New boundaries and dissociation of the mouse hippocampus along the dorsal-ventral axis based on glutamatergic, GABAergic and catecholaminergic receptor densities

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Abstract
In rodents, gene-expression, neuronal tuning, connectivity and neurogenesis studies have postulated that the dorsal, the intermediate and the ventral hippocampal formation (HF) are distinct entities. These findings are underpinned by behavioral studies showing a dissociable role of dorsal and ventral HF in learning, memory, stress and emotional processing. However, up to now, the molecular basis of such differences in relation to discrete boundaries is largely unknown. Therefore, we analyzed binding site densities for glutamatergic AMPA, NMDA, kainate and mGluR2/3, GABAergic GABA_A (including benzodiazepine binding sites), GABA_B, dopaminergic D₁/5 and noradrenergic α₁ and α₂ receptors as key modulators for signal transmission in hippocampal functions, using quantitative in vitro receptor autoradiography along the dorsal-ventral axis of the mouse HF. Beside general different receptor profiles of the dentate gyrus (DG) and Cornu Ammonis fields (CA1, CA2, CA3, CA4/hilus), we detected substantial differences between dorsal, intermediate and ventral subdivisions and individual layers for all investigated receptor types, except GABA_B. For example, striking higher densities of α₂ receptors were detected in the ventral DG, while the dorsal DG possesses higher numbers of kainate, NMDA, GABA_A and D₁/5 receptors. CA1 dorsal and intermediate subdivisions showed higher AMPA, NMDA, mGluR2/3, GABA_A, D₁/5 receptors, while kainate receptors are higher expressed in ventral CA1, and noradrenergic α₁ and α₂ receptors in the intermediate region of CA1. CA2 dorsal was distinguished by higher kainate, α₁ and α₂ receptors in the intermediate region, while CA3 showed a more complex dissociation. Our findings resulted not only in a clear segmentation of the mouse hippocampus along the dorsal-ventral axis, but also provides insights into the neurochemical basis and likely associated physiological processes in hippocampal functions. Therein, the presented data has a high impact for future studies modeling and investigating dorsal, intermediate and ventral hippocampal dysfunction in relation to neurodegenerative diseases or psychiatric disorders.

KEYWORDS
atlas, dorsal-ventral axis, hippocampus, neurotransmitter receptors, receptor autoradiography, rodent
1 | INTRODUCTION

Since the first introduction of the term hippocampus and its anatomical description in the 16th century of Arantius (1564), the hippocampus became one of the most studied brain regions for neuroscientists of all fields (Andersen, 2007). Cytoarchitectonically, the hippocampus consists of the dentate gyrus (DG) and the Cornu Ammonis fields (CA1-4) (Amaral, 1978; Nó, 1934; Ramon y Cajal, 1893; Ramón y Cajal, 1911). Together with the subiculum and the entorhinal cortex, all of these structures belong to the hippocampal formation (HF) (Andersen, 2007; Witter & Amaral, 2004).

The HF is extensively connected to other cortical and subcortical areas that are involved in processing and controlling cognitive functions and plays a prominent role in memory processing, learning, spatial navigation and anxiety and fear (Bannerman et al., 2002; Bannerman et al., 2004; Moser & Moser, 1998; Saxe et al., 2006; van Strien, Cappaert, & Witter, 2009). With increasing evidence for a different distribution of several functions along the dorsal-ventral (septo-temporal) axis of the HF, researchers suggested further separations, that is, that the dorsal hippocampus (posterior in primates) is involved in spatial navigation and memory, whereas the ventral hippocampus (anterior in primates) mediates more anxiety and fear-related responses (Anacker & Hen, 2017; Bannerman et al., 2004; H. W. Dong, Swanson, Chen, Fanselow, & Toga, 2009; Fanselow & Dong, 2010; Kheirbek et al., 2013; Moser & Moser, 1998; Muzzio et al., 2009; Plachti et al., 2019; Strange, Witter, Lein, & Moser, 2014). Up to now, it seems to be that there is no clear cut and a common functional specialization scheme along the dorsal-ventral axis of the mammalian HF is still a matter of debate, very likely depending on different parameters like intrinsic and extrinsic connectivity patterns, neurochemical systems, cell types, adult neurogenesis, vulnerability to ischemia, and discrete genetic domains (Anacker & Hen, 2017; Bienkowski et al., 2018; Cembrowski, Wang, Sugino, Shields, & Spruston, 2016; Fanselow & Dong, 2010).

Considering the general findings that link functions in the HF to specific neurotransmitter systems (Amaral & Witter, 1989; Bliss & Collingridge, 1993; Duman, Sanacora, & Krystal, 2019; Gage & Thompson, 1980; Huang & Kandel, 1995; Lisman & Grace, 2005; Lodge & Grace, 2008; R. Morris, 2007; Osten, Wisden, & Sprengel, 2007; Strange et al., 2014; Vizi & Kiss, 1998; Zinn et al., 2016), we assume that looking at different neurochemical systems and modulatory in- and outputs play a substantial role in understanding dorsal, intermediate and ventral hippocampal functional segmentation. Both, glutamatergic and GABAergic innervation to the HF is ubiquitous and related functions strongly depend on hippocampal intrinsic and extrinsic connectivity with cortical and subcortical areas (Amaral, Scharfman, & Lavenex, 2007; Freund & Buzsáki, 1996; Klausberger & Somogyi, 2008). Whereas the dorsal hippocampus is connected to associative cortical areas and receives polymodal sensory information, the ventral hippocampus is more linked to subcortical structures like the amygdala and the hypothalamus (Bannerman et al., 2014; Fanselow & Dong, 2010; Moser & Moser, 1998; Strange et al., 2014), but also receives key projections from the prefrontal cortex (rats: Swanson, 1981; Jay & Witter, 1991; Verwer, Melier, Van Uum, & Witter, 1997; primates: Barbas & Blatt, 1995). Herein, the glutamatergic inputs to the hippocampus, originating in the entorhinal cortex, or ipsi- and contralateral hippocampal regions, are topographically organized and show laminar selectivity, that is, selectivity for neuronal targets (Buh & Whittington, 2007; Nilssen, Doan, Nigo, Ohara, & Witter, 2019). Vice versa, the septal-hippocampal GABAergic projections show target cell type preferences without laminar selectivity (Freund & Antal, 1988). Additional glutamatergic inputs reach the hippocampus from the thalamus and the amygdala and GABAergic inputs reach the hippocampal subdivisions by further GABAergic interneurons (Buh & Whittington, 2007; Klausberger & Somogyi, 2008). Regarding both systems, synaptic activity is differentially modulated by binding to specific receptor subtypes, co-modulators of synaptic transmission and receptor cross-talks that in turn lead to complex functional contributions (Duman et al., 2019; Duszkiewicz, McNamaara, Takeuchi, & Genzel, 2019; Herold et al., 2018). For one example, both, glutamatergic NMDA and AMPA receptors are particularly involved in long-term potentiation during memory formation (Collingridge, Kehl, & McLennan, 1983; R. G. Morris, Anderson, Lynch, & Baudry, 1986; Tsien, Huerta, & Tonegawa, 1996) and both receptor types also modulate hippocampal place cell activity that have been shown to increase place field size gradually from dorsal to ventral (Jung, Wiener, & McNaughton, 1994; Kjelstrup et al., 2008; Maurer, Vanrhoads, Sutherland, Lipa, & McNaughton, 2005). Indeed, glutamatergic NMDA, AMPA and kainate receptors showed a hippocampal dorsal-ventral gradient of densities in rats (Martens, Capito, & Wree, 1998; Pandis et al., 2006). In addition, changes in densities of glutamatergic and GABAergic receptors in the HF of rodents in relation to learning, hormones, seizures and Morbus Parkinson have been reported (C. M. Cremer et al., 2009; J. N. Cremer et al., 2015; Oermann, Warskutat, Heller-Stibl, Haussinger, & Zilles, 2005; Palomer-Gallagher, Bidmon, & Zilles, 2003; Topic et al., 2007; K. Zilles, Wu, Crusio, & SchwTEGR, 2000). Further, ventral hippocampal GABAergic dysfunctions were reported in schizophrenia, cognitive aging, Alzheimer’s disease, autism, depression and bipolar disorder (Bast, Pezze, & McGarrity, 2017; Benes, 2015; McGarrity, Mason, Fone, Pezze, & Bast, 2017). Other transmitters, or neuromodulators like noradrenaline or dopamine projections reach the dorsal and ventral hippocampus differentially. Noradrenaline sources from the locus coeruleus project along the cingulum, the fornix and the ventral amygdaloid bundle and in sum show a higher density of terminals in the ventral hippocampus (Haring & Davis, 1985). Dopaminergic projections reach the hippocampus through the fimbria, the supracallosal bundle and the ventral amygdaloid bundle. The dopaminergic projections from the ventral tegmental area and the substantia nigra to the dorsal hippocampus are sparse, while ventral hippocampus is densely innervated (Edelmann & Lessmann, 2018; Gasbarri, Sulli, & Packard, 1997). Further, additional routes for dopamine to effect dorsal hippocampal neurons have been described via release from noradrenergic containing neurons from the locus coeruleus (Han et al., 2020; Kempadoo, Mosharov, Choi, Sulzer, & Kandel, 2016; Takeuchi et al., 2016), the midbrain raphe nuclei and the nucleus accumbens (Edelmann & Lessmann, 2018). In addition, dorsal-ventral hippocampus gradients have been reported in...
dopamine receptor gene expression (Gangarossa et al., 2012; Wei et al., 2018), but the functional contribution of these differences is yet not fully clear. Both, hippocampal noradrenaline and dopamine, seem to regulate synaptic plasticity and memory encoding and consolidation via modulation of excitatory glutamatergic and inhibitory GABAergic signaling neurons and receptor crosstalk within these systems and associated networks (Dahl & Savery, 1989; Duszkiewicz et al., 2019; Hansen, 2017; Hu et al., 2007; Pezze & Bast, 2012; Yang, 2000).

