Identification and characterisation of regionally enriched cortex genes in the rat brain

Kari Merete Ersland

Dissertation for the degree philosophiae doctor (PhD)
at the University of Bergen

2012

If the human brain were so simple
that we could understand it,
we would be so simple
that we couldn't.

Emerson M. Pugh

Scientific environment

This work was carried out from 2007 to 2012 in Dr. Einar Martens Research Group for Biological Psychiatry at the Department of Clinical Medicine, University of Bergen, and the Center for Medical Genetics and Molecular Medicine, Haukeland University Hospital. The work was conducted within the framework of the International Graduate School in Integrated Neuroscience (IGSIN).

The presented work was performed with Professor Vidar M. Steen as the main supervisor at the Department of Clinical Medicine, University of Bergen, Norway.

The work was funded by the University of Bergen (PhD grant), the Western Norway Regional Health Authority (Helse Vest RHF), The Research Council of Norway (FUGE program) and Dr. Einar Martens Fund.

Acknowledgements

Many colleagues have contributed to the fulfilment of this thesis, and I thank you all. First and foremost, I would like to express my sincere gratitude to my supervisor Prof. Vidar M. Steen. Thank you for including me in your group, for sharing your profound knowledge of the field and for giving me freedom to explore different aspects of the project. And most of all, thank you for your never-ending enthusiasm and encouragement!

I am also very grateful to my co-supervisors Dr. Christine Stansberg, Dr. Stephanie Le Hellard and Dr. Bjarte Håvik. Christine- you made the foundation that this thesis is based upon, and none of this work would have been possible without you. Thank you for including me in the project and for your invaluable input on manuscripts and this thesis. Stephanie- I thank you for introducing me to the world of genetics and for sharing your expertise with me. I am very thankful for your endless patience and support. I am also indebted to Bjarte, who through his laboratory know-how, great ideas and invaluable discussions strongly contributed to the success of this work.

I would like to thank all the co-authors for their contributions to the papers included in this work. In addition, a special thanks to Dr. Jean M. Hebert at the Albert Einstein College of Medicine for welcoming me in his group, and Dr. Marie Fernandes for teaching me their methods. I also thank Dr. Jonathan Soule for his technical input and valuable discussions.

I am so happy to be a part of the Martens group, and I thank both present and former members for making it fun to go to work every day! I especially thank Dr. Silje Skrede, Dr. soon-to-be Teresa Osland and Dr. to-be Carla Fernandes Neto for our shared joys (and minor depressions) during this joint PhD run. Also, a special thanks to Dr. Andrea Christoforou for all the invaluable help and guidance through the jungle of SNPs and P-values! I also thank Marianne Nævdal for your excellent technical assistance. And to all my co-workers at MGM- thank you for creating a

great working environment! I am also thankful for the technical and infrastructure support provided by the Center for Medical Genetics and Molecular Medicine.

Bergithe, I am so grateful for the friendship we share. Our many coffee breaks have really helped to keep me sane and motivated through these years. Together with Dr. Gro and Masters (of the Universe) Andreas and Åsmund, I thank you for the many enjoyable vacations, visits and good memories. To all my other friends, near and far, I thank you all for the understanding and support during these years. I truly look forward to spending more time with you!

My family has always believed in me and cheered me on, and for this I thank you from the bottom of my heart!

And finally, Christian! Thank you for your never-ending encouragement, high buffer-capacity, patience and love. I would not have made it without you!

Bergen, March 2012

Kari Merete Ersland

List of contents

Abbreviations

BA	Brodmann area
BP	Bipolar affective disorder
C1orf146	Chromosome 1 open reading frame 146
CNS	Central nervous system
CNV	Copy number variation
FMCx	Frontomedial cortex
GSEA	Gene set enrichment analysis
GWAS	Genome-wide association study
HCRTR1	Hypocretin (orexin) receptor 1
IP	Intermediate progenitor
LOC689986	Hypothetical protein LOC689986
OCx	Occipital cortex
ORG	Outer radial glia
OSVZ	Outer sub-ventricular zone
P-FIT	Parieto-frontal integration theory
RG	Radial glia
RORB	RAR-related orphan receptor B
SCZ	Schizophrenia
SNP	Single nucleotide polymorphism
SVZ	Sub-ventricular zone
TCx	Temporal cortex
VZ	Ventricular zone

Summary

The highly complex neocortex is a mammalian specific region of the brain. In humans, several areas of the neocortex have been linked to normal cognitive functioning. Importantly, abnormalities in the neocortex are associated with serious psychiatric disorders, such as schizophrenia and bipolar affective disorder, to which cognitive dysfunctions have been linked.

The specific gene expression in a certain region or organ often reflects the functional specialisation of the given area. However, surprisingly few genes have been demonstrated to display a regional pattern of expression in adult neocortex. In fact, the global gene expression in various functionally and anatomically distinct areas of the neocortex seems to be almost identical across regions. In this work we have used microarray-based global gene expression profiling, and identified 65 regionally enriched genes in the frontomedial-, temporal- and occipital cortices of the adult rat neocortex (30, 24 and 11 genes, respectively). A substantial portion of these genes seemed to be involved in signal transduction processes. In addition, many of them were found to display layer- and cell type specific expression. We proposed that these genes could be important to sustain the normal function in a given cortical area. Based on the importance of the neocortex in normal cognitive functioning, we used these differentially expressed genes, as candidate genes, to mine a large genome-wide association study of healthy adults for association to nine psychometric tests. At the single gene level, we found that several of the 65 candidate genes displayed association to various test measures of cognitive abilities. In addition, we applied gene set enrichment analysis of the candidate genes, and found that the genes differentially expressed in the temporal cortex, as a set, displayed a significant enrichment of association signal to a test measure of non-verbal intelligence. Since cognitive dysfunctions are often observed in patients suffering from serious psychiatric disorders, we also analysed the candidate genes in large genome-wide

association studies of schizophrenia and bipolar affective disorder. However, none of the candidate genes, at the gene set level, seemed to be associated to these illnesses.

In this work we have also partially characterised one of the 65 regionally enriched genes, the unannotated gene *hypothetical protein LOC689986*, which displayed an almost exclusive gene expression in samples from the temporal cortex. Here we report that this unique gene is highly conserved in, and apparently specific for, the vertebrate lineage. The gene displayed a restricted area- and layer specific expression in the parieto-temporal cortex, and in addition we found that the LOC689986 protein was present in the somatosensory cortex and the Purkinje cells of the cerebellar cortex in rodents. The protein was found to localise to neuronal dendrites, and identification of potential protein interaction partners suggests a role for this protein in transcriptional regulation, and possibly the translational machinery.

In summary, we have identified and partially characterised, genes that could be important to sustain the normal function of selected areas of the neocortex. Furthermore, we have analysed such genes for association to cognitive functioning and psychiatric disorders. Our results suggest that genes differentially expressed in the temporal cortex could be involved in mechanisms of non-verbal intelligence.

1. Introduction

The brain is by far the most complex organ in the human body, and stands responsible for our thoughts, memories, feelings, actions and the perception of our surroundings. Based on anatomy and functional aspects, the brain is divided into highly specialised structures. One of these structures, the cerebral cortex, constitutes the largest part of the brain, and in humans the cerebral cortex is estimated to account for as much as 77% of the total volume of the brain [reviewed in 1]. It harbours specialised functional properties, ranging from processing and interpretation of sensory information to the conscious control of motor function. One of the remarkable attributes of the cortex is higher intellectual functioning, which is outstanding in humans, and sets us apart from non-human primates and other mammalian species.

Despite the prominent role of the cortex in cognitive functions, the underlying mechanisms explaining how the numerous cortical neurons are connected and function to make up the highly complex structure, remain elusive. We do, however, have substantial knowledge about the basic organisation, development, evolution and functional organisation of the cerebral cortex.

In the following I will present relevant topics on the mammalian cortex, and how cognitive abilities and psychiatric disorders relate to this extraordinary structure.

1.1 The mammalian neocortex

1.1.1 Basic structural organisation of the mammalian neocortex

The cerebral cortex is located at the anterior part of the mammalian brain (superior orientation in humans), surrounding the cerebrum (the main brain) (Figure 1). Based on cellular lamination, the cerebral cortex can be divided into the neocortex (also called isocortex or homogeneous cortex) and the allocortex (also known as the heterogenic cortex). The highly complex neocortex constitutes the phylogenetically most recent addition to the brain, and has been estimated to contain approximately 20 billion neurons in humans [2]. Anatomically, the neocortex is divided into four main areas, namely the frontal-, parietal-, temporal- and occipital lobes (Figure 1). Each of the four lobes is associated with various functional domains, such as higher mental functioning in the frontal lobe and processing of auditory, visual and sensory information in the temporal-, occipital- and parietal lobe, respectively (Figure 1).

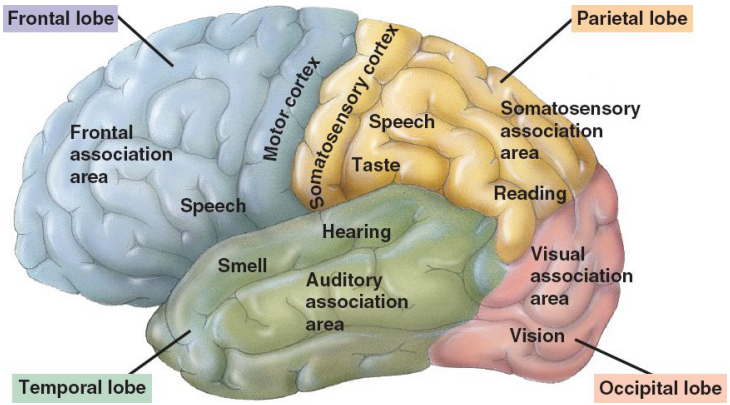

Figure 1: Lobes of the brain. The figure shows a lateral view of the human brain, illustrating the folded neocortex surrounding the left hemisphere of the brain. The four different lobes are illustrated. Blue: frontal lobe, yellow: parietal lobe, green: temporal lobe and pink: occipital lobe. Specific functions associated with the different regions of the neocortex, such as vision, hearing and speech, are indicated. The image is taken from http://headwayballymena.org.uk/page3.html.

In the horizontal dimension, the mature neocortex is organised as a sheet composed of six cellular layers (Figure 2), each of which is comprised of heterogeneous populations of morphological and connectional distinct neurons. In general, these cortical neurons can be divided into two broad classes, namely projection neurons (also known as pyramidal or excitatory neurons) and interneurons (also called inhibitory or local circuit neurons). The projection neurons are glutamatergic (i.e. they use glutamate as a neurotransmitter) and make intra-cortical, sub-cortical and sub-cerebral connections. In contrast, the interneurons normally use γ-aminobutyric acid (GABA) as a neurotransmitter, and make local connections [reviewed in 3]. Of the six layers of the neocortex, layer I (molecular layer) is the outermost layer, mainly made up of dendritic clusters originating from distal pyramidal neurons. A scattered pattern of interneurons is also observed in this layer, as well as many axon terminations [reviewed in 4]. Layer II (external granular layer) comprises mainly stellate neurons, in addition to a scattered pattern of small pyramidal neurons. Layer III (external pyramidal layer) of the neocortex is primarily composed of small projection neurons, while layer IV (internal granular layer) predominantly contains different types of stellate and small pyramidal neurons. Layer V (internal pyramidal layer) consists of large pyramidal neurons, while a great number of small spindle-like pyramidal and multiform neurons, in addition to a few large pyramidal neurons, make up the innermost layer VI (polymorphic/multiform layer) [5].

In the radial dimension, the neocortex is intersected by vertical columns consisting of synaptically linked neurons from layer II-VI (Figure 2) [reviewed in 6]. These vertically organised neurons (minicolumns) are the basic unit of the neocortex. Short range horizontal connections couple several minicolumns together, and thereby make up a cortical column. The cortical columns link both input and output projections, making them highly complex processing and distributing units of the neocortex [reviewed in 6]. The excitatory pyramidal neurons are arranged in the same basic tangential and laminar organisation in all mammals [reviewed in 4]. Afferent thalamic and cortical input signals arrive in cortical layer IV, where the axons of excitatory neurons subsequently project to the superficial layers (II and III).

Pyramidal neurons within the superficial layers project to other cortical areas, and to the deeper layer V which in turn is connected to layer VI. Excitatory neurons in layer VI project to layer IV, closing the neuronal circuit [for review see 4,7].

