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Molecular fingerprinting of principal neurons in the rodent hippocampus: a neuroinformatics approach

DJ Hamilton, CM White, CL Rees, DW Wheeler, and GA Ascoli

Krasnow Institute for Advanced Study, George Mason University, Fairfax, VA, United States

Abstract

Neurons are often classified by their morphological and molecular properties. The online knowledge base Hippocampome.org primarily defines neuron types from the rodent hippocampal formation based on their main neurotransmitter (glutamate or GABA) and the spatial distributions of their axons and dendrites. For each neuron type, this open-access resource reports any and all published information regarding the presence or absence of known molecular markers, including calcium-binding proteins, neuropeptides, receptors, channels, transcription factors, and other molecules of biomedical relevance. The resulting chemical profile is relatively sparse: even for the best studied neuron types, the expression or lack thereof of fewer than 70 molecules has been firmly established to date. The mouse genome-wide in situ hybridization mapping of the Allen Brain Atlas provides a wealth of data that, when appropriately analyzed, can substantially augment the molecular marker knowledge in Hippocampome.org. Here we focus on the principal cell layers of dentate gyrus (DG), CA3, CA2, and CA1, which together contain approximately 90% of hippocampal neurons. These four anatomical parcels are densely packed with somata of mostly excitatory projection neurons. Thus, gene expression data for those layers can be justifiably linked to the respective principal neuron types: granule cells in DG and pyramidal cells in CA3, CA2, and CA1. In order to enable consistent interpretation across genes and regions, we screened the whole-genome dataset against known molecular markers of those neuron types. The resulting threshold values allow over 6000 very-high confidence (>99.5%) expressed/not-expressed assignments, expanding the biochemical information content of Hippocampome.org more than five-fold. Many of these newly identified molecular markers are potential pharmacological targets for major neurological and psychiatric conditions. Furthermore, our approach yields reasonable expression/non-expression estimates for every single gene in each of these four neuron types with >90% average confidence, providing a considerably complete genetic characterization of hippocampal principal neurons.

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Keywords

genetic characterization of hippocampal principal neurons; rodent hippocampal formation; neuron types; morphological and molecular properties; molecular biomarkers; neuroinformatics

1. Introduction

The hippocampus plays a central role in many cognitive functions, including memory consolidation and retrieval, spatial navigation, and context representation (Kumaran et al., 2016). The primary conduits of information processing in the hippocampal formation are granule cells in the dentate gyrus (DG) and pyramidal cells in the Cornu Ammonis (CA) areas. These glutamatergic neurons are densely packed in the principal cell layers, namely stratum granulosum (SG) in DG and stratum pyramidale (SP) in CA1, CA2, and CA3. The open-access online knowledge base Hippocampome.org separates excitatory and inhibitory neurons according to the location of their axons and dendrites across the sub-regions and layers of the hippocampal formation (Wheeler et al., 2015). Granule cells in SG extend dendrites in superficial layers of DG and project axons to CA3. Pyramidal cells in CA1-3 have dendrites spanning all layers within their respective sub-regions and axons projecting to other sub-regions. Hippocampome.org also links neuron types to any published information on biochemical expression or lack thereof. The literature describes over 90 molecular markers for hippocampal neurons types. Granule cells have information for 66 markers, and CA1, CA2, and CA3 pyramidal cells for 63, 49, and 57, respectively.

Although appreciably richer than for most other cell types in mammalian brains, the available chemical characterization of hippocampal principal neurons is still miniscule compared to the ~20,000 genes in the mouse genome. Several recent efforts aimed to expand the evidence base for molecular phenotyping of neurons. Within area CA1 of the rodent hippocampus, single-cell RNA-seq identified major cellular families including glia, pyramidal neurons, and interneurons (Zeisel et al., 2015). Although the morphology was recovered only for a small sample of cells in this work, post-hoc analysis of those results putatively identified several known classes of GABAergic inhibitory neurons based on previously established molecular markers (Harris et al., 2015). A subsequent comprehensive investigation of hippocampal gene expression also relied on RNA-seq in glutamatergic excitatory neurons (Cembrowski et al. 2016b). Molecular classification using state-of-the-art genetic techniques is most prominently advancing in cortical neurons (Tasic et al., 2016), where it has been paired with electrophysiological recording (Cadwell et al., 2016) and single-nucleotide sequencing of individual human cells (Lodato et al., 2015).

