human cortical synapses, and how reliably can human neurons encode high input frequencies in their output?

Neurons are processors that integrate information received in the form of synaptic inputs into timed action potential output. To date, little is known about synaptic communication and information transfer in the human brain. In Chapter 3 of this thesis, we begin by characterising short-term plasticity at synapses between human layer 2/3 cortical pyramidal neurons. We find that human cortical synapses all show short-term depression, similar to rodent synapses. In contrast to rodent synapses however, they recover much more rapidly from depression and are therefore capable of transferring much more information. In the second part of this chapter, we find human neurons can make use of this high information content synaptic input, as human neurons turn out to be well adept at encoding very rapid fluctuations in inputs into the timing of their action potential, an ability supported by the fast onset kinetics of their action potentials.

Chapter 4

Research question: What are the rules and mechanisms of spike timing-dependent plasticity at human cortical synapses?

Information is stored in the brain by activity-dependent modifications in the strength of connections between neurons. An important form of such neuron-level information storage, called spike timing-dependent plasticity, has not been investigated directly at a cellular level in the human brain. In Chapter 4, we therefore set out to identify the rules and underlying mechanisms of spike timing-dependent plasticity at human cortical synapses. We find that human synapses can undergo bidirectional changes in strength throughout adulthood with a wider and reversed temporal window compared to that generally found in juvenile rodents. Employing pharmacological and calcium imaging techniques, we find synaptic potentiation and depression at human synapses are gated by postsynaptic NMDA receptors and that dendritic L-type voltage-gated calcium channels recruited by back-propagating action potentials are important for synaptic strengthening.

Chapter 5

Research question: How are the rules for spike timing-dependent plasticity modulated by acetylcholine in different layers of mouse and human cortex?

Spike timing-dependent plasticity rules are not fixed, but plastic themselves and can be altered by the actions of neuromodulators such as acetylcholine. In Chapter 5 we investigate to what extent layer-specific expression of nicotinic acetylcholine receptors translates to layer-specific modulation of spike timing-dependent plasticity rules in mouse medial prefrontal cortex. We find that endogenous acetylcholine release augments long-term potentiation of glutamatergic synapses on layer 6 pyramidal neurons by activating dendritic nicotinic receptors which amplify back-propagating action potentials. This is in contrast to layer 5 where long-term potentiation was shown before to be suppressed by nicotine and so points to layer-specific control over spike timing-dependent plasticity rules by the cholinergic system. Comparable mechanisms are found to operate in the human neocortex, where functional nicotinic receptors have a similar laminar distribution and cholinergic modulation of synaptic plasticity is opposite in superficial versus deep cortical layers.
DENDRITIC AND AXONAL ARCHITECTURE OF INDIVIDUAL PYRAMIDAL NEURONS ACROSS LAYERS OF ADULT HUMAN NEOCORTEX

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* Equal contribution; ^ shared senior authorship

Publication

The work in this chapter was published in 2015 in Cerebral Cortex:


Contributions

My contribution to this paper was to provide a large volume of biocytin-loaded human and mouse pyramidal neurons for 3D reconstruction. This involved optimising slicing procedures of human tissue to best preserve dendritic arborisations, maximising the nr. of cells filled per session and ensuring our sampling of neurons spanned the entire pia-white matter axis. Further contributions included the dealing with the anonymous patient medical records and commenting on the manuscript. Contributions of all authors in short: Conceived and designed the experiments: HM, MBV, HDM and CPJdK. Biocytin-loading: MBV, NG, CG, GTS and JO; Golgi/DAB/Nissl stainings: HM, RTN and ZSRMB; Computational models: GE; 3D reconstructions: RA, BA and ABCBB, Analysed the data: HM, KKD, GE and SvdS. Contributed materials/analysis tools: JCB and IS. Wrote the paper: HM, HDM and CPJdK.
Chapter 2

ABSTRACT

The size and shape of dendrites and axons are strong determinants of neuronal information processing. Our knowledge of neuronal structure and function is primarily based on brains of laboratory animals. Whether it translates to humans is not known since quantitative data on full human neuronal morphologies are lacking. Here, we obtained human brain tissue during resection surgery and reconstructed basal and apical dendrites and axons of individual neurons across all cortical layers in temporal cortex (Brodmann area 21). We show that human Layer 2 and Layer 3 pyramidal neurons have threefold larger dendritic length and increased branch complexity with longer segments compared to temporal cortex neurons from macaque and mouse. Importantly, morphologies did not correlate to etiology, disease severity or disease duration. Unsupervised cluster analysis classified 88% of human Layer 2 and Layer 3 neurons into human-specific clusters distinct from mouse and macaque neurons. Computational modelling of passive electrical properties to assess the functional impact of large dendrites indicates stronger signal attenuation of electrical inputs compared to mouse. Our quantitative analysis of human neuron morphology suggests human neurons are not “scaled-up” versions of rodent or macaque neurons, but have unique structural and functional properties.

2.1 INTRODUCTION

The cellular organisation of the human brain has been the focus of neuroscience research ever since Ramon y Cajal and Golgi’s ground-breaking work of more than a century ago. From many experimental and computational studies investigating neurons in brains of laboratory animals we now know that a strong interdependence exists between dendritic and axonal morphology and information processing capabilities of a neuron (van Elburg and van Ooyen, 2010; Eyal et al., 2014; Magee, 2000; Mainen and Sejnowski, 1996; Segev and Rall, 1998; Yuste and Tank, 1996). Mammalian dendrites have a rich repertoire of electrical and chemical dynamics, and individual neurons are capable of sophisticated information processing (Yuste and Tank, 1996). Dendritic geometry strongly affects the action potential firing pattern of neurons (Mainen and Sejnowski, 1996). In addition, we recently found that the size of dendritic arbours strongly modulates the shape of the action potential onset at the axon initial segment; it is accelerated in neurons with larger dendritic surface area (Eyal et al., 2014). Action potential onset rapidness is key in determining the capability of the axonal spikes to encode rapid changes in synaptic inputs (Fourcaud-trocme et al., 2003; Ilin et al., 2013). Hence, neurons with larger dendritic arbours have improved encoding capabilities.

Whether structure and function of neurons in brains of laboratory animals such as rodents accurately reflect human brain organisation is only partly known. Techniques commonly used in humans to study brain organisation such as EEG, MEG and MRI lack cellular resolution. Molecular and histological approaches using post-mortem human brain material have limitations to unravel extensive subcellular architecture, since typically, only partial cellular morphologies can be resolved and quantitative analysis is performed on sub-compartments of the apical/basal dendritic tree (Anderson et al., 2009; Braak, 1980; Elston et al., 2001; Jacobs et al., 2001; Ong and Garey, 1990; Petanjek et al., 2011; Rosoklija et al., 2014). Additionally, post-mortem delays to brain tissue fixation may effect morphology of fine cellular structures (Oberheim et al., 2009; de Ruiter and Uylings, 1987; Swaab and Uylings, 1988). Still, multiple studies provide evidence that the cellular organisation of the human cortex may differ substantially from that of laboratory animals (Bianchi et al., 2013; Clowry et al., 2010; Elston et al., 2001; Geschwind
and Rakic, 2013; Luebke et al., 2013; Nimchinsky et al., 1999). First, astrocytes in human temporal cortex are two to three times larger and processes are ten times more complex than their rodent counterparts (Oberheim et al., 2009), second, interneurons are more numerous and diverse in humans (Radonjic et al., 2014), third, absolute numbers for neurons, spines and synapses are highly species-specific and finally, density values for neurons, spines and synapses are also highly species-specific (DeFelipe, 2011; DeFelipe et al., 2003). In a comparison between single subjects, basal dendrites of pyramidal neurons in human prefrontal cortex of a 48-year-old subject were more branched and contained more spines than those in the prefrontal cortex of a 10-year-old macaque and an 18-month-old marmoset monkey (Elston et al., 2001). Comprehensive and quantitative datasets on full human neuronal morphologies including basal dendrites, apical dendrites (with oblique dendrites and distal tuft) and axonal architecture are however lacking. As a consequence, it has never been tested directly whether neocortical pyramidal neurons in the human brain show a larger dendritic structure of both apical and basal dendrites.

Here, we address this gap in our understanding of human brain organisation using intracellular dye loading of individual excitatory neurons in acute, living brain slices of human temporal cortex to avoid potential effects of post-mortem delays on cellular morphology. The dimensions of our living brain slices (350 µm) exceed typical slice dimensions for conventional Golgi-Cox stains on human brain samples (100-200 µm, (Jacobs et al., 2001; Petanjek et al., 2008; Zeba et al., 2008)) and truncation artefacts due to sectioning are therefore relatively small (van Pelt et al., 2014). Using this approach, we tested the hypothesis that total dendritic length of human Layer 2 and L3 pyramidal neurons (including basal, apical oblique dendrites, main apical trunk and distal tuft) are distinct from mouse and macaque pyramidal neurons. We find that the majority of the human temporal cortex pyramidal neurons clustered as a separate class distinct from both mouse and macaque temporal cortex pyramidal neurons. These findings show that human pyramidal neurons differ in their subcellular architecture and suggest that human pyramidal neurons have information processing capabilities distinct from rodent and macaque neurons.

2.2 METHODS

2.2.1 Human and mouse brain slice preparation

All procedures on human tissue were performed with the approval of the Medical Ethical Committee of the VU University Medical Centre, written consent by patients involved, and in accordance with Dutch license procedures and the declaration of Helsinki. Patients were anaesthetised with intravenous fentanyl 1–3 µg/kg and a bolus dose of propofol (2–10 mg/kg) and during surgery this was maintained with remyfentanyl 250 (µg/kg/min) and propofol (4–12 mg/kg). Human brain resection material that had to be removed for the surgical treatment of deeper brain structures typically originated from Gyrus Temporalis Medium (Brodmann area 21, occasionally Gyrus Temp. inferior or Gyrus Temp. superior), 2 – 6 cm posterior with respect to the temporal pole (human-specific speech areas were avoided during resection surgery through functional mapping). We obtained neocortical tissue from 28 patients (16 females, 12 males; age range, 19–66 years) predominantly treated for mesial temporal sclerosis (16 cases), for the removal of a hippocampal tumour (7 cases), epilepsy due to meningitis (2 cases), or cavernoma (3 cases). In all patients, the resected neocortical tissue was located outside the epileptic focus or tumour and displayed no structural/functional abnormalities in preoperative MRI investigations.
Upon surgical resection, the cortical tissue block (several cm$^3$s) was swiftly transferred to ice-cold artificial cerebral spinal fluid containing (in mM): 110 choline chloride, 26 NaHCO$_3$, 10 D-glucose, 11.6 sodium ascorbate, 7 MgCl$_2$, 3.1 sodium pyruvate, 2.5 KCl, 1.25 NaH$_2$PO$_4$, and 0.5 CaCl$_2$ and transported to the neurophysiology laboratory, which is located within a few hundred meters of the operating room. Transition time between resection of tissue and slice preparation was typically less than 10 min. Next, the tissue block was stabilised on a platform and 350 $\mu$m brain slices were prepared on a vibratome slicer as described before (Testa-Silva et al., 2010; Verhoog et al., 2013). Only completely intact, undamaged slices in which cortical layers span from pia to white matter were selected for electrophysiology, stored for 30 min at $34^\circ$C and subsequently left to recover for at least 1 h at room temperature before recording in

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Table 2.1 Patient details

Patient disease history and number of reconstructed neurons for individual patients. CBZ, carbamazepine; CZP, clonazepam; KEP, keppra; LAM, lamictal; LEV, levetiracetam; LTG, lamotrigine; LCS, lacosamide; OXC, oxcarbazepine; TPM, topiramate; VPA, sodium valproate.

Upon surgical resection, the cortical tissue block (several cm$^3$s) was swiftly transferred to ice-cold artificial cerebral spinal fluid containing (in mM): 110 choline chloride, 26 NaHCO$_3$, 10 D-glucose, 11.6 sodium ascorbate, 7 MgCl$_2$, 3.1 sodium pyruvate, 2.5 KCl, 1.25 NaH$_2$PO$_4$, and 0.5 CaCl$_2$ and transported to the neurophysiology laboratory, which is located within a few hundred meters of the operating room. Transition time between resection of tissue and slice preparation was typically less than 10 min. Next, the tissue block was stabilised on a platform and 350 $\mu$m brain slices were prepared on a vibratome slicer as described before (Testa-Silva et al., 2010; Verhoog et al., 2013). Only completely intact, undamaged slices in which cortical layers span from pia to white matter were selected for electrophysiology, stored for 30 min at $34^\circ$C and subsequently left to recover for at least 1 h at room temperature before recording in
artificial cerebrospinal fluid (aCSF) containing (in mM): 125 NaCl, 3 KCl, 1.25 NaH$_2$PO$_4$, 1 MgSO$_4$, 2 CaCl$_2$, 26 NaHCO$_3$, and 10 glucose. All aCSF and slicing solutions were continuously bubbled with carbogen gas (95% O$_2$, 5% CO$_2$), and had an osmolarity of 300 mOsm. Mouse brain slices were prepared using the same procedures; C57Bl/6J mice (7-15 weeks) were decapitated and brains submerged in ice-cold aCSF. Coronal sections (350 µm) including temporal association cortex (TEa, coordinates relative to Bregma: -4.16 mm posterior, 3.85 mm lateral) were then cut.

2.2.2 Neuronal visualisation and reconstruction

Slices in the recording chamber were submerged in aCSF (32–35°C) and whole-cell patch-clamp recordings were made as described in Chapter 3 Methods. Biocytin was supplemented to the intracellular solution and allowed to diffuse throughout the cell during recording. After recordings, slices were fixed in 4% paraformaldehyde and the recorded cells were visualised with chromogen 3,3-diaminobenzidine tetrahydrochloride (DAB) using the avidin–biotin–peroxidase method (Horikawa and Armstrong, 1988). Slices were mounted on slides and embedded in mowiol (Clariant GmbH, Frankfurt am Main, Germany). Neurons without apparent slicing artefacts were reconstructed using Neurolucida software (Microbrightfield, Williston, VT, USA), using a 100x oil objective. To determine shrinkage due to tissue fixation, staining and mounting (Egger et al., 2008), the post-mounting thickness of a subset of 10 human and mouse slices was measured, but no statistical difference was observed (140.7 µm vs 137.8 µm, medians for human and mouse, p>0.05, Mann-Whitney). Considering original slice thickness during in vitro electrophysiology (350 µm), shrinkage in the z-plane is 63±10% (see Egger et al. (2008)). Shrinkage correction of reconstructed morphologies and subsequent quantification of the TDL reveals that TDL increases only by 11±2% after shrinkage correction (n=5 examples) suggesting that most dendritic structures in our digital reconstructions consist of branches traveling in the x-y plane. To estimate layer boundaries, Nissl stainings were performed on a selection of human slices; these were rinsed in Na-acetate buffer (0.54%NaOAc and 0.96% acetic acid) and incubated for 10–15 min in 0.5% Cresyl-violet. Immediately after Cresyl-violet staining, slices were cover-slipped and images were taken at 4x magnification.

We compared our reconstructions of human and mouse pyramidal neurons to pyramidal neuron morphologies of Macaque *fascicularis* and Macaque *mulatta*. These morphologies were selected based on labelling method (intracellular dye injection) and reconstruction quality (400 µm sections were used and distal dendrites included in reconstruction) and files downloaded from Neuromorpho.org (cell IDs *M. fascicularis*: cnic_019-024 and cnic_055-060, *M. mulatta*: cnic_007-012 and cnic_050-054, (Duan et al., 2003)). These morphologies also suffered from shrinkage due to histological processing (Duan et al., 2003), but quantitative analysis was performed without shrinkage correction. It is important to note therefore, that all measurements underrepresent in vivo like dendritic and axonal measures.

2.2.3 Hierarchical agglomerative clustering

Dendrograms were constructed using statistics toolbox in Matlab (Mathworks, Natick, MA, USA). Since the number of branch points and total dendritic length were correlated (Figure 2.6F), only TDL was used for clustering. Neurons were clustered based on the Euclidean distance between their TDL and number of clusters determined by Thorndike procedure (Thorndike, 1953). The dendrograms were validated using their cophenetic correlation coefficient.
2.2.4 Analysis of segment length in apical tuft, apical obliques and basal dendrites
To compare change in segment length as a function of distance from the soma between human and mouse apical oblique, tuft and basal dendrites, we fitted a multi-level model (MLM (Hox, 2002)) using the Mixed Models procedure in IBM SPSS Statistics 21 (IBM corporation, Armonk, NY, USA). MLM can handle the fact that not all cells have the same number of dendritic segments, and that each cell can yield multiple measurements of segments of the same order. In addition, MLM allows the estimation of random effects, i.e., individual differences in the length of the first segment, and in the rate of increase (Aarts et al., 2014). In our analyses, both linear and quadratic terms were included to model the change in segment length as a function of the order of the segments.