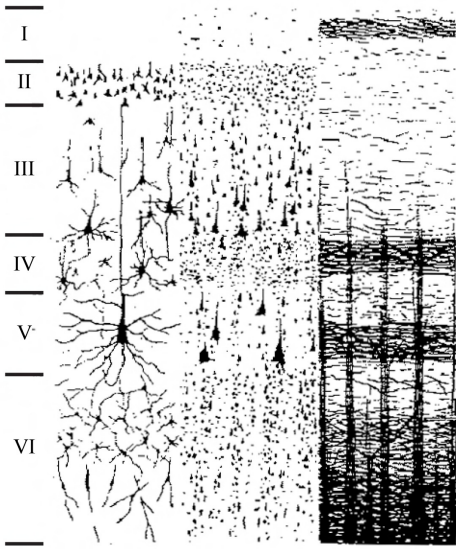

Figure 2: Basic structural organisation of the mammalian neocortex. The neocortex is organised into a six-layered structure in its horizontal dimension (left). The roman numerals indicate the different neocortical layers, from the outermost (layer I) to the innermost layer (layer VI). The neocortex is intersected radially by vertical columns (right). The image is modified from http://www.sbirc.ed.ac.uk/cyril/cp_neurophysio2.html.

1.1.2 Neocortical neurogenesis

All of the numerous projection neurons within the neocortex are generated in transient proliferative zones, situated immediately adjacent to the surface of the lateral ventricles in the dorsal forebrain. The first proliferative zone to emerge during the embryonic development is the ventricular zone (VZ), followed by the sub-ventricular zone (SVZ) and the outer sub-ventricular zone (OSVZ) as the neurogenesis proceeds [reviewed in 3,8]. Several cortical progenitor cells have been

identified, that give rise to all the various neuronal cell types. At the beginning of neurogenesis, neuroepithelial cells mainly undergo symmetric cell divisions, in order to generate a large pool of progenitors. These cells transform into the radial glia (RG) cells, which are situated in the VZ (Figure 3). The radial glia cells constitute the majority of progenitor cells in rodents [9,10], and to some extent in primates [reviewed in 11]. The RG cells project to the cortical surface, and undergo both symmetric and asymmetric division, giving rise to numerous neuronal cell types [12-14]. An important feature of the radial glia cells is that they enable postmitotic neurons to migrate to their appropriate layers (according to the "radial unit hypothesis" [15]), where they bypass the previous layers in a fashion known as the "inside-out gradient of neurogenesis" [reviewed in 16].

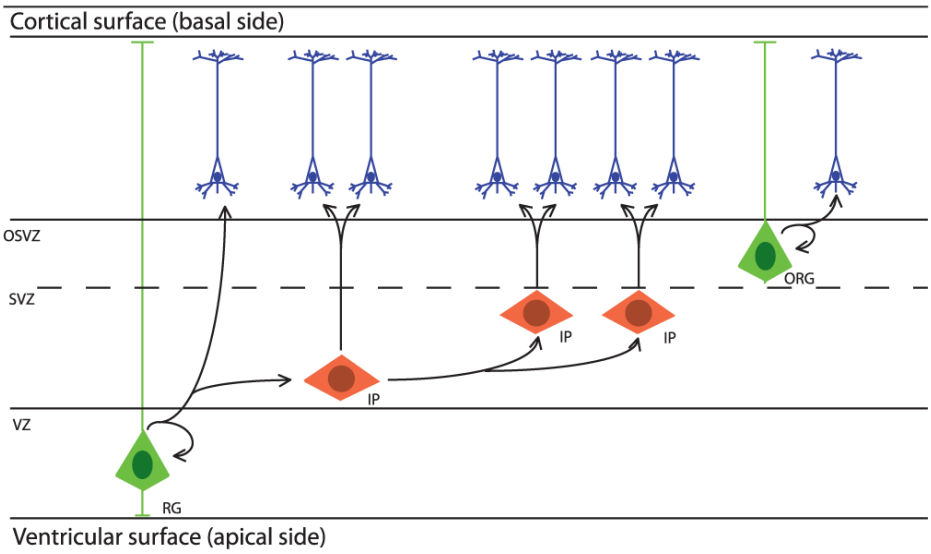

Figure 3: Progenitor cells in the developing neocortex. Radial glia (RG) progenitor cells are situated in the ventricular zone (VZ), making projections towards the cortical surface. RG cells undergo both asymmetric and symmetric division, and provide a scaffold for neuronal migration to the appropriate neocortical layer. Intermediate progenitor (IP) cells reside in the sub-ventricular zone (SVZ) and undergo symmetric division. In primates, outer-radial glial (ORG) progenitor cells are situated in the outer SVZ (OSVZ). ORG cells make projections to the cortical surface and are able to go through both asymmetric and symmetric cell divisions. Similar to the RG cells, the ORG cells provide a scaffold for migrating neurons generated in the OSVZ. The figure is taken with permission from [17].

In addition to providing a scaffold for migrating neurons, the radial glia cells give rise to the intermediate progenitor (IP) cells (Figure 3) [18-21]. These cells migrate to the upper part of the ventricular zone to create a new proliferating zone, namely the sub-ventricular zone. In contrast to RG cells, intermediate progenitor cells are only able to undergo symmetric cell division [18]. However, the IP cells constitute the majority of neuron producing cells compared to the RG cells in rodents [22]. An expansion of the outer region of the sub-ventricular zone (the OSVZ) has been observed in the primate developing neocortex (Figure 3) [reviewed in 8,17]. This region is populated by RG-like cells (called outer-radial glia (ORG) cells) in addition to IP cells. The ORG cells originate from the radial glia cells in the ventricular zone, and constitute a second pool of progenitor cells which are able to go through both symmetric and asymmetric cell divisions, giving rise to neurons and new progenitor cells [23,24]. In contrast to radial glia cells, the ORG cells lack contact with the ventricular surface. However, they project to the cortical plate, functioning as scaffolds for migrating neurons [reviewed in 8].

1.1.3 Arealisation and functional organisation of the neocortex

During embryonic development the mammalian neocortex is divided into specific neocortical areas through a process termed arealisation. This developmental process is crucial for the establishment of the unique functional properties, interactions with other areas of the brain as well as the appropriate size of a given area [reviewed in 25]. Control of the arealisation is thought to involve an interplay between intrinsic genetic regulation of the neocortex and external influence from thalamic projection neurons [reviewed in 26]. Early in neocortical development, morphogens are secreted from telencephalic patterning centres, which in turn initiate anterior–medial and posterior–lateral gradients of transcription factors in progenitor cells in the cortical ventricular zone (i.e. Emx2, Pax6, COUP-TFI, Sp8) [27-38]. The graded expression of various transcription factors is crucial for the area identity of the cortical progenitor cells. This area identity is subsequently conferred to the neuronal progeny which makes up the cortical plate [reviewed in 25]. Moreover, expression of specific

guidance molecules ensures the proper thalamocortical axon innervations of the developing neocortex. It has long been debated whether or not the thalamocortical input projections induces the structural differences in the neocortex ("protocortex" hypothesis [39]). However, it is now generally accepted that intrinsic regulation (i.e. by differential gene expression in progenitor cells in the VZ) is the key factor in defining specific areas ("protomap" hypothesis [15]), and that these areas attract the appropriate thalamocortical axons, rather than being specified by them [reviewed in 40].

In general, the mature neocortex can be divided into four main functional areas in all mammalian species. These areas include the primary visual-, the somatosensory-, the auditory- and the motor area, located within the occipital-, parietal-, temporal- and frontal lobes, respectively (Figure 1). Each of these functionally distinct regions receive specific thalamic input projections [reviewed in 25]. However, several other distinct functional domains can be recognised, based on differences in cytoarchitecture, chemoarchitecture, input and output projections, and patterns of gene expression. At first glance, the cytoarchitecture of the mature neocortex may appear uniform. Nonetheless, there is substantial variability in neocortical areas based on lamination, connectivity and neurochemistry [reviewed in 41]. As an example, more than 50 distinct cytoarchitectonic regions (also called Brodmann Areas) have been distinguished in the human brain [reviewed in 40], each harbouring highly specific functions. Damage to certain regions, such as the Broca's or Wernicke's areas, results in difficulties, or inability to process language and generation of speech [reviewed in 41], clearly illustrating that distinct anatomical regions are responsible for highly specialised functions.

1.1.4 Differential gene expression in the mammalian brain

The global gene expression (transcriptome) in various parts (e.g., neocortex, cerebellum, hippocampus, striatum) of the mammalian central nervous system (CNS), differs substantially and clearly distinguishes distinct regions from each other (Figure 4) [42-46]. Importantly, the regionally enriched gene expression in different brain areas has been demonstrated to reflect the functional activity of the given region [42,45]. In contrast, the transcriptome in sub-areas within the adult neocortex has been shown to be surprisingly similar [42,44,45] (Figure 4), despite the specialised functions attributed to distinct cortical regions. In fact, the gene expression in different neocortical areas of the adult human brain was demonstrated to be more similar within an individual, than between corresponding regions in another individual [44]. It appears that differential gene expression across cortical areas is relatively rare [reviewed in 47]. However, several studies have reported on a limited number of genes displaying highly area specific expression within functionally distinct regions of both primate (e.g. primary sensory, association and motor cortices) [44,48-52] and rodent neocortex [46,52-54].

It has been suggested that the region-specific gene expression is a result of differential distribution of a certain cell type across cortical areas, which specifically expresses a certain gene. This has been demonstrated for a few genes selectively expressed by a certain cell type within a specific cortical layer (e.g. *Nuclear receptor related 1 protein*) [reviewed in 55]. In contrast, it has also been proposed that regionally enriched genes are expressed in a wide range of neuronal cell types, and undergo dynamic regulation during neocortical maturation [reviewed in 47]. The biological significance of such area-specific gene expression in the mature neocortex is slowly emerging. As an example, the products of genes selectively expressed in the primate visual cortex areas (e.g. *occipital 1*- related family genes), have been suggested to be involved in conferring stability on neuronal circuits. This is important since newly acquired information (in this case by visual input) would be lost under

conditions where there is too much plasticity. In contrast, the neuronal circuits would not be formed if the stability was too high [reviewed in 56].

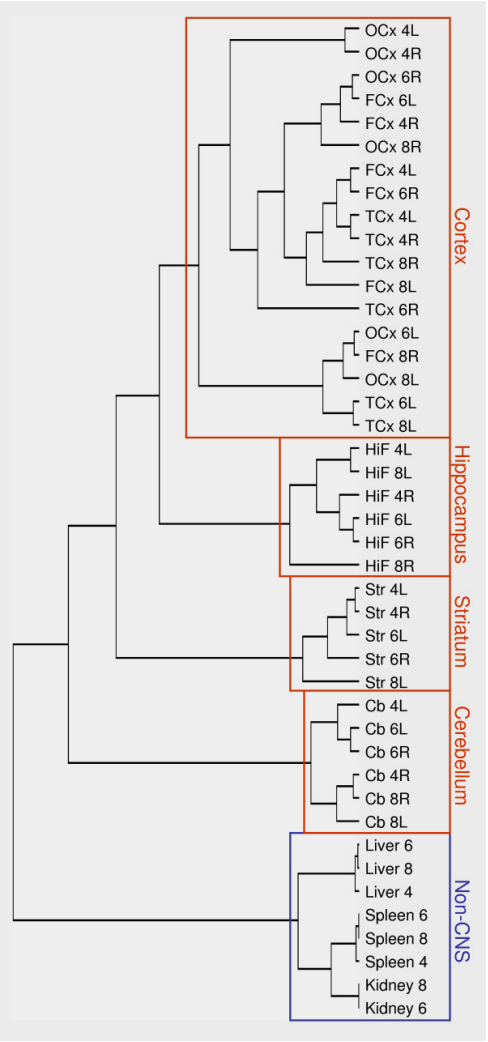

Figure 4: A hierarchical clustering of various brain and non-CNS tissues from the adult rat. The transcriptomes of various brain and non-CNS tissue samples from the adult rat differ substantially from each other. CNS and non-CNS samples are clearly separated (red and blue colour, respectively), as are the different CNS regions (i.e. cortex, hippocampus, striatum and cerebellum). The samples from the three different cortical areas (i.e. FCx, TCx and OCx) do not, however, cluster according to region. Individual rats are indicated by numbers (i.e. 4, 6 and 8), L or R: samples from the left or right hemisphere, respectively, OCx: occipital cortex, TCx: temporal cortex, FCx: frontomedial cortex. The figure is taken with permission from [42].

