

Relationships between neurotransmitter receptor densities and expression levels of their corresponding genes in the human hippocampus

Ling Zhao ^{a,1}, Thomas W. Mühleisen ^{a,b,c,1}, Dominique I. Pelzer ^a, Bettina Burger ^c, Eva C. Beins ^d, Andreas J. Forstner ^{a,d}, Stefan Herms ^{c,d}, Per Hoffmann ^{c,d}, Katrin Amunts ^{a,b}, Nicola Palomero-Gallagher ^{a,b,2,*}, Sven Cichon ^{a,c,e,2,*}

^a Institute of Neuroscience and Medicine (INM-1), Research Centre Jülich, Germany

^b Cécile and Oskar Vogt Institute for Brain Research, Medical Faculty, University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Germany

^c Department of Biomedicine, University Hospital Basel and University of Basel, Switzerland

^d Institute of Human Genetics, University of Bonn, School of Medicine & University Hospital Bonn, Bonn, Germany

^e Institute of Medical Genetics and Pathology, University Hospital Basel, Switzerland

ARTICLE INFO

Keywords:
Rna expression
Receptor density
Human hippocampus
Genomic
Transcriptomic
Receptomic

ABSTRACT

Neurotransmitter receptors are key molecules in signal transmission, their alterations are associated with brain dysfunction. Relationships between receptors and their corresponding genes are poorly understood, especially in humans. We combined *in vitro* receptor autoradiography and RNA sequencing to quantify, in the same tissue samples (7 subjects), the densities of 14 receptors and expression levels of their corresponding 43 genes in the *Cornu Ammonis* (CA) and dentate gyrus (DG) of human hippocampus. Significant differences in receptor densities between both structures were found only for metabotropic receptors, whereas significant differences in RNA expression levels mostly pertained ionotropic receptors. Receptor fingerprints of CA and DG differ in shapes but have similar sizes; the opposite holds true for their “RNA fingerprints”, which represent the expression levels of multiple genes in a single area. In addition, the correlation coefficients between receptor densities and corresponding gene expression levels vary widely and the mean correlation strength was weak-to-moderate. Our results suggest that receptor densities are not only controlled by corresponding RNA expression levels, but also by multiple regionally specific post-translational factors.

1. Introduction

Neurotransmitter receptors are membrane bound proteins or protein complexes which play vital roles in neuronal signaling transmission and thus constitute potential drug targets for the treatment of neurologic and neuropsychiatric disorders (Groc and Choquet, 2020; Zilles and Palomero-Gallagher, 2017). The central dogma of molecular biology postulates the major flow for the transfer of genetic information from DNA through RNA to protein. However, despite over two decades of research, the precise nature of the relationship between gene and protein/receptor expressions remains elusive particularly in the human brain, where only a few studies have been performed due to the limited brain tissue availability, technical challenges and high cost (Buccitelli and Selbach, 2020; Gry et al., 2009; Hansen et al., 2022a, 2022b; Zachlod et al., 2022).

Manifold biological regulation mechanisms drive the relationship between gene and protein expression levels in a complex manner

(Buccitelli and Selbach, 2020; Gry et al., 2009; Hood and Emeson, 2011; Liu et al., 2016; Payne, 2015; Schulz et al., 2017; Vogel and Martotte, 2012). Some examples of these mechanisms are the differential methylation at quantitative trait loci in the human hippocampus (Schulz et al., 2017), or the modification of protein properties by alternative exon selection (Keren et al., 2010). Furthermore, receptor assembly and trafficking (Fig. 1), which are specific features of neurotransmitter receptors (Collingridge et al., 2004; Groc and Choquet, 2020; Malinow and Malenka, 2002; Schwappach, 2008; Stephenson et al., 2008), make studies of the relationships between RNA expression levels and receptor densities most challenging. A notable example is the regulation of the subunit composition of NMDA receptors, encoded by *GRIN* genes, and trafficking at hippocampal synapses involved in plasticity (Ferreira et al., 2017; Paoletti and Neyton, 2007). However, dissecting correlations between genetic factors (e.g., gene transcription and translation) and receptor distributions in the human brain could provide novel insights into understanding the mechanisms of receptor

* Corresponding author at: Institute of Neuroscience and Medicine (INM-1), Research Centre Jülich, Germany.

E-mail addresses: n.palomero-gallagher@fz-juelich.de (N. Palomero-Gallagher), s.cichon@fz-juelich.de (S. Cichon).

¹ These authors contributed equally to this work.

² Joint senior authors.

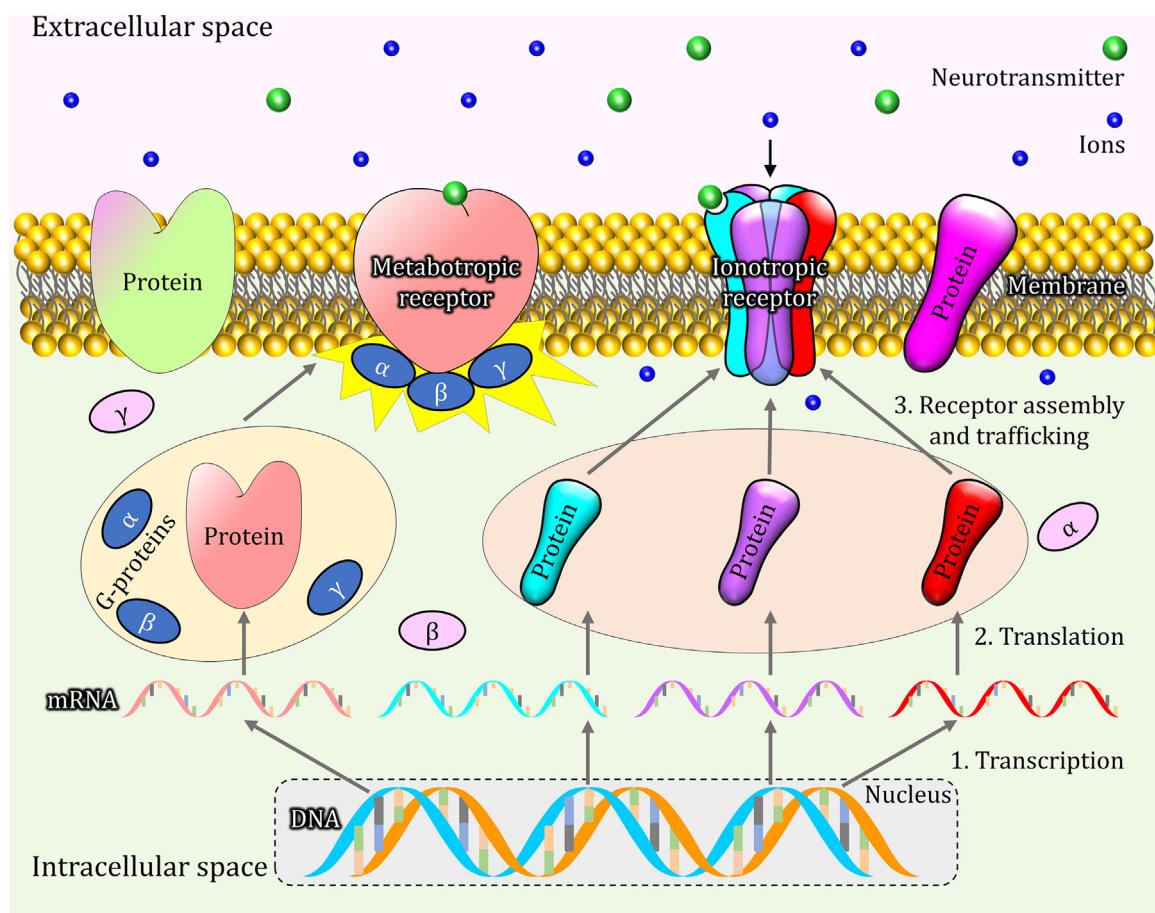


Fig. 1. A schematic diagram of the major steps involved in neurotransmitter receptor expression. The expression mechanisms are tightly controlled by gene transcription, translation, proper protein folding and co-assembly in the endoplasmic reticulum (ER), post-translational modifications, and subsequently trafficking to the appropriate membrane surface (on the cell body, dendritic tree or axonal bouton) along the secretory pathway (Levitz et al., 2016; Schwappach, 2008; Stephenson et al., 2008). Thus, the number of functional receptors is the result of several control steps more than those involved in protein synthesis. These additional steps include the complex control of receptor assembly from the single proteins, transport to the required site (trafficking) and dynamic inclusion in the cellular membrane. Of the receptors analyzed in the present study, the AMPA, NMDA, kainate, and GABA_A belong to the ionotropic category, whereas mGluR2/3, M₁, M₂, M₃, α₁, 5-HT_{1A}, 5-HT₂, D₁, and A₁ are metabotropic.

expressions and span this necessary bridge to further understand the pathogenic mechanisms mediating the effects of genetic risk variants in neurologic and neuropsychiatric disorders.

Neurotransmitter receptors are heterogeneously distributed throughout the brain, and regional differences in receptor densities can be analyzed among others, by means of *in vivo* receptor positron emission tomography or of quantitative *in vitro* receptor autoradiography. While the first approach can be applied *in vivo* and is capable to capture dynamic changes of receptor binding in healthy and patient subjects, the latter method has the advantage of revealing specific regional and laminar distribution patterns of multiple receptors within the same brain sample and with a high resolution (Palomero-Gallagher and Zilles, 2018). Furthermore, *in vivo* neurotransmitter receptor imaging is only accessible for a limited set of receptor types due to both technical and ethical restrictions, which makes the *in vivo* visualization and quantification of multiple receptors in the same subject extremely difficult (Khan et al., 2021).

For receptor autoradiography, there is growing interest in comprehensively decoding the relationships between receptor densities and expression levels of their encoding genes across multiple regions of the human brain (Hansen et al., 2022a, 2022b; Murgaš et al., 2022; Zachlod et al., 2022). We recently performed correlation analyses of receptor densities and corresponding genes across 15 areas of the human primary and early visual, auditory, somatosensory, and motor cortices, and revealed a covariation of receptor densities with areal gene expres-

sion levels (Zachlod et al., 2022). Other studies encompassing 33–38 architectonically identified areas spread throughout the cerebral cortex revealed a lack of correlation for most of the analyzed receptor types (Hansen et al., 2022a; Murgaš et al., 2022). However, the spatial resolution of PET may obscure specific findings in single areas, and the RNA (Hawrylycz et al., 2012) and neurotransmitter receptor data (Zilles and Palomero-Gallagher, 2017) were obtained from different subjects. Inter-subject variability of RNA expression levels and of receptor densities superimposed on the effects of age differences may constitute a natural confounder to explore the relationship between these datasets (Murgaš et al., 2022), which cannot be fully overcome and compensated. Indeed, a pronounced interindividual variability of gene expression in the human hippocampus has been demonstrated in our previous study (Schulz et al., 2017).

The hippocampus plays a crucial role in functions including learning and memory, spatial navigation and emotion processing (Zhong et al., 2020). Notably, the hippocampus is also a key structure in various neurologic and neuropsychiatric disorders such as epilepsy and Alzheimer's disease (Berger et al., 2020; Bernasconi et al., 2003; Bernhardt et al., 2016, 2015; Blümcke et al., 2013; Debski et al., 2020; Hogan et al., 2004; Thom, 2014). The hippocampus consists of the dentate gyrus (DG) and the Cornu Ammonis (CA, with the CA1-CA3 regions) (Palomero-Gallagher et al., 2020). They are parts of the Papez circuit, which was proposed as the neuroanatomical substrate of emotion and memory (Aggleton et al., 2016; Papez, 1937). Information reaches the DG from

the entorhinal cortex through the perforant pathway, is transferred via mossy fibers to the CA3 region, and then via Schaffer collaterals to the major hippocampal output structure, the CA1, which in turn mainly projects back to the entorhinal cortex either directly or via the subiculum (van Strien et al., 2009). Neurotransmitters play a central role in this circuit and trafficking of their receptors is involved in the synaptic plasticity which mediates memory consolidation, i.e., the transfer of information from short-term to long-term memory (Collingridge et al., 2004; Groc and Choquet, 2020; Malinow and Malenka, 2002; Zhong et al., 2020).