Due to the increasing amount of functional studies that take dorsal-ventral differences with more or less precise anatomical borders in the hippocampus into account, we set out to study the receptor densities of glutamatergic, GABAergic, dopaminergic and noradrenergic receptors along the dorsal-ventral axis with autoradiography in combination with a detailed cyto- and myelo-architecture analysis in serial coronal sections of the mouse hippocampus. In addition, we analyzed the detailed receptor architecture in the different layers of dorsal, intermediate and ventral subregions, which has further functional implications to understand the effects of neurotransmitter receptor modulation on synaptic activity depending on different cell types and the position at cellular structures. Thus, we mapped different neurochemical functional domains in the mouse hippocampus, integrated the findings in current maps of dorsal, intermediate and ventral subregions and included these borders and domains into an atlas scheme to provide a robust parcellation for future functional studies.

2 | MATERIAL AND METHODS

2.1 | Animals and tissue preparation

We used 10, adult male C57BL/6 mice that were obtained from CERJ (Janvier Labs, Germany). The number of animals was chosen due to consideration of statistical power as well as previous anatomical/ future functional studies conducting similar analysis. After arrival, animals were housed in groups (five/cage) in an enriched environment, under constant temperature and humidity control in a 12-hour light/dark cycle for 8 weeks. Food pellets and water were ad libitum. All procedures were in accordance with the German law to protect animals and the guidelines of the “Landesamt für Natur, Umwelt und Verbraucherschutz NRW, Germany (LANUV)” as well as with the guidelines of the European Council Directive 2010/63/EU. For the histological procedures and the receptor autoradiography, mice were decapitated, brains were removed from the skull and frozen in isopentane at −40°C. At this timepoint all mice were at the age of 27 weeks (~5 month) and weighed 28–33 g. Unfixed frozen tissue was stored at −80°C until sectioning.

2.2 | Histology and receptor autoradiography

Serial coronal cryosections (16 μm) from one brain hemisphere per mouse (in total n = 10, left and right hemispheres were randomized) were prepared using a cryostat microtome (Leica, Germany). Adjacent glass-mounted cryosections were processed for quantitative in vitro receptor autoradiography with tritiated ligands according to previously described protocols to label receptors for glutamate, γ-aminobutyric acid (GABA), dopamine and noradrenaline (Herold et al., 2014; Palomero-Gallagher, Vogt, Schleicher, Mayberg, & Zilles, 2009; Zilles et al., 2002; Zilles, Schleicher, Palomero-Gallagher, & Amunts, 2002). Additionally, cryosections were treated with a modified silver staining to mark cell bodies or myelo fibers for cyto- and myeloarchitectonic analysis (Gallyas, 1971; Merker, 1983).

For the receptor binding studies the following binding sites were labelled: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor with [3H]-AMPA, kainate receptor with [3H]-kainate, N-methyl-D-aspartate (NMDA) receptor with [3H]-MK-801, group II metabotropic glutamate (mGlu2/3) receptor with [3H]-LY-341495, γ-aminobutyric acid (GABA) receptor GABAA with [3H]-Muscimol, GABAδ receptor with [3H]-CGP-54626, GABAδ associated benzodiazepine (BZ) binding sites with [3H]-Flumazenil, noradrenergic α1 adrenoreceptor with [3H]-Prazosin, noradrenergic α2 adrenoreceptor with [3H]-RX-821002 and dopaminergic D1/D5 receptors with [3H]-SCH 23390. All binding protocols are summarized in supplementary Table 1 (Table S1). Three steps were performed in the following sequence:

1. A preincubation step removed endogenous ligand from the tissue.
2. During the main incubation step binding sites were labeled with the respective tritiated ligand (total binding), or co-incubated with the tritiated ligand and a 1,000-10,000-fold excess of specific non-labeled ligand (displacer) determined non-displaceable, and thus, non-specific binding. Specific binding is the difference between total and non-specific binding. It was less than 5% in all cases.
3. A final rinsing step eliminated unbound radioactive ligand from the sections.
4. Radioactively labeled sections were air-dried overnight and co-exposed with plastic [3H]-standards (Microscales, Amersham, UK) of known radioactivity concentrations against tritium-sensitive films (Hyperfilm, Amersham, UK) for 4–18 weeks.

2.3 | Image analysis

The resulting autoradiographs were processed using densitometry with a video-based image analyzing technique (Zilles, Schleicher, et al., 2002). Autoradiographs were digitized by means of a KS-400 image analyzing system (Kontron, Germany) connected to a CCD camera (Sony, Japan) equipped with a 5-Orthoplanar 60-mm macro lens (Zeiss, Germany). The images were stored as binary files with a resolution of 512 x 512 pixels and 8-bit gray value. The gray value images of the co-exposed microscales were used to compute a calibration curve by nonlinear, least-squares fitting, which defined the relationship between gray values in the autoradiographs and concentrations of radioactivity. This enabled the pixel-wise conversion of the gray values of an autoradiograph into the corresponding concentration of radioactivity. The concentrations of binding sites occupied by a ligand
under incubation conditions are transformed into fmol/mg protein at saturation conditions by means of the equation: 

\[ \frac{K_D + L}{A_S \times L} \]

where \( K_D \) is the equilibrium dissociation constant of ligand-binding kinetics, \( L \) is the incubation concentration of ligand, and \( A_S \) the specific activity of the ligand. The results of these calculations were used for binding site density measurements. The digitized autoradiographic images were color-coded to facilitate the detection of regional differences in binding site densities by visual inspection. Thereby a color bar encodes the receptor density in fmol/mg protein.