1.1.5 Evolution of the neocortex

In general, all mammalian species share some common functional neocortical regions, but there is substantial variability in the size of the neocortical surface area, functional domains and organisation. An obvious example of this is the large difference in size of the primate and rodent neocortex (Figure 5). While the rodent neocortex has a smooth surface (lissencephalic), the human and non-human primate neocortex is organised into a folded structure (gyrencephalic). Gyrencephalic brains are characterised by folds (gyri) separated by depressions (sulci) (Figure 5). Generally, the surface area in gyrencephalic mammals is disproportionally increased relative to the ventricular surface, as compared to lissencephalic mammals [reviewed in 40]. An increase in the number of progenitor cells by symmetric cell division, prior to neurogenesis, has most probably led to this enlargement during evolution. The increased number of progenitor cells in turn led to an increase in the pool of radial glia cells in the ventricular zone, and thereby, ultimately a larger amount of cortical columns [reviewed in 40,57]. In addition, an increase in the number of symmetric intermediate progenitor cell divisions in the sub-ventricular zone, has also been assigned to play a role in the surface area expansion [reviewed in 8]. Moreover, the increase in neocortical surface area in gyrencephalic brains, and particularly in the human neocortex, is related to an expansion of the pool of outer-radial glia founder cells in the outer sub-ventricular zone [23,24,58].

The human neocortex comprises several unique functional domains, that are not present in phylogenetically “lower” mammalian species (e.g. rodents). These areas are thought to be a result of the neocortical surface area expansion during evolution. Among others, these include Broca’s and Wernicke’s area, which are involved in production and processing of language. Most importantly, human brains are characterised by having a large expansion of the prefrontal cortex (Figure 5). The specific functions attributed to the prefrontal cortex in humans are associated with unique mental abilities, which sets humans apart from non-human primates and other mammalian species [reviewed in 40,59].

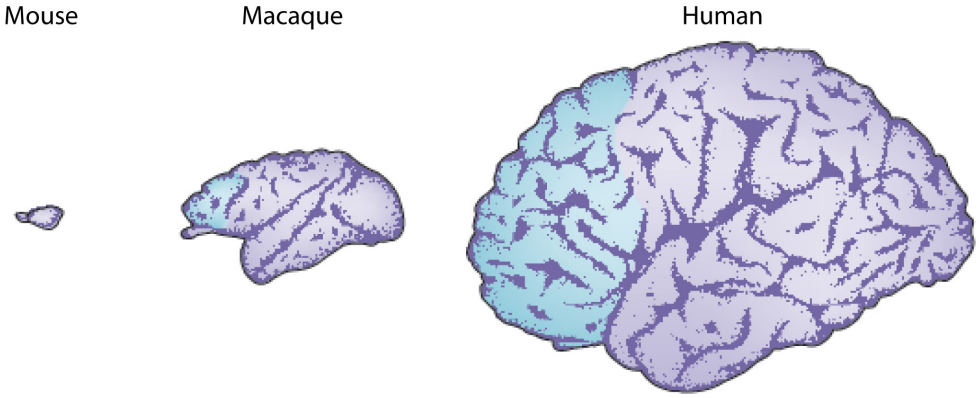

Figure 5: Comparison of the mouse, macaque and human brain. A lateral view of the brain illustrates the difference in size of the brain in mouse, macaque and human (the brains are drawn to approximately the same scale). The mouse brain has a smooth surface, while the primate brains have distinct sulci and gyri. The prefrontal cortex is indicated in blue in the human and macaque brains. The image is modified from [40].

1.2 Cognition

1.2.1 Cognitive abilities

The terms cognitive abilities, intelligence, mental ability and IQ (intelligence quotient) are often used interchangeably in order to describe a wide range of mental processes in humans. These processes include reasoning, working memory, attention, executive functioning and speed of processing. Cognitive abilities are strongly associated with important life outcomes such as education, professional occupation, income and length of life [60, reviewed in 61]. In general, intelligence varies in the healthy population, following an approximately normal distribution. A large battery of psychometric tests is available to measure cognitive abilities, resulting in an estimate of how well an individual performs in solving specific tasks. Such test estimates are not at all independent measures, since people who perform well on one

specific test also tend to do well on the other psychometric tests. This phenomenon can be explained by the "general factor of intelligence" (*g*), which has been estimated to account for 40% (or more) of the observed total variance in cognitive abilities [reviewed in 59].

1.2.2 Cognition and the neocortex

Interestingly, there is a moderate correlation between intelligence and total brain volume in healthy individuals [62]. In addition, a modest positive correlation between estimates of general intelligence and volumes of the frontal-, parietal- and temporal cortices, as well as the hippocampus, has been reported [62-65]. However, how these areas interact, and how they relate to measures of intelligence, remained elusive. Recently, based on several magnetic resonance imaging studies, Jung and Haier proposed that a network consisting of areas in the dorsolateral prefrontal cortex, parietal lobe, anterior cingulate cortex, and areas in the temporal- and occipital lobes are linked to differences in cognitive abilities (the parieto-frontal integration theory (P-FIT)) (Figure 6) [66]. According to this theory, several areas of the brain are thought to influence differences in cognitive abilities between individuals. Regions within the occipital lobe (Brodmann Area (BA) 18 and 19) and the temporal lobe (BA 37) are involved in recognising visual input, and these signals are further processed in areas of the parietal lobe (BA 7, 39 and 40). According to the parieto-frontal integration theory, the parietal regions interact with areas within the frontal lobe (BA 6, 9, 10 , 32, 45 ,46 and 47) in order to generate a working memory network and to select the appropriate task response [reviewed in 59].

The thickness of various cortical regions has also been linked to intelligence differences between healthy individuals [67-70]. The analysis of cortical thickness, as compared to volumetric estimates, may give a more accurate measure of cytoarchitectonic characteristics associated with cognition. The thickness of the frontal-, parietal- and occipital cortices, in addition to multimodal association areas, has been positively correlated with intelligence [70,71]. Interestingly, a longitudinal

study of cortical development in children and adolescents revealed that the relationship between intelligence and cortical thickness changes with age, and that the pattern of cortical growth during development, primarily in the frontal cortex, is related to levels of intelligence [71].

Brain lesion studies have also provided highly specific relationships between neocortical areas and deficits in cognitive abilities. As an example, damage in the left frontal and parietal cortices causes difficulties in performance related to working memory, while lesions in left inferior frontal cortex cause a decrease in verbal comprehension [72].

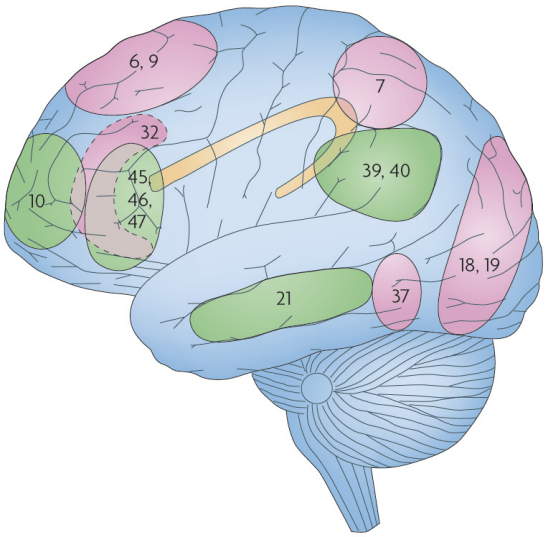

Figure 6: Areas of the brain involved in cognitive abilities. The Parieto-frontal integration theory describes a network of brain regions thought to be involved in cognitive abilities [66]. Brodmann Areas (BA) are indicated in each region. BAs coloured green or pink indicate predominantly left or right hemispheric correlation with intelligence, respectively. The figure is taken from [59].

1.2.3 The heritablity and genetics of human intelligence

Twin- and adoption studies have demonstrated a high heritability for cognitive abilities, with estimates of genetic influence ranging from 30-80%. Interestingly, such estimates have been shown to increase with age [for review see 59,73]. Despite the high heritability, the underlying genetic architecture leading to the observed differences in cognitive functioning between healthy individuals are not well understood. It is generally accepted that intelligence is influenced by variants in a large number of genes. Such variants have traditionally been examined by linkage- and association- studies, and more recently by genome-wide association studies (GWAS). In fact, a recent multi centre GWAS found that thousands of common genetic variants account for ~40-50% of the variation in cognitive abilities [74].

In GWASs, between several hundred thousand and more than a million genomic variants (single nucleotide polymorphisms (SNP)), covering the entire genome, are analysed in thousands of individuals [75]. The aim of genome-wide association studies is to identify SNP variants occurring at a different frequency in cases than in matched controls, and thereby identifying SNPs that are likely to explain differences in for example cognition, or variants that underlie the predisposition for developing disease.

Despite an extensive search for the underlying genetic factors important for normal cognitive abilities, no single genes or genetic regions have so far been identified to be conclusively responsible for the normal variation in cognitive abilities in the healthy population [reviewed in 59,73]. This corresponds well with a highly polygenic underlying mechanism [74]. However, a limited number of genes has been implicated in cognitive abilities. These include reliable association between variants in the *Apolipoprotein E* gene and cognitive abilities and dementia in old age [76,77]. Furthermore, *Catechol-O-methyltransferase* and *Brain-derived neurotrophic factor* are two other examples of genes that have been linked to variation/differences in intelligence [78,79,reviewed in 80].

1.3 Schizophrenia and bipolar affective disorder

Patients suffering from serious psychiatric disorders, such as schizophrenia (SCZ) and bipolar affective disorder (BP), are often characterised by cognitive dysfunctions. Approximately 1% of the general population will be affected by these mental illnesses during their lifetime [reviewed in 81], which seriously influence the behaviour and function of the patients. The disorders affect each individual's life-outcome to a great extent, in addition to imposing large challenges for the society in general.

1.3.1 Clinical symptoms in schizophrenia and bipolar affective disorder

Patients suffering from schizophrenia are characterised by having a mixture of positive and negative symptoms, together with cognitive dysfunctions (e.g., memory, processing speed, verbal fluency, attention) and/or mood symptoms [as reviewed in 82]. The positive symptoms observed in SCZ patients include hallucinations, delusions and other reality distortions, while the negative symptoms range from blunted or inappropriate affect, loss of motivation, to social and emotional withdrawal [reviewed in 82].

Bipolar affective disorder (also called manic-depressive illness) is characterised by episodes of abnormal elevated or irritable mood (manic episode), which results in an impairment of social and/or occupational functioning, or psychosis. In addition, most patients suffering from BP also experience episodes of severe lowering of mood and activity level (depression) [reviewed in 83].

The onset of both SCZ and BP generally occurs during adolescence or early adulthood, and the diagnosis is based on the presence and duration of several of the aforementioned symptoms, as described in the 4th Edition of The Diagnostic and Statistical Manual of Mental Disorders (DMS-IV-TR) [84] or the International

Statistical Classification of Disease and Related Health Problems, 10th Revision (ICD-10) [85].

1.3.2 Morphological brain abnormalities in schizophrenia and bipolar affective disorder

Several morphological brain changes have been linked to SCZ and BP. In patients suffering from SCZ, such changes include reduction of grey matter and cortical thinning in areas of the neocortex, namely frontal-, temporal-, cingulate-, occipital-, parietal- and insular cortex, as compared to healthy matched controls (Figure 7) [86,87]. In BP, there are rather conflicting reports regarding abnormalities in grey matter and cortical thickness. Some studies have reported regional reduction of grey matter in certain areas (e.g. frontal and temporal regions), decrease in global cortical grey matter or thinning of prefrontal cortical areas, while others have shown regional increase, or no grey matter abnormalities in BP relative to control subjects [as reviewed in 88]. However, based on recent meta-analyses in BP, significant grey matter reduction seems to be present in the anterior cingulate and insular cortex [86,89]. In addition, a recent analysis of patients suffering from BP, revealed substantial neocortical thinning in frontal-, temporal- and temporo-parietal regions (Figure 7) [87]. Although morphological changes in the neocortex are apparently more extensive in SCZ patients compared to BP, a substantial overlap of areas displaying cortical abnormalities in both patient groups has been observed (e.g. frontal and temporal lobes, anterior cingulate and bilateral insular cortex) [86,87].

In addition to the morphological changes in areas of the neocortex, measures of global brain volume have revealed a small, but statistically significant reduction of the total brain volume in patients with schizophrenia [90]. In contrast, there are conflicting reports regarding the total brain volume in BP. No apparent reduction of the cerebral volume in patients with bipolar affective disorder has been reported [91,92], while others have shown a significant reduction of whole brain and prefrontal lobe volumes [93]. Moreover, patients suffering from schizophrenia or

bipolar affective disorder may display a substantial, and overlapping pattern of volume reduction in sub-cortical areas (e.g. hippocampus, thalamus, nucleus accumbens and brainstem) [86,87], and an enlargement of ventricular volume [87,92,93].