To obtain a more comprehensive molecular marker fingerprint of hippocampal neurons, we leverage machine-readable repositories such as the mouse genome in situ hybridization (ISH) database of the Allen Brain Atlas (ABA; Lein et al., 2007). The vast majority of cell bodies in the principal layers of the hippocampus correspond to distinct neuron types: granule cells in DG and pyramidal cells in CA1, CA2, and CA3. Thus, the expression profiles of these anatomical parcels can be confidently associated with the corresponding principal neuron types (see ‘Neurobiological rationale’ in Materials and Methods). Here we

describe a novel neuroinformatics approach to obtaining, mining, and interpreting these data. The resulting wealth of information dramatically increases the biochemical content of Hippocampome.org, providing a considerably complete genetic characterization of hippocampal principal neurons. The entire dataset, publicly available online, may clarify the molecular functioning of the hippocampus and provide a better mechanistic understanding of the interaction between drugs and their gene targets in this important brain region.

2. Materials and Methods

The following information-processing workflow establishes a mechanism to meaningfully relate ABA ISH expression levels to Hippocampome.org literature-based molecular markers.

2.1 Map Hippocampome.org proteins of interest to associated genes

Each of the Hippocampome.org protein markers known to be expressed or not expressed in DG granule cells, and CA1, CA2 or CA3 pyramidal cells was cross-referenced to its respective mouse genes using UniProtKB (uniprot.org/uniprot). For instance, substance P receptor is translated from the *Tacr1* gene. In case of multiple results, we consider all genes associated with a specific protein that are included in ABA. For example, Hippocampome.org protein mGluR1 maps to genes *Gprc1a*, *Grm1*, and *Mglur1*. In our analysis, however, we only selected *Grm1* based on ABA ISH expression information availability.

2.2 Extract and parse out ISH expression-energy

The quantified ISH expression values for all of the genes in the ABA dataset were downloaded via its Application Programming Interface (API) by structure (brain-map.org/api). We analyzed the 124 GBytes of pulled XML-formatted metadata locally using a custom utility written in the Python programming language leveraging standard Python XML libraries. First, we extracted the expression-density and expression-energy values (Ng et al., 2007) into a table for analysis. The expression density is defined as the sum of ISH expressing pixels divided by the sum of all pixels in a region of interest (e.g. CA1 stratum pyramidale). The expression energy is computed by multiplying the expression density by the average intensity of all expressing pixels in the region of interest. These values are available in ABA for each gene and region of interest. Most genes were interrogated in multiple experiments yielding multiple values of expression energy. Since the variance across experiments is small, we extracted the mean for every gene in each region of interest.

2.3 Normalize expression values

The expression energy values vary substantially across regions and genes. Cross-regional variability depends on the considerably diverse density and size of cell bodies among other factors. Among different genes, ISH intensity is highly dependent on the specific antisense probe utilized. Thus, in order to compare expression energy in the four hippocampal principal cell layers over all mouse genes, the extracted raw values need to be normalized. To achieve this, we first identified for each gene the minimum and maximum expression energies across all anatomical regions, and then normalized these values to between 0 and 1 for each gene. Second, for each of the four regions of interest, we determined the minimum

and maximum of the obtained values across all genes, and renormalized the results to the 0–1 range.

2.4 Identify Expressed/Not-Expressed thresholds and confidence values

Normalized expression values close to one or zero indicate that the given gene is likely to be expressed or not expressed, respectively, in the considered region. However, intermediate values may be more difficult to interpret. To establish and evaluate suitable numerical thresholds, we leveraged known molecular marker expression for principal neuron types from Hippocampome.org (Table 1). Specifically, the expression state is reported for a total of 229 combinations of molecular marker and principal cell layer (114 expressed, 115 not expressed). Bootstrapping this dataset, we identified three normalized expression thresholds at high (~0.7122), low (0.0245), and intermediate (0.2008) values. All of the Hippocampome.org molecular markers with normalized expression values above the high threshold were known to be expressed; all of the Hippocampome.org molecular markers with normalized expression values below the low threshold were known to be not expressed. Thus, these high and low thresholds predict expression and lack thereof with high confidence (~1/229 = 99.5%). Among the Hippocampome.org molecular markers with normalized expression values between the high and low thresholds, most of those above the intermediate threshold were known to be expressed, while most of those below were known to be not expressed. However, the confidence of these intermediate assignments drops as the normalized expression values approach the intermediate threshold. The minimum confidence (at the intermediate threshold) is 76.9%, meaning that 23.1% of known Hippocampome.org molecular markers are expressed while displaying a normalized expression value below 0.2008 or are not expressed while displaying a normalized expression value above 0.2008.