Previous studies have provided detailed insights into the regional and laminar distribution patterns of multiple neurotransmitter receptors in the human hippocampal regions (Biegton et al., 1982; Palomero-Gallagher et al., 2020; Perry et al., 1993; Szot et al., 2005; Tremblay et al., 1985). The hippocampal expression patterns of genes coding for these neurotransmitter receptors or for their subunits have also been described (Hawrylycz et al., 2015, 2012; Szot et al., 2005). Furthermore, Vogel et al. (2020) performed a transcriptome-wide screening along the longitudinal axis of the human hippocampus by using human gene expression data and macroanatomical labels from the Allen Human Brain Atlas (Hawrylycz et al., 2012), and found a regionally specific anteroventral-posterior dorsal gradient of gene expression profiles. However, no studies have systematically and comprehensively analyzed the relationship between the densities of multiple classical neurotransmitter receptors and the expression levels of their encoding genes in the hippocampus. Since many neurologic and neuropsychiatric disorders such as epilepsy and Alzheimer's disease are associated with both a genetic predisposition and an altered balance in the densities of multiple receptors (Graebenitz et al., 2011; Khan et al., 2021; Palomero-Gallagher et al., 2012; Selkoe, 2001; Symonds et al., 2017), elucidating the relationship between gene expression levels and receptor densities could deepen our understanding of the pathogenesis of such diseases, and provide insights into the specific link between disease-associated genes and the disease itself.

Specific cyto- and receptor architecture as well as gene expression patterns have been related to differences in cortical function (Amunts and Zilles, 2015; Burt et al., 2018; Zilles and Palomero-Gallagher, 2017). Thus, our study aimed to simultaneously quantify the densities of 14 neurotransmitter receptors and the expression levels of their 43 encoding genes separately for the DG and CA hippocampal regions in tissue from the same brain samples, and then map the relationships between the ensuing receptor densities and expression levels of the corresponding genes.

2. Materials and methods

2.1. Tissue collection and processing

We obtained six hippocampus samples from the Netherlands Brain Bank in accordance with ethical and legal declarations (Klioueva et al., 2018) and one sample through the body donor program in compliance with the Ethics Committee of the Department of Anatomy, University of Düsseldorf, Germany. All donors (71 ± 15 years of age; 5 females; 2 males; European origin) did not have a history of neurologic or psychiatric disease, and the postmortem delay of autopsy (postmortem interval) was less than 5 h.

Since chemical fixation alters the physiological properties of receptor subunits, it affects the specific and nonspecific receptor binding, and consequently results in density measurements that do not reflect the true receptor concentrations (Palomero-Gallagher and Zilles, 2018; Zilles et al., 2002). Therefore, unfixed brain tissue was shock-frozen in isopentane at -40°C immediately after autopsy and stored until further processing in air-tight plastic bags at -80°C to protect it from freeze-artifacts.

After quality control by visual examination, the hippocampus samples were serially sectioned coronally by means of a cryostat micro-

tome (Leica CM3050 S) at around -20°C . For each subject's sample, the first three series of $20\ \mu\text{m}$ sections, each comprising a total of 20 sections, were collected for receptor autoradiography and cell-body staining. Then, 10 sections, each $60\ \mu\text{m}$ thick, were obtained for RNA isolation and quantification (Supplementary Fig. S1). Finally, three further series of $20\ \mu\text{m}$ sections, also comprising a total of 20 sections each, were collected for receptor autoradiography and cell-body staining. Each section destined to the visualization of receptor or cell body distribution patterns was thaw-mounted onto a pre-cooled, silanized glass slide upon sectioning, then air dried and stored in air-tight plastic bags at -80°C until further processing. For each section collected for analysis of RNA expression, the CA and DG regions were dissected inside the cryotome with a scalpel upon sectioning, then each region (CA/DG) was separately transferred to a tube filled with QIAzol Lysis Reagent (Qiagen, Hilden, Germany) and stored at -80°C until further RNA extraction in one batch to ensure the same experimental conditions for all samples.

2.2. Quantitative *in vitro* receptor autoradiography

Alternating sections were processed for visualization and quantification of 14 different neurotransmitter receptor binding sites by means of quantitative *in vitro* receptor autoradiography according to previously published protocols and quality controls (Palomero-Gallagher and Zilles, 2018; Zilles et al., 2002), or for the visualization of cell bodies by means of a modified silver cell body staining procedure (Merker, 1983). All sections collected for the visualization of cell bodies or of a given receptor type were processed together. Thus, within a given modality, we could ensure the same experimental conditions for all samples. Histologically processed sections served to confirm the delineation of the CA and DG regions in the receptor autoradiographs. Supplementary Table S1 provides a list of the analyzed receptors together with their classification based on type and mechanism of action, as well as the corresponding binding protocols.

The receptor binding procedure encompassed a preincubation step to rehydrate sections, a main incubation step with a tritiated ligand known to bind specifically to one of the targeted receptor types, and a washing step to rinse off residual tritiated ligand. For each targeted receptor type and in parallel with the main incubation to detect the total binding of each tritiated ligand, which was carried out with five sections per sample, an additional section per sample was incubated with the tritiated ligand plus a non-labeled specific displacer to determine the proportion of the non-displaceable (i.e., non-specific) binding. Specific binding is the difference between total and non-specific binding. However, since non-specific binding was found to be less than 5% of the total binding sites (see below for details), the latter was defined here as specific binding.

The radioactively labeled sections were exposed against tritium-sensitive films together with plastic standards of known concentrations of radioactivity for 9 to 15 weeks (Supplementary Table S1) and the ensuing autoradiographs were digitized with a CCD camera and processed densitometrically for the analysis of receptor densities (Palomero-Gallagher and Zilles, 2018; Zilles et al., 2002). In short, transformation curves, calculated based on the plastic standards, were used to indicate the relationship between gray values in the autoradiographs and receptor densities (fmol/mg protein) in the tissue (Palomero-Gallagher and Zilles, 2018; Zilles et al., 2002). Images were subsequently linearized by means of the transformation curves. For visualization purposes, these linearized images were smoothed, contrast enhanced, and pseudo-color coded in a spectral order to optimize visualization of regional and laminar receptor distribution patterns. Finally, the in-house-developed software AnaRec (Impieri et al., 2019) was used to quantify the receptor densities in architectonically identified hippocampal CA and DG regions in both the sections processed for visualization of total binding sites and those for identification of non-specific binding. Depending on section quality (e.g., presence of technically related artifacts such as tears or folds), total binding was quantified in 3–5 sec-

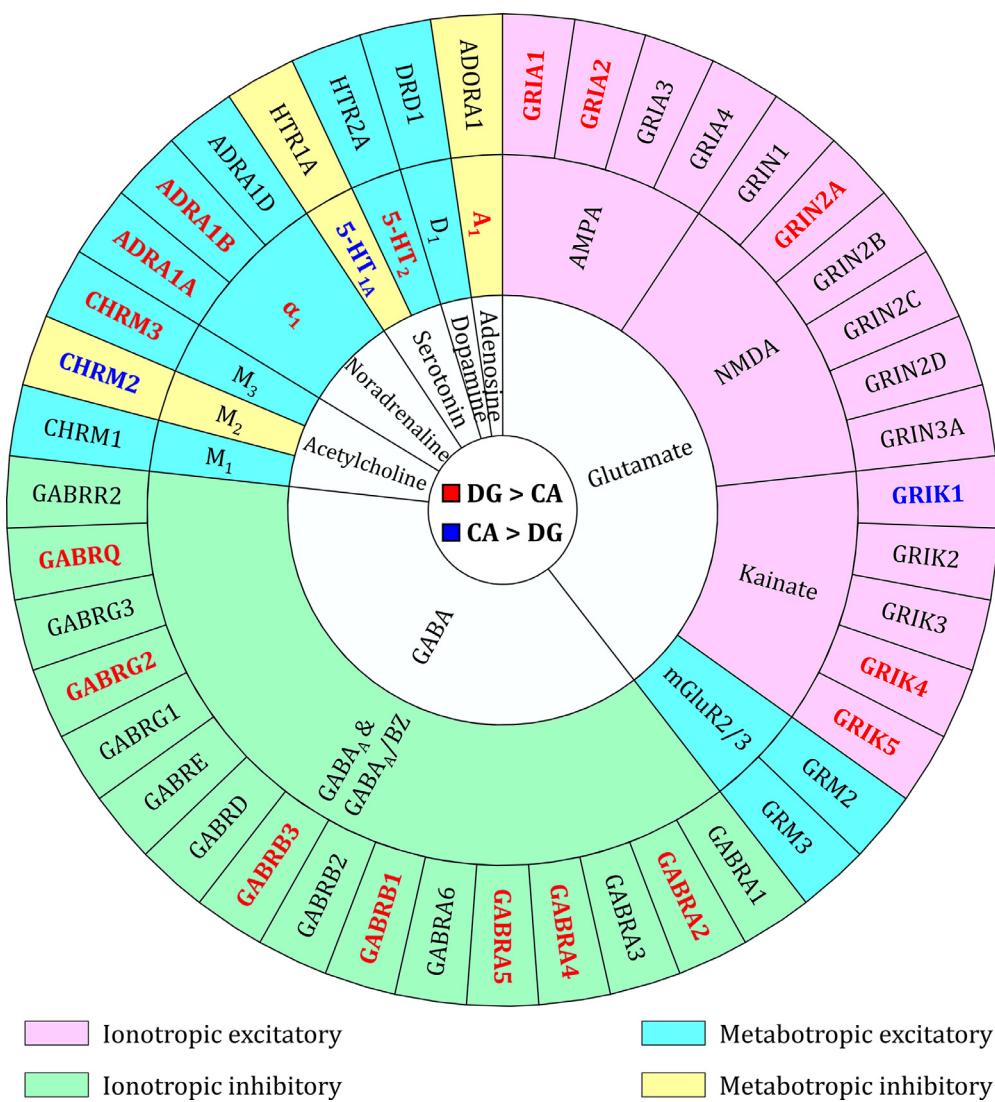


Fig. 2. Neurotransmitter receptors and their corresponding genes. Comprehensive list of the neurotransmitter receptors and their corresponding genes analyzed in the present study. Colors have been used not only to differentiate receptors by their effect (ionotropic or inhibitory) and mechanisms of action (ionotropic or metabotropic), but also to highlight the significant results of our statistical analyses. Names highlighted in red or blue indicate significant differences in receptor densities or gene expression levels between the CA and DG regions after partial Bonferroni correction (Scholtens et al., 2014). Further information is provided in Supplementary Tables S3 and S4.

tions per receptor type and sample. The mean densities of all 14 receptors were visualized for the DG and CA separately in a polar-coordinate plot, and the resulting graph constitutes the “receptor fingerprint” of the region in question (Zilles et al., 2002).

Based on their mechanisms of action, neurotransmitter receptors are classified into ionotropic and metabotropic receptors (Hall and Hall, 2020). Ionotropic receptors consist of different subunits, and each subunit is encoded by a single gene, so that an ionotropic receptor is jointly encoded by a composition of multiple genes (Fig. 1). Conversely, metabotropic receptors are monomeric structures and thus only encoded by one gene, which were further selected to perform across-gene correlation analysis. Of the receptor types analyzed here, AMPA, NMDA, kainate, GABA_A, and GABA_{A/BZ} are ionotropic receptors, whereas mGluR2/3, M₁, M₂, M₃, α₁, 5-HT_{1A}, 5-HT₂, D₁ and A₁ are metabotropic receptors (Supplementary Table S1). However, since the ligands used to label the mGluR2/3 and the α₁ receptors are subtype non-specific antagonists, it was necessary to analyze all encoding genes in parallel for these two metabotropic receptors. Fig. 2 provides an overview of the neurotransmitter receptors analyzed in the present study and their corresponding genes.