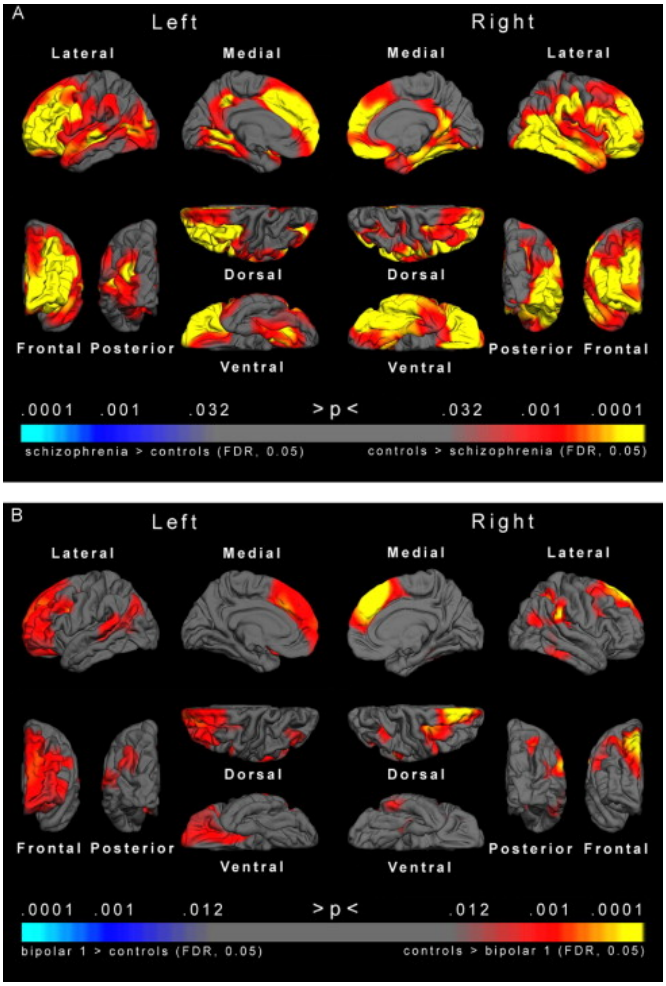

Figure 7: Abnormalities in cortical thickness in psychiatric disorders. The image shows difference in cortical thickness between individuals suffering from schizophrenia (**A**) or bipolar affective disorder (**B**), compared to healthy controls. The orientation of the brains is indicated. Red and yellow indicate areas of cortical thinning. The image is taken from [87].

A comparison between individuals with schizophrenia and bipolar affective disorder demonstrated that the ventricular enlargement was substantially greater in schizophrenia [93]. The overlap in neocortical and sub-cortical structures affected in both schizophrenia and bipolar affective disorder has been suggested to reflect a common underlying pathophysiology in the two illnesses [86,87]. Interestingly, the anterior and insular cortices are connected to the paralimbic structures, which are involved in functional systems linked to emotional regulation [86]. Overall, the morphological changes observed in schizophrenia are apparently more extensive, compared to bipolar affective disorder.

1.3.3 The genetics of schizophrenia and bipolar affective disorder

Schizophrenia and bipolar affective disorder are familial and highly heritable psychiatric disorders. Through family-, twin- and adoption studies, the heritability has been estimated to be as high as 0.7 (i.e. genetic factors explain up to 70% of the variance in risk of developing disease) [94-98]. The remaining risk is assigned to environmental factors (Figure 8) [99]. Family studies have suggested a shared genetic overlap in risk factors between bipolar affective disorder and schizophrenia (Figure 8). The disorders seem to co-aggregate in families, and an increased risk of developing either illness has been observed in individuals with affected first degree relatives [98,100].

Both schizophrenia and bipolar affective disorder are complex genetic disorders, where numerous common genetic variants of very small effect are thought to cause an increase in disease susceptibility [101]. Over the last decades, great efforts have been made to identify genetic factors causing predisposition to illness, mainly through linkage-, association- and, more recently, through genome-wide association studies. Still, the underlying genetic architecture of schizophrenia and bipolar affective disorder remains poorly understood, and so far only a limited number of common genetic variants has been implicated in disease susceptibility.

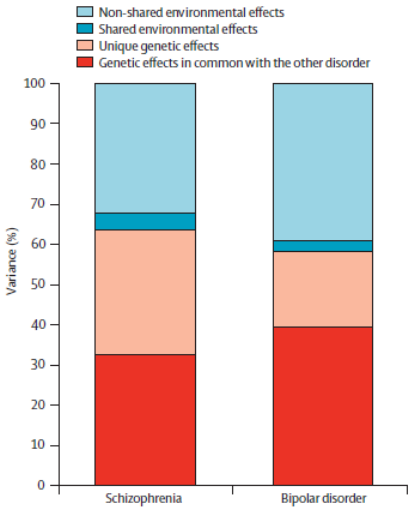

Figure 8: Variance explained by genetic and environmental effects for schizophrenia and bipolar affective disorder. Red colour indicates the variance accounted for by shared genetic effects (% of variance illustrated on the x-axis). Light red illustrates the unique genetic effects attributed to the two disorders. The dark blue and light blue colours indicate shared and non-shared environmental effects, respectively. The image is taken from [98].

These include variants in the major histocompatibility complex (MHC) region located on chromosome 6p, which was reported from three independent genome-wide association studies to display significant association to schizophrenia [101-103]. The most extensive analysis so far combined data from 17 separate schizophrenia GWASs in a recent mega-analysis conducted by the Psychiatric Genomics Consortium [104]. A similar mega-analysis was performed in bipolar affective disorder [105]. By analysing these large samples, 7 and 4 common variants were identified in SCZ and BP, respectively, reaching genome-wide significance for association (i.e. P value $< 5 \times 10^{-8}$) [104,105]. For schizophrenia, five of the seven identified loci had not previously been linked to disease susceptibility, while the remaining 2 loci had already been associated to disease. The results implicated variants linked to microRNA 137 (*MIR137*) and to the *CUB and Sushi multiple domains 1* (*CSMD1*) gene in increased disease susceptibility [104]. For bipolar affective disorder,

association was reported for variants in *ankyrin 3* (*ANK3*), *synaptic nuclear envelope protein 1* (*SYNE1*), *odd Oz/ten-m homolog 4* (*ODZ4*) and a region on chromosome 12 [105]. A combined analysis including both schizophrenia and bipolar affective disorder patients in a large meta-analysis, further strengthened the finding of genetic overlap between the two disorders (i.e. for the genes *CACNA1C*, *ANK3*, and the *ITIH3-ITIH4* region) [104,105].

In addition to common variants of small effect, some rare variants of relatively large effect have been linked to increased disease vulnerability. Such rare variants include copy number variations (CNV), in which large regions of the genome (ranging from ten thousand to five million basepairs) have either been inserted, duplicated or deleted. Patients suffering from schizophrenia have a greater genome-wide burden of such structural variants, compared to healthy matched controls, and also to individuals affected by BP [106-108]. Such CNVs, associated to disease susceptibility, have been identified on chromosomes 1q21.1, 15q13.3, 15q11.2, 16p11.2 and 22q11.2 [106,109,110]. Another example of a structural variance linked to SCZ, is the balanced translocation of the gene *Disrupted in schizophrenia 1* (*DISC1*) [111]. These structural variants point at the likelihood of schizophrenia resulting from a combination of common and rare variants, of small and large effect, respectively [107,112,113].

2. Aims of the study

The overall aim of this study was to identify and characterise differentially expressed genes in the rat neocortex, and subsequently explore their possible role as genetic risk factors in normal intellectual function and serious psychiatric disorders.

Specific aims:

1. Identify and characterise differentially expressed genes in three neocortical regions in the adult rat brain.

2. Search for association between selected regionally enriched cortical genes and cognitive abilities and risk for serious psychiatric disorders.

3. Characterise a hitherto unannotated gene that displays an almost exclusive expression in the parieto-temporal cortex of the adult rat brain.

3. List of publications

The thesis is based on the following papers, and will be referred to by their roman numerals in the text:

Paper I Stansberg C, Ersland KM, van der Valk P, Steen VM. Gene expression in the rat brain: High similarity but unique differences between frontomedial-, temporal- and occipital cortex. BMC Neuroscience 2011 Jan 26; 12:15.

Paper II Ersland KM, Christoforou A, Stansberg C, Espeseth T, Mattheisen M, Mattingsdal M, Hardarson GA, Hansen T, Fernandes CPD, Giddaluru S, Breuer R, Strohmaier J, Djurovic S, Nöthen MN, Rietschel M, Lundervold AJ, Werge T, Cichon S, Andreassen OA, Reinvang I, Steen VM, Le Hellard S. Gene-based analysis of regionally enriched cortical genes in GWAS data sets of cognitive traits and psychiatric disorders. PLoS One. 2012;7(2):e31687. Epub 2012 Feb 22.

Paper III Ersland KM, Håvik B, Rinholm JE, Gundersen V, Stansberg C, Steen VM. *LOC689986*, a unique gene showing specific expression in restricted areas of the rodent neocortex. Manuscript for submission to Cerebral Cortex.

4. Summary of results

Paper I

The mammalian neocortex is divided into anatomically and functionally distinct areas. However, the global gene expression in different regions of the neocortex is strikingly similar. By microarray-based global gene expression profiling of samples from the adult rat brain (i.e. frontomedial-, temporal- and occipital cortex (FMCx, TCx and OCx, respectively), striatum, hippocampus and cerebellum), as well as three non-CNS samples (i.e. kidney, liver and spleen), we were able to identify distinct sets of genes that displayed marked regional enrichment in the three different cortical areas (30, 24 and 11 genes enriched in the FMCx, TCx or OCx, respectively). A majority of these genes was over-represented in certain biological processes, such as signal transduction and developmental processes. Moreover, several of the genes were over-represented in molecular functional categories linking them to receptor-, signalling molecule- and ion channel function. Finally, a majority of the differentially expressed genes displayed enriched expression in neurons, and a laminar pattern of expression was observed for a substantial portion of the genes. Based on the assumption that regionally enriched genes might reflect functional specialisation of a given tissue or organ, we hypothesised that these genes could be important in order to sustain the normal function of their respective neocortical areas.

Paper II

People differ in cognitive performance, but despite its estimated high heritability, the underlying genetic architecture leading to such differences remains poorly understood. Different areas of the neocortex have been shown to play important roles in normal cognitive functioning and impairments. Moreover, it has been suggested

that genes preferentially or specifically expressed in one region or organ might reflect functional specialisation. We previously identified a novel set of genes, displaying differential expression in three cortical regions from the adult rat brain (FMCx, TCx and OCx) (Paper I). By employing a gene-based approach to the analysis, we used these candidate genes to mine a genome-wide association study of the Norwegian Cognitive NeuroGenetics sample for association to nine psychometric tests. We also explored available GWAS data for the serious psychiatric disorders schizophrenia and bipolar affective disorder, to which cognitive impairment is linked. At the single gene level, we found that the TCx enriched gene *RAR-related orphan receptor B* (*RORB*) showed the overall strongest association in the analysis, to a test of verbal intelligence (P= 7.7E-04). We next applied a gene set enrichment analysis (GSEA) approach to search for enrichment of association signal in the Norwegian Cognitive NeuroGenetics GWAS and in three GWASs of bipolar affective disorder and of schizophrenia. The set of genes differentially expressed in the TCx showed a significant enrichment of association signal to a test measure of non-verbal intelligence. In contrast, no reliable enrichment of association signal was observed for the two psychiatric disorders. The gene-based approach suggested a role for *RORB* in verbal intelligence differences, while the genes showing an enriched expression in the TCx could be important to intellectual functions as measured by a test of non-verbal intelligence in the healthy population.

Paper III

The mammalian neocortex is a highly complex structure which is involved in a wide range of tasks, such as processing and interpretation of sensory information and control of motor functions. We previously analysed the global gene expression in three different cortical regions from the adult rat brain (FMCx, TCx and OCx), and identified distinct sets of genes showing regional enrichment (Paper I). One of the genes, the hitherto unannotated *hypothetical protein LOC689986* (*LOC689986*) gene, displayed an almost exclusive expression in samples from the TCx, and only weak, or

no expression in other CNS and non-CNS samples, respectively. Highly or specifically expressed genes within a certain region or organ have been suggested to reflect the functional specialisation of the given region. We therefore aimed at characterising this unannotated gene in detail, in order to gain insight into potential functional roles. Blast searches and phylogenetic analysis revealed that the gene was conserved in, and apparently specific for, the vertebrate lineage. A detailed RT-PCR- and *in situ* RNA hybridisation based analysis of the *LOC689986* gene expression pattern in rat, revealed a highly restricted gene expression in areas of the parieto-temporal cortex, and furthermore, a specific expression in layer 4 of the somatosensory cortex (SCx). By analysing the human orthologous gene (*Chromosome 1 open reading frame 146* (*C1orf146*)) expression in 8 different human brain tissues, we found the highest gene expression level in samples from the frontal pole, in addition to expression in samples from the medulla, hippocampus and cerebellum. Surprisingly, we detected an exclusive *C1orf146* gene expression in samples from testis, when microarray data from 32 different human tissues were explored. By immunoblot analysis of samples from the adult rat, we found that *LOC689986* was translated *in vivo*. The protein seemed to be expressed in samples from the temporal and cingulate cortices, in addition to the hippocampus and cerebellum. Furthermore, we established that the encoded mouse orthologous protein (1700028K03Rik) was present in the SCx, with a rather abrupt border towards the neighbouring motor cortex. In addition, we detected protein expression in the large Purkinje cells of the cerebellar cortex. At the electron microscopic level, we observed protein expression in stem dendrites of neurons, and also in astrocyte cells from the somatosensory cortex. Furthermore, the localisation of both endogenous and recombinant LOC689986 protein was confined to the nucleus and cytosol. Finally, analysis of potential interaction partners indicated a role for this protein in control of gene expression, and also suggested a role in the translational machinery. Our results could indicate a specialised role for LOC689986 in the processing of somatosensory information, and the protein might be involved in normal cerebellar function. Further functional analysis is needed to determine the biological function of LOC689986 in the brain and during CNS development.