2.2.5 Detailed compartment models of mouse and human pyramidal neurons
The electrotonic dendrograms in Figure 2.8B1, B2 were constructed using the trees toolbox (Cuntz et al., 2010), whereas the compartmental modelling simulations of 3D reconstructed neurons shown in Figure 2.8C1-E2 were performed using NEURON 7.2 (Carnevale and Hines, 2006).

2.3 Results
Human brain organisation at sub-cellular level is typically studied in post-mortem brain material obtained from autopsy surgery. The post-mortem delay in these cases is typically 23-26h (Jacobs et al., 1997, 2001; Ong and Garey, 1990), although optimisation protocols have been developed to limit autolysis time and ensure tissue fixation within 2-3h (Elston et al., 2001; Morales et al., 2014; Ravid and Swaab, 1993; de Ruiter and Uylings, 1987). However, even under these conditions, structural changes as a consequence of fixation delay cannot be excluded. To be able to digitally reconstruct in vivo-like apical dendrites, basal dendrites as well as axons belonging to the same neuron, we filled single neurons by dye loading during whole-cell patch clamp recordings from individual human cortical neurons in living brain tissue (Eyal et al., 2014; Testa-Silva et al., 2014; Verhoog et al., 2013). Brain tissue was obtained from patients with drug-resistant epileptic seizures associated with mesiotemporal sclerosis (MTS), subcortical tumours, cavernoma, meningitis or subcortical lesions (n=28 patients, age range 19-66 years, Table 2.1). Only neocortical brain tissue was used that was not part of the disease, but which had to be removed to gain access to deeper brain structures for surgical treatment.

To enable classification and registration of single neuron morphologies to a standard structural reference frame, we performed Nissl stainings on 50 µm sections to reveal layer borders in human temporal cortex (Figure 2.1A,B). First, a sharp increase in cell body densities was observed at 252±7 µm from pia delineating the border between L1 and 2 (Figure 2.1A,C, n=5). Second, increased cell densities (but smaller cell bodies) were encountered from 1201±186 µm to 1582±220 µm, indicative of L4 (Figure 2.1A,C). Finally, the border delineating the white matter (WM) was located at 2772±359 µm (Figure 2.1A,C). This is in line with previous studies that characterised the anatomical and cytoarchitectural structure of the human temporal cortex (DeFelipe, 2011; DeFelipe et al., 2003; Fischl and Dale, 2000; Ong and Garey, 1990).
Intracellular recordings for biocytin loading were performed on neurons with pyramidal shaped cell bodies (Figure 2.1D-F) identified by infrared differential interference contrast microscopy. Out of >500 biocytin filled neurons, 91 neurons across all layers of human temporal cortex were selected for 3D reconstruction (Figure 2.2). To include a neuron for reconstruction, first the biocytin signal had to be dense and uniform throughout distal dendrites. Second, dendritic structures had to show minor cutting artefacts by the slicing procedure and had to be retained in the slice as much as can be expected realistically. These two criteria rejected about 80% of recovered neurons from reconstruction, mainly because of obvious truncation of the apical dendrite. After reconstruction, morphologies were checked for accurate reconstruction in x/y/z planes, dendritic diameter, and no unconnected dendrites. Finally, reconstructions were crosschecked for false positive/false negative dendrites using an overlay in Adobe Illustrator between the Neurolucida reconstruction and Z-stack projection image from Surveyor software (Chromaphor, Oberhausen, Germany).
Dendritic and axonal architecture of human pyramidal neurons

Reconstructed neurons were exclusively spiny, indicative of excitatory nature and were categorised according to pia–soma distance (Figure 2.2). At cortical depths corresponding to L2–L3 in the Nissl stains (Figure 2.1A-C), neurons were encountered with multiple basal dendrites and one apical dendrite projecting towards the pial surface. In superficial L2 and L3, a depth-dependent gradual increase in both basal and apical dendrite length was observed, accompanied by an increase in the number of branch points (Figure 2.3A-E). At the L3–L4 border, a sharp decline in total dendritic length (TDL) was observed for individual neurons due to stellate-like morphologies from which an extensive apical dendrite was missing (Figure 2.2, Figure 2.3A-D). This was not the result of a slice-cutting artefact; dendrites of these neurons were complete within the slice. Deeper layers (L5 and 6) were characterised by relatively heterogeneous morphologies including shorter basal dendrites and irregularly shaped apical dendrites (Figure 2.2, 2.3A-D). The gradual, depth-dependent increase in morphological complexity in superficial layers up to L4 was also reflected in Sholl analysis (Figure 2.3F,G).

Figure 2.2  Dendrite gallery of human temporal cortex neurons.

Representation of 91 3D reconstructed human temporal cortex pyramidal neurons arranged according to somatic depth. First row indicates soma-to-pia distance in μm. Apical dendrites in blue, basal dendrites in red. Capitals (T, C, and M) indicate whether tissue was obtained from patients with subcortical tumour, cavernoma or meningitis. Neurons not labelled originate from patients with mesiotemporal sclerosis (MTS).
Since patient history may influence neuronal morphology, we tested whether patient-related parameters such as age or disease history correlate to morphological parameters such as total dendritic length (TDL) and number of branch points. We first determined the median TDL and number of branch points for individual subjects and used these median values (n=28) to determine correlations to patient-specific parameters. First, we found that both TDL and number of branch points were not significantly correlated to age (Figure 2.4A,B). Soma-pial depth (layer location) versus age was also not correlated (Rho 0.17, Spearman p=0.11), indicating unbiased sampling across layers versus age. Next, we determined whether disease duration (years between epilepsy onset and age at resection surgery) correlated to TDL and/or number of branch points, but did not find a significant relationship (Figure 2.4C,D). Seizure frequency (number of seizures per month) did also not correlate to TDL and number of branch points (Figure 2.4E-F). Finally, the approximate total number of seizures (years of epilepsy * number of seizures/year), was not correlated with either TDL or number of branch points (data not shown, TDL Rho=-0.02, p=0.90, # of branch points Rho=-0.07, p=0.72). Thus, we did not find any significant correlation between disease history and morphological parameters within our patient sample. Additionally, morphological parameters directly tested between the two patient groups (MTS versus tumour/cavernoma/meningitis) did not reveal consistent differences (p=0.28 for TDL, p=0.81 for # of branch points, Wilcoxon rank sum test) and single-cell data was therefore pooled.

In vivo approaches to reconstruct single neuron axonal morphology generate axonal trees that can be an order of magnitude larger compared to in vitro approaches on the same neuronal population (Bruno et al., 2009; Narayanan et al., 2015; Oberlaender et al., 2011). Since axonal morphologies have not been obtained for human neurons before and in vivo approaches are not available for individual human neurons, we reconstructed axons of 20 human neurons

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**Figure 2.4** Disease history does not affect total dendritic length and number of branch points.

A, Total dendritic length versus patient age. B, Number of branch points for individual morphologies versus patient age. C, D, TDL and number of branch points versus years since epilepsy onset. E, F, TDL and number of branch points versus seizure frequency. Each point in the scatterplots represents the median value for a single patient. Spearman’s Rho and p-value are indicated within figure panels.
Dendritic and axonal architecture of human pyramidal neurons sampled across all layers of medial temporal cortex (Figure 2.5A). We first quantified the depth dependence of polarity of axonal arborisations (Figure 2.5B,C, quantified as max/mean for 36 different directions), total axon length (Figure 2.5D), and number of branch points (Figure 2.5E,F). We found that direction selectivity of axonal projections increased with somatic depth (Figure 2.5C), but not axonal length (Figure 2.5D) or number of branch points (Figure 2.5E), suggesting that only polarisation of axonal projections depends on neuron location (for instance cell #17). Total axonal length versus number of branch points was also significantly correlated (Figure 2.5F). Our results may suggest layer-specific axonal architecture and may reflect a trend towards columnar organisation in deeper layers, at least at short intra-cortical distances. Obtaining detailed axonal reconstructions of human neurons in combination with information on dendritic architecture, dendritic spine and axonal bouton densities is in our view an essential prerequisite to “reverse engineer” a realistic human cortical column. However, organisational principles of human pyramidal neuron axons will need to be confirmed by axonal reconstructions in larger pieces of human cortical tissue in the future.

Figure 2.5 Axon gallery and layer specific axon property of human temporal cortex neuron.

A, 3D reconstructed axons with apical and basal dendrites of human temporal cortex neurons. First row indicates somatic depth in µm. Last row indicates ID of the same reconstruction in dendrite gallery if present in both. Axons in yellow, apical dendrite in blue, basal dendrite in red. Capitals (T, M) indicate that tissue was obtained from patients with subcortical tumour or meningitis. B, Polar plots of six example axonal reconstructions illustrating radial orientation of axons with respect to cell body. Top row indicates ID within axon gallery. C, Direction selectivity index of axons versus somatic depth. D, Total axonal length versus depth. E, Number of axonal branch points versus depth. F, Correlation between total axonal length and number of axonal branch points.
Chapter 2

To directly compare the size and complexity of dendritic arborisation across species we reconstructed pyramidal neurons from superficial L2 and L3 of adult mouse temporal cortex using the exact same methodology for slice preparation, dye loading, histological processing and neuronal reconstruction as for the human neurons. We used L2 and L3 pyramidal neurons at comparable cortical depths with respect to the pia-white matter span (n=15, age 7-15 weeks, Figure 2.6A). In addition, we used previously published complete dendritic morphologies from *Macaca fascicularis* (crab-eating macaque) and *Macaca mulatta* (rhesus monkey) L2 and L3 temporal cortex (Ascoli, 2006; Duan et al., 2002, 2003; Neuromorpho.org). Only pyramidal neurons from L2 and L3 temporal cortex were used to compare pyramidal neurons from human, mouse, *M. fascicularis* and *M. mulatta*. TDL was 3-fold larger in human neurons (median 14533 µm, 1st quartile 12595 µm, 3rd quartile 16570 µm, n=60) compared to mouse, *M. fascicularis* or *M. mulatta* neurons (mouse 5317 µm, n=15; *M. fascicularis* 6248 µm, n=12; *M. mulatta* 6081 µm, n=11).

Figure 2.6  Comparison of human, macaque and mouse L2/3 pyramidal neuron morphology.

A, Representative example 3D dendritic reconstructions of one mouse and human temporal cortex neuron. Apical main trunk in dark yellow, apical oblique dendrites in light blue, apical tuft in dark blue, basal dendrites in red, respectively. B, Normalized pia-soma distance of individual L2 and L3 neurons from mouse and human temporal cortex. C, Comparison of total dendritic length (TDL) between L2 and L3 temporal cortex neurons of mouse, human, *M. fascicularis* and *M. mulatta*. D, Comparison of basal, apical oblique and apical tuft length between mouse and human temporal cortex neurons. E, Number of branch points for mouse and human L2 and L3 temporal cortex neurons for basal, apical oblique, and apical tuft. F, Correlation between number of branch points and TDL of human and mouse L2 and L3 temporal cortex neurons. G, Dendrogram based on hierarchical agglomerative clustering of mouse, human, *M. fascicularis* and *M. mulatta* L2 and L3 temporal cortex neurons. Neurons were grouped into two clusters indicated by blue and red dendrograms based on TDL. Note isolation of 88% of human neurons into unique red cluster.
5239 µm, n=11, all medians, p<0.0001, Kruskal-Wallis, Figure 2.6C, Table 2.2). Surprisingly, the macaque pyramidal neurons were more similar in dendritic length to mouse pyramidal neurons than to human pyramidal neurons. Considering the species-specific temporal cortical thickness (human 2773 µm, M. fascicularis 2300 µm (brainmaps.org), mouse 969 µm), TDL apparently does not scale linearly to cortical thickness.

The TDL does not take into consideration that dendritic compartments can have very different functions (Larkum, 2013; Larkum et al., 2009; Spruston, 2008). Thus, we determined dendritic length for apical oblique dendrites, apical tuft and basal dendrites separately (Figure 2.6D, Table 2.2). The apical tuft was quantified as the dendritic length of all segments distal to the main bifurcation of the apical trunk with respect to the soma. The oblique dendrites were taken as the collection of branches originating from the main trunk, proximal to the main bifurcation of the apical trunk with respect to the soma. We found that all individual compartments (basal, main trunk, obliques and tuft) showed larger dendritic length for human L2 and L3 neurons compared to mouse neurons (basal, oblique and tuft: p<0.0001, Mann-Whitney, main trunk: p<0.01, Mann-Whitney, Figure 2.6E, Table 2.2). Since branching pattern is a strong indicator of dendritic complexity (DeFelipe, 2011), we compared the number of branch points between human and mouse neurons. The number of dendritic branch points per neuron was substantially different for basal and oblique dendrites, but not for the apical tuft in human neurons compared to mouse neurons (basal: p<0.01, oblique: p<0.0001, and tuft p=0.18, Mann-Whitney, Table 2.2).

Next, we determined whether these first order structural parameters allow for unsupervised classification of human neurons from a pool of unlabelled morphologies. Since TDL and number of branch points were highly correlated (Figure 2.6F), we only used TDL for cluster analysis. After randomised pooling of all available reconstructions from L2 and L3 temporal cortex of mouse, human, M. fascicularis and M. mulatta (n=98), unsupervised cluster analysis based on Euclidian distance and Thorndike procedure (Methods, (Thorndike, 1953)) resulted in emergence of two major clusters with 53 out of 60 human reconstructions isolated into one (red cluster, Figure 2.6G). In contrast, neurons from mouse, M. fascicularis and M. mulatta clustered together and were thus virtually indistinguishable from each other (blue cluster, Figure 2.6G). This shows that L2 and L3 pyramidal neurons in human temporal cortex have a distinct dendritic architecture compared to mouse, M. fascicularis and M. mulatta.

Table 2.2 Quantitative parameters of L2 and L3 neuronal morphology across species

<table>
<thead>
<tr>
<th>Species</th>
<th>Total</th>
<th>Basal</th>
<th>Apical</th>
<th>Apical trunk</th>
<th>Apical obliques</th>
<th>Apical tuft</th>
<th># of branch points</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Basal</td>
</tr>
<tr>
<td>M. fascicularis</td>
<td>6248</td>
<td>5140</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>(n=12)</td>
<td>7324</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. mulatta</td>
<td>5239</td>
<td>4760</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>(n=11)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human</td>
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<td>6467</td>
<td>8006</td>
<td>209</td>
<td>4121</td>
<td>3643</td>
<td>31</td>
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<td>2512</td>
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<td>1580</td>
<td>22</td>
</tr>
<tr>
<td>(n=15)</td>
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<td>2451</td>
<td>1991</td>
<td>23</td>
<td>50</td>
<td>1333</td>
<td>18</td>
</tr>
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<td></td>
<td>6546</td>
<td>3682</td>
<td>3111</td>
<td>216</td>
<td>1216</td>
<td>2361</td>
<td>29</td>
</tr>
</tbody>
</table>

Median values in bold, first and third quartile listed below, respectively. NA, not available.
To quantify the differences in subcellular morphology between human and mouse neurons, we compared the segment length of both apical and basal dendrites using linear and quadratic polynomials in a multilevel model (MLM). The segment length was determined as path length between branch points and we found that segment length increases significantly with distance from soma in both mouse and human neurons (Figure 2.7A-C, p<0.01 for both linear and quadratic effects, MLM). We also observed a significant difference in the rate of linear increase in segment length between human and mouse neurons (basal: p<0.001, apical obliques: p<0.05; and apical tuft p<0.001, MLM). For basal and apical oblique dendrites, initial mouse and human segment lengths were comparable, but compared to mouse L2 and L3 temporal cortex neurons, human dendrites had significantly longer segments from the 3rd, 2nd and 1st order segments of basal, apical oblique- and apical tuft dendrites, respectively (Figure 2.7A-C, p<0.001, MLM). This shows that human pyramidal neurons are not linearly scaled with respect to mouse pyramidal neurons and scaling is compartment-specific.