2.3. RNA isolation and quantification

The processing of tissue and RNA was done by the same experimenter in one batch for all samples at the core NGS Core Facility of the Uni-

versity of Bonn, Germany. For each of the subjects, tissue samples from the CA or DG regions were placed in different tubes filled with QIAzol solution to lyse the tissue and protect the isolated RNA from degradation. All subsequent steps were performed simultaneously for all probes, to ensure the same experimental conditions for all samples. Then, each lysate was resuspended with a filter-tipped pipette to dissolve remaining fragments. Subsequently, the samples were aliquoted into portions reflecting about 20 mg tissue and stored at -80 °C until isolation. Finally, total RNA was isolated using the Qiagen miRNeasy Micro Kit (Qiagen, Hilden, Germany) according to the manufacturer instructions.

Quality control of total RNA was performed using a TapeStation (Agilent, Santa Clara, USA). Libraries for sequencing were prepared using the QuantSeq 3'mRNA-Seq Library Prep Kit FWD for Illumina (Lexogen, Vienna, Austria) in accordance with manufacturer instructions. Reads were sequenced on a HiSeq 2500 V4 (Illumina, San Diego, USA). Approximately 16 million single-reads of 100 bp length were obtained per sample library.

The quantification of RNA samples was performed using the Lexogen QuantSeq pipeline implemented in the Partek® Flow® Genomic Analysis Software, with small modifications. Briefly, trimmed reads were aligned to the hg38.p13 reference index using STAR - 2.7.3a (deviation from default settings: filtering BySJout; max. read mapping 20; max. mismatches 999; mismatch mapped ratio 0.1; min. intron size 20; min. spliced alignment overhang 8; min. annotated spliced alignment overhang 1). Aligned reads were quantified using Partek E/M against

hg38.p13 - Ensembl Transcript release 103. Read counts for each gene were normalized to counts per million (CPM). One DG sample was identified as an outlier in principal component analysis and excluded from further analysis (Supplementary Fig. S2). For differential gene expression analysis, gene counts were normalized using trimmed mean of M values (TMM) (Robinson and Oshlack, 2010).

Based on information provided by the human gene database (<https://www.genenames.org/>), 47 receptor encoding genes were identified and selected for further analysis. During quality control of RNA-Seq data, four genes (*GRIN3B*, *GABRP*, *GABRR1*, and *GABRR3*) were excluded for further analyses (Fig. 2). The mean expression levels of the 43 analyzed receptor encoding genes were visualized for the DG and CA separately in the form of a polar-coordinate plot. Analogous to the receptor fingerprint, this resulting graph provides a region-specific expression profile of multiple genes, i.e., the “RNA fingerprint” of the area in question.

2.4. Statistical analysis of receptor densities and gene expression levels

Differences in receptor densities and gene expression levels between CA and DG were analyzed with a non-parametric permutation test (10,000 permutations) (Nichols and Holmes, 2002). A partial Bonferroni correction method was used to control for multiple comparisons in a relatively conservative way, while still keeping enough sensitivity (Scholtens et al., 2014). Specifically, the receptor densities (fmol/mg) and normalized RNA counts were separately used for decomposing and processing with principal component analyses (PCA), the four first principal components together were found to explain 95% of receptor and corresponding gene data, respectively (Supplementary Table S2). Thus, the *p* value threshold of multiple comparisons correction for each data was set at 0.0125 (0.05/4). Unless otherwise specified, only corrected *p* values are described in the results section. To ensure the replicability and robustness of the above analyses, a leave-one-observation-out cross-validation (LOOCV) approach (Schurter et al., 2010) was performed by repeating the analyses while one observation data was removed from the corresponding dataset at a time to determine if the result would remain significantly different. Furthermore, the effect size and statistical power were also calculated (Charan and Kantharia, 2013; Cohen, 1992).

In addition, for further comparison of the variability of RNA expressions in hippocampal regions from different subject sample, the RNA expression differences between CA and DG were analyzed using data obtained from the Allen Human Brain Atlas (Hawrylycz et al., 2015, 2012). We used the JuGEx tool, which integrates RNA expression data in the Allen Human Brain Atlas with cytoarchitectonic probability maps from the Julich-Brain Atlas (Amunts et al., 2020) to extract the necessary data (Bludau et al., 2018). A detailed description of this procedure was published in Bludau et al. (2018), and summarized here in the section Supplementary Material and Methods.

The relationship between the density of each receptor type and the expression level of the corresponding gene or subunit gene was analyzed across subjects (within-gene correlation analysis) with a Spearman rank correlation (*r*), which is more robust to outliers than Pearson’s correlation (Schober et al., 2018). Specifically, 7 data points, each representing one of the analyzed samples, were used for calculating the correlation in CA, however 6 data points were used in DG as one DG sample had been identified as an outlier in RNA isolation and quantification and excluded from further analysis. The *r* value threshold of moderate strength of the correlation was set at 0.4–−0.4 (Schober et al., 2018), and the *p* value threshold of multiple correction was also set at 0.0125. In addition, the mean correlation coefficients were calculated based on the different conditions, e.g., mean correlation strength in the entire hippocampus, the CA, or the DG, respectively. For cross-validating the correlation coefficient (*r*), the LOOCV method was conducted by repeating the correlation analysis while one subject data was removed at a time, and then the mean correlation coefficient (r_{Loocv}) was calculated. Furthermore, we performed an across-gene correlation analysis

with the partial metabotropic receptors and their corresponding genes separately in the CA and DG regions, and the detailed information was provided in Supplementary Fig. S3.

In addition, the Spearman correlation coefficient was used as a distance parameter in a multidimensional scaling analysis (MDS) (Cox and Cox, 2008) to map the relationship pattern of the ionotropic receptors and their corresponding subunit genes.

3. Results

3.1. CA and DG differ in their receptor and RNA fingerprints

In the human hippocampus, the analyzed receptors present distinct regional and laminar distribution patterns (Fig. 3). The densities of AMPA, mGluR2/3, GABA_A/BZ, α_1 , and 5-HT₂ receptors are higher in DG than in CA, and the opposite holds true for the NMDA, GABA_A, 5-HT_{1A}, and A₁ receptors, thus resulting in receptor fingerprints of comparable sizes, but very different shapes for the CA and DG regions (Fig. 4A). However, only the differences in α_1 , 5-HT_{1A}, 5-HT₂, and A₁ metabotropic receptor densities were statistically significant and confirmed by the validation analysis (Fig. 2 and Supplementary Table S3). Within CA, the CA1 region presents the highest densities of most of the analyzed receptors, with the notable exception of the kainate, α_1 , and 5-HT₂ receptors, which are present at higher concentrations in CA3 than in CA1 (Fig. 3).

In contrast to the situation described for the receptor fingerprints, the corresponding RNA fingerprints of the CA and DG regions differ in size, but are similar in shape, indicating that both hippocampal regions differ in their absolute gene expression levels, but not in the balance in the expression levels of the analyzed genes (Fig. 4B). The DG presents significantly higher *GRIA1* and *GRIA2* (for the AMPA receptor); *GRIN2A* (NMDA); *GRIK4* and *GRIK5* (kainate); *GABRA2*, *GABRA4*, *GABRA5*, *GABRB1*, *GABRB3*, *GABRG2*, and *GABRQ* (GABA_A and GABA_A/BZ); *CHRM3* (M_3); as well as *ADRA1A* and *ADRA1B* (α_1) expression levels than does CA (Fig. 2 and Supplementary Table S4). Significantly lower gene expression levels in DG than in CA were found only for the kainate encoding gene *GRIK1* and the M_2 encoding gene *CHRM2*. Thus, we identified 17 genes showing significantly different expression levels between CA and DG regions. The results of the validation analysis underline the reproducibility of these statistical findings concerning region-specific differences (Supplementary Table S4). In addition, *GABRA6*, *GABRE*, and *GABR2* present extremely low gene expression levels (Supplementary Table S4), thus they were excluded in the further relationship analyses between receptors and corresponding genes. Interestingly, a comparable analysis using the edited Julich-Brain maps to precisely select independent RNA data from the Allen Human Brain Atlas only revealed a few significantly higher gene expression levels in DG than in CA, and no significantly lower gene expression levels were found in DG than in CA (Supplementary Table S5). Specifically, we only found the expression levels of *GRIA4*, *GRIK2*, *GRM2*, *GABRB3*, and *ADRA1A* to be significantly higher in DG than in CA (Supplementary Table S5).

Of particular interest is the fact that significant differences in receptor densities were found only for metabotropic receptors, but significances in encoding gene expressions mostly pertained to ionotropic receptors. Furthermore, only the α_1 receptor shows both significantly higher receptor densities and encoding gene expression levels in DG than in CA. Interestingly, although 5-HT_{1A}, 5-HT₂, and A₁ receptor densities in CA and DG differed significantly from each other, no significances were found for the expression levels of their different encoding genes (Fig. 2 and Supplementary Tables S3 and S4). Taken together, these results point at a poor covariation expression phenomenon between encoding genes and corresponding receptors in the hippocampus, i.e., for most of the receptors analyzed here differences in their densities between the CA and DG regions do not match the differences between these regions in the expression levels of the corresponding genes.