2.5 Neurobiological rationale

A key conceptual distinction needs to be recognized between anatomical parcels and cell types. Hippocampome.org divides the hippocampal formation (DG, CA3, CA2, CA1, subiculum, and entorhinal cortex) in 26 laminar parcels (Wheeler et al., 2015). The majority of these anatomical subdivisions, illustrated in Fig. 1, are occupied by a broad diversity of neuron types. For example, CA3 stratum lucidum (slu in Fig. 1A) contains somata from at least 10 morphologically distinct neuron types. Since the relative abundance of these cellular types is unknown, the ISH expression pattern of stratum lucidum cannot be related to that of any specific neuron. Recognizing this major confound, this analysis is strictly delimited to the principal cell layers (highlighted in blue and purple in Fig. 1A). These anatomical parcels are >90% occupied by granule cells in DG and pyramidal cells in CA3, CA2, CA1 (Cassell and Brown, 1977; Seress and Pokorny, 1981; Patton and McNaughton, 1995; Rapp and Gallagher, 1996; Jinno and Kosaka, 2010;). Only a minority (<10%) of cells in these principal layers may still be glia and various types of interneurons. This fact provides an essential logical link between anatomy and neuron types. Namely, if one of these parcels is negative (or positive) for a given gene in the Allen Brain Atlas, it is mathematically certain that the corresponding principal neuron types (granule cells in DG and pyramidal cells in CA) would also be negative (or positive) for that same gene.

2.6. Detailed protocol

The entirety of the Allen data that we utilize here comes from in situ hybridization (ISH) experiments and was obtained via Application Programming Interface (API). From the Allen Brain Atlas “Example Queries for Experiment Metadata” listed in the help page, we followed the link “All Mouse Brain ISH experiments in comma delimited format”. In the above, the original example had “num_rows=50”. We changed 50 to 50000, allowing for all 26,069 rows of metadata to be obtained. This yielded ready-for-download comma separated value files such as the following example: “plane, gene, section_data_set_id, coronal, Calb1, 71717640”. Next, a custom-written python program pulled all “structure_unionize” XML files for each of the 26,069 ISH experiments. This was achieved by reading each “section_data_set_id” from the metadata previously obtained, constructing the appropriate URL, and then saving the resultant XML file to a local folder for later post-processing. Since our focus was on principal cell layers of the hippocampal sub-regions DG, CA1, CA2, and CA3, the “structure-id-path” corresponding to these four parcels were used to pull from each of the 26069 XML files into a single CSV file. These core data were then utilized to perform subsequent analyses.

3. Results

From the ABA dataset, we extracted the normalized expression information for 19,934 genes in each of the four principal cell layers (one example is illustrated in Fig. 1). Of the resulting 79,736 values, 1730 were above the high threshold, indicating expression of the corresponding genes in those anatomical parcels with high confidence. Furthermore, 5156 values were below the low threshold, indicating lack of expression of the corresponding genes in those anatomical parcels. In total, this amounted to 6886 highconfidence assignments. Among these, 42 involved Hippocampome.org molecular markers with previously unknown expression status in principal cells (Table 1). For the users’ convenience, all molecular markers pertaining to the high-confidence assignments are linked online to the Gene Ontology (see ‘Molecular markers’ at Hippocampome.org/help).