### TABLE 1

| Subregion/receptor | DG   | CA1  | CA2  | CA3  | CA4/Hilus | Friedman ANOVA (\( \chi^2 \)) |
|--------------------|------|------|------|------|-----------|-----------------------------|
| AMPA               | 2044 ± 134 | 2,709 ± 262 | 2,304 ± 149 | 2,360 ± 141 | 3,402 ± 184 | 31.68***                   |
| Kainate            | 1,108 ± 73  | 619 ± 47 | 506 ± 50 | 1,104 ± 73 | 1,643 ± 85 | 38.08***                   |
| NMDA               | 2,411 ± 273 | 3,873 ± 383 | 3,800 ± 427 | 2,776 ± 284 | 2,209 ± 217 | 35.20***                   |
| mGlu2/3            | 2,882 ± 236 | 3,125 ± 139 | 2,576 ± 171 | 2,000 ± 98  | 1,529 ± 81  | 31.84***                   |
| GABA_A             | 755 ± 39   | 1,185 ± 51 | 962 ± 69  | 625 ± 38  | 707 ± 45  | 39.28***                   |
| GABA_A(D)          | 3,437 ± 262 | 4,605 ± 293 | 4,209 ± 355 | 3,670 ± 233 | 2,819 ± 183 | 33.92***                   |
| GABA_B             | 5,016 ± 495 | 5,095 ± 291 | 4,837 ± 229 | 5,222 ± 338 | 4,936 ± 235 | 8.24                        |
| \( \alpha_1 \)     | 158 ± 7   | 229 ± 15  | 154 ± 13  | 137 ± 13  | 132 ± 6   | 25.04***                   |
| \( \alpha_2 \)     | 729 ± 103 | 982 ± 95  | 689 ± 67  | 426 ± 38  | 231 ± 10  | 26.24***                   |
| D_1/5              | 179 ± 14  | 187 ± 12  | 169 ± 14  | 137 ± 10  | 126 ± 11  | 25.76***                   |

***p < .001.

**FIGURE 1** Coronal sections through the mouse (C57BL/6) HF along the anterior–posterior axis. (a, e, first column) Nissl stained, (b, f, second column) myelin stained and an exemplary original autoradiograph (c, g, third column) of a coronal section around Bregma level – 2.1 mm (a–d) and Bregma level – 2.3mm (e–h). (d) and (h) show the corresponding schematic atlas of the hippocampus at the different Bregma levels. The atlas map is based on the multiscale analysis of the cyto-, myelo-, and receptor-architecture at the corresponding brain levels. Hippocampal subfields and layers are different colour coded (see legend). The subiculum as well as the fibrous alveus and the fimbria are coded in white. alv, alveus; CA1d, Cornu Ammonis area 1, dorsal; CA2d, Cornu Ammonis area 2, dorsal; CA3d, Cornu Ammonis area 3, dorsal; DGd, Dentate gyrus, dorsal; fi, fimbria; sg, granule cell layer; slm, stratum lacunosum-moleculare; slu, stratum lucidum; sr, stratum radiatum; sp, pyramidal layer; so, stratum oriens; Subd, Subiculum, dorsal; mo, molecular layer; po, polymorph layer [Color figure can be viewed at wileyonlinelibrary.com]

### 2.4 | Anatomical identification

The borders and the subregions of the HF were anatomically identified based on our cyto-, myelo- and receptorarchitectonic data (Figures 1-3) in series of sections in the mouse brain and previous cytoarchitectural, connectional and genetic maps from the Allen Brain Atlas (Hong Wei Dong, 2008; Lein et al., 2007), the Franklin and Paxinos atlas (Franklin & Paxinos, 2008), the comparative cytoarchitectonic atlas of the C57BL/6 and 120/Sv mouse brains (Hof, Young, Bloom, & Belichenko, 2000), Thompson and colleagues.
FIGURE 2  Coronal sections through the mouse (C57BL/6) HF along the anterior–posterior axis. (a, e, i, first column) Nissl stained, (b, f, j, second column) myelin stained and an exemplary original autoradiograph (c, g, k, third column) of a coronal section around Bregma level −2.5 mm (a–d), Bregma level −2.7 mm (e–h) and −3.0 mm (i–l). (d), (h), (l) (fourth column) show the corresponding schematic atlas of the hippocampus at the different Bregma levels. The atlas map is based on the multiscale analysis of the cyto-, myelo-, and receptor-architecture at the corresponding brain levels. Hippocampal subfields and layers are differently colour coded (see legend). The subiculum as well as the fibrous alveus and the fimbria are coded in white. The borders between dorsal, intermediate and ventral subfields are indicated by a different color-shading. alv, alveus; CA1d, Cornu Ammonis area 1, dorsal; CA1i, Cornu Ammonis area 1, intermediate; CA2d, Cornu Ammonis area 2, dorsal; CA2i, Cornu Ammonis area 2, intermediate; CA3d, Cornu Ammonis area 3, dorsal; CA3i, Cornu Ammonis area 3, intermediate; CA3v, Cornu Ammonis area 3, ventral; DGd, Dentate gyrus, dorsal; DGv, Dentate gyrus, ventral; sg, granule cell layer; slm, stratum lacunosum-moleculare; slu, stratum lucidum; sr, stratum radiatum; sp, pyramidal layer; so, stratum oriens; Subd, Subiculum, dorsal; mo, molecular layer; po, polymorph layer [Color figure can be viewed at wileyonlinelibrary.com]
Borders of the different subregions were traced on prints of the digitized autoradiographs by projecting the cell body and the myelin stained sections onto the digitized images of the autoradiographs between Bregma level $-0.9$ mm and $-3.8$ mm. The stereoaxial subdivisions into dorsal, ventral and intermediate were identified along the dorsal-ventral (septo-temporal) axis manually by inspection of visible

**FIGURE 3** Coronal sections through the mouse (C57BL/6) HF along the anterior–posterior axis. (a, e, i, first column) Nissl stained, (b, f, j, second column) myelin stained and an exemplary original autoradiograph (c, g, k, third column) of a coronal section around Bregma level $-3.2$ mm (a–d), Bregma level $-3.4$ mm (e–h) and $-3.6$ mm (i–l). (d), (h), (l) (fourth column) show the corresponding schematic atlas of the hippocampus at the different Bregma levels. The atlas map is based on the multiscale analysis of the cyto-, myelo-, and receptor-architecture at the corresponding brain levels. Hippocampal subfields and layers are differently colour coded (see legend). The subiculum as well as the fibrous alveus and the fimbria are coded in white. The borders between dorsal, intermediate and ventral subfields are indicated by a different colour-shading. Alv., alveus; CA1d, Cornu Ammonis area 1, dorsal; CA1i, Cornu Ammonis area 1, intermediate; CA1v, Cornu Ammonis area 1, ventral; CA2d, Cornu Ammonis area 2, dorsal; CA2i, Cornu Ammonis area 2, intermediate; CA2v, Cornu Ammonis area 2, ventral; CA3i, Cornu Ammonis area 3, intermediate; CA3v, Cornu Ammonis area 3, ventral; DGd, Dentate gyrus, dorsal; DGv, Dentate gyrus, ventral; slm, stratum lacunosum-moleculare; sr, stratum radiatum; sp, pyramidal layer; so, stratum oriens; Subd, Subiculum, dorsal; Subv, Subiculum, ventral; mo, molecular layer; sg, granule cell layer; po, polymorph layer [Color figure can be viewed at wileyonlinelibrary.com]
differences in receptor densities due to adjusting optimal density color coding for each area and each receptor type in series of sections for each mouse brain, differences in the myeloarchitecture and according to a set of individual references of stereotactic coordinates including different parameters in terms of measurements (Bienkowski et al., 2018; H. W. Dong et al., 2009; Fanselow & Dong, 2010; Lein et al., 2007; Strange et al., 2014; Thompson et al., 2008). Here, for example, dorsal and ventral DG were distinguished by differences in kainate and GABA_A receptor ligand binding (Figures 4 and 6), dorsal, intermediate and ventral CA3 by AMPA and NMDA receptor ligand binding (Figure 4), dorsal and intermediate CA2 by noradrenergic receptor ligand binding (Figure 8) and dorsal, intermediate and ventral

**FIGURE 4**  Color-coded autoradiographs showing the distribution and density of glutamatergic receptors at different atlas levels in the HF at Bregma level −2.1 mm (a, e, i, m) to −2.8 mm (b, f, j, n), Bregma level −2.7 mm to −3.00 mm (c, g, k, o) and Bregma level −3.2 mm to −3.8 mm (d, h, l, p). (a–d) AMPA receptor expression. (e–h) kainate receptor expression. (i–l) NMDA receptor expression. (m–p) mGlu2/3 receptor expression. The colour scales code for the receptor densities are denoted in fmol/mg protein and are specific for each receptor type. Note that the red end of the scale bar indicates the best fit for the investigated receptors and subregions but not the maximum receptor density [Color figure can be viewed at wileyonlinelibrary.com]
To investigate the chemoarchitectural differences between different main areas of the hippocampus and between dorsal, ventral and intermediate areas, statistical analyses were used. The significance level was set at .05.

### 3 RESULTS

We analysed the receptor architecture of the different subdivisions of the hippocampus in the mouse brain with a focus on dorsal-ventral differences.