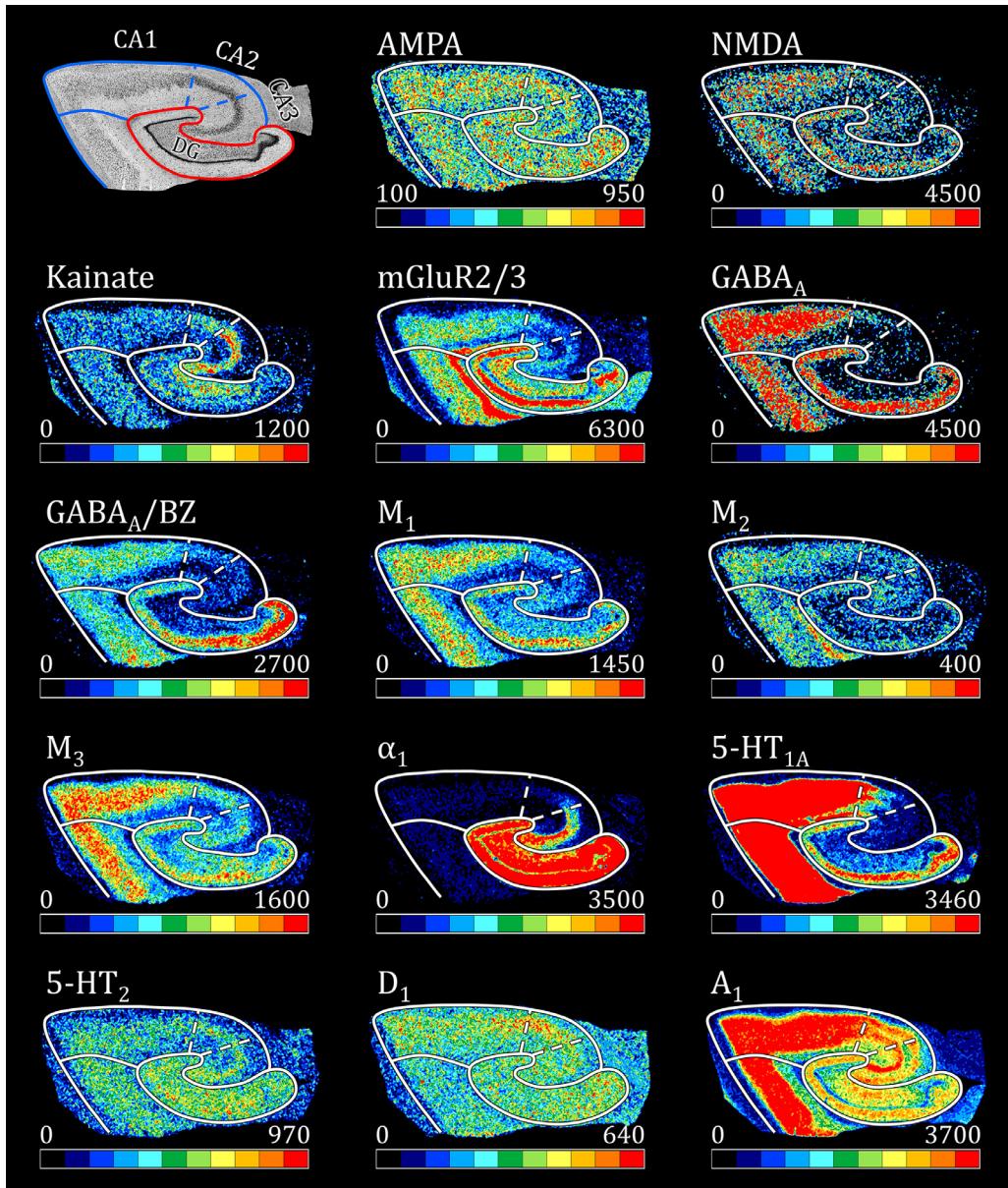


Fig. 3. Regional and laminar distribution patterns of cell bodies (cytoarchitecture) and of neurotransmitter receptors (receptor architecture) in the human hippocampus. The CA1-CA3 subregions of CA are delineated with dashed lines. Color scales code for receptor densities in fmol/mg protein. Abbreviations: CA1-CA3, regions 1–3 of *Cornu Ammonis*; and DG, dentate gyrus.

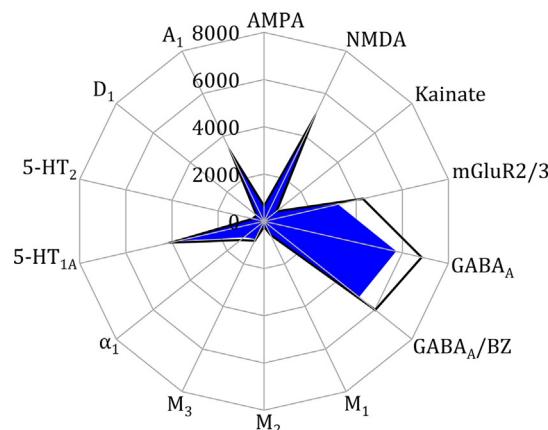
3.2. Correlations between receptor densities and encoding gene expression levels

For the within-gene correlation analysis, the correlation coefficients between receptors and their corresponding genes vary widely from negative to positive, with -0.8286 and 0.9286 being the extreme values (Supplementary Table S6). The results of the LOOO-CV present a high similarity with the above described statistical findings, which further prove their reproducibility and robustness (Supplementary Table S6). However, the mean correlation coefficients are poor for CA (0.09) and DG (0.20). Furthermore, we computed this measure for the entire hippocampus, which yielded a value of 0.15. Considering some receptors with many subunits or subtype may weigh heavily the calculation of the mean correlations, we provide two alternative strategy for cross verification. (1) Assigning each receptor with the mean correlation by averaging the correlation coefficients between receptor density and its main subunit genes (Supplementary Tables S6 and S7), the mean correlation

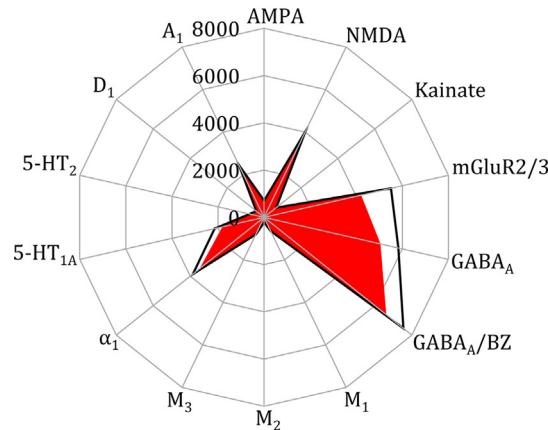
coefficients are 0.16 for CA, 0.11 for DG, and 0.14 for hippocampal formation. (2) Averaging the main subunit gene expressions for a given receptor type for each subject before running the within-gene correlation analysis, the mean correlation coefficients are 0.15 for CA, 0.12 for DG, and 0.14 for hippocampal formation; we further found a poor congruent receptor-gene alignment between CA and DG (Supplementary Fig. S3A and Table S8). Taken together, these results indicate that the mean correlation coefficients for the entire hippocampal formation ranging from 0.14 to 0.15 represent the high reliability. In contrast, the results of the across-gene correlation analysis with the partial metabotropic receptors and their corresponding genes reveal a high correlation in both CA (0.57) and DG (0.71) (Supplementary Fig. S3B). Moreover, we averaged main subunit gene expressions of all subjects to a receptor, then analyzed across-gene correlation analysis with the 14 receptors and found a high robustness results in both CA (0.60) and DG (0.64).

After filtering the moderate-to-strong correlation strength (0.4–1.0) between receptors and their respective encoding genes revealed by the

A. Receptor fingerprints



█ CA █ DG



B. RNA fingerprints

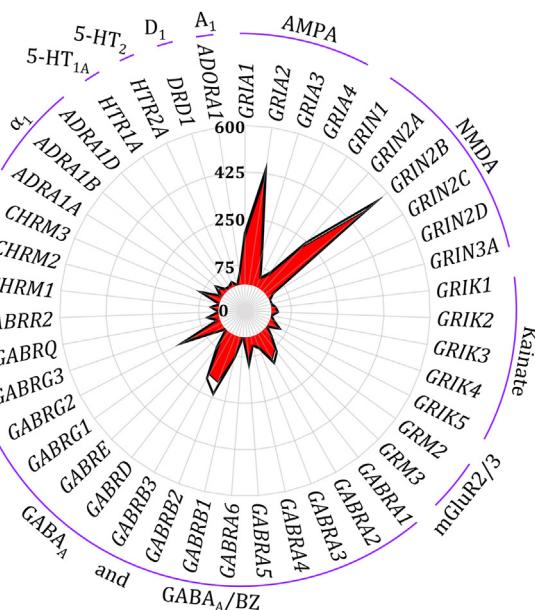
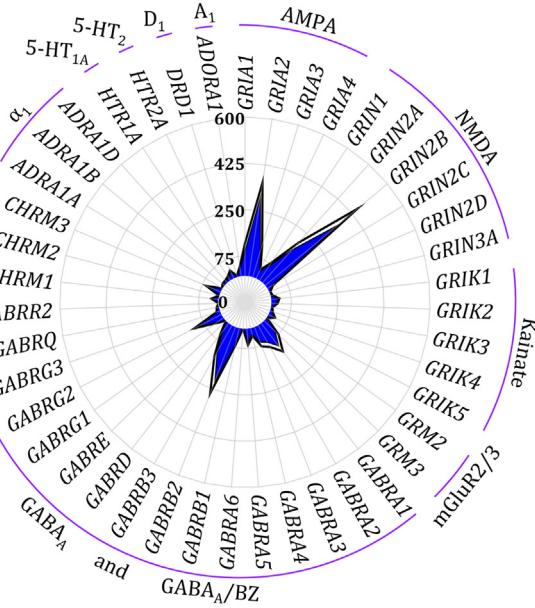


Fig. 4. Fingerprints of receptor and corresponding gene in the human hippocampus. (A) Receptor fingerprints depict the mean densities (thick black line indicates the standard deviation) of all 14 analyzed receptors in the CA and DG regions. (B) Corresponding RNA fingerprints depict the mean expression levels (thick black line indicates the standard deviation) of all 43 analyzed genes in the CA and DG regions. Note, to facilitate visualization of low RNA counts, we have expanded the scale range. The order of receptor and corresponding gene types is identical with Fig. 2. The underlying data are provided in Supplementary Tables S3 and S4, and made freely available to the neuroscientific community via the EBRAINS platform (DOI to be released upon publication).

within-gene correlation analysis, the CA and DG regions display differential patterns both in positive and negative correlations (Fig. 5). Specifically, the DG region presented a higher number of high-strength correlations with corresponding receptors than did the CA region (24 vs. 15 genes, respectively, out of the 43 genes analyzed) (Supplementary Table S9). Interestingly, there was an overlap between the genes showing high-strength correlations and the genes which were found to be expressed at significantly different levels in the CA and in the DG regions, whereby the ratio of overlapping genes was larger for CA (10/15) than for DG (9/24) (Supplementary Fig. S4).

For the moderate-to-strong correlation strength, the only positive correlations present in both CA and DG are those of NMDA with *GRIN1*,

mGluR2/3 with *GRM3*, and *GABA_A* with *GABRG2* (Fig. 5). In addition, CA and DG show no common correlation patterns concerning the negative correlation coefficients (Fig. 5). Interestingly, we found a higher number of correlations between receptors and their encoding genes in DG than in CA. Furthermore, in the CA region we mainly found high-strength correlations between ionotropic receptors and their encoding genes (Fig. 5A), whereas in the DG we also found high-strength positive and negative correlations for multiple metabotropic receptors (Fig. 5B). Finally, in CA we found more positive high-strength correlation coefficients for the *GABA_A* receptor than for the *GABA_A/BZ* binding site, and the opposite holds true for the DG region (Fig. 5), although these two binding sites for GABA share the same encoding genes.