Several of the newly found gene expression assignments are of potential pharmacological interest. In particular, it is important to consider the relationships between specific drugs, their protein targets, the mechanism of interaction, the regional distribution within the brain, and neuron-type specificity (Ferrante et al., 2008). To explore these factors, we queried the Drug Gene Interaction Database (DGIdb.org: Griffith et al., 2013; Wagner et al., 2016) and DrugBank (drugbank.ca: Law et al., 2014) with a sample of the genes found to be expressed in all hippocampal principal neurons (Table 2). Given the importance of the hippocampal formation in memory and cognition, drugs relevant to neurological or psychiatric therapeutic interventions are especially noteworthy. For example, Levetiracetam inhibits Synaptic vesicle glycoprotein 2A (a selective enhancer of low-frequency neurotransmission) and is used as an anti-epileptic to treat partial onset seizures (Law et al., 2014). Phenobarbital (Daniell, 1994) and Acamprosate (Mann et al., 2008), both antagonists of the NMDA-sensitive ionotropic glutamate receptor 1 (a key regulator of synaptic and developmental plasticity) are prescribed respectively to prevent convulsion and relapse in alcoholism.

For other drugs used in diseases not traditionally related to the hippocampal formation, the presence of their effectors in hippocampal principal cells raises the possibility of cognitive side effects. For example, Baclofen is an agonist of metabotropic GABA receptors used to alleviate spasticity in multiple sclerosis (Rask-Andersen et al., 2011). Tetracycline inhibits the prion protein, prolonging survival of animals with Creutzfeldt-Jakob disease (De Luigi et al., 2008). Nicotinamide, a binder of the poly-ADP-ribosepolymerase 1 (a transcription regulator), has been shown to increase endurance in mice (Chen et al., 2002). The same considerations of possible hippocampal-mediated collateral effects apply to several anticancer agents, such as Docetaxel, a binder of nuclear receptor transcription activators (Ikezoe et al., 2004) and Dasatinib, which targets a tyrosine-protein kinase proto-oncogene (Nam et al., 2005).

In addition to the high-confidence (>99.5%) assignments of expressed and non-expressed genes in the four principal cell layers, an increasing number of assignments can be estimated with decreasing confidence by relaxing the threshold. For example, as many as 35,704 assignments (8688 expressed, 27,016 not expressed), or nearly 45% of the total mouse genome, can be called with >95% confidence (Figure 2). The full dataset, searchable and sortable by gene, normalized expression value, anatomical parcel, assignment, and confidence, is publicly available for download and re-analysis in plain comma-separated-value format (hippocampome.org/GeneParcelExpressionConfidence). Furthermore, to facilitate the investigation of the expression confidence by gene and hippocampal principal neuron type, we freely provide the custom-developed Gene Expression Analyzer, a web-based interactive tool (hippocampome.org/genexan). Moving the sliders allows the user to observe the impact of threshold settings on characterization of expressed/not-expressed levels and the associated confidence computed from the concordance with Hippocampome.org molecular marker knowledge (Figure 3).

In the extreme, all 19,934 genes can be called in each hippocampal principal neuron type (yielding 33,713 expressed and 46,023 non-expressed assignments) by using the single intermediate threshold, with an average confidence of 91.3%. Although these data must clearly be treated as exploratory trends only, they nonetheless provide a potentially valuable source of interesting hypotheses and speculations that can be useful in designing new neurochemical experiments. For example, while for the majority (>75%) of genes the ‘likely’ assignment is uniform throughout the hippocampal principal neuron types (i.e., either expressed in all or not expressed in all), as many as 4829 genes appear to ‘single out’ one of the principal cell layers as an expressing or non-expressing outlier.

In particular, 375 genes are likely expressed specifically in DG granule cells and not in CA pyramidal cells. Among these, *Zfp90*, *Lct*, *Mcm6*, and *Prox1* all stand out at relatively high assignment confidence (>95%). Moreover, 361 genes display the opposite pattern (expressed in CA pyramidal cells and absent in DG granule cells). Similarly, 541 genes (most notably *Zfp551*, *Epb4.115*, and *Pgcp*) are specifically expressed in CA3 among all hippocampal principal cells, while only 39 are expressed throughout the hippocampus except CA3 pyramidal cells. Pyramidal cells in CA2 can be distinguished by up to 414 specifically expressed genes (including *Zfp616*, *Cldn21*, *Rfc4*, *Leo1*, and *Kcp*), and as many as 734 non-expressing markers. Lastly, 810 genes appear to be expressed exclusively in CA1 pyramidal

cells (e.g. *Csf2*, *Gdf5*, *Ptafr*, *Fsd2*, and *Hoxc13*) against 234 following the opposite distribution.