### 2.5 Statistics

| Subregion/receptor | DGd | DGv | CA1d | CA1i | CA1v | CA2d | CA2i | CA3d | CA3i | CA3v | Friedman ANOVA ($\chi^2$) |
|-------------------|-----|-----|------|------|------|------|------|------|------|------|--------------------------|
| AMPA              | 2.139 ± 479 | 1.950 ± 136 | 2.972 ± 279 | 2.852 ± 296 | 2.294 ± 227 | 2.349 ± 167 | 2.259 ± 173 | 2.058 ± 109 | 2.617 ± 157 | 2.526 ± 181 | 53.23*** |
| Kainate           | 1.310 ± 59 | 0.889 ± 99 | 3.530 ± 44 | 2.687 ± 44 | 7.71 ± 50 | 4.43 ± 44 | 7.50 ± 60 | 10.29 ± 83 | 1.140 ± 88 | 1.165 ± 62 | 7.743*** |
| NMDA              | 3.832 ± 457 | 1.545 ± 265 | 4.533 ± 440 | 3.995 ± 389 | 3.081 ± 326 | 3.960 ± 400 | 3.640 ± 493 | 2.866 ± 288 | 3.031 ± 318 | 2.402 ± 262 | 7.811*** |
| mGlu2/3           | 2.277 ± 98 | 3.038 ± 419 | 3.479 ± 157 | 3.318 ± 152 | 2.537 ± 156 | 2.533 ± 188 | 2.621 ± 238 | 1.933 ± 107 | 1.890 ± 136 | 2.182 ± 115 | 53.54*** |
| GABA_A            | 1.512 ± 66 | 3.758 ± 33 | 1.421 ± 51 | 1.275 ± 70 | 0.859 ± 47 | 0.912 ± 72 | 0.920 ± 122 | 0.638 ± 40 | 0.692 ± 46 | 0.744 ± 34 | 53.21*** |
| GABA_B            | 1.581 ± 194 | 3.287 ± 357 | 5.655 ± 324 | 4.467 ± 317 | 3.694 ± 262 | 4.276 ± 287 | 4.141 ± 441 | 3.658 ± 251 | 3.822 ± 257 | 3.531 ± 219 | 54.87*** |
| α1               | 1.517 ± 60 | 1.311 ± 11 | 1.86 ± 11 | 2.48 ± 15 | 2.47 ± 28 | 1.124 ± 9 | 1.951 ± 14 | 1.124 ± 14 | 1.166 ± 6 | 1.144 ± 9 | 58.74*** |
| α2               | 4.373 ± 31 | 1.018 ± 19 | 0.905 ± 65 | 1.185 ± 125 | 0.856 ± 122 | 0.940 ± 60 | 0.886 ± 100 | 0.301 ± 26 | 0.481 ± 60 | 0.514 ± 45 | 54.02*** |
| D1/5             | 2.373 ± 14 | 0.119 ± 18 | 0.202 ± 11 | 0.189 ± 14 | 0.168 ± 14 | 0.164 ± 12 | 0.176 ± 17 | 0.144 ± 10 | 0.117 ± 10 | 0.149 ± 11 | 62.66*** |

Note: The results of the Friedman ANOVA display the regional differences for all subregions for each receptor type (all $N = 10$, df = 9).

**$p < .001$.**
with 10 different neurotransmitter receptor 3[H]-ligands and used a multimodal comparison of cyto-, myelo- and receptor-architecture to achieve an accurate mapping of the structures and layering schemes of the HF (Figures 1-3). Quantitative measurements of receptor densities in fmol/mg protein are depicted to show the differences between multireceptor density profiles for each area (Figures 4-10 and S1-3, Tables 1 and 2).

3.1 Combined analysis of the cyto-, myelo-, and receptor-architecture of the HF

Figures 1-3 show representative brain sections of the HF in Nissl-stained (first column), myelin-stained (second column) and 3[H]- receptor ligand labeled (third column) sections from anterior to posterior Bregma coordinates \(-2.1\) mm to \(-3.8\) mm. For a detailed overview, different atlas levels are shown, depicting the outlines and surrounding structures of the HF (fourth column) that were used to identify the subregions in the receptor autoradiographs. The HF is divided into the main structures designated as cornu ammonis (CA1 – CA4/hilus) and the dentate gyrus (DG).

The DG is a three-layered C-shaped structure within the HF consisting of the principle granule cell layer (sg), containing densely packed granule cells, nicely resolved in Nissl-stained sections, an overlying molecular layer (mo) and a subjacent polymorph layer of cells (po; Figures 1-3, first column). The surrounding structure of the HF is formed by the alveus that is particularly visible in myelin-stained sections reflecting the outermost subependymal fiber layer of the HF (Figures 1-3, second column). The hippocampal fields are formed by a characteristic cellular layer, the stratum pyramidale (sp), clearly visible in Nissl-stained tissues (Figures 1-3, first column). Between the alveus and the stratum pyramidale, the stratum oriens (so) builds the outer

**FIGURE 5** Histograms of the mean receptor densities for glutamatergic AMPA, kainate, NMDA, mGlu2/3 in hippocampal regions. (a), the main regions of the HF DG, CA1, CA2, CA3, CA4/hilus. (b), dorsal, intermediate and ventral subregions. Densities are provided in fmol/mg protein. Error bars represent standard errors of the means. Line bars between different regions and in between dorsal, intermediate and ventral subregions represent significant differences between structures of an examined region (all \(p < .01\); except AMPA: DG/CA2; CA1d/CA1i; CA3d/CA3v; kainate: CA2d/CA2i; CA3d/CA3i; CA3d/CA3v; mGlu2/3: CA1/CA2; all \(p < .05\); Wilcoxon-rank test) [Color figure can be viewed at wileyonlinelibrary.com]
layer of the hippocampus. The inner layers are divided into the stratum radiatum (sr) and the stratum lacunosum-moleculare (slm). CA3 contains an additional layer that is absent in CA4/hilus, CA2 and CA1, the stratum lucidum (slu).

The borders to map the spatial distribution of the main regions DG and CA1-4 along the dorsal-ventral axis followed previous cytoarchitectural and imaging studies based on different parameters (Bienkowski et al., 2018; H. W. Dong et al., 2009; Fanselow & Dong, 2010; Franklin & Paxinos, 2008; Lein et al., 2007; Strange et al., 2014; Thompson et al., 2008), and our new determined borders in the dorsal-ventral axis based on a highly significant data set for the analyzed receptor classes are described below. Therefore, first, we will briefly outline the general differences in receptor densities in the HF and most important, along the dorsal-ventral axis. Second, we will present the detailed analysis of specific layers in this context. The combined analysis of all data was integrated into the presented atlas scheme (Figures 1-3, fourth column).
3.1.1 Quantitative analysis of hippocampal subdivisions along the dorsal-ventral axis

Quantitative receptor data of the HF is presented in form of color-coded autoradiographs for each receptor at different atlas levels of a series of coronal sections with a gap of approximately 192 μm between each serial slice to highlight the regional differences in receptor expression in the dorsal-ventral axis and the overall analysis of the densities is presented in the corresponding bar graphs. The detailed results and statistics are further summarized in Tables 1 and 2 or provided in the figure legends and description of the results.

Glutamatergic receptors

The glutamatergic receptors were individually expressed at different density levels in specific subdivisions (Tables 1 and 2). Here, AMPA receptors showed the highest levels in CA1 and CA4/hilus (Figures 4a–d, 5a and S1), kainate in DG, CA3 and CA4/hilus (Figures 4e–h, 5a and S1), NMDA in CA1 and CA2 (Figures 4i–l, 5a and S1) and mGlu2/3 receptors in DG, CA1 and CA2 (Figures 4m–p, 5a and S1). In relation to the dorsal-ventral axis, AMPA receptors discriminate particularly between CA1d/i and CA1v with higher levels in CA1d/i and vice versa, between CA3 d and CA3i/v with lower levels in CA3d (Figures 4a–d, 5b and S2). Kainate receptors were 1.5 times higher expressed in DGd compared to DGv. In contrast, densities increased from CA1d to CA1i to CA1v, from CA2d to CA2i and from CA3d to CA3i/v (Figures 4e–h, 5b and S2). NMDA receptors showed two-fold higher density levels in DGd compared to DGv. The highest levels of NMDA receptors, and further the highest levels of all analyzed glutamatergic receptors, were observed in CA1d, with a stepwise decrease to CA1v. Additionally, differences were detected between CA3i and CA3d/v (Figures 4i–l, 5b and S2). The analysed mGlu2/3 receptors showed higher densities in CA1d/i compared to CA1v, while lower levels were found in CA3 d/i compared to CA3v (Figures 4m–p, 5b and S2).