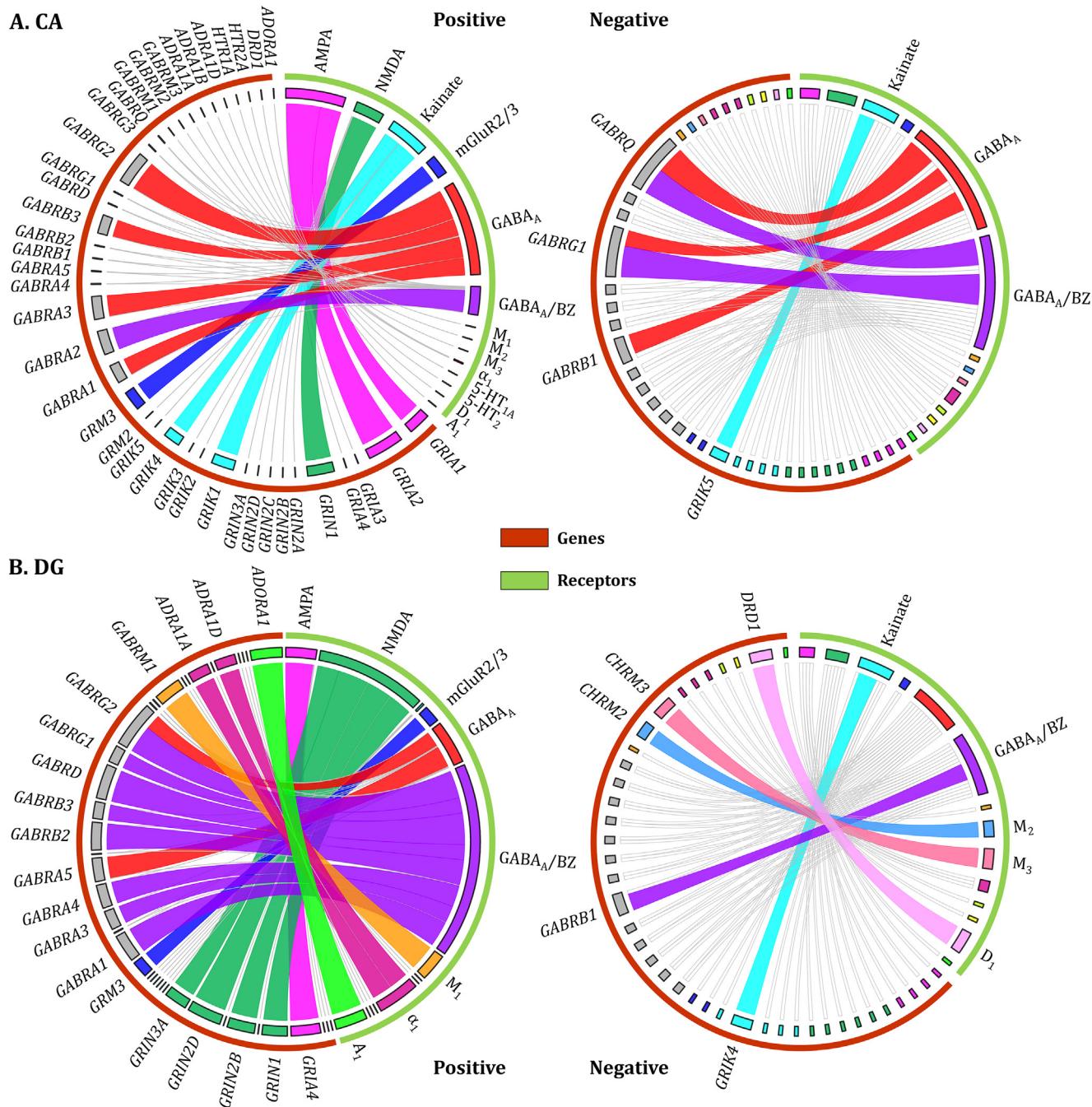


Fig. 5. Correlation patterns of receptor densities and corresponding gene expression levels in the human hippocampus. (A) Correlations in the CA region. (B) Correlations in the DG region. For both regions the positive correlations are shown in the left plot and negative ones in the right plot. The ribbons show the correlation coefficient at moderate-to-strong strength (0.4–1.0), while the gray lines indicate the correlation coefficient at weak strengths (0.0–0.4). Further information concerning correlation coefficients is provided in Supplementary Table S6.

3.3. Multidimensional scaling analysis

The correlation coefficients between genes and corresponding receptors were found to be significant in the case of the AMPA receptor with *GRIA2* and *GABA_A* with *GABRG2* in the CA region, as well as for NMDA with *GRIN2D* and *GABA_{A/BZ}* with *GABRD* in the DG region (Supplementary Table S6). However, only the correlation coefficient between the AMPA receptor and *GRIA2* in CA remains significant after partial Bonferroni correction (Fig. 6A). Interestingly, the MDS revealed opposing relationships between the AMPA receptor and its encoding subunit genes in the CA and DG regions, since in CA *GRIA2* presented the strongest and *GRIA4* the weakest corre-

lation coefficients with AMPA, whereas the opposite holds true for DG (Fig. 6B).

4. Discussion

We analyzed the regional densities of 14 neurotransmitter receptors and expression levels of their corresponding 43 encoding genes in the CA and DG hippocampal regions from the same brain samples. Neurotransmitter receptor densities presented distinct regional and laminar distribution patterns, and the CA and DG also differed in their gene expression levels. Significant differences in receptor densities between CA and DG were found only for metabotropic receptors, but in encoding

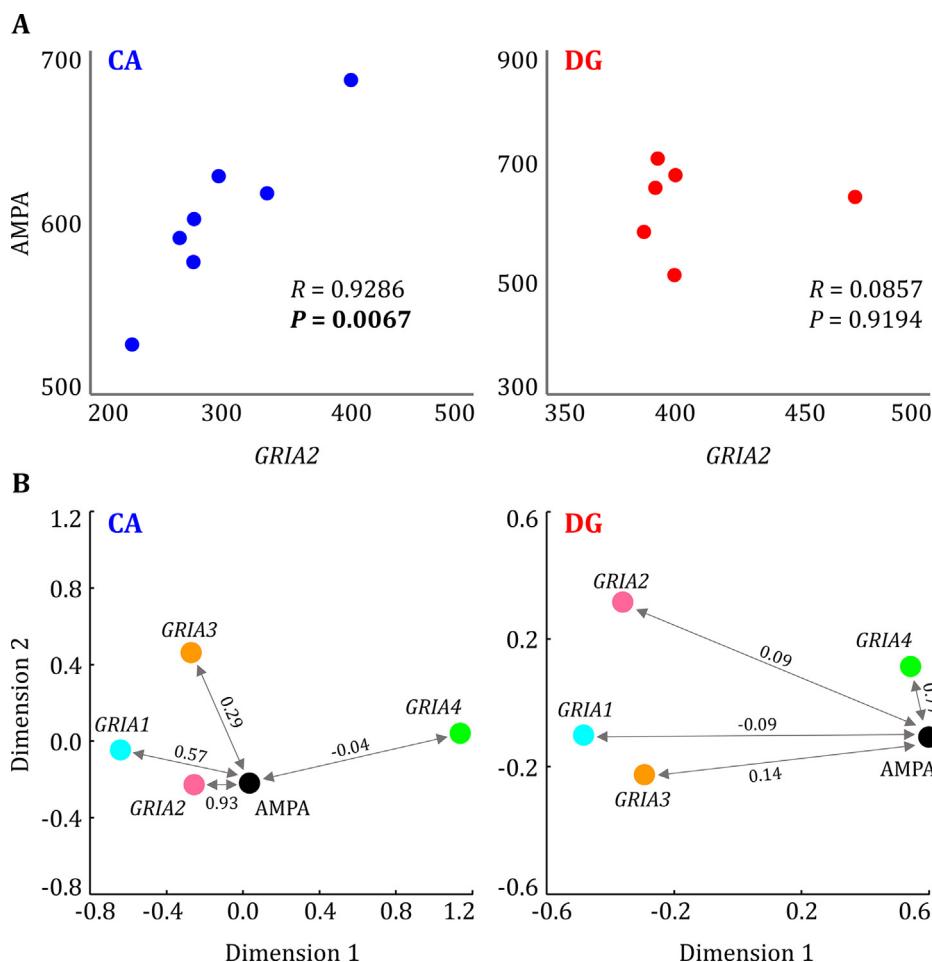


Fig. 6. Relationships between the AMPA receptor and corresponding genes in the human hippocampus. (A) Significant correlation coefficient between AMPA receptor density and *GRIA2* expression level in CA but not in DG. Plots depicting the relationships between all other analyzed receptors and their corresponding genes are provided in Supplementary Fig. S5. (B) Multidimensional analyses (MDS), based on the Spearman rank correlation coefficients between the AMPA receptor and its corresponding subunit genes, reveal opposite distribution patterns of relationships in CA and DG regions. Further information concerning MDS between other analyzed receptors and their corresponding subunit genes is provided in Supplementary Fig. S6.

gene expressions mostly for ionotropic receptors. Furthermore, the receptor fingerprints of the CA and DG regions differ in shape but have a similar size, however the opposite holds true for the RNA fingerprints. These results provide evidence that expression levels of encoding genes and densities of the corresponding receptors do not covariate, which was further characterized by their correlation analyses. The correlation coefficients between receptors and their encoding genes vary widely in strength, and the mean correlation coefficient only presents a weak strength. It is worth noting that a significant correlation was found only for AMPA and *GRIA2* in the CA region, and the relationships between the AMPA receptor and its corresponding subunit genes display diametrically opposite trends in CA and DG.

Functional differentiation of the cortex has been correlated with specific regional differences in cytoarchitecture, receptor architecture and gene expression levels (Amunts and Zilles, 2015; Burt et al., 2018; Hawrylycz et al., 2015, 2012; Zilles and Palomero-Gallagher, 2017). The distinctive regional and laminar receptor distribution patterns of hippocampal regions in the present study are in accordance with our previous research (Palomero-Gallagher et al., 2020), however to the best of our knowledge this is the first report of differential expression levels of corresponding receptor-encoding genes in the CA and DG regions from the same brain. The densities of the 14 analyzed receptor types and the expression levels of the 43 corresponding genes were visualized for each region as its receptor fingerprint and RNA fingerprint, respectively. Fingerprints depict the region-specific balance of multi-receptor densities or gene expressions and thus reveal the locally specific neurochemical or transcriptional organization at the receptor or gene level, respectively. Interestingly, receptor fingerprints of the CA and DG regions differ in shape but have a similar size, but the opposite holds true for the RNA

fingerprints. Thus, CA and DG regions present a comparable balance in the expression levels of the analyzed genes, but not in the densities of the receptors they encode. These differences suggest a poor covariation between genes expression levels and receptor densities in the CA and DG regions, which was further verified by the correlation analyses.

This poor covariation phenomenon could be partially explained by the complex gene-encoding-receptor expression mechanisms (Levitz et al., 2016; Payne, 2015; Schwappach, 2008; Stephenson et al., 2008), which can result in nuanced relationships between different receptor types and their encoding genes. The complexities of regulation mechanisms are governed by gene transcription, translation, proper protein folding and co-assembly in the endoplasmic reticulum, post-translational modifications, and subsequent trafficking to the target membrane surface along the secretory pathway (Fig. 1), as well as by remarkably different degradation rates for mRNA (minutes) and protein/receptor (hours to years) and different tissue situation between postmortem and alive (Buccitelli and Selbach, 2020; Payne, 2015). The complexity of biological regulation mechanisms seems to make the relationship between receptors and corresponding genes unpredictable (Buccitelli and Selbach, 2020; Payne, 2015). In accordance with this assumption, our results strongly suggest that in the human hippocampus region-specific receptor densities cannot simply be derived from the expression levels of corresponding genes, as reflected by their widely varying correlation coefficients. Furthermore, the mean correlation coefficient for the entire hippocampus ($r=0.36$) based on within-gene correlation analysis is weak and consistent with the correlation coefficients between genes and proteins reported in previous studies (Battle et al., 2015; Gry et al., 2009; Vogel et al., 2010; Zhang et al., 2014). In contrast, the across-gene correlation

analyses display relatively high correlations in CA ($r=0.57$) and DG ($r=0.71$), which further provide evidence for the fact that across-gene correlations are generally higher than within-gene correlations as the variation between genes largely exceeds the variation within genes (Buccitelli and Selbach, 2020; Fortelny et al., 2017). It is worth noting that only one previous study performed across-gene correlation analysis between mRNA and protein expression levels in human brain tissue, and described a moderate correlation ($r=0.54$, $r^2 = 0.29$) (Wang et al., 2019). This relatively low r^2 value means that the variability in mRNA levels explains less than 30% of the variability in protein levels in human brain tissue (Wang et al., 2019). This is in agreement with previous studies (de Sousa Abreu et al., 2009; Schwanhäußer et al., 2011; Vogel et al., 2010; Vogel and Marcotte, 2012), which demonstrated that around 40% of the variation in protein concentration may be explained by knowing mRNA abundances by using mammalian cell lines. Multiple post-transcriptional, translational, and protein degradation regulations play critical roles in contributing to explain the remaining more than 60% of the variation (Buccitelli and Selbach, 2020; Liu et al., 2016; Schwanhäußer et al., 2011; Vogel et al., 2010; Vogel and Marcotte, 2012). The relatively low explanatory power (2%) in the present study not only arises from the fact that we performed a within-gene correlation analysis, which generally yields lower values than across-gene analyses (Buccitelli and Selbach, 2020), but also from the important fact that mechanisms governing receptor expression are even more complex than those of proteins, since they additionally involve, e.g. receptor assembly and trafficking steps (Levitz et al., 2016; Schwappach, 2008; Stephenson et al., 2008). Taken together, gene expression levels in the CA and DG regions cannot be directly used as proxies for receptor densities in the respective hippocampal regions.