5. General discussion

Morphological, functional and volumetric studies of the neocortex have clearly linked this important brain structure to intellectual functions, as well as to psychiatric disorders in which cognitive decline is a prominent symptom. Based on the importance of the neocortex in both health and disease, we have analysed different cortical regions in order to identify potential regionally enriched gene expression, and used such genes as candidates to mine data from large genome-wide association studies on cognition and psychiatric disorders.

5.1 Differentially expressed genes in the neocortex

5.1.1 Regionally enriched cortical genes in the rat brain

The global gene expression has been demonstrated to be highly similar across different areas of the adult mammalian neocortex (Paper I and [42,44,45]). However, specific functions are attributed to distinct cortical areas, suggesting the existence of patterned gene expression in order to support the functional divergence. In paper I, we identified 65 genes that displayed regionally enriched expression in the adult rat frontomedial-, temporal- or occipital cortices (30, 22 and 11 genes, respectively). These genes were identified by using two independent microarray platforms, in addition to manual re-inspection of gene expression profiles, and verification of the gene expression by real-time PCR (RT-PCR) analysis on a sub-set of the genes. The RT-PCR analyses were performed by using sequence specific probes (i.e. TaqMan® probes) which provide significantly less background and reduces the risk of detecting false positives, as compared to other and less expensive reporter molecules, such as DNA binding dyes (e.g. SYBR®Green). In agreement with our findings, some of the 65 genes had previously been shown to display a patterned expression in both developing and adult neocortex (e.g., *Latexin*, *RAR-related orphan receptor B*,

Nuclear receptor subfamily 2 group F member 1, *LIM domain only 4*, *Odd Oz/ten-m homolog 3 (Drosophila)*) [114-118]. It is important to note that the genes were found to display an enriched expression in the given cortical area. This notion should not be mistaken for an exclusive expression, since most of the genes were also found to be expressed in other areas of the brain, outside of the neocortex (i.e. samples from the cerebellum, hippocampus or striatum). In addition, some of the genes that were enriched in a certain region of the neocortex also displayed expression in the other examined neocortical areas, though to a lesser extent. This was observed for some of the genes enriched in the frontomedial- and occipital cortex. The co-expression of genes in these two regions could possibly reflect a graded expression in the anterior-posterior (or posterior-anterior) direction, rather than an exclusive region specific expression. In contrast, the strongest region specific gene expression was observed for the temporal cortex enriched genes. However, it is possible that the enrichment of genes in this region reflects a graded expression in the lateral part of the neocortex, rather than being restricted to the temporal cortex. Since neither regions from the medial, nor more regions from the lateral part of the neocortex were examined, the existence of such graded expression of the genes has to be explored in future studies.

5.1.2 Functional roles of regionally enriched cortex genes

The regionally enriched cortical genes identified from rat, and their human orthologs, were functionally annotated using the Panther classification system (Paper I and II, respectively). As expected, the regionally enriched genes displayed similar over-representations of both biological processes and molecular functions in rat and human, including signal transduction, developmental processes and receptor activity. In support of our findings, genes involved in signal transduction have previously been reported to account for the main group of genes differing between various cortical regions in the adult human and primate brain [44]. In addition, strikingly many of the enriched genes had already been annotated, which could reflect their overall biological importance (Paper I and II). The significant over-representation of the genes in certain biological and molecular processes suggests that the regionally

enriched genes could be important in order to sustain and maintain specialised functions within the neocortex. By re-analysis of a data set, examining the differential gene expression in neurons, astrocytes and oligodendrocytes [119], we observed that a substantial portion of the regionally enriched genes were expressed in neurons. Furthermore, by searches in the Allen Mouse Brain Atlas [46], we observed that approximately two thirds of the genes displayed a laminar pattern of expression (e.g. *FXYD domain-containing ion transport regulator 6* and *LIM domain only 4*) (Paper I). Interestingly, genes displaying a layer-specific pattern of expression were recently demonstrated to be more likely to encode proteins involved in processes such as synaptic transmission or ion transport. Genes displaying an evenly distributed expression pattern across cortical layers, on the other hand, were more likely to encode cellular "housekeeping" proteins [120]. The cell and/or layer specific gene expression for a majority of the regionally enriched genes could therefore further suggest functional specificity conferred by certain neuronal subtypes or layers in a given neocortical region.

5.1.3 Neocortical organisation: rat versus human

The neocortices of various mammalian species differ substantially with regard to surface area, connectivity and cortical fields. Although some similarities in the broad neocortical organisation and gene expression patterns are observed between mammalian species, there are cortical areas that are not well conserved in all mammals. The substantial surface area expansion of the human neocortex, compared to non-human primates and rodents, is thought to account for the additional functional domains found in humans [reviewed in 40]. The enlarged human prefrontal cortex is especially important for the cognitive abilities which set us apart from non-human primates and other mammalian species [reviewed in 40]. In addition, no homologous regions for the areas of the human brain involved in language processing and production (i.e. Broca's and Wernicke's area) have been observed in other mammalian species. However, some conserved features of neocortical organisation are observed in major groups of mammals, including areas

within the OCx (i.e. primary and secondary visual areas), somatosensory cortex and TCx (primary auditory area) [reviewed in 121,122]. Genetic factors, that specify regional identity, are thought to cause the broad similarities in cortical field organisation [reviewed in 122]. In fact, it was recently demonstrated that the genetically influenced neocortical regionalisation in the human brain shares similarities with the regionalisation in rodents [123]. Also, a comparison of the gene expression in the adult mouse anterior cortex, striatum and cerebellum, and the anatomically and functionally homologous human brain regions, revealed a very similar pattern of expression [52]. We were not able to determine any differential expression of the human orthologs to the regionally enriched rat genes in selected and corresponding areas of the human neocortex by RT-PCR (Paper I). However, gene expression searches in the Allen Brain atlas clearly demonstrated that at least a sub-set of the genes was expressed in corresponding neocortical areas (i.e. frontal-, temporal- or occipital lobes) in the human brain (Paper II).

Given the obvious medical, ethical and practical limitations in using samples from the human brain to study different aspects of neurobiology, rodents have become a well established model system. We demonstrated that the inter-individual variation in cortical gene expression is very low in rodents (Paper I and [42]). In contrast, the global gene expression in different cortical regions has been shown to vary more between human individuals, than among such regions within one individual [44]. Furthermore, the inter-individual variation is apparently larger among humans than chimpanzees [44]. Based on these observations, rats could therefore serve as a useful animal model for the identification of regionally enriched genes in the adult neocortex.

5.2 The identification of genetic factors underlying complex traits

5.2.1 The search for susceptibility genes in serious psychiatric disorders

Although both schizophrenia and bipolar affective disorder are known to be highly heritable mental illnesses [94-98], our understanding of the underlying genetic susceptibility factors remains poor. Genome-wide association studies are currently the most common method to examine large numbers of single nucleotide polymorphisms in thousands of affected individuals in comparison to matched healthy controls. The underlying assumption for GWASs is the "common disease-common variant" hypothesis. According to this theory, common diseases can be explained by allelic variants that are present in more than 1-5% of the population [reviewed in 124]. Several hundreds, or even thousands of genes, each of a very small effect size, are thought to be involved in the susceptibility of complex common disorders. Only a decade ago it was generally believed that the GWAS approach would resolve the genetic background of complex diseases, such as schizophrenia and bipolar affective disorder. However, so far, no more than a handful of genes (genetic loci) has been linked to these serious psychiatric disorders, accounting for approximately 23% of the observed heritability [125]. With an estimated heritability of up to 70%, much of the underlying genetic architecture of schizophrenia and bipolar affective disorder remains "missing" or "hidden". This could at least in part be explained by rare genetic variants of larger effect size, which would be difficult to detect by current GWAS genotyping [reviewed in 126]. Such rare variants include CNVs (i.e. micro-duplications or micro-deletions), which have been found to increase susceptibility to SCZ [106,109,110].

5.2.2 Convergent functional genomics approach in psychiatric genetics

In this work we have used a functional genomics approach in order to search for genetic association between a set of candidate genes and certain complex traits. The term "convergent functional genomics" refers to the integration of multiple lines of evidence, in order to prioritise and integrate candidate genes for complex disorders [127]. In paper I, we examined areas in the adult rat neocortex, which corresponded to regions in the human neocortex that had previously been linked to cognitive functioning and psychiatric disorders. In paper II, we integrated these gene expression data from the animal model, and mined the differentially expressed genes in GWASs from a large sample of healthy adult individuals characterised by nine psychometric tests of cognitive functioning (i.e. test measures of intellectual functioning, memory, executive attention and attention). Analysis of these biologically relevant candidate genes, at the single gene level, demonstrated significant association between several of them and the test measures of cognitive abilities (Paper II). The temporal cortex enriched gene *RORB* showed the overall strongest association in the analysis, namely to a test measure of verbal intelligence. *RORB* has been shown to be involved in circadian rhythms and has also been suggested to play a role in processing of sensory information [128]. Although this gene had not previously been linked to cognitive functioning, it is worth noting that the gene was recently ranked as a top candidate for susceptibility to bipolar affective disorder [129,130], in which disturbance of the circadian clock has been reported [reviewed in 131]. We also observed marked association to the test measure of verbal intelligence for the *Huntingtin-associated protein 1* gene, in addition to nominal association to the estimated full-scale intelligence. Even though no association between this gene and cognitive functioning had previously been reported, it is noteworthy that dysfunction of this gene had been linked to the neuropathology in Huntington disease, a disease which is often characterised by marked cognitive decline and psychiatric symptoms [reviewed in 132]. Three of the candidate genes (i.e. *Complement component 1 q subcomponent-like 3*, *Hypocretin (orexin) receptor 1* (*HCRTR1*) and *Calcium binding protein 1*) displayed association to all test measures

of attention. A link to attentional processing had previously only been reported for Hcrtr1, which was shown to activate the basal forebrain cholinergic system in rats [reviewed in 133]. Interestingly, an association between *HCRTR1* and major mood disorder was also recently reported [134]. Although several of the candidate genes showed association to cognitive functioning, none of them met the conservative experiment-wide threshold of significance ($P = 0.00014$). This threshold corrects for the number of traits and genes tested, which may be too conservative, given the *a priori* hypothesis conferred by these candidate genes.

As an alternative approach to search for association between single genes and cognitive abilities, we also analysed the cortical candidate genes as gene sets, using gene set enrichment analysis (Paper II). GSEA is a powerful analytical method, originally developed for the interpretation of gene expression data [135], that has recently been implemented successfully in the analysis of GWAS data [136,137]. The gene sets are defined on the basis of prior biological knowledge, and the GSEA method is used to determine whether or not the members of a gene set tend to cluster towards the top (or bottom) of a pre-ranked list of all the genes. We found that the genes differentially expressed in the temporal cortex, as a set, showed significant enrichment of association signal in a test measure of non-verbal intelligence. According to the parieto-frontal integration theory, differences in cognitive performance, including a test measure of non-verbal intelligence, have been suggested to involve a network of areas in the dorsolateral prefrontal-, parietal-, anterior cingulate-, temporal- and occipital cortices [66]. It is possible that the temporal cortex genes play a role in this network, but the functional importance of the genes in cognitive abilities remains to be explored. We also found that the set of genes showing differential expression in the occipital cortex, displayed enrichment of association signal to one of the test measures of attention.