The size of the dendritic tree, the number of branch points and dendrite thickness strongly determine the passive electrical properties of dendrites. To determine how the increased morphological complexity of human neurons affects passive dendritic signal propagation, we used a detailed computational model. For both mouse and human populations, we took the most representative morphology (median TDL for the population; cell #44 for human). The study of the effect of dendritic architecture on passive attenuation in L2 and L3 neurons was performed by using the same specific values for membrane (R_m) and axial (R) resistivity for reconstruction of the electrotonic dendrogram for both mouse and human neurons (R_m=15000 Ωcm² and R=150 Ωcm, Figure 2.8B1,B2, (Sarid et al., 2007)). In addition to these specific parameters, passive attenuation in dendrites depends also on segment length, dendritic diameter (space constant (λ = \sqrt{(R_m/R)} \times \text{diam}^4)) as well as on the boundary conditions at the ends of this dendritic segment (Rall, 1959). With these factors incorporated into the computational model, human neurons show much longer electrotonic means than mouse neurons (Figure 2.8C1,C2).
Electrotone structure and voltage attenuation in human and mouse L2 and L3 pyramidal cells.

**A1,A2.** Morphology of human and mouse temporal cortex example neurons, respectively. Red denotes basal tree whereas blue the apical tree, respectively. 

**B1,B2.** Dendrogram in cable units for the neurons in **A1** and **A2** respectively. In both cases, $R_m=15,000 \, \text{Ω} \cdot \text{cm}^2$ and $R=150 \, \text{Ω} \cdot \text{cm}$. 

**C1,C2.** Normalized soma voltage resulting from steady state current injection in the corresponding dendritic sites for the example neurons in **A1** and **A2** respectively. Each line represents a path from the soma to a dendritic terminal. Note the 1.5 fold increase in the average voltage attenuation for the human vs. the mouse cell and the enhance attenuation from the distal apical tuft in the human neuron.

**D1,D2.** Steady state voltage attenuation from the dendrites to soma (taking into account local dendritic responses to current injection) for the neurons in **A1** and **A2** respectively. Note the 2.5 fold increase in the average attenuation for the human vs. the mouse neuron. 

**E1,E2.** Steady state voltage attenuation from the soma to the dendrites for the neurons in **A1** and **A2** respectively.

A direct consequence of extended and more ramified dendrites was that forward- and backward spread of electrical signals showed larger attenuation in human compared to mouse (Figure 2.8C-E). More specifically, when comparing the soma voltage response for steady current injected at the dendrites (normalised to the soma response for somatic current injection), we found an average of 1.5 fold increase in the dendritic-to-soma (forward) voltage attenuation factor for the human vs. the mouse L2 and L3 neuron (Figure 2.8C1,C2). Steady state voltage attenuation from dendrites to soma (taking into account local dendritic responses to current injections) shows a 2.5 fold increase in the average attenuation factor for the human vs. the mouse neuron (Figure 2.8D1,D2). Finally, backward propagating (from soma-to-dendrites) signals following steady-state current injection into the soma was also characterised by higher attenuation factor in the human neuron compared to mouse neuron (Figure 2.8E1,E2). Active conductance mechanisms to propagate signals have been well documented in rodents, but we aimed at studying the direct effect of dendritic architecture on signal conduction. In addition, the vast array of potential active mechanisms that a neuron adopts to conduct signals would directly or indirectly depend on the inherent passive properties associated with its dendritic structure. Thus, the cellular architecture directly impacts passive electrical properties and behaviour of human neurons, and affects both forward- and backward spread of electrical signals in dendrites.
2.4 Discussion

The human neocortex contains cell types that are not represented in the rodent neocortex (Nimchinsky et al., 1999; Oberheim et al., 2009; Radonjic et al., 2014). In this study, we asked the question whether the most abundant cell type of the neocortex, the pyramidal neuron, is morphologically similar or distinct in human, mouse and macaque temporal cortex. Thereto, we made 3D dendritic and axonal reconstructions of human temporal cortex pyramidal neurons. Because L2 and L3 holds a relatively homogenous class of pyramidal neurons, in contrast to deeper cortical layers (Andjelic et al., 2009; Arimatsu et al., 2003; Bortone et al., 2014; Groh et al., 2010; Harris and Shepherd, 2015; Marx and Feldmeyer, 2013), we restricted our study to L2 and L3 pyramidal neurons. By directly comparing with temporal cortex L2 and L3 pyramidal neurons of adult mice and macaque monkeys, we find that basic structural properties such as dendritic length, the number of branch points and segment length are different in human neurons. Human L2 and L3 pyramidal neurons have three times more dendrite than their mouse and macaque counterparts. Both basal dendrites and apical oblique dendrites (but not apical tufts) have more segments and in particular the distal segments are substantially longer in human pyramidal neurons. Cluster analysis showed that the majority of human L2 and L3 pyramidal neurons are morphologically distinct from mouse and macaque L2 and L3 pyramidal neurons.

Several studies have made direct comparisons of molecular, laminar, developmental and cellular morphology between rodents, monkeys and humans (DeFelipe, 2011; Elston et al., 2001, 2011; Johnson et al., 2009; Kwan et al., 2012; Zeng et al., 2012). These studies highlight that complexity of neurons and astrocytes is substantially larger in human prefrontal and temporal cortex (DeFelipe et al., 2003; Elston et al., 2001; Oberheim et al., 2009). Defelipe et al. (2003) reported that also other subcellular neuronal features, such as the shape of dendritic spines, the number of excitatory and inhibitory synapses received by neurons, as well as cell densities can differ substantially between human and rodent neocortex. From these studies it is estimated that a human L2/L3 pyramidal neuron receives more than thirty thousand synapses, approximately twice as much as is received by rodent L2/L3 pyramidal neurons (DeFelipe, 2011). We found that human L2/L3 pyramidal neurons have three times more dendrite. This large dendritic tree can well accommodate a doubling of the number of synapses.

2.4.1 Caveats of human tissue

In this study, we used brain tissue from twenty-eight patients that underwent surgical treatment. This is brain tissue that is not part of the disease, but had to be removed to gain access to deeper brain structures for surgical treatment. We found no systematic differences in dendrite morphology between neurons obtained from patients with different disease backgrounds. Also, we found no correlation between morphology (TDL and # of branch points) and disease etiology, disease duration since onset, disease intensity (# of seizures per month) and disease severity (total number of seizures; Figure 2.4). Since more elongated and more complex dendritic morphology of human temporal cortex neurons was consistent across patient populations, this is most likely not the result of a specific disease history.

The only alternative method for studying the cellular organisation of human brain is to obtain human tissue from post-mortem brain. This approach suffers from other shortcomings, such as a drop in spine and dendrite densities that is observed already 1.5 hours post-mortem (Oberheim et al., 2009; de Ruiter and Uylings, 1987; Swaab and Uylings, 1988). Preliminary observations comparing basal dendrites of human temporal cortex L2 and L3 pyramidal neurons in surgical resection tissue and post-mortem tissue showed that pyramidal neurons in post-mortem tissue had 30% less basal dendrite length (Guy Eyal, Idan Segev, unpublished...
observations). The outcome of this specific comparison could be due to ischemia-induced neurotoxic processes in the time delay from death to brain fixation, or to differences in the age of subjects used which was higher for the post-mortem brain tissue. The average age of patients in our study was 36.0±12.4 years. One of the strengths of our approach is that the comparison between human and mouse pyramidal neurons was done using the exact same methods of single neuron labelling in acute, living brain slices of adult temporal cortex in the same lab.

Important to note however is that standardisation protocols for rodent brain research are much more advanced compared to human brain research. Increasingly difficult to control for in human brain research are genetic and non-genetic factors that could contribute to neuronal morphology. The genetic background of the human population is highly diverse and non-genetic factors influencing fine-scale cortical organisation are not controlled for. For instance, pre-surgical medication with anti-epileptic drugs is highly subject-specific (Table 2.1) and lifetime use of alcohol, nicotine or caffeine is largely unknown. It seems highly unlikely though that those genetic and environmental factors could have such a consistent and profound effect on cellular morphology explaining the 3-fold difference in TDL between mouse and human. Future studies and increasing information on subcellular organisation of human brain will be the only framework in which the interdependence between neuronal morphology in human brain and genetic/environmental factors can be investigated.

2.4.2 Functional consequences for distal synapses

With larger dendritic trees and more input synapses, human L2 and L3 pyramidal neurons seem to be larger integration centres compared to rodent L2 and L3 pyramidal neurons. Yet, larger dendrites and more branch points will mean that input synapses on distal segments run the risk of having little impact on the cell body membrane potential. Using computational modelling of the cable properties of L2 and L3 neurons, we showed that the morphological differences between mouse and human directly affect the electrical properties of dendrites. Both forward- and backward propagation of electrical signals are affected and are strongly attenuated in human dendrites. This first step in computationally modelling the electrical behaviour of human neurons may suggest that distal dendrites of human neurons can act electrically more independently from the soma. Alternatively, additional synaptic amplification or non-linear dendritic mechanisms may exist in human dendrites that could aid dendritic propagation of electrical signals (Chen et al., 2011; Lavzin et al., 2012). We have recently shown that action potentials evoked at the soma of human temporal cortex neurons induce sharp calcium transients in the dendrite that can be blunted by L-type calcium channel blockers (Verhoog et al., 2013). How active human dendrites are and whether the distribution of dendritic voltage-dependent conductances resemble those in rodent pyramidal neurons (Larkum et al., 1999b), awaits direct dendritic recordings.
HIGH BANDWIDTH SYNAPTIC COMMUNICATION AND FREQUENCY TRACKING IN HUMAN NEOCORTEX

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Contributions
My contribution to this paper was the acquisition of the high-resolution action potential data from human and mouse pyramidal neurons to analyse and compare action potential (onset) kinetics, and writing the associated methodology section. Contributions of all authors in short: Conceived and designed the experiments: GT-S, MG and HDM. Performed the experiments: GT-S, MBV, and DL. Analysed the data: GT-S, MBV, DL, MG, and HDM. Contributed reagents/materials/analysis tools: GT-S, DL, MG, CIZ, CPJK, JCB, and RMM. Wrote the paper: GT-S, MG, CPJK, JCB, CIZ, RMM, and HDM.
Chapter 3

Abstract

Neuronal firing, synaptic transmission, and its plasticity form the building blocks for processing and storage of information in the brain. It is unknown whether adult human synapses are more efficient in transferring information between neurons than rodent synapses. To test this, we recorded from connected pairs of pyramidal neurons in acute brain slices of adult human and mouse temporal cortex and probed the dynamical properties of use-dependent plasticity. We found that human synaptic connections were purely depressing and that they recovered three to four times more swiftly from depression than synapses in rodent neocortex. Thereby, during realistic spike trains, the temporal resolution of synaptic information exchange in human synapses substantially surpasses that in mice. Using information theory, we calculate that information transfer between human pyramidal neurons exceeds that of mouse pyramidal neurons by four to nine times, well into the beta and gamma frequency range. In addition, we found that human principal cells tracked fine temporal features, conveyed in received synaptic inputs, at a wider bandwidth than for rodents. Action potential firing probability was reliably phase-locked to input transients up to 1000 cycles/s because of a steep onset of action potentials in human pyramidal neurons during spike trains, unlike in rodent neurons. Our data show that, in contrast to the widely held views of limited information transfer in rodent depressing synapses, fast recovering synapses of human neurons can actually transfer substantial amounts of information during spike trains. In addition, human pyramidal neurons are equipped to encode high synaptic information content. Thus, adult human cortical microcircuits relay information at a wider bandwidth than rodent microcircuits.

3.1 Introduction

Human cognitive abilities clearly stand out from those of other mammals (Shettleworth, 2012). Evolutionary development of brain size, encephalisation, neocortical thickening, and specialisation of cortical circuits (DeFelipe, 2011; Rakic, 2009) most likely underlie the superior human mental capacity, but other factors may contribute as well (Varki et al., 2008). Cognitive functions rely on appropriate relay and filtering of information and on efficient communication between brain areas. Ultimately, neuronal firing and synaptic transmission between neurons form the building blocks for coding, processing, and storage of information in the brain (Salinas and Sejnowski, 2001). Synapses in particular are fundamental computational units (Abbott and Regehr, 2004; Fuhrmann et al., 2002; Shepherd, 2003), and the increased complexity of synaptic protein networks was recently put forward as a potential correlate of mammalian cognitive ability (Bayés et al., 2012; Nithianantharajah et al., 2013; Ryan and Grant, 2009). Given the vast number of synapses in the brain, in the order of a trillion per cubic centimetre (Drachman, 2005), even a slight increase in efficacy of synaptic information processing could potentially translate into a substantial elevation of the brain’s overall computational performance (Rieke et al., 1999). Whether human synapses are more efficient in transferring information between neurons is not known and has not been tested directly.

Here, we addressed this question and studied the properties of signal transfer at unitary synaptic connections between pyramidal neurons of adult human and mouse neocortex. We then applied an information theory approach to calculate synaptic transfer performance (Borst and Theunissen, 1999; Fuhrmann et al., 2002, 2004). We focused on the short-term
High bandwidth synaptic communication in human neocortex

dynamics of transmission, as synapses are not passive conveyers of information. Instead, they display prominent use-dependent plasticity, which has important roles in information processing (Abbott and Regehr, 2004; Klug et al., 2012). Following chemical signal transduction at a single synapse, postsynaptic signals appear as selectively filtered versions of the train of action potentials (APs) that the presynaptic neuron generates (Abbott et al., 1997; Tsodyks and Markram, 1997). Amplitudes of successive postsynaptic potentials are in fact transiently and reversibly attenuated or amplified by the context of previous pre- and postsynaptic activation. Whether or not a postsynaptic neuron fires in response to an individual presynaptic AP thus depends on the instantaneous AP frequency, on the short-term dynamical properties of each synapse, and on the previous history (Abbott and Regehr, 2004; Abbott et al., 1997; Markram et al., 1998b; Zucker and Regehr, 2002). We found that human cortical synapses recover faster from depression than rodent cortical synapses, resulting in a substantially higher information transfer rate than in rodent synapses. In addition, we directly observed that human pyramidal neurons are equipped to encode such a high information content synaptic transmission in their output, unlike rodent pyramidal neurons, by their dynamical excitability properties.

3.2 Methods

3.2.1 Human and mouse neocortical slice preparation
Tissue was obtained from patients (aged 18–61 years) and slices were prepared using procedures described in Chapter 2 Methods. All procedures for mouse neocortical brain slice preparation were approved by the VU University’s Animal Experimentation Ethics Committee and by the Ethics Committee of the Department of Biomedical Sciences of the University of Antwerp. Brain slices of C57Bl6 mice (2–11 weeks of age) were prepared as described in Chapter 2 Methods, using the same solutions and basic procedures as for human slices. Coronal slices (300–450 mm thickness) were cut from the pre-limbic region of the medial prefrontal cortex (mPFC; P12–36) or the temporal association cortex (TC; 8–11 weeks).

3.2.2 Electrophysiology
Neocortical slices were visualised using either infrared differential interference contrast (IR-DIC) microscopy or Hoffman modulation contrast. All experiments were performed at 32-35°C. None of the neurons recorded from showed spontaneous epileptic-form spiking activity. Recordings were made using Multiclamp 700A/B amplifiers (Axon Instruments, CA, USA) sampling at intervals of 4 to 100 µs, and low-pass filtered at 10 to 30 kHz. Recordings were digitised by pClamp software (Axon), by LCG software (Destexhe et al., 2001), or custom written scripts in Igor Pro, and later analysed off-line using custom Matlab scripts (The Mathworks, Natick MA, USA). Patch pipettes (3-5 MΩ) were pulled from standard-wall borosilicate capillaries and filled with intracellular solution containing (in mM): 110 K-gluconate; 10 KCl; 10 HEPES; 10 K-phosphocreatine; 4 ATP-Mg; 0.4 GTP, pH adjusted to 7.2-7.3 with KOH; 280-290 mOsm., 5 mg/ml biocytin. Post-hoc visualisation and neuron identification using biocytin labelling was performed as described previously (van Aerde et al., 2009; Nemenman et al., 2008) and in Chapter 2 Methods. Pyramidal neurons were classified based on morphological and electrophysiological criteria. Input resistances were calculated from the steady state response
to hyperpolarising current pulses (mean±SEM): human $R_{\text{in}} = 70 \pm 6$ MΩ ($n=27$), young mouse $R_{\text{in}} = 84 \pm 3$ MΩ ($n=45$), adult mouse $R_{\text{in}} = 102 \pm 7$ MΩ ($n=26$) (adult mice significantly different from human, $p<0.01$). Resting membrane potentials (not corrected for liquid junction potentials; mean±SEM): human $V_{\text{rest}} = -73.2 \pm 0.8$ mV ($n=27$), young mouse $V_{\text{rest}} = -69.6 \pm 0.7$ mV ($n=45$), adult mouse $V_{\text{rest}} = -74.5 \pm 1.1$ mV ($n=26$) (adult mice and human significantly different from young mice ($p<0.02$), but not different from each other). These numbers were taken into account when injecting current to test whether human pyramidal neurons can time their AP firing to high frequency inputs. The baseline current injected was set to keep iso-frequency firing close to $10 – 15$ Hz.