Interestingly, the range of correlation coefficients between receptors and their corresponding genes varied widely depending on the combination of receptor type and hippocampal region. Furthermore, the genes presenting moderate-to-strong correlations with corresponding receptors in CA have a striking overlap with the genes which display significantly different expression levels when comparing CA with DG. Importantly, we also demonstrated a differential pattern of high-strength correlation between receptor densities and expression levels of their respective encoding genes in the CA and DG regions. This different expression pattern between CA and DG regions may closely associate with their respectively specific principal cell types, physiological properties, and regional functional differentiation. The DG region, with its cellular layer consisting of granule cells, is the primary gateway of the trisynaptic loop, and plays a crucial role in code transformation between cortical afferents and the hippocampus, as well as in pattern separation (Acsády and Káli, 2007; Dieni et al., 2016). The CA region, with its pyramidal cell layer, possesses an unusual autoassociative memory network in the CA3 region and displays extensive interconnectivity among the CA3, CA2, and CA1 regions, which is critical for short-term and long-term potentiation (Acsády and Káli, 2007). Its unique synaptic plasticity underlying learning and memory occurs dynamically in the CA and DG regions, which necessarily requires the high participation of specific receptor trafficking (Collingridge et al., 2004; Groc and Choquet, 2020; Malinow and Malenka, 2002). Therefore, the different high-correlation patterns in CA and DG may imply that the receptor expression mechanisms are not only controlled by genetic information, but also associated with specific physiological properties of different cell types as well as with modifications of regional functional differentiation.

Concerning the AMPA receptor and its corresponding subunit genes, the hippocampus is enriched with GluA1, GluA2, and GluA3 receptor subunit expression, while it has a lower GluA4 expression (Yadav et al., 2017). This is in agreement with the expression levels we demonstrated here for the corresponding genes. Interestingly, the AMPA receptor and its corresponding subunit genes present completely opposite distribution patterns in the CA and DG regions. It is worth noting that there is a significant positive correlation between the AMPA receptor density with *GRIA2* subunit gene expression level in the CA region. Previ-

ous studies have demonstrated that the pyramidal cells of the CA region express AMPA receptors predominantly composed of the GluA2 subunit, which is encoded by *GRIA2*, in complex with either GluA1 or GluA3 subunits (Greger et al., 2017; Haselmann et al., 2018). In addition, the GluA2 subunit regulates the critical biophysical properties of the AMPA receptor, strongly impacts receptor assembly and trafficking, and plays critical roles in synaptic transmission and long-term synaptic plasticity (Greger et al., 2017; Haselmann et al., 2018; Isaac et al., 2007). Thus, the expressions of GluA2 and encoding gene *GRIA2* can be selectively changed in response to specific pattern stimuli at different regional synapses (Haselmann et al., 2018; Yadav et al., 2017). These findings further indicate that the relationships between gene expression levels and receptor densities are mediated and modified by multiple post-translational specific-regional factors (Battle et al., 2015).

Surprisingly, we observed some differences in gene expression levels between data obtained from our brain samples and data derived from the Allen Brain. This could be explained in part by the fact that different approaches were used to obtain the tissue/cell samples for RNA extraction, which resulted in the analysis of different cellular populations. Samples from the Allen Brain were obtained by “laser ablation” specifically from the granular layer of the DG and from the pyramidal layer of the CA region. Thus, these samples contained the cell bodies of the main neuronal types of these two regions, i.e., of granular and pyramidal cells, respectively. In contrast, we obtained our RNA expression levels from homogenates of the DG and CA regions. The DG homogenate encompassed the molecular, granular, and polymorph layers of the fascia dentata, as well as the CA4 region, while the CA homogenate contained the oriens, pyramidal, radiatum and lacunosum-molecular layers of the CA1-CA3 regions and the lucidum layer of CA3. Since the overall cellular organization of the DG and CA regions is more complex than hitherto thought and hippocampal layers differ in their cellular composition (Yao et al., 2021), each of these homogenates not only contained cell bodies of the principal neuronal type of each region, but also those of other cell types such as the radiatum giant cells or ivy cells in the radiatum layer (Gulyás et al., 1998; Somogyi et al., 2012), the neurogliaform, Cajal-Retzius, or RADI cells of the lacunosum-molecular layer (Fuentelba et al., 2010), or the oriens-lacunosum-moleculare interneuron of the oriens layer (Klausberger et al., 2003).

The present study provides novel insights into the relationships between neurotransmitter receptor densities and expression levels of corresponding genes by analyzing tissue acquired from the same brain samples. Thus, we have overcome the limitation of previous studies which analyzed gene (Hawrylycz et al., 2012) and receptor (Zilles and Palomero-Gallagher, 2017) data obtained from different donors (Hansen et al., 2022a; Murgaš et al., 2022; Zachlod et al., 2022). However, there are a few other limitations to note. The first one is the rather small sample size due to the constraints resulting from the scarcity of human brain samples adequately fulfilling the technical requirements for autoradiography (e.g., unfixed fresh brain tissue (Palomero-Gallagher and Zilles, 2018)) and RNA quantification (e.g., postmortem interval (Lipska et al., 2006)). Of note, all tissue samples were processed in one batch to minimize potential bias through technical factors. The results of the effect size, statistical power, and cross validation confirmed the robustness and reliability of our findings. Given the relatively small sample size, follow-up analyses using additional non-overlapping samples would be required to further support our findings. Another technically-related limitation arises from the fact that the available radioligands cannot specifically label the subunits of ionotropic receptors or, in some cases, distinguish different subtypes of partial metabotropic receptors (Murgaš et al., 2022). For example, [³H]-LY 341,495 labels both the mGluR2 and the mGluR3 receptors (Johnson et al., 1999), or [³H]-prazosin does not distinguish between α_1 subtypes (Zilles et al., 1991). These issues motivate the development of novel ligands for the visualization of specific receptor subtypes, as well as of advanced methods to specifically identify the subunits of ionotropic receptors. A further limitation in the chosen approach for gene expression analysis is

that 3'mRNA-Seq reads mostly map to the last exon of genes and are thus not suited to determine transcript isoform expression (Ma et al., 2019), which might be differentially regulated between hippocampal regions (Park and Farris, 2021). Moreover, the present analysis has targeted the CA-region as a whole, although it can be further subdivided into areas CA1, CA2 and CA3, which differ in their receptor architecture, e.g. regarding the densities of AMPA, GABA_A and GABA_B receptors (Palomero-Gallagher et al., 2020). These regionally specific differences in densities may have an influence on the rather weak correlation found here. Such a combination into one region was necessary, however, since it is not possible to accurately identify and dissect the CA1, CA2 and CA3 regions on cryosections inside the cryotome. Finally, spatial transcriptomics would conserve the spatial information of cells within a tissue and would display gene expression profiles of individual cells, which provide a powerful method for the further study.

Concluding, using data obtained from the same brain samples, we could demonstrate that the regional-specific densities of neurotransmitter receptors and expression levels of their corresponding genes show a poor covariation in the human hippocampal CA and DG regions. Correlation coefficients between receptors and their corresponding genes vary widely depending on the different combinations between the receptor-gene types and the hippocampal regions. These data suggest that receptor expression is not only controlled by the corresponding RNA levels, but also mediated and modified by regionally specific functional differentiation, as well as by other multiple post-translational factors. Finally, the neurotransmitter receptor and corresponding gene data from the same brain samples constitute a valuable open resource for future studies and are made available via the EBRAINS platform (<https://doi.org/10.25493/6BT0-BB1>).

Data availability

All data are available in the main text, the supplementary materials, or the EBRAINS platform. Additional data related to this paper may be requested from the authors.

Declaration of Competing Interest

Authors declare that they have no competing interests.

Credit authorship contribution statement

Ling Zhao: Software, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Thomas W. Mühlleisen:** Formal analysis, Writing – original draft, Writing – review & editing, Visualization. **Dominique I. Pelzer:** Investigation, Writing – original draft, Writing – review & editing, Visualization. **Bettina Burger:** Investigation, Writing – review & editing. **Eva C. Beins:** Investigation. **Andreas J. Forstner:** Investigation, Writing – review & editing. **Stefan Herms:** Investigation. **Per Hoffmann:** Investigation. **Katrin Amunts:** Conceptualization, Resources, Writing – review & editing, Funding acquisition. **Nicola Palomero-Gallagher:** Conceptualization, Resources, Data curation, Writing – review & editing, Project administration, Funding acquisition. **Sven Cichon:** Conceptualization, Resources, Writing – review & editing, Project administration, Funding acquisition.

Acknowledgments

This article is dedicated to Professor Dr. med. Dr. h.c. Karl Zilles, deceased the 26th of April 2020. He was fully involved in the planning of the project, and we are greatly indebted to his neuroscientific legacy.

Funding

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under the Specific

Grant Agreement 945539 (Human Brain Project SGA3), and from the Helmholtz Association's Initiative and Networking Fund through the Helmholtz International BigBrain Analytics and Learning Laboratory (HIBALL) under the Helmholtz International Lab grant agreement InterLabs-0015 and from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under grant agreement 491111487.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.neuroimage.2023.120095](https://doi.org/10.1016/j.neuroimage.2023.120095).