Cognitive abilities are often affected in patients suffering from schizophrenia and bipolar affective disorder, and can be regarded as an endophenotype for these psychiatric illnesses [138]. The concept of endophenotypes (also known as

intermediate biological phenotypes) was introduced 40 years ago, and describes an internal phenotype or trait that is associated with the expression of the illness [139,140]. The study of endophenotypes has recently gained a lot of attention in the search for genetic susceptibility factors in psychiatric disorders. In paper II we therefore also analysed the differentially expressed cortical genes, as gene sets, for enrichment of association signal in several large GWASs of schizophrenia and bipolar affective disorder. We observed an enrichment of association signal for the OCx gene set in one of the schizophrenia GWASs. However, no association was observed in the other schizophrenia GWAS data sets, which makes it hard to conclude on the validity of this finding.

The reliability of our findings from the GSEA, in both cognition and psychiatric disorders, was addressed by three different approaches, namely by analysis of the cortical gene sets in GWASs of six non-psychiatric phenotypes [141], by exploring a gene set comprised of "housekeeping" genes across all phenotypes [142] and finally, by analysis of randomly generated gene sets mimicking the significant gene sets. No significant association was observed by these three approaches, which strongly supports the validity of our findings. However, the observed enrichment of association signal for the OCx gene set could be false positive findings, since the risk of generating a false positive result increases for small gene sets ($n < 10$ genes).

5.3 Characterisation of the *hypothetical protein LOC689986* gene

One of the 65 regionally enriched genes identified in paper I, the *hypothetical protein LOC689986* gene, was demonstrated to display the overall strongest patterned gene expression in the analysis. This unannotated gene showed an almost exclusive expression in samples from the adult rat temporal cortex, with only weak expression being detected in the other examined CNS samples (i.e. FMCx, OCx, striatum, cerebellum and hippocampus), and no apparent expression in the non-CNS samples (i.e. liver, kidney and spleen) (Paper I and [42]). Interestingly, the control of highly area- and/or layer specific gene expression seems to be linked to the mechanisms underlying normal function of cortical regions in primates [47]. Based on the observed *LOC689986* gene expression in a restricted neocortical region, we speculated that this gene could be important for the normal function in the temporal cortex and related areas of the rodent brain.

In paper III we reported that *LOC689986* is highly conserved in vertebrate species, and that no orthologous gene is present in yeast or in invertebrates. This observation renders it likely that the gene is specific for the vertebrate lineage. Moreover, the high degree of sequence conservation, and the observation that the gene was located in a large syntenic block suggests an important role for this gene in supporting functional specialisation in vertebrates. In general, the structure and function of the vertebrate CNS is by far more complex than the invertebrate nervous system. It is possible that the gene could be involved in vertebrate specific functions related to the increased complexity of the nervous system.

Detailed analysis of the *LOC689986* gene expression in the adult rat parietal-, temporal- and occipital cortex, revealed a restricted and specific pattern of expression in the parieto-temporal cortex, with the overall strongest expression in the primary somatosensory area (Paper III). In addition, high levels of expression were also observed in the secondary somatosensory cortex. By *in situ* RNA hybridisation, we

confirmed the region specific expression, and revealed that the gene expression seemed to be confined to cortical layer IV in rats. Moreover, the distribution of the mouse ortholog (1700028K03Rik) of the LOC689986 protein was analysed by immunohistochemistry at three different postnatal stages. The mouse ortholog was detected in the somatosensory cortex, with a rather abrupt border towards the neighbouring motor cortex (at postnatal stage 5), clearly demonstrating the regional specificity of the protein expression. The somatosensory cortex in rodents is characterised by distinct barrel fields, which are located in cortical layer IV. This area receives tactile sensory information originating from the whiskers, and each of the whiskers corresponds topographically to a distinct area of the barrel cortex [143]. In a recent global gene expression profiling of the primary somatosensory cortex in rat, *LOC689986* was found to be one of several genes that were up-regulated in response to an enriched environment [144]. Interestingly, functional characterisation of these up-regulated genes linked them to various functional categories, including synaptic plasticity, gene expression and regulation of metabolic processes [144]. The up-regulation of *LOC689986* in response to experience-dependent plasticity indicated that LOC689986 could be important in the regulation of somatosensory information [144]. In addition to the strong *LOC689986* gene expression in the somatosensory cortex, we also observed expression in cortical regions corresponding to the primary and secondary auditory cortex by the detailed RT-PCR based gene expression analysis. These cortical areas receive and process auditory signal, and the observed gene expression in areas of the temporal cortex corresponded well with the original data from the microarray analysis.

Based on transcriptome data from 32 different human tissues, we observed an almost exclusive expression of the human orthologous gene (*C1orf146*) in samples from testis. No apparent expression was detected in samples from the fetal and adult human brain. However, these samples were comprised of whole brain, and given that the human orthologous gene is expressed in similar restricted pattern as observed in rat, it is possible that the number of transcripts was too low to be detected. Indeed, we

observed an enriched expression of the human gene in samples from the human frontal pole in a second gene expression analysis.

While gene expression analyses via mRNA detection is an important method for the examination of regional and spatial expression of a gene, the pattern and expression level of mRNA and protein may differ. In order to characterise the predicted protein encoded by the *LOC689986* gene, it was therefore also important to analyse the distribution of protein expression. We used a custom made peptide-antibody that recognised an epitope in the C-terminal end of the protein (Paper III). Immunoblot analysis of transiently transfected cells over-expressing the recombinant protein confirmed the specificity of the antibody. In addition, we detected a robust protein band in samples from the temporal cortex by immunoblot analysis of various tissue lysates from the adult rat, using the custom made peptide-antibody. In contrast, only weak expression was detected in samples from the frontomedial- and occipital cortices. This finding suggested a similar patterned protein expression, as was observed at the gene expression level, and further indicated a specific binding of the peptide-antibody. However, the detected protein band from the immunoblot analysis of tissue lysates was approximately 4kDa larger than the predicted size of LOC689986. It is possible that the observed difference in size is due to post-translational modifications, or it could reflect a different endogenous splice variant. Surprisingly, we also detected a protein band in tissue samples from the cingulate cortex, hippocampus and cerebellum. Although these data did not correspond well with the observed gene expression, it is possible that the gene is in fact expressed in these areas, however, at a level that was too low for our detection limit. Indeed, by immunocytochemistry analysis we found that LOC689986 was present in the Purkinje cells of the cerebellar cortex in mice. In the initial microarray analysis, we observed very low *LOC689986* gene expression in whole cerebellar tissue samples from rats. Given that the protein is expressed in a similar cell type specific pattern in the rat cerebellum, it is possible that the low level of detected gene expression reflects the heterogeneous population of cell types analysed. The transcript would in that case be rather diluted, which could explain the failure in detecting higher levels of gene

expression in the initial analysis. The Purkinje cells are among the largest neurons in the brain, and are linked to motor function, learning and cognitive abilities. In the human brain, a reduction of these cells accompanied by morphological changes in the axons, has been implicated in the neurological disorder essential tremor [reviewed in 145]. The expression of LOC689986 in the Purkinje cells is intriguing, and further investigation will be required to gain insight into the functional role of the protein within these cells.

At the sub-cellular level, the LOC689986 protein was found to localise to both the nucleus and cytosol. Moreover, the endogenous LOC689986 protein clearly localised to dendrites, which could suggest a role for this protein in signalling pathways activated in response to an electrical or chemical synapse.

In order to gain insight into potential functional roles of LOC689986, we also performed yeast-2-hybrid (Y2H) screens in adult and embryonic mouse brain libraries. Among the, in total, seven identified interaction partners of LOC689986, two components of the Mi-2/NuRD complex (Chd3 and Chd4) [146-149] were detected. This complex has been shown to bind acetylated histone tails, which induces transcriptional repression by remodelling of the chromatin structure. Interestingly, the complex displays subunit heterogeneity, varying with cell type and physiologic signals within a tissue. The functional specialisation of the Mi-2/NuRD complex has been suggested to be influenced by the subunit composition [reviewed in 150]. Given that the interaction between LOC689986 and components of the Mi-2/NuRD complex can be verified, it is possible that LOC689986 could be a so far unknown subunit, leading to functional specialisation in distinct areas of the brain (e.g. somatosensory cortex). The mechanisms linking LOC689986 to experience dependent plasticity in rodents [144], might involve chromatin remodelling and transcriptional regulation in response to sensory information.

6. Conclusions

The following conclusions can be drawn from this work:

- A unique set of genes display differential gene expression in the rat frontomedial-, temporal- and occipital cortices (Paper I).

- Several of the regionally enriched cortical genes displayed association to intellectual function (e.g. *RORB* associated to a test measure of verbal intelligence) (Paper II).

- Differentially expressed genes in the temporal cortex could be involved in intellectual functions, as measured by a test of non-verbal intelligence in the healthy adult population (Paper II).

- The regionally enriched neocortical genes might not be linked to disease susceptibility for major psychiatric disorders, at the gene set level (Paper II).

- LOC689986 is a hitherto unannotated but highly conserved vertebrate-specific gene, displaying distinct expression patterns in the rodent brain (Paper III).

7. Future perspectives

In order to further examine the potential role of the differentially expressed temporal cortex genes in cognitive functioning, replication studies in large independent GWAS samples are warranted. A requirement for this replication study will be that the individuals included in the GWAS are characterised by similar psychometric tests, as the ones used in this study.

Further functional characterisation of LOC689986 is required to establish the biological relevance of this gene. First of all, by resolving the LOC689986 protein structure, major structural similarities (folds) might be identified, and possibly give hints to shared functional roles with proteins of known function. Secondly, the validation of identified potential protein interaction partners is of outmost importance, in order to determine the functional role of LOC689986. Further work should focus on exploring the predicted interactions in tissue lysates from parieto-temporal cortex and cerebellum, using immunoprecipitation analysis. Should the interactions be verified, co-localisation analysis of LOC689986 with its potential interaction partners in neuronal cell cultures, could give further indications as to functional roles. Third, cell specific localisation of the expressed protein should be established. The results from the immunogold cytochemistry analysis revealed protein expression in neuronal dendrites and astrocyte cells. In order to explore the protein expression in more detail, analysis of the endogenous and recombinant protein expression in primary neuronal- and astrocyte cell cultures will be required. Finally, generation of “knock-out” or “knock-down” models would be required to obtain information about the possible role of the gene during development.

8. References

1. Swanson LW (1995) Mapping the human brain: past, present, and future. Trends in neurosciences 18: 471-474.
2. Pakkenberg B, Gundersen HJ (1997) Neocortical neuron number in humans: effect of sex and age. The Journal of comparative neurology 384: 312-320.
3. Molyneaux BJ, Arlotta P, Menezes JR, Macklis JD (2007) Neuronal subtype specification in the cerebral cortex. Nature Reviews Neuroscience 8: 427-437.
4. Douglas RJ, Martin KA (2004) Neuronal circuits of the neocortex. Annual review of neuroscience 27: 419-451.
5. Schuenke M, Schulte E, Schumacher U (2007) Head and Neuroanatomy (Thieme Atlas of Anatomy).
6. Mountcastle VB (1997) The columnar organization of the neocortex. Brain : a journal of neurology 120 (Pt 4): 701-722.
7. Shipp S (2007) Structure and function of the cerebral cortex. Current biology : CB 17: R443-449.
8. Lui JH, Hansen DV, Kriegstein AR (2011) Development and evolution of the human neocortex. Cell 146: 18-36.
9. Hartfuss E, Galli R, Heins N, Gotz M (2001) Characterization of CNS precursor subtypes and radial glia. Developmental biology 229: 15-30.
10. Noctor SC, Flint AC, Weissman TA, Wong WS, Clinton BK, et al. (2002) Dividing precursor cells of the embryonic cortical ventricular zone have morphological and molecular characteristics of radial glia. The Journal of neuroscience : the official journal of the Society for Neuroscience 22: 3161-3173.
11. Rakic P (2003) Developmental and evolutionary adaptations of cortical radial glia. Cerebral cortex 13: 541-549.
12. Malatesta P, Hartfuss E, Gotz M (2000) Isolation of radial glial cells by fluorescent-activated cell sorting reveals a neuronal lineage. Development 127: 5253-5263.
13. Miyata T, Kawaguchi A, Okano H, Ogawa M (2001) Asymmetric inheritance of radial glial fibers by cortical neurons. Neuron 31: 727-741.
14. Noctor SC, Flint AC, Weissman TA, Dammerman RS, Kriegstein AR (2001) Neurons derived from radial glial cells establish radial units in neocortex. Nature 409: 714-720.
15. Rakic P (1988) Specification of cerebral cortical areas. Science 241: 170-176.
16. Leone DP, Srinivasan K, Chen B, Alcamo E, McConnell SK (2008) The determination of projection neuron identity in the developing cerebral cortex. Current opinion in neurobiology 18: 28-35.
17. Gaspard N, Vanderhaeghen P (2011) Laminar fate specification in the cerebral cortex. F1000 biology reports 3: 6.
18. Noctor SC, Martinez-Cerdeno V, Ivic L, Kriegstein AR (2004) Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. Nature neuroscience 7: 136-144.
19. Noctor SC, Martinez-Cerdeno V, Kriegstein AR (2008) Distinct behaviors of neural stem and progenitor cells underlie cortical neurogenesis. The Journal of comparative neurology 508: 28-44.
20. Haubensak W, Attardo A, Denk W, Huttner WB (2004) Neurons arise in the basal neuroepithelium of the early mammalian telencephalon: a major site of neurogenesis. Proceedings of the National Academy of Sciences of the United States of America 101: 3196-3201.
21. Miyata T, Kawaguchi A, Saito K, Kawano M, Muto T, et al. (2004) Asymmetric production of surface-dividing and non-surface-dividing cortical progenitor cells. Development 131: 3133-3145.