4.0 Discussion

The industrial-scale adoption of brain-wide, full-genome, cellular-level, high-throughput ISH, on which the Allen Brain Atlas is based (Ng et al., 2009), was originally developed and demonstrated in the mouse hippocampus (Lein et al., 2004). The present study to molecularly profile the principal cells of the hippocampal formation is thus a natural application of available neuroinformatics techniques. This utilization of a well-established source ensures homogeneous experimental conditions (e.g. histological protocol), but prevents analysis of the gene that can vary by activity state. While gene expression assessment by ISH colorimetry remains a semi-quantitative measurement, we described here a novel approach to derive stringent thresholds from normalized expression levels in principal cell layers by direct comparison with Hippocampome.org molecular marker evidence for the corresponding principal neuron types. Such a bootstrapping operation relies on the interpretation of the molecular designations reported in Hippocampome.org as the ‘gold standard.’ This knowledge is gleaned by manually mining peer-reviewed literature largely based on immuno-histochemical protein detection.

This strategy provides high-confidence expression/non-expression predictions for thousands of genes. Furthermore, because the glutamatergic, excitatory, projecting principal cells considered here constitute the most numerous neuronal populations in the hippocampus, the resulting molecular profiling is applicable to the vast majority (~90%) of hippocampal neurons. Although comprehensive biochemical phenotyping of neuron types is still rare, the scientific literature provides abundant evidence indirectly corroborating these new findings. For example, multiple investigations ratify the expression of glutamate receptor channel throughout all principal cells in the mouse hippocampus (e.g. Seeburg et al., 2001). Furthermore, the selective expression of *Cfp* in DG granule cells is consistent with previously reported observations (Encinas et al., 2011). Similarly, *Dsp* is lacking in CA pyramidal cells (Fig. 1), but expressed in DG (Hagihara et al., 2009). This same earlier study also describes *Tyro3* expression in the principal layers of “Ammon’s horn” (i.e. CA1-3), again in line with our results. Other findings likewise describe an abundance of *Stathmin* in the CA1 and CA3 sub-regions of the mouse hippocampus (Gozal et al., 2002), also confirmed by our data.

Although the principal neurons of the mammalian hippocampus are densely packed in their respective layers, they do not necessarily constitute molecularly homogeneous populations. Instead, gradients of genetic expression might exist in both the longitudinal and transverse axes (Cembrowski et al., 2016a; Leonardo et al., 2006). More generally, while most studies to date focused on the dorsal hippocampus, substantial cognitive (e.g. fear vs. declarative learning), connectivity (Ropireddy and Ascoli, 2011; Cardoso-Cruz et al., 2014; Krook-Magnuson et al., 2012; Lee et al., 2014), and biophysical (Graves et al., 2012; Malik et al., 2016; Marcelin et al., 2012b; Marcelin et al., 2012a) differences exist in the septotemporal directions towards the posterior and ventral regions. Given the explicit anatomical mapping of the ISH intensity and density values across the rostro-caudal extent in the ABA, the

present study could in principle be expanded in the future to account for these gradients, possibly refining the neuron type classification of Hippocampome.org.

Given such knowledge, it may appear surprising that the machine-readable data available through the Allen Brain Atlas only provides an integral view across the septo-temporal hippocampus axis.

Specifically, “structure_unionize” does not differentiate rostral-caudal information, but instead summarizes expression-density and expression-energy across the entire rostral-caudal extent. Thus, any extension of the findings reported here would require substantial manual involvement to save and mine the section-by-section data, or custom code development to scrape the values from the database. Similarly, all other major available neuroinformatics resources (e.g. BrainInfo, SenseLab, NeuroLex, BAMS, etc.) also always discriminate between hippocampal regions and among layers, but typically not across the rostral-caudal gradient. This is because the cell-type differences both in the transverse (e.g. DG vs. CA3 vs CA1) and in the laminar (e.g. oriens vs pyramidal vs radiatum layers) dimensions are generally considered more substantial than in the longitudinal (rostral-caudal) direction. Neuron types appear to change categorically (e.g. in terms of circuitry) between CA3 and CA1 and across layers, but only gradually from the septal to the temporal pole (Bernard and Wheal, 1995; Freund and Buzsáki, 1996; Deadwyler and Hampson, 1999; Andersen et al., 2007; Houser, 2007; Cutsuridis et al., 2010).