References

- Acsády, L., Káli, S., Scharfman, H.E., 2007. Models, structure, function: the transformation of cortical signals in the dentate gyrus. In: *Progress in Brain Research*. Elsevier, pp. 577–599.
- Aggleton, J.P., Pralus, A., Nelson, A.J.D., Hornberger, M., 2016. Thalamic pathology and memory loss in early Alzheimer's disease: moving the focus from the medial temporal lobe to Papez circuit. *Brain* 139, 1877–1890.
- Amunts, K., Mohlberg, H., Bludau, S., Zilles, K., 2020. Julich-brain: a 3D probabilistic atlas of the human brain's cytoarchitecture. *Science* 369, 988–992.
- Amunts, K., Zilles, K., 2015. Architectonic mapping of the human brain beyond Brodmann. *Neuron* 88, 1086–1107.
- Battle, A., Khan, Z., Wang, S.H., Mitrano, A., Ford, M.J., Pritchard, J.K., Gilad, Y., 2015. Impact of regulatory variation from RNA to protein. *Science* 347, 664–667.
- Berger, T., Lee, H., Young, A.H., Aarsland, D., Thuret, S., 2020. Adult hippocampal neurogenesis in major depressive disorder and Alzheimer's disease. *Trends Mol. Med.* 26, 803–818.
- Bernasconi, N., Bernasconi, A., Caramanos, Z., Antel, S.B., Andermann, F., Arnold, D.L., 2003. Mesial temporal damage in temporal lobe epilepsy: a volumetric MRI study of the hippocampus, amygdala and parahippocampal region. *Brain* 126, 462–469.
- Bernhardt, B.C., Bernasconi, A., Liu, M., Hong, S.J., Caldaírou, B., Goubran, M., Guiot, M.C., Hall, J., Bernasconi, N., 2016. The spectrum of structural and functional imaging abnormalities in temporal lobe epilepsy. *Ann. Neuro.* 80, 142–153.
- Bernhardt, B.C., Hong, S.J., Bernasconi, A., Bernasconi, N., 2015. Magnetic resonance imaging pattern learning in temporal lobe epilepsy: classification and prognostics. *Ann. Neurol.* 77, 436–446.
- Biegton, A., Rainbow, T.C., Mann, J.J., McEwen, B.S., 1982. Neurotransmitter receptor sites in human hippocampus: a quantitative autoradiographic study. *Brain Res.* 247, 379–382.
- Bludau, S., Mühlleisen, T.W., Eickhoff, S.B., Hawrylycz, M.J., Cichon, S., Amunts, K., 2018. Integration of transcriptomic and cytoarchitectonic data implicates a role for MAOA and TAC1 in the limbic-cortical network. *Brain Struct. Funct.* 223, 2335–2342.
- Blümcke, I., Thom, M., Aronica, E., Armstrong, D.D., Bartolomei, F., Bernasconi, A., Bernasconi, N., Bien, C.G., Cendes, F., Coras, R., Cross, J.H., Jacques, T.S., Kahane, P., Mathern, G.W., Miyata, H., Moshé, S.L., Oz, B., Özkarla, Ç., Perucca, E., Sisodiya, S., Wiebe, S., Spreafico, R., 2013. International consensus classification of hippocampal sclerosis in temporal lobe epilepsy: a Task Force report from the ILAE commission on diagnostic methods. *Epilepsia* 54, 1315–1329.
- Buccinelli, C., Selbach, M., 2020. mRNAs, proteins and the emerging principles of gene expression control. *Nat. Rev. Genet.* 21, 630–644.
- Burt, J.B., Demirtaş, M., Eckner, W.J., Navejar, N.M., Ji, J.L., Martin, W.J., Bernacchia, A., Anticevic, A., Murray, J.D., 2018. Hierarchy of transcriptomic specialization across human cortex captured by structural neuroimaging topography. *Nat. Neurosci.* 21, 1251–1259.
- Charan, J., Kantharia, N.D., 2013. How to calculate sample size in animal studies? *J. Pharmacol. Pharmacother.* 4, 303–306.
- Cohen, J., 1992. A power primer. *Psychol. Bull.* 112, 155.
- Collingridge, G.L., Isaac, J.T., Wang, Y.T., 2004. Receptor trafficking and synaptic plasticity. *Nat. Rev. Neurosci.* 5, 952–962.
- Cox, M.A.A., Cox, T.F., Chen, C.H., Härdle, W., Unwin, A., 2008. Multidimensional scaling. In: *Handbook of Data Visualization*. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 315–347.
- de Sousa Abreu, R., Penalva, L.O., Marcotte, E.M., Vogel, C., 2009. Global signatures of protein and mRNA expression levels. *Mol. Biosyst.* 5, 1512–1526.
- Debski, K.J., Ceglia, N., Ghestem, A., Ivanov, A.I., Brancati, G.E., Broer, S., Bot, A.M., Müller, J.A., Schoch, S., Becker, A., Loscher, W., Guye, M., Sassone-Corsi, P., Lukasiuk, K., Baldi, P., Bernard, C., 2020. The circadian dynamics of the hippocampal transcriptome and proteome is altered in experimental temporal lobe epilepsy. *Sci. Adv.* 6, eaat5979.
- Dieni, C.V., Panichi, R., Aimone, J.B., Kuo, C.T., Wadicke, J.I., Overstreet-Wadicke, L., 2016. Low excitatory innervation balances high intrinsic excitability of immature dentate neurons. *Nat. Commun.* 7, 11313.
- Ferreira, J.S., Papouin, T., Ladépêche, L., Yao, A., Langlais, V.C., Bouchet, D., Dulong, J., Mothe, J.-P., Sacchi, S., Pollegioni, L., Paoletti, P., Oliet, S.H.R., Groc, L., 2017. Co-agonists differentially tune GluN2B-NMDA receptor trafficking at hippocampal synapses. *Elife* 6, e25492.
- Fortelny, N., Overall, C.M., Pavlidis, P., Freue, G.V.C., 2017. Can we predict protein from mRNA levels? *Nature* 547, E19–E20.

- Fuentealba, P., Klausberger, T., Karayannis, T., Suen, W.Y., Huck, J., Tomioka, R., Rockland, K., Capogna, M., Studer, M., Morales, M., Somogyi, P., 2010. Expression of COUP-TFII nuclear receptor in restricted GABAergic neuronal populations in the adult rat hippocampus. *J. Neurosci.* 30, 1595–1609.
- Graebenitz, S., Kedo, O., Speckmann, E.J., Gorji, A., Panneck, H., Hans, V., Palomero-Gallagher, N., Schleicher, A., Zilles, K., Pape, H.C., 2011. Interictal-like network activity and receptor expression in the epileptic human lateral amygdala. *Brain* 134, 2929–2947.
- Greger, I.H., Watson, J.F., Cull-Candy, S.G., 2017. Structural and functional architecture of AMPA-type glutamate receptors and their auxiliary proteins. *Neuron* 94, 713–730.
- Groc, L., Choquet, D., 2020. Linking glutamate receptor movements and synapse function. *Science* 368, eaay4631.
- Gry, M., Rimini, R., Strömberg, S., Asplund, A., Pontén, F., Uhlén, M., Nilsson, P., 2009. Correlations between RNA and protein expression profiles in 23 human cell lines. *BMC Genom.* 10, 1–14.
- Gulyás, A.I., Tóth, K., McBain, C.J., Freund, T.F., 1998. Stratum radiatum giant cells: a type of principal cell in the rat hippocampus. *Eur. J. Neurosci.* 10, 3813–3822.
- Hall, J.E., Hall, M.E., 2020. Guyton and Hall textbook of Medical Physiology, 14th ed. Elsevier Health Sciences, Philadelphia, PA.
- Hansen, J.Y., Markello, R.D., Tuominen, L., Nørgaard, M., Kuzmin, E., Palomero-Gallagher, N., Dagher, A., Misic, B., 2022a. Correspondence between gene expression and neurotransmitter receptor and transporter density in the human brain. *Neuroimage* 264, 119671.
- Hansen, J.Y., Shafiei, G., Markello, R.D., Smart, K., Cox, S.M.L., Nørgaard, M., Beliveau, V., Wu, Y., Gallezot, J.-D., Aumont, É., Servaes, S., Scalza, S.G., DuBois, J.M., Wainstein, G., Bezin, G., Funk, T., Schmitz, T.W., Spreng, R.N., Galovic, M., Koepf, M.J., Duncan, J.S., Coles, J.P., Fryer, T.D., Aigbirhio, F.I., McGinnity, C.J., Hammers, A., Soucy, J.P., Baillet, S., Guimond, S., Hietala, J., Bedard, M.A., Leyton, M., Kobayashi, E., Rosa-Neto, P., Ganz, M., Knudsen, G.M., Palomero-Gallagher, N., Shine, J.M., Carson, R.E., Tuominen, L., Dagher, A., Misic, B., 2022b. Mapping neurotransmitter systems to the structural and functional organization of the human neocortex. *Nat Neurosci.*
- Haselmann, H., Mannara, F., Werner, C., Planagumà, J., Miguez-Cabello, F., Schmidl, L., Grünewald, B., Petit-Pedrol, M., Kirmse, K., Classen, J., Demir, F., Klöcker, N., Soto, D., Doose, S., Dalmau, J., Hallermann, S., Geis, C., 2018. Human Autoantibodies against the AMPA receptor subunit GluA2 induce receptor reorganization and memory dysfunction. *Neuron* 100, 91–105 e109.
- Hawrylycz, M., Miller, J.A., Menon, V., Feng, D., Dolbeare, T., Guillozet-Bongaarts, A.L., Jegga, A.G., Aronow, B.J., Lee, C.K., Bernard, A., Glasser, M.F., Dierker, D.L., Menche, J., Szafer, A., Collman, F., Grange, P., Berman, K.A., Mihalas, S., Yao, Z., Stewart, L., Barabási, A.L., Schulkin, J., Phillips, J., Ng, L., Dang, C., Haynor, D.R., Jones, A., Van Essen, D.C., Koch, C., Lein, E., 2015. Canonical genetic signatures of the adult human brain. *Nat. Neurosci.* 18, 1832–1844.
- Hawrylycz, M.J., Lein, E.S., Guillozet-Bongaarts, A.L., Shen, E.H., Ng, L., Miller, J.A., van de Lagemaat, L.N., Smith, K.A., Ebbert, A., Riley, Z.L., Abajian, C., Beckmann, C.F., Bernard, A., Bertagnoli, D., Boe, A.F., Cartagena, P.M., Chakravarty, M.M., Chapin, M., Chong, J., Dalley, R.A., Daly, B.D., Dang, C., Datta, S., Dee, N., Dolbeare, T.A., Faber, V., Feng, D., Fowler, D.R., Goldy, J., Gregor, B.W., Haradon, Z., Haynor, D.R., Hohmann, J.G., Horvath, S., Howard, R.E., Jeromin, A., Jochim, J.M., Kinnunen, M., Lau, C., Lazarz, E.T., Lee, C., Lemon, T.A., Li, L., Li, Y., Morris, J.A., Overly, C.C., Parker, P.D., Parry, S.E., Reding, M., Royall, J.J., Schulkin, J., Sequeira, P.A., Slaughterbeck, C.R., Smith, S.C., Sodt, A.J., Sunkin, S.M., Swanson, B.E., Vawter, M.P., Williams, D., Wohnoutka, P., Zielke, H.R., Geschwind, D.H., Hof, P.R., Smith, S.M., Koch, C., Grant, S.G.N., Jones, A.R., 2012. An anatomically comprehensive atlas of the adult human brain transcriptome. *Nature* 489, 391–399.
- Hogan, R.E., Wang, L., Bertrand, M.E., Willmore, L.J., Buchholz, R.D., Nassif, A.S., Csernansky, J.G., 2004. MRI-based high-dimensional hippocampal mapping in mesial temporal lobe epilepsy. *Brain* 127, 1731–1740.
- Hood, J.L., Emeson, R.B., 2011. Editing of neurotransmitter receptor and ion channel RNAs in the nervous system. *Curr. Top. Microbiol. Immunol.* 61–90.
- Impieri, D., Zilles, K., Niu, M., Rapan, L., Schubert, N., Galletti, C., Palomero-Gallagher, N., 2019. Receptor density pattern confirms and enhances the anatomic-functional features of the macaque superior parietal lobule areas. *Brain Struct. Funct.* 224, 2733–2756.
- Isaac, J.T.R., Ashby, M.C., McBain, C.J., 2007. The role of the gluR2 subunit in AMPA receptor function and synaptic plasticity. *Neuron* 54, 859–871.
- Johnson, B.G., Wright, R.A., Arnold, M.B., Wheeler, W.J., Ornstein, P.L., Schoepp, D.D., 1999. [3H]-LY341495 as a novel antagonist radioligand for group II metabotropic glutamate (mGlu) receptors: characterization of binding to membranes of mGlu receptor subtype expressing cells. *Neuropharmacology* 38, 1519–1529.
- Keren, H., Lev-Maor, G., Ast, G., 2010. Alternative splicing and evolution: diversification, exon definition and function. *Nat Rev Genet* 11, 345–355.
- Khan, A.F., Adewale, Q., Baumeister, T.R., Carbonell, F., Zilles, K., Palomero-Gallagher, N., Iturria-Medina, Y., Alzheimer's Disease Neuroimaging, I., 2021. Personalized brain models identify neurotransmitter receptor changes in Alzheimer's disease. *Brain* awab375.
- Klausberger, T., Magill, P.J., Márton, L.F., Roberts, J.D., Cobden, P.M., Buzsáki, G., Somogyi, P., 2003. Brain-state- and cell-type-specific firing of hippocampal interneurons *in vivo*. *Nature* 421, 844–848.
- Klioueva, N.M., Rademaker, M.C., Huitinga, I., Huitinga, I., Webster, M.J., 2018. Design of a European code of conduct for brain banking. In: *Handbook of Clinical Neurology*. Elsevier, pp. 51–81.
- Levitz, J., Habrian, C., Bharill, S., Fu, Z., Vafabakhsh, R., Isacoff, E.Y., 2016. Mechanism of assembly and cooperativity of homomeric and heteromeric metabotropic glutamate receptors. *Neuron* 92, 143–159.
- Lipska, B.K., Deep-Soboslay, A., Weickert, C.S., Hyde, T.M., Martin, C.E., Herman, M.M., Kleinman, J.E., 2006. Critical factors in gene expression in postmortem human brain: focus on studies in schizophrenia. *Biol. Psychiatry* 60, 650–658.
- Liu, Y., Beyer, A., Aebersold, R., 2016. On the dependency of cellular protein levels on mRNA abundance. *Cell* 165, 535–550.
- Ma, F., Fuqua, B.K., Hasin, Y., Yukhtman, C., Vulpe, C.D., Lusis, A.J., Pellegrini, M., 2019. A comparison between whole transcript and 3' RNA sequencing methods using Kapa and Lexogen library preparation methods. *BMC Genom.* 20, 9.
- Malinow, R., Malenka, R.C., 2002. AMPA receptor trafficking and synaptic plasticity. *Annu. Rev. Neurosci.* 25, 103–126.
- Merker, B., 1983. Silver staining of cell bodies by means of physical development. *J. Neurosci. Methods* 9, 235–241.
- Murgaš, M., Michenthaler, P., Reed, M.B., Gryglewski, G., Lanzenberger, R., 2022. Correlation of receptor density and mRNA expression patterns in the human cerebral cortex. *Neuroimage* 256, 119214.
- Nichols, T.E., Holmes, A.P., 2002. Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum. Brain Mapp.* 15, 1–25.
- Palomero-Gallagher, N., Kedo, O., Mohlberg, H., Zilles, K., Amunts, K., 2020. Multimodal mapping and analysis of the cyto- and receptorarchitecture of the human hippocampus. *Brain Struct. Funct.* 225, 881–907.
- Palomero-Gallagher, N., Zilles, K., Huitinga, I., Webster, M.J., 2018. Cyto- and receptor architeconic mapping of the human brain. In: *Handbook of Clinical Neurology*. Elsevier, pp. 355–387.
- Paoletti, P., Neyton, J., 2007. NMDA receptor subunits: function and pharmacology. *Curr. Opin. Pharmacol.* 7, 39–47.
- Papez, J.W., 1937. A proposed mechanism of emotion. *Arch. Neurol. Psychiatry* 38, 725–743.
- Park, J., Farris, S., 2021. Spatiotemporal regulation of transcript isoform expression in the hippocampus. *Front. Mol. Neurosci.* 14, 694234.
- Payne, S.H., 2015. The utility of protein and mRNA correlation. *Trends Biochem. Sci.* 40, 1–3.
- Perry, E.K., Court, J.A., Johnson, M., Smith, C.J., James, V., Cheng, A.V., Kerwin, J.M., Morris, C.M., Piggott, M.A., Edwardson, J.A., Birdsall, N.J.M., Turner, J.T., Perry, R.H., 1993. Autoradiographic comparison of cholinergic and other transmitter receptors in the normal human hippocampus. *Hippocampus* 3, 307–315.
- Robinson, M.D., Oshlack, A., 2010. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol.* 11, R25.
- Schober, P., Boer, C., Schwarte, L.A., 2018. Correlation coefficients: appropriate use and interpretation. *Anesth. Analg.* 126, 1763–1768.
- Scholtens, L.H., Schmidt, R., de Reus, M.A., van den Heuvel, M.P., 2014. Linking macroscale graph analytical organization to microscale neuroarchitectonics in the macaque connectome. *J. Neurosci.* 34, 12192.
- Schulz, H., Ruppert, A.-K., Herms, S., Wolf, C., Mirza-Schreiber, N., Stegle, O., Czamara, D., Forstner, A.J., Sivalingam, S., Schoch, S., Moebus, S., Pütz, B., Hillmer, A., Fricker, N., Vatter, H., Müller-Myhsok, B., Nöthen, M.M., Becker, A.J., Hoffmann, P., Sander, T., Cichon, S., 2017. Genome-wide mapping of genetic determinants influencing DNA methylation and gene expression in human hippocampus. *Nat. Commun.* 8, 1511.
- Schurter, A., Pereira, F., Treisman, A., Cohen Jonathan, D., 2010. Reproducibility distinguishes conscious from nonconscious neural representations. *Science* 327, 97–99.
- Schwanhäusser, B., Busse, D., Li, N., Dittmar, G., Schuchhardt, J., Wolf, J., Chen, W., Selbach, M., 2011. Global quantification of mammalian gene expression control. *Nature* 473, 337–342.
- Schwappach, B., 2008. An overview of trafficking and assembly of neurotransmitter receptors and ion channels (Review). *Mol. Membr. Biol.* 25, 270–278.
- Selkoe, D.J., 2001. Alzheimer's disease: genes, proteins, and therapy. *Physiol. Rev.* 81, 741–766.
- Somogyi, J., Szabo, A., Somogyi, P., Lamsa, K., 2012. Molecular analysis of ivy cells of the hippocampal CA1 stratum radiatum using spectral identification of immunofluorophores. *Front. Neural Circuits* 6.
- Stephenson, F.A., Cousins, S.L., Kenny, A.V., 2008. Assembly and forward trafficking of NMDA receptors (Review). *Mol. Membr. Biol.* 25, 311–320.
- Symonds, J.D., Zuberi, S.M., Johnson, M.R., 2017. Advances in epilepsy gene discovery and implications for epilepsy diagnosis and treatment. *Curr. Opin. Neurol.* 30.
- Szot, P., White, S.S., Greenup, J.L., Leverenz, J.B., Peskind, E.R., Raskind, M.A., 2005. $\alpha 1$ -Adrenoreceptor in human hippocampus: binding and receptor subtype mRNA expression. *Brain Res. Mol. Brain Res.* 139, 367–371.
- Thom, M., 2014. Review: hippocampal sclerosis in epilepsy: a neuropathology review. *Neuropathol. Appl. Neurobiol.* 40, 520–543.
- Tremblay, E., Represa, A., Ben-Ari, Y., 1985. Autoradiographic localization of kainic acid binding sites in the human hippocampus. *Brain Res.* 343, 378–382.
- van Strien, N.M., Cappaert, N.L.M., Witter, M.P., 2009. The anatomy of memory: an interactive overview of the parahippocampal–hippocampal network. *Nat. Rev. Neurosci.* 10, 272–282.
- Vogel, C., de Sousa Abreu, R., Ko, D., Le, S.Y., Shapiro, B.A., Burns, S.C., Sandhu, D., Boutz, D.R., Marcotte, E.M., Penalva, L.O., 2010. Sequence signatures and mRNA concentration can explain two-thirds of protein abundance variation in a human cell line. *Mol. Syst. Biol.* 6, 400.
- Vogel, C., Marcotte, E.M., 2012. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nat. Rev. Genet.* 13, 227–232.