### Frequency-dependent short-term synaptic depression

The model of Tsodyks & Markram (Markram et al., 1998a; Tsodyks and Markram, 1997) was employed to quantitatively characterize use-dependent short-term depression (STD) of EPSPs amplitude in response to defined trains of presynaptic APs. This description refers to the existence of generic resources for neurotransmission, without distinguishing between presynaptic (e.g., the ready-releasable pool of vesicles) and postsynaptic biophysical components (e.g., desensitisation of AMPA receptors). The model is identified by 5 numerical parameters (Castillo and Katz, 1954): $A$ – the absolute synaptic efficacy; $U$ – the fraction of resources consumed by each AP; $\tau_{\text{rec}}$ – the time constant of recovery from exhaustion of available resources; $\tau_{\text{inac}}$ – the synapses’ time constant to transit between active and inactive states; $\tau_{\text{mem}}$ – the membrane time constant, as defined in a leaky integrate-and-fire model. The peak amplitude of the n-th postsynaptic response, indicated by $E_n$, is given by $E_n = (A U R)$, where the dynamical variable $R$ is the running value of the available resources. Indicating the times of successive AP as $t_1, t_2, \ldots, t_n$, the model responses $E_1, E_2, \ldots, E_n$ are obtained by $R_1, R_2, \ldots, R_n$ upon numerical iteration:

$$R_n = 1 + [(1 - U) R_{n-1} - 1] \exp[-(t_n - t_{n-1})/\tau]$$

The same numerical method was employed for both simulating model responses, as well as to search for parameters ($A, U, \tau$) that optimally reproduce the experimental data after least-square fitting.

### Novelty-detection in presynaptic firing rate by a population of synaptic afferents

A passive R-C circuit was mathematically defined to mimic temporal integration of postsynaptic responses in a point-neuron with membrane time-constant of 10 msec. Then, 1500 identical synaptic afferents impinging on this neuron were activated, each by an independent realisation of an identical Poisson point-process. The mean frequency of this random process was step-changed from $f_1$ to $f_2$, after several seconds of simulation lifetime. Each individual model synapse relayed the occurrence of a presynaptic AP in a use-dependent manner, according to the Tsodyks-Markram model described above. Without losing any generality, simulated postsynaptic responses were expressed and plotted in arbitrary units, normalising voltage responses to the product of the (unspecified) neuronal input resistance and maximal synaptic efficacy $A$.
3.2.5 Quantifying temporal information transfer at a single synapse

As an alternative to the Tsodyks-Markram model, we considered its non-deterministic formulation, which combines the classical quantal model (Allen and Stevens, 1994; Castillo and Katz, 1954) with use-dependent short-term depression as in Fuhrmann et al. (2002). We considered \( n=5 \) release sites and the average quantal content \( A/N \), with \( A \) being the maximal synaptic efficacy of the deterministic Tsodyks-Markram model. The last choice implies that on the average of many repeated trials, the non-deterministic model responses quantitatively correspond to the predictions of the Tsodyks-Markram deterministic formulation. The coefficient of variation of the quantal content was set to 0.4, choosing its standard deviation as 0.4 \( A/N \). The coefficient of variation's value was taken from an example in the literature (Fuhrmann et al., 2002) and its numerical value scales proportionally the mutual information calculations and thus does not affect our conclusions. To demonstrate the previous statement we explored different values of \( CV \) of the simulated quantal content (i.e., 0.2, 0.4, 0.6, and 0.8) (see Figure 3.5.2). The parameter has, therefore, no qualitative effect, but only a scaling effect. As opposed to a classic quantal model, the probability of release is non-stationary, and it is computed as the product between the fixed probability that a release site contains a vesicle \( (U) \) and the probability \( P_v(t) \) that a vesicle is available at a given time \( t \). In the lack of any presynaptic AP, \( P_v(t) \) recovers exponentially to 1 with a recovery time-constant \( \tau \), while immediately after an AP this probability is decreased by a proportional amount, \( P \rightarrow (1-U)P \). This model allows one to apply information theoretical methods (Borst and Theunissen, 1999), extended to probabilistic synaptic transmission, and quantify mutual information between the set of postsynaptic responses to a train of presynaptic spikes, and the corresponding set of interspike intervals (Fuhrmann et al., 2002). The last are assumed to act as a source of (arbitrary) temporal information.

Several average presynaptic firing frequencies were considered (0.01–100 Hz). For each average frequency, a realisation of a Poisson point-process was generated to simulate the time of occurrence of 10,000 presynaptic spikes fired at such an average frequency. The marginal probability density of the postsynaptic amplitudes was estimated, and the corresponding conditional probability density, given the instantaneous probability of release, derived under the same assumptions of Fuhrmann et al. (2002). Conditional entropies were computed according to the definition of Shannon (1948), and mutual information computed as their difference.

3.2.6 Modulation of firing by noise + sine injection and fit of the transfer function

To evaluate the dynamical transfer properties of neuronal discharge in response to rapidly varying inputs, a sinusoid of amplitude \( I_1 \) and frequency \( F \) (1-1000 cycles/sec) was applied simultaneously to a DC baseline \( I_0 \) and to a randomly fluctuating waveform, under current-clamp stimulation:

\[
I(t) = I_0 + I_1 \sin(2\pi Ft) + I_{\text{noise}}(t)
\]  

(1)

The fluctuating component \( I_{\text{noise}}(t) \) was synthesised as an exponentially filtered Gaussian white-noise realisation, mimicking at the soma the consequences of a barrage of balanced back-
ground excitatory and inhibitory irregular synaptic inputs, as described previously (Benavides-Piccione et al., 2012; Destexhe et al., 2001; Wang et al., 2006). $I_{\text{noise}}(t)$ had zero-mean, variance $s^2$ and correlation length $\tau_I=5$ ms, and was generated by means of the LCG software (Linaro et al., 2014) iterating the following expression at the same rate of the sampling interval (i.e. $1/dt=20$kHz),

$$I_{\text{noise}}(t+dt) = (1 - dt/\tau_I)I_{\text{noise}}(t) + s \sqrt{2dt/\tau_I} \xi_t$$

(2)

Where $\{ \xi_t \}$ is a sequence of independent pseudo-random numbers with normal distribution (Press et al., 1992). Depending on the cell input resistance and rheobase, the current DC baseline $I_0$ and the random fluctuation amplitude $s$ were adjusted so that for $I_1=0$ pA neurons responded (i) with low mean rate (~10-15Hz), (ii) highly irregular inter-spike intervals, and (iii) subthreshold voltage random fluctuations (5-10 mV) as observed in cortical recordings \textit{in vivo} (Destexhe et al., 2001). Finally, $I_1$ was chosen as a fraction of $I_0$ (e.g. $I_1=50$ pA, $I_1=400$ pA) and each stimulation $I(t)$ lasted 50 s and was followed by a long recovery time of at least 50 s. Raw voltage traces were recorded for different values of $F$ and offline processed in MATLAB (The Mathworks). Individual spike times $\{t_k\}$, $k=1, 2, 3, \ldots$, occurring across subsequent input oscillation cycles, were extracted by a peak-detection algorithm and then normalised to the corresponding oscillation period: i.e. $t_k \rightarrow (t_k \% F)^{-1}$, where $\%$ indicates the remainder of integer division. Peristimulus time histograms (PSTH) with 30 bins, were then computed and normalised to represent the instantaneous discharge rate. Three free parameters $r_0$, $r_1$ and $\Phi$ of the sinusoidal function:

$$r(t) = r_0 + r_1 \sin(2\pi F t - \Phi)$$

(3)

were optimised to best-fit in the least-squares sense each PSTH by $r(t)$, through the Levenberg-Marquardt algorithm (Press et al., 1992). The same procedure was repeated on surrogate spike-train data, obtained by randomly shuffling the interspike-intervals $\{(t_{k+1} - t_k)\}$ to obtain the minimal level of significance for the estimates of $r_1$ and $\Phi$.

To fit experimentally measured amplitude and phase response data, we used a linear model, as described in (Köndgen et al., 2008), reminiscent of a passive analogue electronic filter. Briefly, the modulation depth $r_1(F)/r_0$ and the phase $\Phi(F)$ were taken as the magnitude and phase of the impulse response of a linear dynamical system described, in the Fourier domain, by the following rational complex function:

$$H(j\omega) = A \frac{\prod_{i=1}^{M} (j\omega - p_i)}{\prod_{i=1}^{N} (j\omega - z_i)}$$

(4)

where the polynomials roots $\{p_i\}$ and $\{z_i\}$ are known as the “poles” and “zeros” cut-off of the transfer function, respectively, and $A$ is the low-frequency gain. The transfer function in Equation 4 was used to fit the magnitude and phase responses over the population of cells, as shown in \textbf{Figure 3.4E}, and the fit was weighted by the inverse of the standard deviation of each data point. The two datasets, corresponding to human and adult mouse cells, were fit with functions containing a single zero (i.e., $M=1$), and either two or three poles (i.e., $N=2–3$). In both cases, $N>M$ accounts for the power-law decay of the magnitude of the transfer function at frequencies above the cut-off frequency according to the relation:

$$\|H(2\pi if)\| \approx f^{-\alpha}$$

(5)
with $\alpha = N - M$. To model the fact that the phase response does not saturate for high Fourier frequencies at integer multiples of $\pi$ (Arsiero et al., 2007), we included in our formulation a constant propagation delay $\Delta T$, which takes into account, among other things, the time it takes for the spike to travel from its originating zone to the point at the soma where it is recorded (see (Arsiero et al., 2007; Köndgen et al., 2008) for a discussion).

### 3.2.7 Analysis of action potential waveform

In experiments aimed at examining AP-waveform (Figure 3.5, Figure 3.S3), data were acquired with 4 or 10 µs sampling intervals, low-pass filtered at 30 kHz, and filtered offline at 15 kHz. Bridge balance was adjusted manually and pipette capacitance was compensated for. Recordings were excluded if bridge balance exceeded 12 MΩ. For analysis, all APs with instantaneous firing frequencies up to 30 Hz were pooled and binned for all recordings from a cell. Traces with resting membrane potentials above 260 mV and APs where the linear fit to obtain onset rapidity had $R^2$ values <0.95 were excluded from analysis. The various AP parameters were calculated for each AP in a train, as follows: the AP threshold was defined as the membrane potential at the point that the velocity of the AP exceeded 10 mV/ms (Naundorf et al., 2006). The AP peak voltage was determined as the absolute membrane potential measured at the peak of the AP waveform. The AP amplitude was calculated as the difference in membrane potential between the AP threshold and the AP peak voltage. Maximum rate of rise was defined as the maximum $dV/dt$ value reached during the AP (calculated between adjacent points). Onset rapidity was defined as the slope of a linear fit to the AP phase plot ($dV/dt$ versus $V$, with unit 1/ms) at $dV/dt=30$ mV/ms. The first AP in a train was considered a single AP. For all APs that followed, the instantaneous AP firing frequency was calculated as: $1/(\text{time since previous spike})$. For subsequent analysis, APs with instantaneous firing frequencies up to 30 Hz were binned in frequency bins of 5 Hz. For each neuron, the mean value of a given AP parameter in a frequency bin was then obtained by averaging over all APs falling in that frequency bin. AP amplitude adaptation was calculated by dividing the mean amplitude of APs in a frequency bin by the mean amplitude of single APs. Maximum rate of rise adaptation was calculated by dividing the mean maximum rate of rise of APs in a frequency bin by the mean maximum rate of rise of single APs. Threshold variance was calculated as the standard deviation of AP thresholds for all APs within a frequency bin. Differences in AP features between human and mouse neurons were tested for significance using independent samples t-tests, with a Bonferroni corrected p-value to account for family-wise error rate.

### 3.2.8 Connectivity and connection probability

The surgeon obtained tissue samples from human temporal cortex in variable forms, in a patient-dependent manner. We could reliably determine the location of the pia and the white matter to adjust the slice angle to maintain the apical dendritic tree within the slice, but had less control of the slicing orientation on the coronal/sagittal axis and relative to individual gyri (unlike in mouse brain, where landmarks such as midline or corpus callosum help in positioning the sample). Given these factors, we have not conducted a systematic analysis of connection probability between mouse and man to provide reliable estimates and comparisons of synaptic connectivity ratios between species. Rather, we focused only on finding direct monosynaptic connections for investigation and subsequent analysis.
3.3 Results and Discussion

In the rodent brain, unitary connections between neocortical pyramidal neurons show frequency-dependent short-term synaptic depression, in response to a sequence of APs (Markram et al., 1998a; Testa-Silva et al., 2012; Tsodyks and Markram, 1997). To test whether excitatory connections between adult human neocortical pyramidal neurons show short-term plasticity, and whether this quantitatively resembles that in mouse neocortex, we made whole-cell recordings from synaptically connected layer 2/3 pyramidal neurons of non-pathological samples of cortex from adult human patients (see Methods) (Figure 3.1A) (Testa-Silva et al., 2010; Verhoog et al., 2013) and mouse neocortex (medial prefrontal cortex of young mice of 12–36 days old, and temporal association cortex of adult mice of 8–11 weeks old). Human monosynaptic connections showed no facilitation but only frequency-dependent depression, whose occurrence resembled that of mouse synapses (Figure 3.1A–D). The amount of depression during a 30 Hz presynaptic AP train did not differ between human and mouse synapses.
To gain a full quantitative comparison of mouse and human short-term synaptic depression and recovery, we used the mathematical minimal description of activity-dependent short-term synaptic plasticity first proposed by Tsodyks and Markram (Loebel et al., 2009; Tsodyks and Markram, 1997), and extracted best-fit model parameters for each recording (see Methods). Two out of five fitted parameters were similar between mouse and human synapses (Figure 3.1A,C). Those parameters that differed between human and mouse unitary synapses included a higher $U$, which reflects the probability of synaptic release (Figure 3.2A; 0.45±0.03 in human versus 0.25±0.02 and 0.29±0.03 in young and adult mice, respectively; p<0.001 and p<0.05), and the cell membrane time constant, as calculated directly from the experimental traces and by the Tsodyks-Markram model (Figure 3.1C,D). It is relevant to mention that the membrane time constant directly measured from experimental traces and the one calculated by the model differ slightly in definition. The model-driven observable, as extracted by the Tsodyks-Markram model, is obtained by fitting a set of two passive differential equations to the decay of the last EPSP for the sole aim of compensating the passive temporal summation of the successive EPSPs. Its link to the membrane biophysical properties is only indirect, its estimate confidence lower, and it has been included for the purpose of completeness. By far, the largest difference between adult mouse and human synapses was a shorter first-order kinetic time constant, which reflects the recovery from short-term synaptic depression (Figure 3.2B; 144±13 ms in human versus 536±40 ms and 483±91 ms in young...
and adult mice, respectively; p<0.001). These data indicate that human synapses recover at least three times faster from use-dependent synaptic depression. Similarly fast time constants of recovery have only been reported for facilitating synapses in the ferret prefrontal cortex (Wang et al., 2006). Instead, purely depressing synapses in ferret neocortex also have long time constants of recovery of 500 ms up to 900 ms (Wang et al., 2006), similar to those in mouse and rat neocortex (Markram et al., 1998a; Tsodyks and Markram, 1997). Furthermore, the time constant of recovery from synaptic depression in adult human synapses (average age 45±11 years) did not change with age during adulthood (Figure 3.2C; Pearson’s correlation coefficient rho=-0.36, p=0.1).