Many of the >6,000 novel high-confidence assignments successfully derived from the genome-wide mouse brain atlas starting from the baseline of knowledge provided by Hippocampome.org can be further interpreted in light of their potential pharmacological applications. In addition to this capability, we showed that the expression (or lack thereof) can also be reasonably estimated for the rest of genes falling between the stringent high- and low-thresholds, along with an a priori confidence computed as the relative concordance with known Hippocampome.org highly curated molecular markers. The field of neuroscience, in general, benefits greatly by having readily available on-line access to shared experimental evidence, as demonstrated here. In the same spirit, we publicly released online both the full analyzed dataset and the web-based Gene Expression Analyzer for interactive evaluation of these data. Integration of these functionalities in Hippocampome.org conforms to the standards of the Linked Open Drug Data task force within the World Wide Web Consortium's Health Care and Life Sciences Interest Group (Samwald et al., 2011).

Altogether, these tools and data provide the ability to estimate the expression of any and all mouse genes in the principal neuron types of the hippocampus with confidence ranging from ~77% to >99% (average 91%). From this extension, several potential molecular markers emerge for each of the principal cell layers. While the notion of redefining hippocampal anatomy by gene expression is not new (Lein et al., 2005), these results nonetheless lend themselves to potentially fruitful drug-mining. According to prominent theories of learning and memory, the principal neurons of DG, CA3, CA2, and CA1 serve distinctly dissociated computational functions (Kumaran et al., 2016). Particularly, granule cells are responsible for pattern separation by orthogonalization; CA3 pyramidal cells perform pattern completion by auto-association; CA2 are less plastic and appear involved in social cognition; and CA1

pyramidal cells are tasked with signal recognition by comparing the hippocampal output with the cortical input (Marr, 1971; McClelland et al., 1995; Kumaran et al., 2016). Thus, the identification of psychotropic and neuroactive substances selectively interacting with the proteins specifically expressed in each of these neuron types could have exquisitely fine-tuned effects on complex disorders often associated with the hippocampal formation, including amnesia, epilepsy, Alzheimer's disease, autism, and schizophrenia.

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Highlights

- Neurons classified by morphological and molecular properties
- Online knowledge base Hippocampome.org
- Allen Brain Atlas provides a wealth of gene expression data
- Our approach yields reasonable expression/non-expression estimates for every gene in each of four hippocampal principal neuron types with >90% average confidence
- Considerably complete genetic characterization
- Newly identified biomarkers are potential pharmacological targets for major neurological and psychiatric conditions

Figure 1. (A) Allen Brain Atlas (ABA) anatomical outline. The principal layers are highlighted (DG-sg: purple; CA3sp, CA2sp, and CA1sp: blue) to distinguish them from the rest of the hippocampal formation in which no individual neuron type dominates; (B) sample raw ABA ISH image & (C) gene Dsp expressing in DG-sg, not expressing in CA3sp, CA2sp, and CA1sp

Figure 2.

Expression- and non-expression-thresholds for normalized ISH values, proportion of gene assigned to the four principal neuron types (out of $19,934 \times 4 = 79,736$ possible combinations), and corresponding confidence computed as concordance with known Hippocampome.org molecular markers (the full dataset is publicly available at hippocampome.org/GeneParcelExpressionConfidence)

Figure 3.
Interactive Gene Expression Analyzer (hippocampome.org/genexan)

Expressed (+) and not-expressed (–) (–) molecular markers in hippocampal principal layers (green highlight: new high-confidence findings)