22. Kowalczyk T, Pontious A, Englund C, Daza RA, Bedogni F, et al. (2009) Intermediate neuronal progenitors (basal progenitors) produce pyramidal-projection neurons for all layers of cerebral cortex. Cerebral cortex 19: 2439-2450.
23. Hansen DV, Lui JH, Parker PR, Kriegstein AR (2010) Neurogenic radial glia in the outer subventricular zone of human neocortex. Nature 464: 554-561.
24. Fietz SA, Kelava I, Vogt J, Wilsch-Brauninger M, Stenzel D, et al. (2010) OSVZ progenitors of human and ferret neocortex are epithelial-like and expand by integrin signaling. Nature neuroscience 13: 690-699.
25. O'Leary DD, Sahara S (2008) Genetic regulation of arealization of the neocortex. Current opinion in neurobiology 18: 90-100.
26. O'Leary DD, Chou SJ, Sahara S (2007) Area patterning of the mammalian cortex. Neuron 56: 252-269.
27. Bishop KM, Goudreau G, O'Leary DD (2000) Regulation of area identity in the mammalian neocortex by Emx2 and Pax6. Science 288: 344-349.
28. Mallamaci A, Muzio L, Chan CH, Parnavelas J, Boncinelli E (2000) Area identity shifts in the early cerebral cortex of Emx2-/- mutant mice. Nature neuroscience 3: 679-686.
29. Hamasaki T, Leingartner A, Ringstedt T, O'Leary DD (2004) EMX2 regulates sizes and positioning of the primary sensory and motor areas in neocortex by direct specification of cortical progenitors. Neuron 43: 359-372.
30. Leingartner A, Thuret S, Kroll TT, Chou SJ, Leasure JL, et al. (2007) Cortical area size dictates performance at modality-specific behaviors. Proceedings of the National Academy of Sciences of the United States of America 104: 4153-4158.
31. Armentano M, Chou SJ, Tomassy GS, Leingartner A, O'Leary DD, et al. (2007) COUP-TFI regulates the balance of cortical patterning between frontal/motor and sensory areas. Nature neuroscience 10: 1277-1286.
32. Zhou C, Qiu Y, Pereira FA, Crair MC, Tsai SY, et al. (1999) The nuclear orphan receptor COUP-TFI is required for differentiation of subplate neurons and guidance of thalamocortical axons. Neuron 24: 847-859.
33. Sahara S, Kawakami Y, Izpisua Belmonte JC, O'Leary DD (2007) Sp8 exhibits reciprocal induction with Fgf8 but has an opposing effect on anterior-posterior cortical area patterning. Neural development 2: 10.
34. Zembrzycki A, Griesel G, Stoykova A, Mansouri A (2007) Genetic interplay between the transcription factors Sp8 and Emx2 in the patterning of the forebrain. Neural development 2: 8.
35. Simeone A, Gulisano M, Acampora D, Stornaiuolo A, Rambaldi M, et al. (1992) Two vertebrate homeobox genes related to the Drosophila empty spiracles gene are expressed in the embryonic cerebral cortex. The EMBO journal 11: 2541-2550.
36. Liu Q, Dwyer ND, O'Leary DD (2000) Differential expression of COUP-TFI, CHL1, and two novel genes in developing neocortex identified by differential display PCR. The Journal of neuroscience : the official journal of the Society for Neuroscience 20: 7682-7690.
37. Bishop KM, Rubenstein JL, O'Leary DD (2002) Distinct actions of Emx1, Emx2, and Pax6 in regulating the specification of areas in the developing neocortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 22: 7627-7638.
38. Muzio L, DiBenedetto B, Stoykova A, Boncinelli E, Gruss P, et al. (2002) Emx2 and Pax6 control regionalization of the pre-neuronogenic cortical primordium. Cerebral cortex 12: 129-139.
39. O'Leary DD (1989) Do cortical areas emerge from a protocortex? Trends in neurosciences 12: 400-406.
40. Rakic P (2009) Evolution of the neocortex: a perspective from developmental biology. Nature Reviews Neuroscience 10: 724-735.
41. Job C, Tan SS (2003) Constructing the mammalian neocortex: the role of intrinsic factors. Developmental biology 257: 221-232.

42. Stansberg C, Vik-Mo AO, Holdhus R, Breilid H, Srebro B, et al. (2007) Gene expression profiles in rat brain disclose CNS signature genes and regional patterns of functional specialisation. BMC genomics 8: 94.
43. D'Souza CA, Chopra V, Varhol R, Xie YY, Bohacec S, et al. (2008) Identification of a set of genes showing regionally enriched expression in the mouse brain. BMC neuroscience 9: 66.
44. Khaitovich P, Muetzel B, She X, Lachmann M, Hellmann I, et al. (2004) Regional patterns of gene expression in human and chimpanzee brains. Genome research 14: 1462-1473.
45. Roth RB, Hevezi P, Lee J, Willhite D, Lechner SM, et al. (2006) Gene expression analyses reveal molecular relationships among 20 regions of the human CNS. Neurogenetics 7: 67-80.
46. Lein ES, Hawrylycz MJ, Ao N, Ayres M, Bensinger A, et al. (2007) Genome-wide atlas of gene expression in the adult mouse brain. Nature 445: 168-176.
47. Watakabe A, Komatsu Y, Nawa H, Yamamori T (2006) Gene expression profiling of primate neocortex: molecular neuroanatomy of cortical areas. Genes, brain, and behavior 5 Suppl 1: 38-43.
48. Watakabe A, Fujita H, Hayashi M, Yamamori T (2001) Growth/differentiation factor 7 is preferentially expressed in the primary motor area of the monkey neocortex. Journal of neurochemistry 76: 1455-1464.
49. Tochitani S, Liang F, Watakabe A, Hashikawa T, Yamamori T (2001) The occ1 gene is preferentially expressed in the primary visual cortex in an activity-dependent manner: a pattern of gene expression related to the cytoarchitectonic area in adult macaque neocortex. The European journal of neuroscience 13: 297-307.
50. Komatsu Y, Watakabe A, Hashikawa T, Tochitani S, Yamamori T (2005) Retinol-binding protein gene is highly expressed in higher-order association areas of the primate neocortex. Cerebral cortex 15: 96-108.
51. Yamamori T, Rockland KS (2006) Neocortical areas, layers, connections, and gene expression. Neuroscience research 55: 11-27.
52. Strand AD, Aragaki AK, Baquet ZC, Hodges A, Cunningham P, et al. (2007) Conservation of regional gene expression in mouse and human brain. PLoS genetics 3: e59.
53. Hawrylycz M, Bernard A, Lau C, Sunkin SM, Chakravarty MM, et al. (2010) Areal and laminar differentiation in the mouse neocortex using large scale gene expression data. Methods 50: 113-121.
54. Stansberg C, Ersland KM, van der Valk P, Steen VM (2011) Gene expression in the rat brain: high similarity but unique differences between frontomedial-, temporal- and occipital cortex. BMC neuroscience 12: 15.
55. Watakabe A (2009) Comparative molecular neuroanatomy of mammalian neocortex: what can gene expression tell us about areas and layers? Development, growth & differentiation 51: 343-354.
56. Yamamori T (2011) Selective gene expression in regions of primate neocortex: implications for cortical specialization. Progress in neurobiology 94: 201-222.
57. Rakic P (1995) A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. Trends in neurosciences 18: 383-388.
58. Smart IH, Dehay C, Giroud P, Berland M, Kennedy H (2002) Unique morphological features of the proliferative zones and postmitotic compartments of the neural epithelium giving rise to striate and extrastriate cortex in the monkey. Cerebral cortex 12: 37-53.
59. Deary IJ, Penke L, Johnson W (2010) The neuroscience of human intelligence differences. Nature Reviews Neuroscience 11: 201-211.
60. Gottfredson LS (1997) Why g Matters: The Complexity of Everyday Life. INTELLIGENCE 24: 79-132.
61. Batty GD, Deary IJ, Gottfredson LS (2007) Premorbid (early life) IQ and later mortality risk: systematic review. Annals of epidemiology 17: 278-288.
62. McDaniel MA (2005) Big-brained people are smarter: A meta-analysis of the relationship between in vivo brain volume and intelligence. Intelligence 33: 337-346.
63. Flashman LA, Andreasen NC, Flaum M, Swayze VW (1997) Intelligence and Regional Brain Volumes in Normal Controls. Intelligence 25: 149-160.

64. MacLullich AM, Ferguson KJ, Deary IJ, Seckl JR, Starr JM, et al. (2002) Intracranial capacity and brain volumes are associated with cognition in healthy elderly men. Neurology 59: 169-174.
65. Andreasen NC, Flaum M, Swayze V, 2nd, O'Leary DS, Alliger R, et al. (1993) Intelligence and brain structure in normal individuals. The American journal of psychiatry 150: 130-134.
66. Jung RE, Haier RJ (2007) The Parieto-Frontal Integration Theory (P-FIT) of intelligence: converging neuroimaging evidence. The Behavioral and brain sciences 30: 135-154; discussion 154-187.
67. Hulshoff Pol HE, Schnack HG, Posthuma D, Mandl RC, Baare WF, et al. (2006) Genetic contributions to human brain morphology and intelligence. The Journal of neuroscience : the official journal of the Society for Neuroscience 26: 10235-10242.
68. Narr KL, Woods RP, Thompson PM, Szeszko P, Robinson D, et al. (2007) Relationships between IQ and regional cortical gray matter thickness in healthy adults. Cerebral cortex 17: 2163-2171.
69. Choi YY, Shamosh NA, Cho SH, DeYoung CG, Lee MJ, et al. (2008) Multiple bases of human intelligence revealed by cortical thickness and neural activation. The Journal of neuroscience : the official journal of the Society for Neuroscience 28: 10323-10329.
70. Karama S, Ad-Dab'bagh Y, Haier RJ, Deary IJ, Lyttelton OC, et al. (2009) Positive association between cognitive ability and cortical thickness in a representative US sample of healthy 6 to 18 year-olds. Intelligence 37: 145-155.
71. Shaw P, Greenstein D, Lerch J, Clasen L, Lenroot R, et al. (2006) Intellectual ability and cortical development in children and adolescents. Nature 440: 676-679.
72. Glascher J, Tranel D, Paul LK, Rudrauf D, Rorden C, et al. (2009) Lesion mapping of cognitive abilities linked to intelligence. Neuron 61: 681-691.
73. Deary IJ, Johnson W, Houlihan LM (2009) Genetic foundations of human intelligence. Human genetics 126: 215-232.
74. Davies G, Tenesa A, Payton A, Yang J, Harris SE, et al. (2011) Genome-wide association studies establish that human intelligence is highly heritable and polygenic. Molecular psychiatry 16: 996-1005.
75. Neale BM, Ferreira MA, Medland SE, Posthuma D, editors (2008) Statistical Genetics: Gene Mapping Trough Linkage and Association.
76. Small BJ, Rosnick CB, Fratiglioni L, Backman L (2004) Apolipoprotein E and cognitive performance: a meta-analysis. Psychology and aging 19: 592-600.
77. Wisdom NM, Callahan JL, Hawkins KA (2011) The effects of apolipoprotein E on non-impaired cognitive functioning: a meta-analysis. Neurobiology of aging 32: 63-74.
78. Miyajima F, Ollier W, Mayes A, Jackson A, Thacker N, et al. (2008) Brain-derived neurotrophic factor polymorphism Val66Met influences cognitive abilities in the elderly. Genes, brain, and behavior 7: 411-417.
79. Barnett JH, Scoriels L, Munafo MR (2008) Meta-analysis of the cognitive effects of the catechol-O-methyltransferase gene Val158/108Met polymorphism. Biological psychiatry 64: 137-144.
80. Payton A (2009) The impact of genetic research on our understanding of normal cognitive ageing: 1995 to 2009. Neuropsychology review 19: 451-477.
81. Wittchen HU, Jacobi F, Rehm J, Gustavsson A, Svensson M, et al. (2011) The size and burden of mental disorders and other disorders of the brain in Europe 2010. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology 21: 655-679.
82. Tandon R, Nasrallah HA, Keshavan MS (2009) Schizophrenia, "just the facts" 4. Clinical features and conceptualization. Schizophrenia research 110: 1-23.
83. Barnett JH, Smoller JW (2009) The genetics of bipolar disorder. Neuroscience 164: 331-343.
84. American Psychiatric Association (2000) Diagnostic and statistical manual of mental disorders (4th ed., text revision). Washington, DC.
85. World Health Organization (1999) International Statistical Classification of Disease and Related Health Problems, 10th Revision (ICD-10). Geneva: World Health Organization.