A 3-fold faster recovery from frequency-dependent depression of synaptic connections is likely to affect information transfer between two connected neurons, when repeatedly activated during spike trains (Abbott and Regehr, 2004; Klug et al., 2012; Klyachko and Stevens, 2006). In the neocortex of awake primates, neurons fire irregularly and the instantaneous frequency of each AP varies (Destexhe et al., 2003; Softky and Koch, 1993). We therefore tested whether fast recovery from depression would improve information transfer between two neurons during irregular AP trains with variable firing frequencies (Figure 3.3). In synaptically connected pairs of pyramidal neurons in mouse neocortex, repeated firing of the presynaptic neuron resulted in a marked reduction of the amplitude resolution by which individual EPSPs could be discerned (Figure 3.3A). Consequently, some presynaptic APs resulted in very weak postsynaptic voltage changes (Figure 3.3A,C). In contrast, in connected pairs of human cortical pyramidal neurons, all presynaptic APs led to corresponding EPSPs during repeated firing (Figure 3.3B,C), and each EPSP peak amplitude remained well defined during the AP train. Plotting the relative EPSP amplitude at the same AP in the train for mouse and human synapses shows that the peak amplitude of human EPSPs remains better resolved throughout the AP train than mouse EPSPs (Figure 3.3C). Using the mathematical model and the best-fit parameters, obtained from the short EPSP trains (Figure 3.2A,B), we simulated the response to the irregular synaptic transmission with the exact same AP sequence as applied in the actual recordings: the reduction of synaptic resolution observed in the mouse experiments was replicated (Figure 3.3A,B). With a faster time constant of recovery (144 ms instead of 500 ms), the simulated postsynaptic response resembled the human unitary synaptic responses, whose peak amplitude resolution was maintained throughout the AP train (Figure 3.3B).

Synapses with faster recovery from depression respond more reliably to presynaptic APs during trains of activity (Figure 3.3A,B), and they may also have a larger dynamic range when signalling abrupt variations in presynaptic firing rate. To investigate this, we tested in model simulations whether fast recovering synapses show larger responses to sudden changes in the frequency of presynaptic AP trains. We simulated 1,500 identical, independent excitatory synaptic afferents impinging on the same postsynaptic neuron, which was modelled as a passive membrane compartment (Abbott et al., 1997; Tsodyks et al., 1998). These virtual synapses were activated asynchronously by independent homogenous point processes to engage short-term synaptic plasticity. Subsequently the average activation frequency was step-changed as in a burst, to test how well synapses would detect and respond to phasic presynaptic activity (Tsodyks et al., 1998). Synapses with fast recovery from depression indeed conferred a higher dynamic range of synaptic transmission, as well as an increased sensitivity to small changes in presynaptic network activity time course (Figure 3.3D–F). As we swept through different intra- (Figure 3.3E) and inter-burst frequencies (Figure 3.3F), the faster recovery from depression always provided the postsynaptic neurons with a larger sensitivity to their synaptic inputs. These results indicate that synapses that recover faster from depression, as we observed in human neocortical synapses, are equipped to relay fine variations in the instantaneous firing frequency, more reliably than synapses that slowly recover.
Figure 3. Fast recovering synapses transfer more information.

**A,B,** Unitary EPSPs in synaptically connected pyramidal neurons of mouse (**A**, blue) and human (**B**, red) neocortex generated by a presynaptic Poisson spike train (black). Inset shows enlargement of postsynaptic responses (average of 15 repetitions) to individual presynaptic APs (black dots). Lower traces show model simulations based on the Tsodyks-Markram model with average parameters from experiments in Figure 1. **C,** Normalized EPSP amplitudes in human versus mouse pyramidal neurons in response to each corresponding AP in the Poisson spike train in **A** and **B.** Grey line has unitary slope, whereas the slope of the fit is 1.33±0.13. Differences are significant with p<0.05. Open circle represents the mean and standard deviation (smaller than circle diameter). **D-F,** The steady-state membrane potential $V_{st}$ or its transient change $D$ was simulated for human and rodent synapses (**D**), in response to a step increase of the average APs frequency of afferents, active as independent Poisson spike trains. The value of $D$ is plotted for increasing values of $f_2$ (with $f_1=5$ Hz) **E,** while the value of $V_{st}$ is plotted for increasing values of $f_1$ (with $f_1=f_2$) **F,** as predicted by the Tsodyks-Markram model with parameters identified from the experiments. **G,H,** Combining the quantal release model of synaptic transmission with the Tsodyks-Markram description, the transfer properties of short-term depressing synapses can be quantified by information theory. Over a broad range of firing frequencies, the mutual information, calculated between peak EPSP amplitudes and presynaptic interspike intervals, reveals a peak at an optimal firing frequency **G;** Dividing the mutual information by the firing frequency, the information rate was plotted **H.**
As synapses that recover quickly from depression operate with an increased bandwidth during repeated activation, they may be able to relay more information than synapses that slowly recover from depression. To test this, we numerically calculated the mutual information between the amplitude of the postsynaptic membrane potentials and the length of the interspike intervals of a train of corresponding presynaptic APs. Using the mathematical model and the best-fit parameters (Figure 3.2), the Shannon’s formalism of information theory (Shannon, 1948) applied to depressing synapses (Fuhrmann et al., 2002) provided a quantitative measure for the information transfer through a synapse (see Methods). We found that synapses that recover quickly from depression convey approximately four times more information at peak levels (Figure 3.3G). The average presynaptic firing frequency, corresponding to the optimal information transfer (Fuhrmann et al., 2002), was higher in quickly recovering synapses (9.1 Hz) compared with slowly recovering synapses (4.5 Hz). Quickly recovering synapses were consequently able to sustain larger information transfer rates at higher firing frequencies (Figure 3.3H), and information transfer rate saturated less prominently at higher frequencies than for slowly recovering synapses. These findings suggest that human neocortical depressing synapses that show fast recovery from depression may relay more information than depressing neocortical synapses found in the mouse brain.

Adult human neocortical neurons receive thousands of excitatory synapses, with estimates for adult layer 2/3 pyramidal neurons as high as 30,000, about twice as many as rodent layer 2/3 pyramidal neurons (Benavides-Piccione et al., 2012). When each of these synapses operates with high resolution at high bandwidth and maintains reliability during bursts of activity, as our findings suggest, the question arises whether human pyramidal neurons can actually encode fast-varying temporal inputs in the AP train. To test whether human pyramidal neurons can precisely time their AP firing to rapidly changing inputs, we measured the temporal modulation of the neuronal output firing probability of human pyramidal neurons, during somatic injection of sinusoidal currents in whole-cell recordings (Figure 3.4) (Arsiero et al., 2007; Ilin et al., 2013; Köndgen et al., 2008). Neurons simultaneously received an additional, randomly fluctuating, current component (Figure 3.4A; see Material and Methods) that induced an irregular firing regime with low average rate (13.3±1.6 Hz, CVISI=1.06±0.02, n=13 human and 11.9±0.6 Hz, CVISI=0.8±0.02, n=14 adult mouse neurons). While the fluctuating component per se resulted in a uniform probability of AP firing in time, the superimposed weak amplitude small sinusoidal currents modulated in time the instantaneous firing probability, with the same period of the input (Figure 3.4B-D). Under these conditions, the timing of AP firing in human neurons was more strongly modulated both by large and small input periods, going up to 1,000 cycles/s (Figure 3.4E), indicating that human neurons could encode finer and rapidly changing temporal features of their input into AP timing.

The data in Figure 3.4E represent typical band-pass behaviour for both human (n=13) and murine cells (n=14), where the continuous black and grey lines represent an equivalent passive analogue electronic filter. The pass band of human neurons was shifted to higher Fourier frequencies (low frequency “pole” and “zero” cut-off located at 52 and 82 Hz, respectively, compared to 9 and 20 Hz for mouse cells) showing a higher level of selectivity (high frequency “pole” cut-off located at 524 and 565 Hz in humans and rodents, respectively). Additionally, the multiplicity but not the location of the high Fourier frequency pole differed between human and mouse cells: a value of 2 for the latter group implies that the negative slope of the Fourier transfer function, above a cut-off frequency of <500 Hz, is larger in mouse than in human neurons. This transfer function for adult temporal cortex mouse neurons is consistent with what was previously found for layer 5 pyramidal cells of the primary somatosensory cortex of juvenile rats (Köndgen et al., 2008). Taken together, these results suggest that human neurons are more selectively tuned for high Fourier frequency components of their inputs and that their attenuation while relaying very fast signals, with Fourier components beyond 500 cycles/s, decays significantly less rapidly than in adult mouse cells.
Figure 3.4  Human pyramidal neurons track input signals beyond 500 cycles/s.

A, Top trace (grey): weak amplitude sinusoidal currents, injected with distinct oscillation periods (1–1,000 cycles/s). Middle trace (black): injected randomly fluctuating additional current component. Lower trace: AP firing by human pyramidal neuron in response to the total injected current. B–D, Across successive cycles and for distinct sine input oscillations (10, 100, 1,000 cycles/s), APs fired by a human pyramidal neuron are displayed in raster diagrams (B–D, upper panels) and are quantified by a peristimulus time histogram (PSTH, lower panels), which estimates the instantaneous firing probability. E, Modulation depth (i.e., peak modulation magnitude over the mean rate, M/R) as a function of Fourier frequency for human (n=13, black) and mouse cells (n=14, grey). Larger frequencies imply faster input oscillations. Markers indicate mean±SEM, while the thick solid traces are fits obtained with a rational complex function. The red line indicates the significance level for the data, obtained by computing the modulation depth over surrogate data, obtained shuffling the interspike intervals.
Modelling studies have predicted that tracking of fast input frequencies by neurons depends on the rate of onset of APs (Fourcaud-trocme et al., 2003; Ilin et al., 2013). We tested whether human neocortical pyramidal neurons have substantially faster AP onset kinetics than mouse pyramidal neurons. Single APs of human (26–47 years, n=23 neurons) and adult mouse (10–11 weeks, n=12 neurons) temporal cortex pyramidal neurons had similar waveform and duration, but different kinetic features (Figure 3.5A). However, APs fired in trains with varying instantaneous frequencies showed strong differences between human and mouse pyramidal neurons. In particular, the rising phase of APs fired by mouse neurons slowed down more with increasing firing frequency than APs generated by human neurons (Figure 5B, S3E, and S3F; p<0.005). At higher firing frequencies the threshold for AP generation was elevated in mouse pyramidal neurons compared to human neurons (Figure 3.5E). Importantly, at instanta-
neous firing frequencies of 1 to 30 Hz, mouse APs had reduced onset rapidity compared to APs fired by human pyramidal neurons (Figure 3.5D,F, p<0.005 for all frequencies). A recent study reported that in order for neurons to track fast varying inputs, with Fourier components up to 1,000 cycles/s, the AP onset rapidity needed to be above 30 mV/ms per mV (Ilin et al., 2013). APs fired by human pyramidal neurons had mean onset rapidity values above 32 mV/ms per mV for all firing frequencies tested (Figure 3.5E). These results show that APs generated by human neurons have a sufficiently fast onset to account for the ability of these neurons to track very fast inputs, with Fourier components up to 1,000 cycles/s.

Our findings show that synaptic communication between human neocortical pyramidal neurons has higher bandwidth due to fast recovery from depression and that these neurons are equipped to track fast input Fourier components and encode these into timing of their spikes. Transfer of information between neurons through chemical synaptic transmission is elementary to cognition, and processes of short-term plasticity at these synapses encode information (Klug et al., 2012). Studies on rodent excitatory cortical synapses show that short-term facilitation of synaptic strength may optimize information transfer in particular during spike bursts (Klyachko and Stevens, 2006; Rotman et al., 2011). Based on findings in the rodent brain, it is assumed that purely depressing synapses may be better suited to transmit information for single spikes or short bursts rather than for trains of APs (Klug et al., 2012). In contrast to these observations, we show here that purely depressing synapses in the human brain can actually transfer substantial amounts of information during spike trains, because recovery from depression is fast. We find that information transfer at depressing synapses with fast recovery is optimal at alpha band frequencies (8–12 Hz), and information transfer rate increases well into the beta and gamma band frequency range, suggesting that these synapses can be involved in active cortical computation, during cognition. This may unveil a fundamental difference with purely depressing synapses that slowly recover from depression in the neocortex of mice and other laboratory animals, which we find to have optimal frequencies of information transfer in the lower theta band range with no increase in information transfer rate at higher frequencies. These synapses may be better suited for a different range of cortical processes (Hasselmo and Howard Eichenbaum, 2005).

In our study, we did not include polysynaptic events that have been described previously (Molnár et al., 2008), and we restricted our attention to monosynaptic connections between pyramidal cells from L2/3 in the anterior medial temporal cortex, where polysynaptic events did not seem to play a prominent role. In rodent synapses, the amount and speed of short-term synaptic depression and its recovery are dependent on temperature as well as divalent ion concentrations (Dittman and Regehr, 1998; Klyachko and Stevens, 2006). It is at present unknown to what extent synaptic depression and recovery in human synapses depend on temperature and divalent ion concentrations. Combined with a lack of information on the actual calcium and magnesium concentrations and temperature at synapses in the brain of awake behaving mice and humans, it is not feasible to predict what the speed of recovery from depression in mouse and human cortical synapses will be in the intact brain during behaviour. Nevertheless, we show here that under defined experimental conditions in which temperature and extracellular divalent ion concentrations are controlled, human and mouse temporal cortex synapses show marked differences in the speed of recovery from synaptic depression. This suggests that differences in protein complexity of synaptic protein networks between mouse and human synapses (Bayés et al., 2012) may translate into different functional properties of short-term synaptic plasticity.
Postsynaptically, the outcome of short-term synaptic plasticity processes is translated into AP firing to relay information (Rieke et al., 1999). The brain not only keeps track of the number of spikes occurring in large windows of time, but spike timing can have meaning down to millisecond precision (Nemenman et al., 2008). Spike timing with a temporal resolution smaller than the time scales of sensory and motor signals, even at sub-millisecond levels, can encode significant amounts of visual information (Nemenman et al., 2008). Cortical pyramidal neurons time their AP firing in relation to the timing of synaptic input (van Aerde et al., 2009; Mann et al., 2005). However, populations of rodent pyramidal neurons fail to time their spiking based on rapidly varying inputs components that change faster than 200–300 cycles/s. This may suggest that, during ongoing synaptic membrane potential fluctuations, rodent neurons do not regularly encode and transmit downstream information with sub-millisecond precision. We find that populations of human pyramidal neurons can regularly time AP firing with sub-millisecond precision and that these APs maintain rapid onset kinetics, which can account for such precision of spike timing. Rapid onset kinetics of somatic APs are predicted by Hodgkin-Huxley-based models for AP generation when the spatially extended morphology of neurons and AP propagation from the axon initial segment to the soma are taken into account (Yu et al., 2008). The observed fast onset rapidity of APs in adult human neurons can indeed partly be explained by human pyramidal neuron morphology (Eyal et al., 2014). In particular, the electrical load imposed by the large dendritic tree of adult human layer 2/3 pyramidal neurons compared to adult rodent pyramidal neurons on the axon initial segment induces a larger onset rapidity of the AP and higher frequency tracking capabilities. In conclusion, our data show that elementary circuits in the human neocortex of connected pyramidal neurons that underlie cognition can operate at a substantially higher bandwidth and temporal resolution for information encoding than rodent neurons in response to the high information content synaptic transmission they receive.
### 3.4 Supplementary figures

**Figure 3.51 Tsodyks-Markram model parameter comparisons for mouse and human synapses.**

**A**, Absolute synaptic efficacy, proportional to the amplitude of the first evoked EPSP. (mean±SEM) 3.7±0.5 mV human, 5.6±1.1 mV for young mouse synapses and 1.6±0.4 mV for adult mouse synapses, p>0.05; human n=27 (14 from tumour patients and 13 from epilepsy patients, see Methods; young mouse n=35; adult mouse n=11). **B**, Inactivation time constant. (mean±SEM) 2.2±0.3 ms human, 1.9±0.1 ms for young mouse synapses and 2.1±0.3 ms for adult mouse synapses, p>0.05; human n=27 (14 from tumour patients and 13 from epilepsy patients, see Methods; young mouse n=35; adult mouse n=11). **C**, Membrane time constant measured from membrane potential deflection upon short current pulse injection (mean±SEM) 19.5±1.0 ms human, 25.1±0.4 ms for young mouse synapses and 24.6±2.0 ms for adult mouse synapses, adult and young mice have significantly higher membrane time constants than humans (p<0.05); human n=27; young mouse n=27; adult mouse n=15. **D**, Membrane time constant, as in a leaky integrate-and-fire neuron model, (mean±SEM) 28±3 ms human, 22±1 ms for young mouse synapses and 42±4 ms for adult mouse synapses, adult mice have significantly higher membrane time constants than humans and young mice (p< 0.05 and p<0.001, respectively); human n=27 (14 from tumour patients and 13 from epilepsy patients, see Methods; young mouse n=35; adult mouse n=11). **E**, More examples of the time course of synaptic depression on a 30 Hz train of EPSPs. 8 pulses+1 recovery pulse 500 ms after the 8th pulse for mouse connections and 300 ms after the 8th pulse for a human connection (of the n=27 pairs measured in human slices, six were probed with 500 ms and 21 with 300 ms between the 8th and 9th pulse).
Figure 3.52 Different values of CV of the simulated quantal content (i.e., 0.2, 0.4, 0.6, and 0.8) only scale the mutual information.