FIGURE 7 Histograms of the mean receptor densities for GABAergic GABA_A, GABA_A(BZ), GABA_B in hippocampal regions. (a), the main regions of the HF DG, CA1, CA2, CA3, CA4/hilus. (b), dorsal, intermediate and ventral subregions. Densities are provided in fmol/mg protein. Error bars represent standard errors of the means. Line bars between different regions and in between dorsal, intermediate and ventral subregions represent significant differences between structures of an examined region (all p < .01; except GABA_A: CA3/Hilus/CA4; GABA_A(BZ): CA1/CA2; CA2/CA3; all p < .05; Wilcoxon-rank test) [Color figure can be viewed at wileyonlinelibrary.com]
FIGURE 8  Color-coded autoradiographs showing the distribution and density of catecholaminergic receptors at different atlas levels of the HF at Bregma level −2.1 mm (a, e, i) to −2.8 mm (b, f, j), Bregma level −2.7 mm to −3.00 mm (c, g, k) and Bregma level −3.2 mm to −3.8 mm (d, h, l). (a–d) $\alpha_1$ receptor expression. (e–h) $\alpha_2$ receptor expression. (i–l) D$_{1/5}$ receptor expression. The colour scales code for the receptor densities are denoted in fmol/mg protein and are specific for each receptor type. Note that the red end of the scale bar indicates the best fit for the investigated receptors and subregions but not the maximum receptor density [Color figure can be viewed at wileyonlinelibrary.com]
GABAergic receptors

While the two types of GABA\(_{\text{A}}\) receptor bindings sites tested separated the subdivisions of the HF, GABA\(_{\text{A}}\) receptors showed an equal distribution in all subdivisions (Tables 1 and 2). The highest amounts of GABA\(_{\text{A}}\) receptors were detected in CA1 followed by decreasing amounts in CA2, CA4/hilus, DG and CA3 (Figures 6a–d, 7a and S1). Benzodiazepine binding sites showed only a slightly different picture. Here, the highest densities were detected in CA1 and CA2 that were distinguished from CA3 and DG, with the lowest amounts in CA4/hilus (Figures 6e–h, 7a and S1). Along the dorsal-ventral axis, the density of GABA\(_{\text{B}}\) receptors was three times higher in DGd compared to DGv (Figures 6a–d, 7b and S2). A stepwise decrease in densities was observed from CA1d to CA1i to CA1v, while both, CA3d and CA3i showed higher densities compared to CA3v (Figures 6a–d, 7b and S2). GABA\(_{\text{A}}\) densities differed between CA1d, CA1i and CA1v and showed a 1.5-fold higher density in CA1d compared to CA1v. In addition, GABA\(_{\text{A}}\) receptors were higher expressed in CA3i compared to CA3v (Figures 6e–h, 7b and S2). Further, none of the GABA\(_{\text{A}}\) receptors showed detectable differences between CA2d and CA2i.

Catecholaminergic receptors

In comparison to the densities of glutamatergic and GABAergic receptors, catecholaminergic receptors were relatively low expressed in the HF (Tables 1 and 2). Thereby, densities of noradrenergic \(\alpha_2\) and dopaminergic D\(_{1/5}\) showed similar values, while for example, noradrenergic \(\alpha_2\) receptor densities were five times higher expressed in CA1 if compared to \(\alpha_1\) and D\(_{1/5}\) levels. Further, the highest densities of \(\alpha_2\) receptors were detected in CA1, while the lowest were found in CA4/hilus (Figures 8a–d, 9a and S1). The \(\alpha_2\) receptors were also high expressed in CA1 and showed a stepwise decrease to CA2 to CA3 to CA4/hilus. The DG showed comparable amounts of \(\alpha_2\) receptors to CA2 but differed to all other subdivisions (Figures 8e–h, 9a and S1). D\(_{1/5}\) receptors showed higher densities in DG, CA1, and CA2 if compared to CA3 and CA4/hilus (Figures 8i–l, 9a and S1). Comparable to glutamate receptors, all investigated catecholaminergic receptors exhibited different densities along the dorsal-ventral axis. \(\alpha_2\) receptors are lower expressed in CA1d compared to CA1i, CA2d compared to CA2i, while in CA3i higher densities were detected compared to CA3d/v (Figures 8a–d, 9b and S2). \(\alpha_2\) receptors showed remarkable differences in densities in the DGv compared to DGd and CA2i compared to CA2d. In the ventral and intermediate compartments, the concentration was two-fold higher. Further considerably higher densities were observed in CA1i compared to CA1d/v, as well as in CA3i/v compared to CA3d (Figures 8e–h, 9b and S2). Vice versa, D\(_{1/5}\) receptors were two-fold higher expressed in DGd compared to DGv. In the CA fields, CA1i/d exhibited a higher density compared to CA1v and CA3i compared to CA3d/v (Figures 8i–l, 9b and S2).

Taken together, the densities and distribution of the 10 investigated neurotransmitter receptors, with one exception (GABA\(_{\text{A}}\)), clearly separate the individual subdivisions of the HF and can be used as neurochemical markers. Beside the common regions, additionally the CA4/hilus region was separated from neighboring regions DG (8/10 receptor densities) and CA3 (7/10 receptor densities). Further, the CA2 region is explicitly distinguished from neighboring CA1 and CA3 (both 8/10 receptor densities). Along the dorsal-ventral axis, further differences support a refined parcellation of the HF. Thereby, the DGd is defined by a higher density of kainate, NMDA, GABA\(_{\text{A}}\) and D\(_{1/5}\) receptor expression and a substantially lower expression of \(\alpha_2\) receptors compared to DGv. CA1d, CA1i and CA1v differed in 22 cases. The most expressive differences to demarcate CA1 along the dorsal-ventral axis were detected for GABA\(_{\text{A/B}}\) receptors, which showed the highest differences in receptor concentrations between all three compartments CA1d, CA1i and CA1v, with a stepwise decrease from dorsal to ventral components. CA1i can be further identified by a very high density of \(\alpha_2\) receptors, and CA1v by substantial lower amounts of AMPA, NMDA and mGlu\(_{2/3}\) receptors. The CA2 region showed higher levels of kainate, \(\alpha_1\) and \(\alpha_2\) receptors in CA2i compared to CA2d. For CA3d, CA3i and CA3v, the receptor densities differed 18 times. For example, AMPA receptors were substantially lower expressed in CA3d, while NMDA receptors were higher expressed in CA3d if compared to CA3v. CA3i can be specifically distinguished by high densities of \(\alpha_2\) receptors and low densities of D\(_{1/5}\) receptors.

3.1.2 Qualitative analysis of receptor densities in different layers and subdivisions of the HF along the dorsal-ventral axis

Besides the neurochemical dissimilarities that yielded into a refined atlas including borders of dorsal, intermediate and ventral subregions in the dorsal-ventral axis of the HF, we further observed differences between individual layers that support the idea of functional and layer specific distinct subdivisions in the mouse HF along the dorsal-ventral axis. Thereby, the analysis of the receptor dense and sparse layers resulted in individual fingerprints for each receptor type that are visualized in Figure 10. These receptor fingerprints provide a detailed overview of different receptor types and the corresponding densities in each layer (vertical columns) in the subdivisions of the HF along the dorsal-ventral axis (horizontal columns/rows). In addition to the dissimilarities, common or parallel patterns of distinct receptor types that are co-expressed in the same layers become visible. For overview, the data of all measured layers is further presented in a heat map (Figure S3).