Table 1

Hippocampome Protein	Gene Checked	Granule	CA3 Pyr	CA2 Pyr	CA1 Pyr
5HT - 3	Htr3a	–	–		–
alpha - actinin - 2	Actn2	+	+	+	+
AMIGO2	Amigo2	–		+	–
BDNF	Bdnf	+	+	+	+
Bok	Bok	–	+	–	–
Caln	Cabin1	+	+	+	+
CaM	Calm2	+	+	+	+
CB	Calb1	+	–		
CB1	Cnr1	–	+	–	+
CCK	Cck		+	–	+
CGRP	Calca	–	–	–	–
CoupTF II	Nr2f2	–		–	–
CR	Calb2	–	–	–	–
Ctip2	Rit1				–
Disc1	Disc1	+	+	+	+
DYN	Pdyn	+			
EAAT3	Slc1a1	+	+	+	+
ENK	Penk	–	–	–	–
ErbB4	ErbB4	–	–	–	–
GABA - B1	Gabbr1	+	+	+	+
GABAA alpha1	Gabra1	+	+	+	+
GABAA alpha2	Gabra2	+	+	+	+
GABAA alpha3	Gabra3	–	–	–	+
GABAA alpha4	Gabra4	+	+	+	+
GABAA alpha5	Gabra5	+	+	+	+
GABAA alpha6	Gabra6	–	–	–	–
GABAA beta1	Gabbrb1	+	+	+	+

Hippocampome Protein	Gene Checked	Granule	CA3 Pyr	CA2 Pyr	CA1 Pyr
GABAA beta2	Gabrb2	+	+	+	+
GABAA beta3	Gabrb3	+	+	+	+
GABAA delta	Gabrd	+	-	-	-
GABAA gamma1	Gabrg1	-	-	-	-
GABAA gamma2	Gabrg2	+	+	+	+
GAT - 1	Slc6a1	-	-	-	-
GAT - 3	Slc6a11	-	-	-	-
GluA1	Gria1	+	+	+	+
GluA2	Gria2	+	+	+	+
GluA3	Gria3	+	+	+	+
GlyT2	Slc6a5	-	-	-	-
Id - 2	Id2	-	-	-	-
Kv3.1	Kcnc1	+	-	-	-
Man1a	Man1a	-	+	-	+
Math - 2	Neurod6	-	+	+	+
mGluR1	Grm1	+	+	+	+
mGluR2	Grm2	+	-	-	-
mGluR3	Grm3	+	-	-	+
mGluR4	Grm4	-	-	+	-
mGluR5	Grm5	+	+	+	+
MOR	Oprm1	-	-	-	-
Mus1R	Chrm1	+	+	+	+
Mus2R	Chrm2	-	-	-	-
Mus3R	Chrm3	+	+	+	+
Mus4R	Chrm4	-	-	-	+
Neuropilin2	Nrp2	+	+	-	-
NG	Nrgn	+	+	+	+
NKB	Tac2	+	-	-	-
nNOS	Nos1	-	+	-	+

Hippocampome Protein	Gene Checked	Granule	CA3 Pyr	CA2 Pyr	CA1 Pyr
NPY	Npy	-	-	-	-
p - CREB	Creb1	+	+		+
PCP4	Pcp4	+	-	+	-
PPTA	Tac1	-	-	-	-
Prox1	Prox1	+	-	-	-
PSA-NCAM	Ncam1	+			
PV	Pvalb	-	-	-	-
RLN	Reln	-	-	-	-
SATB1	Satb1		-		
SATB2	Satb2				+
SCIP	Pou3f2	-	+	+	+
SOM	Sst	-	-	-	-
SPO	Synpr	+			
Sub P Rec	Tacr1	-	-	-	-
TH	Th	-	-	-	-
vAChT	Slc18a3	-	-	-	-
vGAT	Slc32a1	-	-	-	-
vGluT1	Slc17a7	+	+	+	+
vGluT2	Slc17a6	-		-	-
vGluT3	Slc17a8			-	
VILIP	Vsnl1	+	+		+
VIP	Vip	-	-	-	-
Y1	Npy1r	+			
Y2	Npy2r		+		

Table 2

Drugs interacting with genes expressed in all principal cell layers of the hippocampus