From E, we can conclude that in the model the CV parameter has no qualitative effect. Changing its value results in a scaling effect only. The figure was obtained with a smaller number of simulated Poisson spikes, it therefore appears noisier than Figure 3.3G and 3.3H.
Figure 3.53  Action potential parameter comparisons for mouse and human neurons.

A–F, Quantification of various AP features of human (red) and mouse (blue) neurons, displayed versus firing frequency. Data are presented as mean±SEM. for single APs, and APs fired in trains binned in 5 Hz bins according to instantaneous firing frequency. Asterisks indicate p<0.005. A, AP amplitude, in mV from threshold. B, AP peak voltage. C, AP amplitude adaptation. D, Threshold variability, presented as the standard deviation of AP thresholds. E, Maximum rate of rise. F, Maximum rate of rise adaptation. For more details on how AP features were calculated, see Methods.
Mechanisms underlying the rules for associative plasticity at adult human neocortical synapses

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Contributions

Conceived and designed the experiments: MBV, NAG, and HDM; Performed the experiments: MBV, NAG, JO, IS, and RMM; Contributed reagents/materials/analysis tools: JJIH, GT-S, JCB, and CPJdK; Analysed the data: MBV, NAG, JO, and RMM; Wrote the paper: MBV, NAG, and HDM.
ABSTRACT

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. Here, we made whole-cell recordings of human pyramidal neurons in slices of brain tissue resected during neurosurgery to investigate spike timing-dependent synaptic plasticity in the adult human neocortex. We find human cortical synapses can undergo bidirectional modifications in strength throughout adulthood. Both long-term potentiation and long-term depression of synapses was dependent on postsynaptic NMDA receptors. Interestingly, we find that human cortical synapses can associate pre- and postsynaptic events in a wide temporal window and that rules for synaptic plasticity in human neocortex are reversed compared to what is generally found in the rodent brain. We show this is caused by dendritic L-type voltage-gated Ca$^{2+}$ channels that are prominently activated during action potential firing. Activation of these channels determines whether human synapses strengthen or weaken. These findings provide a synaptic basis for the timing rules observed in human sensory and motor plasticity in vivo, and offer insights into the physiological role of L-type VGCCs in the human brain.

4.1 INTRODUCTION

One of the leading questions in neuroscience is how memories are formed and stored in the human brain. Learning and memory critically depend on long-term modifications in the strength of synaptic connections (Bliss and Collingridge, 1993; Sjöström and Häusser, 2006). Changes in synaptic strength in response to timed action potential firing of connected neurons, called spike timing-dependent plasticity (STDP), resemble typical features of associative learning and are thought to underlie several forms of memory (Caporale and Dan, 2008; Hebb, 1949; Kampa et al., 2007; Letzkus et al., 2007). Depending on the millisecond timing and temporal order of pre- and postsynaptic spiking, synaptic strength can either increase (long-term potentiation, LTP) or decrease (long-term depression, LTD) (Bi and Poo, 1998; Levy and Steward, 1983; Markram et al., 1997). Despite the important role attributed to synaptic plasticity in normal brain function, it is still unknown to what extent STDP plays a part in the human brain, and if so, what mechanisms govern the timing windows and whether it persists at human synapses into adulthood.

There is accumulating evidence that coincident millisecond timing of activity may indeed govern synaptic changes in humans as well (Cooke and Bliss, 2006; Müller-Dahlhaus et al., 2010). A timing-dependent form of plasticity of motor-evoked potentials (MEPs) can be induced in human subjects by pairing peripheral nerve stimulation (PNS, analogous to presynaptic stimulation) with a transcranial magnetic stimulation (TMS, analogous to postsynaptic stimulation) of the motor cortex (De Beaumont et al., 2012; Lu et al., 2012; Stefan et al., 2000, 2002; Wolters et al., 2003). Such paired associative stimulation (PAS) can induce both LTP-like increases and LTD-like decreases in MEP amplitude, with the sign of change depending on the relative timing of associated stimuli (De Beaumont et al., 2012; Conde et al., 2013; Koch et al., 2013; Lu et al., 2012; Thabit et al., 2010; Wolters et al., 2003, 2005). These changes have been suggested to be of cortical origin and related to STDP at synapses in upper cortical layers (Conde et al., 2013; Koch et al., 2013; Lu et al., 2012; Stefan et al., 2000; Thabit et al., 2010; Wolters et al., 2003, 2005). However, how TMS-induced changes in MEP amplitude reflect plasticity processes at the level of human synapses remains unclear.
To directly test what mechanisms govern the timing rules at neocortical synapses, and to understand the synaptic basis for plasticity-like changes in corticospinal excitability observed in vivo, we investigated STDP induced by single pre- and postsynaptic action potentials in pyramidal neurons of the adult human medial temporal cortex. We find that human neocortical synapses can bidirectionally change strength in response to timed pre- and postsynaptic activity, at least up to 65 years of age. Postsynaptic L-type voltage gated calcium channels play a central role in human STDP, deciding between synaptic strengthening or weakening of the synapse and thus shaping the rules for synaptic plasticity at adult human neocortical synapses.

4.2 METHODS

4.2.1 Electrophysiology in acute human and rat neocortical slices

Neocortical tissue was obtained from 31 patients (18 females, 13 males, aged 18–65 years) treated for medial temporal lobe epilepsy (24 cases), or for the removal of a hippocampal tumour (4 cases), cavernoma (2 cases) or cele (1 case). Slices were made using procedures described in Chapter 2 Methods. Rat brain slices were prepared from adult male and female Wistar rats (Harlan, NL; 10-15 weeks old) in accordance with Dutch licence procedures and with permission from the VU University ethical committee for animal experimentation. Whole-cell recordings were made using procedures and solutions described in Chapter 3 Methods. Pyramidal neurons in human and rat slices were readily identified using differential interference contrast microscopy by the prominent apical dendrite and pyramid-shaped cell bodies. Cells were filled with biocytin (4-5 mg/ml) and then processed for post hoc cell identification. In humans, pyramidal neurons were recorded throughout layers 2-6. In rats, layer 5 pyramidal neurons were targeted. Slices of human cortical tissue were of good quality and showed no sign of spontaneous epileptiform activity. Recordings could be made without difficulty from apparently viable, healthy neurons for up to 24 hours after slicing, but recordings included in the analysis were all made within 15 hours of slice preparation. Recordings were made using Multiclamp 700A/B amplifiers (Axon Instruments, CA, USA), sampling at 10 kHz and low-pass filtering at 3 kHz. Recordings were digitised with an Axon Digidata 1440A and acquired using pClamp software (Axon).

4.2.2 Spike-timing-dependent plasticity

Excitatory postsynaptic potentials (EPSPs) were evoked every 7 s (0.14 Hz) using bipolar stimulating electrodes in glass pipettes filled with ACSF positioned approximately 100–150 μm along the cell’s somatodendritic axis (Figure 4.1A). Duration (50 μs) and amplitude (40-80 μA) of extracellular stimulation were controlled by Isoflex stimulators (A.M.P.I., Jerusalem, Israel). After obtaining a stable baseline of 30-60 EPSPs, presynaptic extracellular stimulations were paired to a single postsynaptic action potential (50 times, 0.14 Hz), evoked by whole-cell current injection. Timing of EPSPs and action potentials was controlled by a Master-8 stimulator (A.M.P.I.). Pairing intervals were measured from the onset of the evoked EPSP to the onset of the postsynaptic AP. Negative intervals up to -130 ms and positive intervals up to +60 ms were used to explore the temporal window for STDP. On a few occasions, extracellular stimulation evoked network activity that could sustain for tens of milliseconds, which in appearance was very similar to the ‘complex events’ reported before in human neocortex, and could be triggered by the firing of a single pyramidal neuron (Molnar et al., 2008). These events disappeared when the stimulus duration was reduced to 30 μs and only single synaptic events remained.
The slope of the initial 2 ms of the EPSP was analysed to ensure that the data reflected only the monosynaptic component of each experiment. In general, EPSP slope had stabilised to plateau levels at 25-30 minutes after pairing. Change in synaptic strength was therefore measured as the percent change in EPSP slope 25–30 min after pairing compared to baseline. When recordings lasted less than 20 min after pairing, the whole post-pairing period was compared to baseline. Cell input resistance was monitored by applying a −100 pA, 200–500 ms hyperpolarising pulse at the end of each sweep. Criteria for inclusion of recordings in STDP dataset were: (1) a stable baseline EPSP slope, (2) less than 30% change in input resistance, (3) no more than 5 mV depolarisation from baseline resting membrane potential over the course of the experiment, and (4) baseline resting membrane potential was <-60 mV. All experiments were performed in the absence of blockers of GABAergic synaptic transmission. To block L-type VGCCs in STDP experiments, nifedipine (10 µM; Sigma-Aldrich, MO, USA) was bath-applied 3-4 min prior to and during pairing procedure, and washed out after pairing. To block postsynaptic NMDRs, the use-dependent NMDA receptor blocker MK801 (1 mM; Sigma-Aldrich) was added to the pipette medium (Bender et al., 2006; Rodriguez-Moreno and Paulsen, 2008).

4.2.3 2-photon Ca\(^{2+}\) imaging

In imaging experiments, the fluorescent dyes Alexa594 (80 µM, Invitrogen) and Fluo-4 (100 µM, Invitrogen) were added to the intracellular solution to visualise morphology and measure [Ca\(^{2+}\)]\(_i\) changes respectively. After whole-cell break-in, dyes were allowed to diffuse into the cell for at least 20 minutes before line scans were made. To induce back-propagating action potentials (bAPs), single APs or bursts APs were triggered by a somatic current injection (1-2 nA), lasting 3 ms (single AP) or 15ms (AP burst, 80-150 Hz). bAP-induced Ca\(^{2+}\) influx was assessed by the fluorescence change in the Fluo-4 signal relative to the corresponding constant Alexa594 signal. Fluorescence was measured using a LEICA RS2 two-photon laser scanning microscope with a 40x (0.8NA) or 63x (0.9 NA) water immersion objective and a Ti:Sapphire laser tuned to 830 nm excitation at a bi-directional scanning frequency of 8 kHz. Line scans (500 ms duration, 8 bit signal) synchronised with AP stimulation were made at the dendritic region of interest (ROI). To assess the effect of the L-type VGCC blocker, nifedipine (10 µM) was bath-applied and identical stimulus protocols and line scans at the ROI were repeated. At the end of the experiment, a z-stack of the dendritic ROI was made, based on the Alexa594 signal, to calculate distance of back-propagation from the soma. 3 to 6 line scans were made per stimulus protocol per ROI and were averaged for analysis. Amplitude, rise, decay and area of fluorescence signal were extracted from the fluorescence trace by fitting a double exponential function.

4.2.4 Data analysis

STDP recordings were Analysed using custom written Matlab scripts (Mathworks). AP waveforms were analysed in Clampfit 10.2. Statistical tests indicated in text were performed in IBM SPSS Statistics 20.0 or Sigmaplot 9.0. To assess whether EPSP-AP pairing induced a significant increase (LTP) or decrease (LTD) in synapse strength for individual STDP experiments, paired samples \(t\)-tests were used to compare baseline EPSP slope to EPSP slope 25-30 min after pairing, with bonferroni corrected p-value. To extract the trend from STDP data (Fig 2E), we used locally weighted polynomial regression (Cleveland and Devlin, 1988), which provides an unbiased estimation of the trend, referred to as a loess curve, based on local smoothing with tricube weighting and polynomial regression. Different polynomial degrees (\(\lambda=1-3, \alpha=0.2\)) were used to determine the switch in sign, which ranged from +6.6 to +7.2 ms. 2D smoothed running averages (sampling proportion 0.05-0.15) accounted for unequally spaced data by using the nearest neighbour method and indicated a switch in sign between +7.3 and +8.6 ms. Summary quantifications in main text, figures and tables are presented as mean±SEM throughout the paper and p<0.05 was taken as level of significance for statistical comparisons.
Table 1. Electrophysiological properties of human and rat temporal cortex pyramidal neurons.

<table>
<thead>
<tr>
<th></th>
<th>Human Total (71 cells)</th>
<th>MTS epilepsy patients (50 cells)</th>
<th>Tumour or other patients (21 cells)</th>
<th>Rat Total (47 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{rest}}$ (mV)</td>
<td>-68.4 ± 0.4</td>
<td>-68.5 ± 0.5</td>
<td>-68.1 ± 0.8</td>
<td>-68.3 ± 0.7</td>
</tr>
<tr>
<td>Change in Vm (mV)†*</td>
<td>0.9 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>0.0 ± 0.5</td>
<td>0.2 ± 0.5</td>
</tr>
<tr>
<td>Input resistance (MΩ)‡</td>
<td>86.1 ± 8.1</td>
<td>79.6 ± 6.3</td>
<td>102.4 ± 23.6</td>
<td>63.8 ± 4.5</td>
</tr>
<tr>
<td>Change in Ri (%)†‡</td>
<td>-2.9 ± 1.5</td>
<td>-2.8 ± 1.9</td>
<td>-3.0 ± 2.0</td>
<td>1.8 ± 3.4</td>
</tr>
<tr>
<td>EPSP amplitude (mV)</td>
<td>7.9 ± 0.3</td>
<td>7.9 ± 0.3</td>
<td>8.0 ± 0.6</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>EPSP slope (mV/ms)</td>
<td>2.6 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>2.9 ± 0.2</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>EPSP decay time (ms)</td>
<td>34.2 ± 1.7</td>
<td>36.1 ± 2.1</td>
<td>30.3 ± 2.7</td>
<td>24.7 ± 2.5</td>
</tr>
<tr>
<td>ADP peak amp. (mV)</td>
<td>16.6 ± 0.8</td>
<td>16.7 ± 0.9</td>
<td>16.5 ± 1.7</td>
<td>19.0 ± 1.4</td>
</tr>
<tr>
<td>ADP duration (ms)</td>
<td>62.2 ± 3.1</td>
<td>64 ± 3.8</td>
<td>58.1 ± 5.0</td>
<td>35.1 ± 4.7</td>
</tr>
<tr>
<td>ADP area (mV*ms)</td>
<td>513.7 ± 64.8</td>
<td>517.1 ± 83.7</td>
<td>505.1 ± 92.3</td>
<td>309.9 ± 43.2</td>
</tr>
</tbody>
</table>

Data from neurons used for control STDP recordings, presented as mean ± s.e.m. Statistical testing for differences in electrophysiological properties of human neurons from two patient groups was done using independent samples t-tests, unless data was not normally distributed or showed inhomogeneous variance between groups, in which case an independent samples Mann-Whitney U test was used (indicated with ‡). † = 20-25 min. after pairing compared to baseline. Asterisk indicates significant difference between two patient groups.
4.3 Results

To investigate synaptic plasticity in human neocortex, we made whole-cell recordings from pyramidal neurons in acute slices of healthy medial temporal cortex (MTC) of adult humans (31 subjects, 18-65 years of age, see Methods section). Pyramidal neurons were readily identified by their prominent apical dendrite and pyramid-shaped cell bodies, which was confirmed post hoc by inspection of neuronal morphology visualised by biocytin labelling (Figure 4.1A). Recorded neurons had stable membrane potentials of -68.4±0.4 mV and had regular-firing properties (Figure 4.1B, Table 4.1). Although the majority of patients were treated for epilepsy (24 out of 31 patients), we found no signs of epileptiform activity in these slices; in general, spontaneous excitatory inputs to pyramidal neurons and interneurons were of small amplitude (pyramidal neurons: 14.1±0.2 pA, n=5; interneurons: 12.4±1.1 pA, n=3) and occurred at low frequencies (pyramidal neurons: 4.2±0.1 Hz, n=5; interneurons: 5.5±0.3 Hz, n=3).