Glutamatergic receptors

AMPA receptors peaked in the stratum pyramidale (sp) of CA1d/i, CA2d/i and CA3 i/v, while high densities in the dorsal molecular layer (mo), the dorsal stratum granulosum (sg) and in the ventral polymorph layer (po) were detected in DGd and therefore broaden the shape of the fingerprint in the upper half (Figure 10, AMPA row). A similarly structured fingerprint of the DG is only observed for the distribution of \(\alpha_1\) receptors (Figure 10, DG column). Kainate receptors were prominent in the stratum oriens (so) of CA1v, in the stratum lacunosum-moleculare of CA2i and peaked in the stratum lucidum (slu) of CA3d, which also showed high levels in CA3i/v compared to other layers.
and gave the fingerprint the shape of a three-pronged asterisk, which is unique compared to the other receptor distributions in CA3 (Figure 10, Kainate row and CA3 column). NMDA receptors showed high levels in the stratum radiatum (sr) of CA1d, CA2d/i, CA3i and in m oDGd (Figure 10, NMDA row). The very high density of NMDA receptors in moDGd and vice versa the low density in the sgDGd turned the fingerprint into a characteristic shape of a hexagon with one elongated side that is close to the distribution of kainate, GABA A (BZ) and GABA B receptors in DGd and DGv (Figure 10, DG column). In contrast to the other determined receptors, mGlu2/3 showed specific high peaks in the same layers of all the CA fields and were densely expressed in slmA1d/i, slmA2d/i and slmA3d. Further the moDGd showed higher levels of mGlu2/3 receptors in DGd and DGv, which resulted in a stretch of the hexagonal fingerprint (Figure 10, mGlu2/3 row).

**GABAergic receptors**

GABA A receptors showed a relatively homogeneously distribution in all layers of CA1 but were higher expressed in all layers of CA1d and CA1i. The same applies for CA2 with a slightly higher density in spCA2d and spCA2i (Figure 10, GABA A row). The shape of the fingerprint for the CA2 region is therefore comparable to the AMPA receptor distribution in CA2 (Figure 10, CA2 column). High densities in slmCA3d, srCA3i and srCA3v shape the fingerprint for CA3. In the DG, GABA A receptors were highly expressed in moDGd and sgDGd that was quite distinctive from the ventral layers (Figure 10, GABA A row). Regarding CA1 and CA2, GABA A(BZ) receptors were even more homogeneously distributed than GABA A receptors. In addition to slmCA3d, srCA3i and srCA3v, the concentration was high in soCA3v. DGd expressed high amounts in mo (Figure 10, GABA A(BZ) row). Comparable to the analysis of the subfields, GABA B receptors showed no remarkable differences between layers (Figure 10, GABA B row).

**Catecholaminergic receptors**

Noradrenergic α 1 receptors were generally higher expressed in the all ventral layers of CA1v and CA2i but were relatively homogeneously distributed in all layers (Figure 10, α 1 row). The shape of the CA3 compartments resembles those of the GABA A(BZ) and GABA B receptors (Figure 10, CA3 column), with peaks in slmCA3d, srCA3i and srCA3v. Further, in moDGd α 1 receptors were expressed at high levels and in relation to the other determined receptors sgDGd showed a high concentration too (Figure 10, α 1 row and DG column). The α 2 receptor densities peaked out in slmA1d and slmA1i, with higher densities in CA1i. CA2i is also characterized by a peak in slm. In CA3 the highest densities were recognized in slmCA3d and soCA3v. Thereby the ventral layers showed a generally higher concentration compared to corresponding layers in intermediate and dorsal regions (Figure 10, α 2 row). A high density of α 2 receptors is also observed in moDGv, which distinguished the shape of the DG fingerprint from all other fingerprints of the DG (Figure 10, DG column). Dopaminergic D 3 receptors showed a small dip in srCA1v and spCA1v and were otherwise relatively homogeneously distributed between layers. In CA2i so and slm exhibited only slightly higher levels, while in CA3d a peak was observed in slmA3d. DGd showed generally higher levels in all layers compared to DGv (Figure 10, D 1/5 row).

To sum up, individual receptor density patterns for the different subdivisions along the dorsal-ventral axis are additionally reflected in specific patterns of receptor densities in layers.

### 4 | DISCUSSION

By using receptor-, cyto- and myelo-architectural analyses, we were able to reliably distinguish dorsal, intermediate and ventral parts of the mouse hippocampus, its subregions and layers. We propose a refined comprehensive atlas as upgrade to previously established hippocampal maps. The detailed mapping of receptor densities showed a complex molecular architecture defined by highly combinatorial density patterns of 10 different receptors that might be interpreted as anatomically/functionally dissimilar or even identical. The receptor density patterns defined individual compartments up to single layers of a compartment in each subregion of the HF. Together with data from previous studies (Bienkowski et al., 2018; Dong et al., 2009; Lein, Zhao, & Gage, 2004; Schultz & Engelhardt, 2014; Thompson et al., 2008; Zhao et al., 2001), our results provide a high-resolution scheme of neurochemically discrete structures that can be used for future hippocampal structural and functional studies. Here, our analyses showed a specific regional receptor distribution along the dorsal-ventral axis of the hippocampus that adds to the multiscale nature of the different subdivisions.

#### 4.1 | Neurochemical profiles in hippocampal subdivisions

While the basic anatomy of the HF has been well established by Ramón y Cajal, (1911), Lorente de Nó (1934), and Blackstad (1956), new findings and methods have refined our knowledge about the anatomy of the HF in different species (Blackstad, 1956; Nó, 1934; Ramón y Cajal, 1911). Thereby, the hippocampus is regarded as a brain structure with highly conserved functions, since this region is considered homologous among amniotes like mammals (Amrein & Slomianka, 2010; Bannerman et al., 2002; Hong Wei Dong, 2008; Fanselow & Dong, 2010; Lein et al., 2007; Lowe et al., 2015; Moser & Moser, 1998; Plachtí et al., 2019; Swanson & Cowan, 1977; Thompson et al., 2008; Wittr & Amaral, 2004; Zeineh et al., 2017; Zhao et al., 2001), birds (Atoji & Wild, 2006; Colombo & Broadbent, 2000; Fanselow & Dong, 2010; Lein et al., 2007; Lowe et al., 2015; Moser & Moser, 1998; Plachtí et al., 2019; Swanson & Cowan, 1977; Thompson et al., 2008; Wittr & Amaral, 2004; Zeineh et al., 2017; Zhao et al., 2001), reptiles and teleost fish (Rodriguez et al., 2002; Striedter, 2016; Tosches et al., 2018). Here, the multireceptor data indicated sharp boundaries between the different subregions of the mouse hippocampus DG, Hilus/CA4, CA3, CA2 and CA1 in line with earlier receptor binding studies and maps in rodents (Cremer, Lubke, Palomero-Gallagher, & Zilles, 2011; Cremer et al., 2009; Cremer et al., 2015; Dean, Scarr, & McLeod, 2005; Palomero-Gallagher et al., 2003; Topic et al., 2007; K. Zilles et al., 2000), rabbits (Tocco
et al., 1991), primates including human (Camps, Kelly, & Palacios, 1990; Kraemer et al., 1995; Palomero-Gallagher, Kedo, Mohlberg, Zilles, & Amunts, 2020) and birds (Herold et al., 2014; Herold et al., 2015). However, although functional differences between the subunits of the Ammon’s horn were described and analyzed by further anatomical and physiological techniques, CA2 in mammals, and particularly in mice, often remained elusive, sometimes referred to as a separate region and sometimes merely as a mixture of CA1 and CA3 cells (Grove & Tole, 1999; Woodhams, Celio, Ulfig, & Witter, 1993). Since cytoarchitectural boundaries cannot be visualized consistently by using standard histological staining, the differentiation of CA2 from the adjacent areas CA1 and CA3 is still often ignored. In line with our data, recent studies showed that CA2 in mice is a separate anatomical and functional region (Lein et al., 2004; Lein, Callaway, Albright, & Gage, 2005; Tole, Christian, & Grove, 1997), while the strongest arguments for a distinct CA2 region in the primate HF is the molecular expression of calcium-binding proteins parvalbumin and calbindin (Leranth & Ribak, 1991). Our data support these findings, showing that CA2 is a distinct region that could be further subdivided into a dorsal and intermediate portion, which is even spared in the more precise work based on genetic markers and connectivity in the hippocampus gene expression atlas (HGEA atlas; Bienkowski et al., 2018). Despite the controversies in various publications, here, distinct boundaries to the neighboring structures CA1 and CA3 were established. The most impressive differences were observed between the AMPA receptor distribution in CA1 and CA2 and kainate receptor distribution in CA3 and CA2. Despite further differences in receptor densities, both glutamate receptor types indicate a clear border between these regions. Therefore, we would argue to include CA2 in mice as separate structure in all future studies. Besides, recently published multi-receptor in the human hippocampus showed a comparable receptor profile for CA2 (Palomero-Gallagher et al., 2020).