Gene	Protein	Protein Role	Drug	Drug Use	Drug Gene Interaction Type	Reference
Atp1a1	Sodium/potassium transporting ATPase subunit alpha-1	catalyzes the hydrolysis of ATP coupled with the exchange of sodium and potassium ions across the plasma membrane	Trichlormethiazide	antihypertensive agent	inhibitor	Takahashi et al., 1995
Camk2g	Calcium/calmodulindependent protein kinase type II subunit gamma	may function in dendritic spine and synapse formation and neuronal plasticity	Bosutinib	treatment of chronic, accelerated, or blast phase Philadelphia chromosomepositive (Ph+) chronic myelogenous leukemia (CML) with resistance or intolerance to prior therapy in adult patients	inhibitor	Somaiah et al., 2012
Faa	Fatty acid amide hydrolase	catalysis of the hydrolysis of various bonds	Acetaminophen	analgesic and antipyretic agent with weak antiinflammatory action	inhibitor	Högstätt et al., 2005
Gabbr2	Gamma-aminobutyric acid type B receptor subunit 2	component of a heterodimeric G-protein coupled receptor for GABA, formed by GABBR1 and GABBR2	Thiopental	general anesthetic	inhibitor	Patel et al., 2003
Grin1	Glutamate receptor ionotropic NMDA 1,	synaptic plasticity, synaptogenesis, excitotoxicity, memory acquisition and learning	Baclofen	alleviation of signs and symptoms of spasticity resulting from sclerosis multiple	agonist	Rask - Andersen et al., 2011
Kcnj3	G protein - activated inward rectifier potassium channel 1	potassium channel is controlled by G proteins	Acamprosate	efficacious in relapse prevention of alcoholism	antagonist	Mann et al., 2008
Kynu	Kynureninase	catalyzes the cleavage of L - kynurenine (LKyn) and L-3- hydroxykynurenine (L- 3OHKyn) into anthranilic acid (AA) and 3hydroxyanthranilic acid (3-OHAA), respectively	Phenobarbital	anesthetic and convulsant activities	antagonist	Daniell, 1994
Map2k1	Dual specificity mitogenactivated protein kinase 1	dual specificity protein kinase which acts as an essential component of the MAP kinase signal transduction pathway	Halothane	general inhalation anesthetic used for induction and maintenance of general anesthesia	binder	Rask - Andersen et al., 2011
Nr112	Nuclear receptor subfamily 1 group 1 member 2	transcriptional activator activity, RNA polymerase II transcription regulatory region sequence-specific binding	Pyridoxal Phosphate	for nutritional supplementation and for treating dietary shortage or imbalance	cofactor	Law et al., 2014
			Bosutinib	treatment of chronic, accelerated, or blast phase Philadelphia chromosomepositive (Ph+) chronic myelogenous leukemia (CML) with resistance or intolerance to prior therapy in adult patients	inhibitor	Somaiah et al., 2012
			Trametinib	treatment of unresectable or metastatic melanoma with BRAF V600E or V600K mutations	inhibitor	Yeh et al., 2013
			Docetaxel	anticancer effects	binder	Ikezoe et al., 2004

Gene	Protein	Protein Role	Drug	Drug Use	Drug Gene Interaction Type	Reference
Purp1	Poly [ADP 1 - ribose] polymerase	Positively regulates the transcription of MTUS1 and negatively regulates the transcription of MTUS2/TIP150	Nicotinamide	anti - anxiety, increases the endurance of mice	binder	Chen et al., 2002
Pmp	Major prion protein	neuronal development, synaptic plasticity, myelin sheath maintenance	Tetracycline	prolonging survival of Creutzfeldt-Jakob diseased animals	inhibitor	De Luigi et al., 2008
Src	Neuronal protooncogene tyrosineprotein kinase Src	activated following engagement of receptors and plays a role in activation of other protein tyrosine kinase (PTK) families	Dasatinib	shown to inhibit growth of Bcr-Abl-dependent chronic myeloid leukemia xenografts in nude mice	multitarget	Nam et al., 2005
Sv2a	Synaptic vesicle glycoprotein 2A	role in control of regulated secretion in neural and endocrine cells, enhancing selectively low-frequency neurotransmission	Levetiracetam	adjunctive therapy in the treatment of partial onset seizures in adults and children 4 years of age and older with epilepsy	inhibitor	Law et al., 2014
Tubb3	Tubulin beta - 3 chain	major constituent of microtubules	Ixabepilone	treatment of aggressive metastatic or locally advanced breast cancer	binder	Rask - Andersen et al., 2011
Vdac1	Voltage-dependent anion-selective channel protein 1	forms channel through the mitochondrial outer membrane and also the plasma membrane, allows diffusion of small hydrophilic molecules	Dihydroxyaluminium	antacids perform a neutralization reaction, ie. they buffer gastric acid, raising the pH to reduce acidity in the stomach	inhibitor	Law et al., 2014