- Vogel, J.W., La Joie, R., Grothe, M.J., Diaz-Papkovitch, A., Doyle, A., Vachon-Presseau, E., Lepage, C., Vos de Wael, R., Thomas, R.A., Iturria-Medina, Y., Bernhardt, B., Rabinovici, G.D., Evans, A.C., 2020. A molecular gradient along the longitudinal axis of the human hippocampus informs large-scale behavioral systems. *Nat. Commun.* 11, 960.
- Wang, D., Eraslan, B., Wieland, T., Hallström, B., Hopf, T., Zolg, D.P., Zecha, J., Asplund, A., Li, L.H., Meng, C., Frejno, M., Schmidt, T., Schnatbaum, K., Wilhelm, M., Ponten, F., Uhlen, M., Gagneur, J., Hahne, H., Kuster, B., 2019. A deep proteome and transcriptome abundance atlas of 29 healthy human tissues. *Mol. Syst. Biol.* 15, e8503.
- Yadav, R., Dravid, S.M., Yuan, H., Traynelis, S.F., 2017. AMPA Receptors: Molecular Biology and Pharmacology☆. The Curated Reference Collection in Neuroscience and Biobehavioral Psychology. Elsevier Science Ltd.
- Yao, Z., van Velthoven, C.T.J., Nguyen, T.N., Goldy, J., Sedeno-Cortes, A.E., Baftizadeh, F., Bertagnoli, D., Casper, T., Chiang, M., Crichton, K., Ding, S.L., Fong, O., Garren, E., Glandon, A., Gouwens, N.W., Gray, J., Graybuck, L.T., Hawrylycz, M.J., Hirschstein, D., Kroll, M., Lathia, K., Lee, C., Levi, B., McMillen, D., Mok, S., Pham, T., Ren, Q., Rimorin, C., Shapovalova, N., Sulc, J., Sunkin, S.M., Tieu, M., Torkelson, A., Tung, H., Ward, K., Dee, N., Smith, K.A., Tasic, B., Zeng, H., 2021. A taxonomy of transcriptomic cell types across the isocortex and hippocampal formation. *Cell* 184, 3222–3241 e3226.
- Zachlod, D., Bludau, S., Cichon, S., Palomero-Gallagher, N., Amunts, K., 2022. Combined analysis of cytoarchitectonic, molecular and transcriptomic patterns reveal differences in brain organization across human functional brain systems. *Neuroimage* 257, 119286.
- Zhang, B., Wang, J., Wang, X., Zhu, J., Liu, Q., Shi, Z., Chambers, M.C., Zimmerman, L.J., Shaddox, K.F., Kim, S., Davies, S.R., Wang, S., Wang, P., Kinsinger, C.R., Rivers, R.C., Rodriguez, H., Townsend, R.R., Ellis, M.J.C., Carr, S.A., Tabb, D.L., Coffey, R.J., Slebos, R.J.C., Liebler, D.C., Carr, S.A., Gillette, M.A., Klauser, K.R., Kuhn, E., Mani, D.R., Mertins, P., Ketchum, K.A., Paulovich, A.G., Whiteaker, J.R., Edwards, N.J., McGarvey, P.B., Madhavan, S., Wang, P., Chan, D., Pandey, A., Shih, I.-M., Zhang, H., Zhang, Z., Zhu, H., Whiteley, G.A., Skates, S.J., White, F.M., Levine, D.A., Boja, E.S., Kinsinger, C.R., Hiltke, T., Mesri, M., Rivers, R.C., Rodriguez, H., Shaw, K.M., Stein, S.E., Fenyo, D., Liu, T., McDermott, J.E., Payne, S.H., Rodland, K.D., Smith, R.D., Rudnick, P., Snyder, M., Zhao, Y., Chen, X., Ransohoff, D.F., Hoofnagle, A.N., Liebler, D.C., Sanders, M.E., Shi, Z., Slebos, R.J.C., Tabb, D.L., Zhang, B., Zimmerman, L.J., Wang, Y., Davies, S.R., Ding, L., Ellis, M.J.C., Reid Townsend, R.the, N.C., National Cancer Institute Clinical Proteomics Tumor Analysis, C., 2014. Proteogenomic characterization of human colon and rectal cancer. *Nature* 513, 382–387.
- Zhong, S., Ding, W., Sun, L., Lu, Y., Dong, H., Fan, X., Liu, Z., Chen, R., Zhang, S., Ma, Q., Tang, F., Wu, Q., Wang, X., 2020. Decoding the development of the human hippocampus. *Nature* 577, 531–536.
- Zilles, K., Gross, G., Schleicher, A., Schildgen, S., Bauer, A., Bahro, M., Schwendemann, G., Zech, K., Kolassa, N., 1991. Regional and laminar distributions of $\alpha 1$ -adrenoceptors and their subtypes in human and rat hippocampus. *Neuroscience* 40, 307–320.
- Zilles, K., Palomero-Gallagher, N., 2017. Multiple transmitter receptors in regions and layers of the human cerebral cortex. *Front. Neuroanat.* 11, 78.
- Zilles, K., Schleicher, A., Palomero-Gallagher, N., Amunts, K., Toga, A.W., Mazziotta, J.C., 2002. Quantitative analysis of cyto- and receptor architecture of the human brain. In: *Brain Mapping: The Methods (Second Edition)*. Academic Press, San Diego, pp. 573–602.