**FIGURE 9** Histograms of the mean receptor densities for catecholaminergic $\alpha_1$, $\alpha_2$, $D_1/5$ receptors in hippocampal regions. (a), the main regions of the HF DG, CA1, CA2, CA3, CA4/hilus. (b), dorsal, intermediate and ventral subregions. Densities are provided in fmol/mg protein. Error bars represent standard errors of the means. Line bars between different regions and in between dorsal, intermediate and ventral subregions represent significant differences between structures of an examined region ($p < .01$; except $\alpha_2$: CA2/CA3; DG/Hilus/CA4; CA3d/CA3i; CA3i/CA3v; $\alpha_2$: DG/CA1; CA1d/CA1i; CA3d/CA3i; $D_1/5$: CA1/CA2; CA2/Hilus/CA4; CA1d/CA1v; CA1i/CA1v; CA3i/CA3v; all $p < .05$; Wilcoxon-rank test) [Color figure can be viewed at wileyonlinelibrary.com]
Additionally, in our atlas scheme Hilus/CA4 is an independent region that was further separated from DG and CA3 but would most likely correspond to DGpod/CA3dd in the study of Bienkowski et al. (2018). Up to now, the Hilus/CA4 region of the mouse hippocampus is often omitted in anatomical studies, and if presented, sometimes referred to as hilar region of the DG or included into the CA3 region, while functional studies differentiate hilar neurons from the rest of the DG (Danielson et al., 2017; Scharfman & Myers, 2012; Swaminathan, Wichert, Schmitz, & Maier, 2018; Tong et al., 2015; S. Zhao, Chai, Forster, & Frotscher, 2004). Here we detected overall substantial differences in the receptor architecture of the Hilus/CA4 region compared to the neighbouring CA3 and DG. Therefore, the combined analysis of receptor densities and the inspection of the cellular and fiber architecture resulted into a delineation of the Hilus/CA4 region and the inclusion of a small Hilus/CA4 subdivision as an individual region in the presented atlas. Based on our analysis we would argue that CA4/hilus is a transitional region between DG and CA3, with an individual chemoarchitecture that reflects its functional diversification. This would be further in line with the high amount of different cell types in this region (Amaral, 1978; No, 1934; Ramón y Cajal, 1911; Scharfman & Myers, 2012) and again matches the multi-receptor profile of human CA4 (Palomero-Gallagher et al., 2020). Besides, recent evidence further supports the idea to think of the hippocampus as a bilateral structure with functional specializations of subfields in either the right or left hemisphere that are accompanied by the asymmetrical presence of neurotransmitter receptor subunit compositions in these subfields and strata (Jordan, 2020; Kawakami et al., 2003; Shinohara et al., 2008; Shipton et al., 2014). Therefore, we cannot exclude that an additional lateralization of the chemoarchitecture of hippocampal subfields per se and in the dorsal-ventral axis exists, which was not in the focus of this study, but should be considered for future studies.

4.2 | Neurochemical differences in dorsal, ventral and intermediate hippocampus

Beyond the more multi-receptor differences in the mouse hippocampus per se, the analysis of receptor densities provided a comprehensive view of neurotransmitter targets at the protein level in order to clarify subdivision- or sublayer specific density patterns in dorsal, intermediate and ventral hippocampus. Here, our data not only complements gene expression studies (Bienkowski et al., 2018; Cembrowski et al., 2016; H. W. Dong et al., 2009; Lein et al., 2007; Palomero-Gallagher et al., 2020).
Thompson et al., 2008), but shows neurochemical heterogeneity in line with different functional connectivity along the dorsal-ventral axis (Anacker & Hen, 2017; Bannerman et al., 2014; Bast, Wilson, Witter, & Morris, 2009; Kheirbek et al., 2013; Lee, Kim, Cho, Kim, & Park, 2017; Moser & Moser, 1998; Strange et al., 2014). Further, our findings of different as well as indifferent (co-) distributions of specific neurotransmitter receptors along the dorsal-ventral axis may offer a link between the dichotomy of functionally segregated subfields with precise borders on the one hand and a more gradual organization of processing functions on the other and show that both views are not exclusive (Bast et al., 2009; Brun et al., 2008; Lee, Rao, & Knierim, 2004; Leutgeb, Leutgeb, Moser, & Moser, 2005; Leutgeb, Leutgeb, Moser, & Moser, 2007; McHugh et al., 2007; Neunuebel & Knierim, 2014; Strange et al., 2014; Vogel et al., 2020). Despite ubiquitous glutamatergic and GABAergic inputs to all hippocampal subdivisions (Amaral et al., 2007; Freund & Buzsáki, 1996; Klausberger, 2009; Klausberger & Somogyi, 2008; Kouvaros & Papatheodoropoulos, 2016) we could separate dorsal, intermediate and ventral subdivisions by disseminative quantities of AMPA, Kainate, NMDA, mGlu2/3, GABA_A, and GABA_A(BZ) binding sites, likely reflecting different types of cells and/or cellular properties in these areas in addition to different outputs to other cortical and subcortical areas as well as intra-hippocampal connectivity (Besnard, Miller, & Sahay, 2020; Bienkowski et al., 2018; Cembrowski et al., 2016; Nakashiba, Young, McHugh, Buhl, & Tonegawa, 2008; Nakazawa, McHugh, Wilson, & Tonegawa, 2004; Scharfman & Myers, 2012; Wheeler et al., 2015; Witter & Amaral, 2004). Our data is partly in line with a binding and hybridization study of AMPA and NMDA receptors and different subunits in the hippocampus of rats, which showed lower levels in ventral DG, CA3, CA2 and CA1 of both receptor types (Pandis et al., 2006). However, we detected no differences for AMPA receptor densities between DGd and DGv, and higher amounts in CA3i/v, which is in line with an earlier receptor binding study in rats (Martens et al., 1998). In addition, we detected lower levels of kainate receptors in DGv and higher levels in CA1v, CA2i and CA3v, while Martens et al. (1998) observed no differences along the septotemporal axis. Beside advanced methods and more precise maps used in this study, it is possible that species differences between mice and rats had led to these discrepancies. Further, mGlu2/3 receptors, which serve as autoreceptors at glutamatergic terminals (Shigemoto et al., 1997), showed lower levels in CA1v and higher levels in CA3v, which we think has not been reported yet but is in line with different treatment effects in these areas due to different stimulation of mGlu2/3 expression in dorsal, intermediate and ventral hippocampus (Dubovyk & Manahan-Vaughan, 2019). With respect to the lower GABA_A receptor densities in DGv, CA1v, CA3v our findings are in line with data obtained from the ventral DG, CA1 and CA3 of rats (Sotiriou, Papatheodoropoulos, & Angelatou, 2005). Additionally, we found a stepwise decrease from CA1d to CA1i of both, GABA_A receptor and GABA_A receptor benzodiazepine binding sites.

Further, noradrenergic α_1 and α_2, as well as dopaminergic D_{1/5} receptors showed different densities along the dorsal-ventral axis along with the reported source inputs implying a stronger input to ventral hippocampal areas (Edelmann & Lessmann, 2018; Gasbarri et al., 1997; Gasbarri, Packard, Campana, & Pacitti, 1994; Haring & Davis, 1985; Verney et al., 1985). Here, in line with higher noradrenergic line sources, higher levels of α_1-receptors discriminated CA1i/v, CA2i and CA3i/v from dorsal CA regions and higher levels of α_2-receptors discriminated DGv, CA2i and CA3i/v from dorsal subregions, while the highest density was detected in CA1i, separating CA1i against CA1d and CA1v. Vice versa, although dopaminergic input was reported to be higher to the ventral regions, higher D_{1/5} receptors levels defined DGd and CA1d/i, while CA3d and CA3v showed higher levels compared to lower levels in CA3i. Higher levels of D_{1/5} receptors in dorsal DG granular cell layer have been reported earlier in dorsal DG as defined in Fremeau Jr. et al. (1991) but showed no overall differences if compared to ventral (Fremeau Jr. et al., 1991), while other studies reported equal or higher levels in the ventral CA regions via in situ hybridization of D_1 or D_2 receptor genes or binding to D_{1/5} (Edelmann & Lessmann, 2018; Fremeau Jr. et al., 1991; Gangarossa et al., 2012; Khan et al., 2000; Lazarov, Schmidt, Wanner, & Pilgrim, 1998; Wei et al., 2018). Despite these controversies, stimulation of D_{1/5} receptors in dorsal hippocampus has been shown to promote spatial learning and memory (Kempadoo et al., 2016), and recently different functional aspects of D_{1/5} receptors in the dorsal hippocampus with respect to novelty and memory consolidation in line with the different inputs from the VTA and the LC have been discussed (Duszkiewicz et al., 2019).