Excitatory postsynaptic potentials (EPSPs) were evoked by extracellular stimulation 100-150 μm away from the soma along the apical dendrite (Figure 4.1A). To have a substantial dynamic range to observe both increases and decreases in synaptic strength, EPSPs were evoked at half-maximal stimulation intensity, which resulted in EPSPs of 7.9±0.3 mV (n=71; Figure 4.1C, Table 4.1). The amplitude and slope of EPSPs evoked at 0.14 Hz remained stable for at least 50 minutes of recording time (2.5 mV/ms baseline slope versus 2.4 mV/ms slope 25-35 minutes after baseline, paired samples t-test: p>0.05, n=12; Figure 4.1D). Other electrophysiological properties of human neocortical pyramidal neurons, such as input resistance and resting membrane potential equally changed little over the course of experiments (Table 4.1) and thus, the human brain slice preparation provided a good background for plasticity experiments.

4.3.1 STDP in adult human neocortex

To induce STDP, EPSPs were repeatedly paired with a single postsynaptic action potential, after which EPSPs were monitored for 30-40 min (Figure 4.2A-C). To characterize the temporal requirements for the induction of LTP and LTD at human cortical synapses, we varied the order and time-interval between presynaptic (EPSPs) and postsynaptic (APs) activation in separate experiments. Similar to rodent synapses, we found that when presynaptic stimulation preceded the postsynaptic AP by up to 5 ms (a ‘pre-before-post’ paradigm, hereafter referred to as pairing at ‘positive intervals’), robust LTP could be elicited (Figure 4.2A, D). In juvenile rodent cortical synapses, LTD is induced when postsynaptic neurons fire before the presynaptic stimulation (‘post-before-pre’ paradigm, hereafter referred to as pairing at ‘negative intervals’) and the temporal window for depression at proximal synapses typically stretches from 0 to around -20 ms (Bell et al., 1997; Bi and Poo, 1998; Caporale and Dan, 2008; Levy and Steward, 1983; Magee and Johnston, 1997; Markram et al., 1997; Wittenberg and Wang, 2006). Interestingly, we found that in contrast to juvenile rodent synapses, induction protocols with negative timing intervals induced LTP at human neocortical synapses (4. Figure 2B, D). The amount of LTP obtained at negative intervals was similar for neurons recorded in superficial and deep layers (126.0±5.6% (superficial layers 2-3) versus 127.5±7.8% (deep layers 4-6) for intervals from 0 to -40 ms, and 112.6±7.2% (superficial) versus 119.2±13.5% (deep) for intervals from -40 to -80 ms). The window for LTP stretched far into the negative intervals, with cases of significant LTP observed even when APs preceded EPSPs by up to 100 ms during pairing (Figure 4.2D).
STDP at human cortical synapses

**Figure 4.2**  Human cortical synapses show inverse timing rules for STDP.

**A**, Slope data from STDP experiment with +5 ms pairing interval. Top left: EPSP-AP pairing protocol used for STDP induction. Scale bars: 40mV, 40ms. Top middle: EPSP waveform before (black) and 25-30 min after pairing (blue). Scale bars: 3mV, 50ms. Top right: rising phase of EPSPs before (black) and after (blue) pairing. Scale bar: 5 ms. Middle panel: EPSP slope over the course of an experiment normalised to baseline. (●) 1 EPSP, (●) mean of 7 EPSPs. Shaded area indicates plasticity induction period. Bottom panels: input resistance and membrane potential over the course of the experiment. Solid line indicates mean baseline value. **B**, As **A**, for an experiment with -40 ms pairing interval. Scale bars equal to **A**, except vertical scale bar top middle and right traces (5 mV), and horizontal scale bar of right trace (3 ms). **C**, As **A**, for an experiment with +10 ms pairing interval. Scaling as in **A**.

**D**, STDP window of adult human temporal cortex synapses. Change in synaptic strength (% change in EPSP slope compared to baseline) versus pairing interval (Δt in ms). (○) EPSP slope change in individual experiments. Blue bars: mean±SEM for 30 ms-wide bins. Red curves: single exponential fit to data with pairing intervals ≤ +5 ms, and ≥10 ms. Insets: examples of EPSP-AP pairings at positive and negative intervals. **E**, Magnification of STDP window around switch in sign from LTP to LTD. Blue bars: mean±SEM for 5 ms-wide bins. Note no net change in synaptic strength occurs between +5 and +10 ms, and that both running average (green) and loess curve (red, dashed) switch in sign within this range of pairing intervals. **F**, STDP window of adult rat temporal association cortex synapses. Data presented as in **D**. Red curves: single exponential fit to STDP data points with pairing intervals <0 ms, and >0 ms. Insets: example of EPSP-AP pairing at negative intervals in rat neuron. Scale bars: 40 mV, 30 ms.
To address the question whether these synapses can also decrease in strength, we tested a wider range of positive intervals. Pairing intervals beyond 8 ms induced significant LTD in the majority of cells (total: n=20, LTP: n=1, LTD: n=13, p>0.05: n=6; Figure 4.2C, D). The switch in sign from LTP to LTD occurred at pairing intervals between +5 and +10 ms. Within this range of timing intervals, EPSP slope did not change compared to baseline (98.4±13.8%, paired samples t-test: p>0.05, n=5; Figure 4.2E). A running average and a Loess curve obtained from locally weighted polynomial regression of STDP data switched in sign at +7.3 ms and +6.6 ms, respectively (Figure 4.2E). These data show that human neocortical synapses can bidirectionally change strength in response to timed pre- and postsynaptic activity and that human cortical synapses follow a reversed STDP rule compared to what is usually found in juvenile rodent cortical synapses (Bi and Poo, 1998; Froemke and Dan, 2002; Tzounopoulos et al., 2004).

Since the majority of STDP recordings were made in tissue obtained from patients with medial temporal lobe epilepsy, we questioned whether STDP results may be influenced by their disease history. We therefore compared STDP data recorded from 13 medial temporal lobe
epilepsy patients to that from patients treated for a hippocampal tumour (3 patients), cele (1 patient) or cavernoma (1 patient). We found no apparent differences in STDP magnitude and sign between patient groups; the amount of LTP and LTD obtained when pairing at intervals ranging from -30 to +5 ms and +8 to +30 ms, respectively, were both not significantly different (independent samples t-tests: p>0.05 for LTP and LTD intervals; Figure 4.3A). These results suggest that the observed inverse window for STDP is not restricted to epilepsy and may apply to human neocortical synapses in general.

The ability of synapses to change strength decreases with age in laboratory animals, such that in older animals, synapses tend to require stronger induction protocols for plasticity to occur (Meredith et al., 2003). Studies in human subjects have also shown a considerable reduction in the amount of PAS-induced plasticity in older individuals (Müller-Dahlhaus et al., 2008; Sawaki et al., 2003). The tissue used in this study was collected from patients that ranged in age from 18 to 65 years. Both in young and older patients, LTP was induced in about 70% of cells in pairing intervals from -80 to +5 ms (Fig 3B). The proportion of cells undergoing LTP was not significantly different between age-groups (<35yrs: 73.7% LTP, n=19; >35yrs: 68.4% LTP, n=19; Chi square test of independence: χ²(1)=0.13, p>0.05), suggesting no age-dependent decline in the efficacy of LTP at human synapses. In pairing intervals >8 ms, LTD was induced in about 65% of cells in both age groups (n=20; Fig 3C). The proportion of cells undergoing LTD was not different between groups (<35yrs: 61.5% LTD, n=13; >35yrs: 71.4% LTD, n=7; Fig 3C). These results suggest no age-dependent decline in the efficacy of STDP at human synapses.

### 4.3.2 STDP in adult rat temporal cortex

The STDP window we report here for human cortical synapses is quite at odds to what is generally reported for rodent synapses. However, most rodent studies on STDP have been conducted in juvenile animals around 2-4 weeks of age in brain areas other than temporal cortex. To test whether STDP rules observed in adult human synapses are similar to STDP rules in adult rodent synapses, we recorded from layer 5 pyramidal neurons of adult rat temporal association cortex (10-15 weeks old). Most passive and active electrical properties of human and rat pyramidal neurons were similar, but human EPSPs did on average have larger amplitudes, faster rise times and slower decay times than rat EPSPs (Table 4.1). Testing the temporal requirements for STDP in rats, we found that similar to humans, adult rat synapses showed robust LTP when pairing at negative intervals (Figure 4.2F). In contrast to human cells however, very few adult rat synapses showed LTD at positive intervals. LTD occurred in only 4 of 32 neurons tested, whereas LTP occurred in 24 of 32 neurons at these intervals. This is in line with previous reports from other brain areas in rats, where the capacity of rodent cortical synapses to undergo LTD, either in response to spike-timing-dependent plasticity paradigms (Banerjee et al., 2009) or to low-frequency stimulation (Bear and Abraham, 1996; Dudek and Bear, 1993), has been shown to decline and disappear with age.
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4.3.3 STDP at human synapses requires NMDA receptors and L-type VGCCs

Neocortical synaptic plasticity depends on intracellular Ca\(^{2+}\) signals to trigger the intracellular machinery involved in changing synaptic strength (Couey et al., 2007; Meredith et al., 2007). Two important sources of Ca\(^{2+}\) in this respect are N-methyl-D-aspartate receptors (NMDARs) and voltage-gated calcium channels (VGCCs). To test whether NMDARs are involved in STDP in adult human synapses, we added the use-dependent NMDAR blocker MK801 (1 mM) to the intracellular solution of the recording pipette (Bender et al., 2006; Rodríguez-Moreno and Paulsen, 2008). Intracellular MK801 (iMK801) did not affect the amplitude or rise time of human EPSPs, but reduced EPSP decay time (Figure 4.4A). Pairing EPSPs to APs at intervals between -15 and +5 ms induced clear LTP in control neurons (121.0±7.0%; paired samples t-test: p=0.019, n=10; Figure 4.4B, C), but not in neighbouring iMK801-loaded neurons (94.3±4.2%; paired samples t-test: p>0.05, n=5; Figure 4.4B, C), indicating that LTP at human synapses is dependent on postsynaptic NMDA receptors.

Recent reports have shown that LTD at a number of cortical synapses in the rodent brain is dependent on presynaptic NMDARs (Banerjee et al., 2009; Rodríguez-Moreno and Paulsen, 2008; Rodríguez-Moreno et al., 2010; Sjöström et al., 2003; Verhoog and Mansvelder, 2011). To test whether this holds true in adult human synapses as well, we tested whether LTD would persist when postsynaptic NMDARs were blocked by iMK801. Pairing pre and postsynaptic activity at intervals ranging from +8 to +15 ms, which induced LTD in interleaved control experiments (79.1±6.8%; paired samples t-test: p=0.028, n=9; Figure 4.4D, F), failed to produce a significant decrease in EPSP slope in neurons loaded with iMK801 (101.3±3.1%; paired samples t-test, p>0.05, n=8; Figure 4.4D, F). This shows that postsynaptic NMDA receptors gate both potentiation and depression at human synapses.

In rodent cortical synapses, activation of L-type VGCCs is required for STDP (Bi and Poo, 1998; Meredith et al., 2007; Nevian and Sakmann, 2006; Remy and Spruston, 2007; Rubin et al., 2005). To test the involvement of L-type VGCCs in human cortical STDP, we blocked these channels with bath-applied nifedipine (10 µM). Nifedipine did not affect the amplitude or slope of EPSPs (Figure 4.4E). Interestingly, pairing at ‘LTP intervals’ between -15 and +5 ms while L-type VGCCs were blocked abolished LTP and resulted in LTD in all cells tested (83.6±3.5%; paired samples t-test: p=0.045, n=5; Figure 4.4B, C). These findings suggest that L-type VGCCs can act as a molecular switch for the polarity of synaptic plasticity at human neocortical synapses. To test whether L-type VGCCs are also necessary for LTD at human synapses, we blocked L-type VGCCs and paired activity at intervals between +8 and +15 ms. Despite L-type VGCCs being unavailable during pairing, significant LTD was induced (80.5±8.6%; paired samples t-test: p=0.028, n=5; Figure 4.4D, F). Thus, L-type VGCCs are not involved in LTD of human neocortical synapses.

STDP in adult rats also involved NMDARs and L-type VGCCs; LTP at negative intervals (in a range of -15 to 0 ms) no longer occurred in neurons loaded with iMK-801 or in the presence of nifedipine (control: 167.5±18.2%, paired samples t-test: p<0.001, n=6; iMK801: 114±10.0%, paired samples t-test: p>0.05, n=6; nifedipine: 104.6±5.3%, paired samples t-test: p>0.05, n=5; Figure 4.4G). At positive intervals (in a range of 0 to +15 ms), LTP equally relied on postsynaptic NMDA receptors and L-type VGCCs (control: 165.2±18.9%, paired samples t-test, p<0.001, n=5; iMK801: 101±3.5%, paired samples t-test: p>0.05, n=7; nifedipine: 109.8±13.9%, paired samples t-test: p>0.05, n=4; Figure 4.4H). In contrast to human synapses, blocking L-type VGCCs did not uncover LTD at adult rat synapses. These data show that STDP in both adult human and adult rat synapses require postsynaptic NMDAR activity. L-type VGCC activation seems to be a common mechanism enabling adult human and rat synapses to potentiate in response to post-before-pre activation. Particular to human synapses however, is the conversion of LTP into LTD when these channels are blocked.
**Figure 4.4** STDP at human cortical synapses requires postsynaptic NMDA receptors and L-type VGCCs.

A, iMK801 reduces human EPSP decay time. Black and grey traces: EPSPs recorded within first 5 minutes and >10 minutes after whole-cell break-in, respectively. Scale bars: 2 mV, 20 ms. Inset: Effect of iMK801 on EPSP decay time in different experiments. Consistent with use-dependent blockade of NMDARs, EPSP decay time is significantly reduced after 10 minutes of EPSP stimulation (paired samples t-test: p=0.002, n=10).

B, Mean normalised slope of human control STDP recordings (▲) and recordings where postsynaptic NMDARs were blocked by iMK801 (□) or where nifedipine was present during pairing (▼), for -15 to +5 ms intervals.

C, Summary histograms of human pharmacology data. Bars represent average EPSP slope change induced using pairing intervals from -15 to +5 ms for control recordings, for recordings with iMK801 and for recordings with bath-applied nifedipine. Differences between experimental groups were tested by one-way ANOVA (F(2,19)=9.036, p=0.002). Asterisks: significant with Tukey’s HSD post-hoc test (Ctrl vs iMK: p=0.031, Ctrl vs Nif.: p=0.003).

D, As C, for +8 to +15 ms intervals. Differences between groups were tested by one-way ANOVA (F(2,21)=4.552, p=0.024).

E, Nifedipine did not affect the slope of human EPSPs that were not paired to APs, or where APs were evoked >400ms after the EPSP (paired samples t-test: n.s.). Black trace: baseline EPSP, red traces: 20-30 minutes after baseline. Inset: EPSP slope during baseline versus slope 20-30 after baseline for different experiments. Scale bars: 3 mV, 20ms.

F, As B, for +8 to +15 ms intervals.

G, Summary histogram of rat pharmacology data for pairing intervals from -15 to 0 ms. Differences between groups were tested by one-way ANOVA (F(2,16)=6.750, p=0.009). Asterisks: significant with Tukey’s HSD post-hoc test (Ctrl vs iMK: p=0.026, Ctrl vs Nif.: p=0.013).