86. Ellison-Wright I, Bullmore E (2010) Anatomy of bipolar disorder and schizophrenia: a meta-analysis. Schizophrenia research 117: 1-12.
87. Rimol LM, Hartberg CB, Nesvag R, Fennema-Notestine C, Hagler DJ, Jr., et al. (2010) Cortical thickness and subcortical volumes in schizophrenia and bipolar disorder. Biological psychiatry 68: 41-50.
88. Emsell L, McDonald C (2009) The structural neuroimaging of bipolar disorder. International review of psychiatry 21: 297-313.
89. Bora E, Fornito A, Yucel M, Pantelis C (2010) Voxelwise meta-analysis of gray matter abnormalities in bipolar disorder. Biological psychiatry 67: 1097-1105.
90. Ward KE, Friedman L, Wise A, Schulz SC (1996) Meta-analysis of brain and cranial size in schizophrenia. Schizophrenia research 22: 197-213.
91. Hoge EA, Friedman L, Schulz SC (1999) Meta-analysis of brain size in bipolar disorder. Schizophrenia research 37: 177-181.
92. McDonald C, Zanelli J, Rabe-Hesketh S, Ellison-Wright I, Sham P, et al. (2004) Meta-analysis of magnetic resonance imaging brain morphometry studies in bipolar disorder. Biological psychiatry 56: 411-417.
93. Arnone D, Cavanagh J, Gerber D, Lawrie SM, Ebmeier KP, et al. (2009) Magnetic resonance imaging studies in bipolar disorder and schizophrenia: meta-analysis. The British journal of psychiatry : the journal of mental science 195: 194-201.
94. Dean K, Stevens H, Mortensen PB, Murray RM, Walsh E, et al. (2010) Full spectrum of psychiatric outcomes among offspring with parental history of mental disorder. Arch Gen Psychiatry 67: 822-829.
95. Lichtenstein P, Bjork C, Hultman CM, Scolnick E, Sklar P, et al. (2006) Recurrence risks for schizophrenia in a Swedish national cohort. Psychol Med 36: 1417-1425.
96. Sullivan PF, Kendler KS, Neale MC (2003) Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry 60: 1187-1192.
97. Cardno AG, Marshall EJ, Coid B, Macdonald AM, Ribchester TR, et al. (1999) Heritability estimates for psychotic disorders: the Maudsley twin psychosis series. Archives of general psychiatry 56: 162-168.
98. Lichtenstein P, Yip BH, Bjork C, Pawitan Y, Cannon TD, et al. (2009) Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: a population-based study. Lancet 373: 234-239.
99. Demjaha A, Maccabe JH, Murray RM (2011) How Genes and Environmental Factors Determine the Different Neurodevelopmental Trajectories of Schizophrenia and Bipolar Disorder. Schizophrenia bulletin.
100. Mortensen PB, Pedersen CB, Melbye M, Mors O, Ewald H (2003) Individual and familial risk factors for bipolar affective disorders in Denmark. Archives of general psychiatry 60: 1209-1215.
101. The International Schizophrenia Consortium (2009) Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 460: 748-752.
102. Shi J, Levinson DF, Duan J, Sanders AR, Zheng Y, et al. (2009) Common variants on chromosome 6p22.1 are associated with schizophrenia. Nature 460: 753-757.
103. Stefansson H, Ophoff RA, Steinberg S, Andreassen OA, Cichon S, et al. (2009) Common variants conferring risk of schizophrenia. Nature 460: 744-747.
104. Ripke S, Sanders AR, Kendler KS, Levinson DF, Sklar P, et al. (2011) Genome-wide association study identifies five new schizophrenia loci. Nature genetics 43: 969-976.
105. Sklar P, Ripke S, Scott LJ, Andreassen OA, Cichon S, et al. (2011) Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4. Nature genetics 43: 977-983.
106. The International Schizophrenia Consortium (2008) Rare chromosomal deletions and duplications increase risk of schizophrenia. Nature 455: 237-241.
107. Grozeva D, Kirov G, Ivanov D, Jones IR, Jones L, et al. (2010) Rare copy number variants: a point of rarity in genetic risk for bipolar disorder and schizophrenia. Arch Gen Psychiatry 67: 318-327.

108. Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, et al. (2008) Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. Science 320: 539-543.
109. Stefansson H, Rujescu D, Cichon S, Pietilainen OP, Ingason A, et al. (2008) Large recurrent microdeletions associated with schizophrenia. Nature 455: 232-236.
110. McCarthy SE, Makarov V, Kirov G, Addington AM, McClellan J, et al. (2009) Microduplications of 16p11.2 are associated with schizophrenia. Nature genetics 41: 1223-1227.
111. Millar JK, Wilson-Annan JC, Anderson S, Christie S, Taylor MS, et al. (2000) Disruption of two novel genes by a translocation co-segregating with schizophrenia. Human molecular genetics 9: 1415-1423.
112. Lee KW, Woon PS, Teo YY, Sim K (2012) Genome wide association studies (GWAS) and copy number variation (CNV) studies of the major psychoses: what have we learnt? Neuroscience and biobehavioral reviews 36: 556-571.
113. Owen MJ, Williams HJ, O'Donovan MC (2009) Schizophrenia genetics: advancing on two fronts. Curr Opin Genet Dev 19: 266-270.
114. Sansom SN, Hebert JM, Thammongkol U, Smith J, Nisbet G, et al. (2005) Genomic characterisation of a Fgf-regulated gradient-based neocortical protomap. Development 132: 3947-3961.
115. Sun T, Patoine C, Abu-Khalil A, Visvader J, Sum E, et al. (2005) Early asymmetry of gene transcription in embryonic human left and right cerebral cortex. Science 308: 1794-1798.
116. Arimatsu Y (1994) Latexin: a molecular marker for regional specification in the neocortex. Neuroscience research 20: 131-135.
117. Hirokawa J, Watakabe A, Ohsawa S, Yamamori T (2008) Analysis of area-specific expression patterns of RORbeta, ER81 and Nurr1 mRNAs in rat neocortex by double in situ hybridization and cortical box method. PloS one 3: e3266.
118. Li H, Bishop KM, O'Leary DD (2006) Potential target genes of EMX2 include Odz/Ten-M and other gene families with implications for cortical patterning. Molecular and cellular neurosciences 33: 136-149.
119. Cahoy JD, Emery B, Kaushal A, Foo LC, Zamanian JL, et al. (2008) A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. The Journal of neuroscience : the official journal of the Society for Neuroscience 28: 264-278.
120. Belgard TG, Marques AC, Oliver PL, Abaan HO, Sirey TM, et al. (2011) A transcriptomic atlas of mouse neocortical layers. Neuron 71: 605-616.
121. Krubitzer L (1995) The organization of neocortex in mammals: are species differences really so different? Trends in neurosciences 18: 408-417.
122. Krubitzer L (2007) The magnificent compromise: cortical field evolution in mammals. Neuron 56: 201-208.
123. Chen CH, Panizzon MS, Eyler LT, Jernigan TL, Thompson W, et al. (2011) Genetic influences on cortical regionalization in the human brain. Neuron 72: 537-544.
124. Psychiatric GWAS Consortium Coordinating Committee (2009) Genomewide association studies: history, rationale, and prospects for psychiatric disorders. The American journal of psychiatry 166: 540-556.
125. Lee SH, Decandia TR, Ripke S, Yang J, Sullivan PF, et al. (2012) Estimating the proportion of variation in susceptibility to schizophrenia captured by common SNPs. Nature genetics 44: 247-250.
126. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, et al. (2009) Finding the missing heritability of complex diseases. Nature 461: 747-753.
127. Niculescu AB, 3rd, Segal DS, Kuczenski R, Barrett T, Hauger RL, et al. (2000) Identifying a series of candidate genes for mania and psychosis: a convergent functional genomics approach. Physiological genomics 4: 83-91.

128. Schaeren-Wiemers N, Andre E, Kapfhammer JP, Becker-Andre M (1997) The expression pattern of the orphan nuclear receptor RORbeta in the developing and adult rat nervous system suggests a role in the processing of sensory information and in circadian rhythm. The European journal of neuroscience 9: 2687-2701.
129. Le-Niculescu H, Patel SD, Bhat M, Kuczenski R, Faraone SV, et al. (2009) Convergent functional genomics of genome-wide association data for bipolar disorder: comprehensive identification of candidate genes, pathways and mechanisms. Am J Med Genet B Neuropsychiatr Genet 150B: 155-181.
130. McGrath CL, Glatt SJ, Sklar P, Le-Niculescu H, Kuczenski R, et al. (2009) Evidence for genetic association of RORB with bipolar disorder. BMC Psychiatry 9: 70.
131. Murray G, Harvey A (2010) Circadian rhythms and sleep in bipolar disorder. Bipolar disorders 12: 459-472.
132. Wu LL, Zhou XF (2009) Huntingtin associated protein 1 and its functions. Cell adhesion & migration 3: 71-76.
133. Fadel J, Burk JA (2010) Orexin/hypocretin modulation of the basal forebrain cholinergic system: Role in attention. Brain research 1314: 112-123.
134. Rainero I, Ostacoli L, Rubino E, Gallone S, Picci LR, et al. (2011) Association between major mood disorders and the hypocretin receptor 1 gene. Journal of affective disorders 130: 487-491.
135. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, et al. (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 102: 15545-15550.
136. Wang K, Li M, Bucan M (2007) Pathway-based approaches for analysis of genomewide association studies. American journal of human genetics 81: 1278-1283.
137. Wang K, Zhang H, Kugathasan S, Annese V, Bradfield JP, et al. (2009) Diverse genome-wide association studies associate the IL12/IL23 pathway with Crohn Disease. American journal of human genetics 84: 399-405.
138. Leboyer M, Bellivier F, Nosten-Bertrand M, Jouvent R, Pauls D, et al. (1998) Psychiatric genetics: search for phenotypes. Trends in neurosciences 21: 102-105.
139. Gottesman, II, Shields J (1973) Genetic theorizing and schizophrenia. The British journal of psychiatry : the journal of mental science 122: 15-30.
140. Gottesman I, Shields J (1972) Schizophrenia and Genetics: A Twin Study Vantage Point. New York: Academic Press, Inc.
141. WTCCC (2007) Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 447: 661-678.
142. Warrington JA, Nair A, Mahadevappa M, Tsyganskaya M (2000) Comparison of human adult and fetal expression and identification of 535 housekeeping/maintenance genes. Physiol Genomics 2: 143-147.
143. Fox K (2008) Barrel cortex: Cambridge University Press.
144. Valles A, Boender AJ, Gijsbers S, Haast RA, Martens GJ, et al. (2011) Genomewide analysis of rat barrel cortex reveals time- and layer-specific mRNA expression changes related to experience-dependent plasticity. The Journal of neuroscience : the official journal of the Society for Neuroscience 31: 6140-6158.
145. Louis ED, Faust PL, Vonsattel JP (2011) Purkinje cell loss is a characteristic of essential tremor. Parkinsonism & related disorders 17: 406-409.
146. Tong JK, Hassig CA, Schnitzler GR, Kingston RE, Schreiber SL (1998) Chromatin deacetylation by an ATP-dependent nucleosome remodelling complex. Nature 395: 917-921.
147. Wade PA, Jones PL, Vermaak D, Wolffe AP (1998) A multiple subunit Mi-2 histone deacetylase from Xenopus laevis cofractionates with an associated Snf2 superfamily ATPase. Current biology : CB 8: 843-846.
148. Xue Y, Wong J, Moreno GT, Young MK, Cote J, et al. (1998) NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. Molecular cell 2: 851-861.

149. Zhang Y, LeRoy G, Seelig HP, Lane WS, Reinberg D (1998) The dermatomyositis-specific autoantigen Mi2 is a component of a complex containing histone deacetylase and nucleosome remodeling activities. Cell 95: 279-289.
150. Bowen NJ, Fujita N, Kajita M, Wade PA (2004) Mi-2/NuRD: multiple complexes for many purposes. Biochimica et biophysica acta 1677: 52-57.