H, As G, for 0 to +15 ms intervals. Differences between groups were tested by one-way ANOVA (F(2,15)=9.124, p=0.003). Asterisks: significant with Tukey’s HSD post-hoc test (Ctrl vs iMK: p=0.003, Ctrl vs Nif.: p=0.021).
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To test the contribution of L-type VGCCs to dendritic action potential back propagation (bAP), we used 2-photon Ca\(^{2+}\) imaging in human apical dendrites (Figure 4.5). Pyramidal neurons were loaded with Fluo-4 (100 μM) and Alexa594 (80 μM), and line scans of apical dendrites were made 100-150 μm from the soma (Figure 4.5A). Single APs and bursts of APs reliably evoked Fluo-4 fluorescence transients (Figure 4.5A-C). To probe the contribution of L-type VGCCs to AP-evoked dendritic Ca\(^{2+}\)-influx, we next bath-applied nifedipine (10 μM), which induced a significant decrease in amplitude of single AP-induced Ca\(^{2+}\)-influx (amplitude: 2.9±0.7 %ΔG/R (aCSF) versus 2.1±0.4 %ΔG/R (nifedipine); paired samples t-test (log-transformed values): p=0.035 and p<0.001 for single APs and bursts, respectively). Nifedipine also significantly reduced the area of Ca\(^{2+}\) transients triggered by bursts of APs (Ca\(^{2+}\) transient area measured over the first 300 ms after AP initiation; paired samples t-test (log-transformed values): p=0.007). E, Overlay of ADP waveforms following single APs concurrently recorded in soma during imaging experiment in aCSF conditions (black) and in presence of nifedipine (red). Inset shows portion of AP-ADP waveform that has been magnified. Note reduction in both amplitude and area (shaded) of the ADP in the presence of nifedipine. Scale bars: 10 mV, 20 ms.

Figure 4.5  L-type VGCCs contribute to dendritic Ca\(^{2+}\) influx evoked by single APs and bursts.

A, Top: 2D z-stack of patched human neuron visualised by 2-photon excitation of morphology dye Alexa594. Scale bar: 20 μm. Bottom: Pseudo-coloured 500 ms line scan of apical dendrite at ROI boxed in yellow above. Line scan is the average of 5 trials where a single AP was evoked, (▲) marks time AP was triggered. Scale bar: 100 ms. B, Top traces: Ca\(^{2+}\) transient traces evoked by single APs in neuron shown in A in aCSF conditions (left, corresponds to line scan in (a)), and in the presence of nifedipine (right). Light coloured traces are means of 4-6 trials, dark trace is 30 ms running average. (▲) marks time AP was triggered. Scaling as in C. C, As B, for burst of APs in neuron shown in A. Scale bars: 10 %ΔG/R, 200 ms. D, Ca\(^{2+}\) transient amplitude and area in the presence of nifedipine as percentage of that in aCSF conditions, for single APs and bursts of APs (80-150 Hz). Nifedipine significantly reduced the amplitude of Ca\(^{2+}\) transients triggered by single APs and bursts of APs (paired samples t-test (log-transformed values): p=0.035 and p<0.001 for single APs and bursts, respectively). Nifedipine also significantly reduced the area of Ca\(^{2+}\) transients triggered by bursts of APs (Ca\(^{2+}\) transient area measured over the first 300 ms after AP initiation; paired samples t-test (log-transformed values): p=0.007).

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4.3.4 AP after-depolarisations shape the LTP time window through L-type VGCC activation

Action potentials in human neocortical neurons showed prominent after-depolarising potentials (ADP) that lasted 62.2±3.1 ms (Figure 4.6, Figure 4.7A,B, and Table 4.1). The ADP is considered a somatic readout of dendritic calcium spike initiation and possibly reflects active dendritic action potential propagation (Larkum et al., 1999b, 2001), which is crucial for LTP induction (Couey et al., 2007; Fuenzalida et al., 2010; Kampa et al., 2006; Magee and Johnston, 1997). Since L-type VGCCs are recruited during action potential back-propagation (Figure 4.5), blocking these channels may affect the ADP waveform. Indeed, during most imaging experiments shown in Figure 4.5, application of nifedipine led to a reduction in the size of the ADP of the somatic APs triggered to elicit action potential back-propagation (Fig 5E). Since these results may have been influenced by the Ca²⁺ buffering capacities of the intracellularly present Ca²⁺ indicator Fluo-4, we quantified the effect of nifedipine on the ADP of human neurons in experiments with standard intracellular (Figure 4.6). ADP amplitude and area markedly decreased upon L-type VGCC blockade to respectively 88% and 79% of their original values in aCSF conditions (paired samples t-test, p=0.021 and p=0.040, for ADP amplitude and area respectively, n=9), suggesting activation of these receptors by single somatic action potentials (Figure 4.6A). In contrast, blocking postsynaptic NMDARs with iMK801 had no effect on ADP waveform (Figure 4.6B).

Can L-type VGCC activation during the ADP explain the wide temporal window for LTP at negative pairing intervals in human cortical pyramidal neurons? When pairing at negative intervals, EPSPs coincide with the downward slope of the ADP (Figure 4.7A,B), which may support LTP induction at negative timing intervals (Testa-Silva et al., 2010). We therefore investigated whether the long-lasting depolarisation of the ADP is important for the LTP window at negative intervals. First, we manipulated the levels of the ADP during pairing by injecting a 100 ms long negative current (-600 to -1,000 pA) 2 ms after evoking the postsynaptic spike during STDP induction (Bender et al., 2006) (Figure 4.7C). Induction of LTP was completely blocked in all human neurons where the ADP was eliminated (interleaved control experiments: 123.9±7.9%, n=9; hyperpolarisation: 100.8±6.3%, n=9, independent samples t-test: p=0.042; Figure 4.7D,E). Rat APs were similarly followed by ADPs, albeit of smaller area and duration (area: 309.9±43.2 mV*ms, duration: 35.1±4.7 ms; Figure 4.2F (inset), Table 4.1). Eliminating the ADP by hyperpolarisation during pairing also completely blocked LTP in rat neurons (control: 151.3±20.7 %, n=7; hyperpolarisation: 101.4±8.0 %, n=9, independent samples t-test: p=0.020; Figure 4.7E).
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Figure 4.7  LTP at negative timing intervals depends on the AP following the AP.

A, AP and ADP waveform recorded from 3 different human neurons at similar membrane potentials (-69 to -71 mV). Inset: Cumulative fraction of ADP areas. Scale bars: 40 mV, 10 ms. B, Schematic showing ADP quantification methods on a magnification of the area boxed in inset AP. Black trace is AP recorded without EPSP, blue trace is an AP + EPSP recorded from the same cell. Scale bars: 10 mV, 20 ms. C, ADP hyperpolarisation protocol. Top: Example traces of EPSP-AP pairing for human neuron in control conditions (blue trace) and where the ADP was eliminated by hyperpolarising current injection (grey trace). Bottom: corresponding current injections. Scale bars: 25 mV (top vertical), 2 nA (bottom vertical) and 25 ms. D, Mean normalised EPSP slope in time for recordings from human neurons where ADP was eliminated by hyperpolarising current injection (□) and control recordings from same patients (▲). Pairing intervals used ranged from -10 to -30 ms. E, Summary quantifications of ADP-hyperpolarisation experiments. Eliminating the ADP by hyperpolarisation blocked LTP in human and in rat neurons (independent samples t-test: p=0.042 and p=0.020, respectively). F, EPSP slope change in individual STDP experiments paired at negative intervals versus ADP amplitude at EPSP onset. Regression line is shown in blue. G, Summarised STDP results for neurons with ADP areas above (▲) and below (●) the median ADP area, in pairing interval bins of 40 ms. At pairing intervals between -40 and -80 ms, neurons with large ADPs potentiated significantly more than neurons with smaller ADPs (independent samples t-test, p=0.01). Inset: example traces of AP-ADP waveforms recorded from neuron with large (blue) and from neuron small (black) ADP area. H, ADP area plotted against somatic depth (measured as distance from pia) and corresponding layers of recorded human neurons. Horizontal blue bars show mean±SEM ADP area per layer; (*) data points corresponding to reconstructed neurons to the right. Asterisks indicate dendrite is cut at slice surface.
Next, we correlated the change in EPSP slope to the amplitude of the ADP at the time the EPSP was triggered during plasticity induction (Figure 4.7B,F). ADP amplitude at EPSP onset showed a weak but significant correlation with EPSP slope change (humans: $R^2=0.17$, $p<0.01$, $n=42$; Figure 4.7F; rats: $R^2=0.19$, $p=0.03$, $n=25$). The effect of the ADP was also evident when comparing EPSP slope changes between neurons with ADP areas above and below the median ADP area of human neurons, which was ~400mV*ms. Comparing three bins of pairing intervals (0 to -40 ms, -40 to -80 ms, and <-80ms; Figure 4.7G), we found that neurons with ADP areas above 400 mV*ms potentiated significantly more than neurons with ADP areas smaller than 400mV*ms at intervals ranging from -40 to -80 ms (independent samples t-test, $p=0.01$, $n=6$ for both groups). Importantly, ADP area was not related to other electrophysiological or morphological characteristics measured; ADP areas were not significantly different between cortical layers (one-way ANOVA: $p>0.05$; Figure 4.7H), nor did ADP area correlate with apical, basal or total dendritic length (not shown). ADP area also showed no relationship with other physiological parameters, including input resistance and membrane time constant. Although the somatic ADP-waveform showed a clear voltage dependence within neurons, across neurons, we found no significant correlation between ADP area and somatic resting membrane potential ($r=-0.21$, $p>0.05$, $n=42$). Taken together, these findings show that L-type VGCC activation during the large, long-lasting ADP following the action potential endows adult cortical synapses with the ability to associate pre and postsynaptic activity at negative timing intervals over long stretches of time, resulting in a wide temporal window for STDP at these synapses.

4.4 Discussion

In this study, we show that adult human neocortical synapses can bi-directionally change strength in response to spike timing at least up to 65 years of age, and uncovered mechanisms underlying human synaptic plasticity rules. The temporal window for associative changes in strength in human synapses is wide (±100 ms) and shows LTP at negative pairing intervals and LTD at positive intervals, with a switch from LTP to LTD occurring between +5 and +10 ms. The depolarisation following the action potential, the ADP, is necessary for LTP at negative timing intervals. L-type VGCCs are activated during the ADP and are required for LTP induction, but not for LTD induction. Adult rodent MTC synapses show similar features of STDP, but do not show LTD at positive timing intervals. The ADP following the action potential and L-type VGCC activation are common mechanisms enabling adult human and rat synapses to associate pre and postsynaptic activity in an exceptionally wide temporal window.

Inverse, anti-Hebbian STDP rules have been reported for synapses between layer 4 spiny stellate neurons in rat barrel cortex, which appear to have a symmetric depression window (Egger et al., 1999). In the cerebellum-like structure of electric fish, pairing at positive intervals induces LTD and at negative intervals induces LTP (Bell et al., 1997). In our previous investigations of STDP at adult human hippocampal synapses, LTP was also observed at negative timing intervals; EPSPs following a postsynaptic burst of APs by up to 80 ms potentiated, while LTD was only observed if EPSPs followed the burst by more than 80 ms (Testa-Silva et al., 2010). Interestingly, in vivo studies in humans subjects observed STDP-like changes in corticospinal excitability following anti-Hebbian plasticity rules similar to those reported in the present study (Conde et al., 2013; Koch et al., 2013; Thabit et al., 2010). Moreover, the timing window for association of stimuli was wide, with significant changes in MEP amplitude occurring when TMS was delivered from 50 ms before to 100 ms after voluntary movement (Thabit et al., 2010). The extent to which changes in MEP amplitude reflect synaptic plasticity processes remains unclear, but the reversed and wide STDP window for human temporal cortex reported here is strikingly similar to that of paired association of TMS and voluntary movement (Conde et al., 2013; Koch et al., 2013; Thabit et al., 2010).
The shape and form of STDP windows are dictated by the intracellular machinery in postsynaptic dendrites and spines and presynaptic terminals (Caporale and Dan, 2008; Sjostrom et al., 2008). Variations in these mechanisms over different cell-types and brain areas, perhaps combined with other factors such as the actions of neuromodulators and/or the levels of GABAergic inhibition, can lead to a variety of synaptic learning rules (Babadi and Abbott, 2010; Fino and Venance, 2010; Meredith et al., 2003). Reports on the involvement of inhibition in shaping synaptic plasticity rules are ubiquitous, but it has perhaps been most dramatically demonstrated in cortico-striatal STDP. There, a reversed STDP window was reported with LTP following post-before-pre induction protocols, and LTD following pre-before-post protocols, which could be completely reverted to the classic STDP window when GABAergic inhibition was blocked during induction (Fino et al., 2010). In vivo studies in humans have shown how inhibition can also shape human associative plasticity (De Beaumont et al., 2012; Conde et al., 2013; Elahi et al., 2012); increased GABA\(_B\)-mediated inhibition in concussed athletes leads to a suppression of LTP- and LTD-like plasticity (De Beaumont et al., 2012), and interhemispheric inhibition of the sensorimotor network during PAS has been shown to result in anti-Hebbian STDP-like plasticity rules (Conde et al., 2013). A role for inhibition in forming the reversed STDP window observed in human synapses in the present study cannot be ruled out, as GABAergic transmission was not blocked in our experiments. Inhibition may act to reduce postsynaptic depolarisation and thereby reduce the Ca\(^{2+}\) signals that would otherwise trigger potentiation (Couey et al., 2007). However, it is not straightforward to understand how a reduction of a synaptically-induced depolarisation by inhibition would favour potentiation over depression of synapses when pairing at negative intervals, as we observed. Recently, in rodent striatum, depolarising actions of GABA in distal dendrites have been suggested to mediate the switch from LTD to LTP in cortico-striatal synapses paired at negative intervals (Paille et al., 2013). Such mechanisms may contribute to shaping the human cortical STDP window, but other factors will have to be considered as well (Sjostrom et al., 2008).

An alternative cause for the reversed STDP observed in humans may be the location of recruited synapses on the dendritic tree of the recorded neuron (Froemke et al., 2005; Letzkus et al., 2006; Sjöström and Häusser, 2006; Sjostrom et al., 2008). Within one neuron, proximal synapses can display the classic Hebbian learning rule, while distal synapses display inverse, anti-Hebbian plasticity rules (Letzkus et al., 2006). Dendritic location of synapses was proposed as an explanation for anti-Hebbian cortico-cortical STDP-like plasticity observed in vivo (Koch et al., 2013). In our study, synaptic inputs were evoked by extracellular stimulation 100-150 \(\mu\)m from the soma, which would hardly qualify as distal inputs (Letzkus et al., 2006). So, unless the stimulated fibres actually impinge on the dendrite at more distal locations than our stimulating electrode, or else that synaptic plasticity rules in human neurons have quite a different distance-dependency than in rodents, it seems unlikely that this synapse location-dependent form of anti-Hebbian STDP is dominating our experiments.

In the present study on human neocortical synapses and in our previous study on human hippocampal synapses (Testa-Silva et al., 2010), we observed that EPSPs coincide with the falling flank of the ADP when pairing at negative intervals. The synaptic potentiation that follows such pairing protocols in human neocortex and hippocampus may result from activating synapses concurrently with the dendritic calcium dynamics induced by the back-propagating action potential. In rodents, apical dendrites and spines express several types of VGCCs, including L-type VGCCs, which are activated during dendritic action potential propagation (Meredith et al., 2007; Sabatini and Svoboda, 2000). In human dentate gyrus cells, AP firing was shown to recruit VGCCs; increases in ADP area when bursting above critical frequency were blocked by the N-type VGCC antagonist NPY (Hamilton et al., 2010). In our experiments in human neocortex, blocking L-type VGCCs with nifedipine caused a prominent reduction of the ADP in
most neurons, indicating a substantial recruitment of L-type VGCCs by single APs. Individual differences in ADP area may therefore in part reflect different degrees of L-type VGCC activation. Pairing at negative intervals in these conditions no longer induced LTP, but resulted in LTD instead. In rodents, L-type VGCCs are also involved in STDP, but have a markedly different role than in adult humans (Meredith et al., 2007; Remy and Spruston, 2007; Sjostrom et al., 2008). In juvenile mouse prefrontal cortex, blocking L-type VGCCs prevented LTP induction (Meredith et al., 2007), but it did not unmask LTD, which is in line with our findings in adult rat MTC. In hippocampal and somatosensory cortical pyramidal neurons, blockade of L-type calcium channels strongly reduced the extent of spike-timing-dependent LTD (Bi and Poo, 1998; Nevian and Sakmann, 2006). In human subjects, systemic application of an L-type VGCC antagonist altered the sign of metaplasticity of evoked peripheral endplate potentials and corticospinal excitability evoked by TMS stimulation (Wankerl et al., 2010). The authors suggested that L-type postsynaptic VGCCs might act at cortical synapses. Here, we directly show that at the synaptic level, L-type VGCCs decide between spike-timing-dependent potentiation and depression and set the timing window for activity-dependent plasticity in adult human cortical synapses.