Overall, we could not establish a border between DGd, DGi, and DGv based on our receptor data in the mouse hippocampus compared to the recent HGEA atlas (Bienkowski et al., 2018) and therefore divided the DG only in DGd and DGv. Although we inspected the most posterior hippocampal slices at the point the DG blades merge, based on our analysis of the receptor architecture in serial frontal sections, no differences were observed, and the DG of the study from Bienkowski and colleagues is mostly included into the dorsal DG. Nevertheless, herein, our data is in line with respect to a septotemporal organization of inputs to the DG, without considering differences in the internal fiber network organization of the DG mentioned in the HGEA atlas or a strict dissociation based on stereotaxic coordinates. This may also imply that the DG is functionally more gradually organized, which future studies may investigate in more detail.

### 4.3 | Specific receptor profiles in layers of dorsal, intermediate and ventral hippocampus

The different multi-receptor maps for the dorsal, intermediate and ventral CA subdivisions were accompanied by specific receptor density peaks in the hippocampal layers and co-distributions of receptors, which is in line with the heterogeneity of pyramidal cell types and associated functions, for example in place field modulation, along the dorsal-ventral axis in CA3 and CA1 found by comprehensive mapping of genomic-wide in situ hybridization data and electrophysiological
Here, the different glutamatergic receptors revealed an individual and specific pattern for each receptor type, with high levels of AMPA receptors in spCA1d/i and spCA3i/v, Kainate receptors in slmCA1i, socA1v, slmCA1v, slmCA2i and sluCA3 with overall higher levels in CA3i/v in all layers, NMDA receptors in srCA1d and srCA3i and mGlu2/3 receptors in slmCA1d/i, slmCA3d but overall higher levels in all layers of CA3v, which is in line with the observed selectivity for neuronal targets (Buhl & Whittington, 2007; Nilssen et al., 2019). GABAergic GABA<sub>A</sub> receptors showed a more homogenously distribution pattern in all layers of CA1d and peaked only in srCA3i/v. Noradrenergic α<sub>1</sub>-receptors were enhanced in all layers of CA1v/i and CA2i and peaked in srCA3i/v, while α<sub>2</sub>-receptors specifically peaked in slmCA1i and slmCA2i and soCA3v. Dopaminergic D<sub>1/5</sub> receptors were highly distributed in all layers of CA1d that differed from the observed peak in slmCA3d. Co-distribution of high levels were detected in srCA3i/v for α<sub>1</sub>, GABA<sub>A</sub> and NMDA receptors, in slmCA3d for D<sub>1/5</sub> and mGlu2/3, in slmCA1i for α<sub>2</sub>, kainate and mGlu2/3 receptors and in slmCA2i for α<sub>2</sub> and kainate receptors. Therefore, we would argue, that the discriminative pattern of individual layers can be further used to define different hippocampal components in line with specific cellular properties for future functional studies.

In DG, noradrenergic α<sub>2</sub>-receptors showed the highest density in all layers of DGv, while the D<sub>Gd</sub> exhibited the highest receptor concentrations in the moDGd and in particular in kainate, NMDA, GABA<sub>A</sub> and D<sub>1/5</sub> receptors if compared to ventral layers. The moDG is a relative cell-free layer occupied by dendrites of dentate granule cells, fibres of the perforant path originating in the entorhinal cortex and a small number of interneurons, while sgDG mainly contains granule cells and poDG includes different cell types, but the most prominent is the mossy cell (Anacker et al., 2007). Here we can assume that α<sub>2</sub>-receptors play a pivotal role in modulating the functions of the ventral DG, particularly with respect to the noradrenergic modulation of stress-response and depression related behavioral measures that are further linked to adult neurogenesis and disturbed pattern separation in the ventral DG (Anacker et al., 2018; Anacker & Hen, 2017; Hu et al., 2007). Vice versa, the co-occurrence of high NMDA, GABA<sub>A</sub> and D<sub>1/5</sub> receptor levels in moDGd may point to a more complex dissociation. Both, NMDA and GABA<sub>A</sub> receptors can interact with D<sub>1/5</sub> Receptor, and this interaction can shape the signal transfer along dendrites by potentional as well as depression of currents via different mechanisms (Duszkiewicz et al., 2019; Lee et al., 2002; Liu et al., 2000; Varela, Hirsch, Chapman, Leverich, & Greene, 2009; Yang, 2000). Future functional and electrophysiological studies as well as detailed cell type analysis and further co-expression analysis may target this complex dissociation.

### 4.4 Conclusion

The present study provides a detailed neurochemical receptor map of the mouse hippocampus in the dorsal-ventral axis. Therefore, the combination of the neurochemical profile and cytoarchitectural analysis resulted in a refined parcelling of the mouse HF based on quantitative molecular measurements. This allowed us not only to specify borders between different subdivisions along the dorsal-ventral axis but also to show that CA2 in mice exists as an independent area with a dorsal and an intermediate component. Furthermore, by confirming and complementing previous studies, we were able to create a map that should contribute to a comprehensive understanding of the principle organization of the hippocampus and to serve as a basis for subsequent functional and molecular studies testing dorsal, intermediate and ventral components as individual structures.

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### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest in the manuscript.

### DATA AVAILABILITY STATEMENT

Data availability statement: The data that support the findings of this study is available in the main text, tables and figures.

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### REFERENCES

Amaral, D. G. (1978). A Golgi study of cell types in the hilar region of the hippocampus in the rat. The Journal of Comparative Neurology, 182(4 Pt 2), 851–914. https://doi.org/10.1002/cne.901820508

Amaral, D. G., Scharfman, H. E., & Lavenex, P. (2007). The dentate gyrus: Fundamental neuroanatomical organization (dentate gyrus for dummies). Progress in Brain Research, 163, 3–22. https://doi.org/10.1016/s0079-6123(07)63001-5

Amaral, D. G., & Witter, M. P. (1989). The three-dimensional organization of the hippocampal formation: A review of anatomical data. Neuroscience, 31(3), 571–591. https://doi.org/10.1016/0306-4522(89)90424-7

Amrein, I., & Slomianka, L. (2010). A morphologically distinct granule cell type in the dentate gyrus of the red fox correlates with adult hippocampal neurogenesis. Brain Research, 1328, 12–24. https://doi.org/10.1016/j.brainres.2010.02.075

Anacker, C., & Hen, R. (2017). Adult hippocampal neurogenesis and cognitive flexibility - linking memory and mood. Nature Reviews. Neuroscience, 18(6), 335–346. https://doi.org/10.1038/nrn.2017.45

Anacker, C., Luna, V. M., Stevens, G. S., Millette, A., Shores, R., Jimenez, J. C., ... Hen, R. (2018). Hippocampal neurogenesis
confers stress resilience by inhibiting the ventral dentate gyrus. Nature, 559(7712), 98–102. https://doi.org/10.1038/s41586-018-0262-4

Andersen, P., Morris, R., Amaral, D., Bliss, T., & O'Keefe, J. (2007). The hippocampus book. Oxford; New York: Oxford University Press. https://doi.org/10.1093/acprof:oso/9780195100273.001.0001

Atoji, Y., & Wild, J. M. (2006). Anatomy of the avian hippocampal formation. Reviews in the Neurosciences, 17(1–2), 3–15. https://doi.org/10.1515/reveneuro.2006.17.1-2.3

Bannerman, D. M., Deacon, R. M., Offen, S., Friswell, J., Grubb, M., & Rawlins, J. N. (2002). Double dissociation of function within the hippocampus: Spatial memory and hyponeophagia. Behavioral Neuroscience, 116(5), 884–901. https://doi.org/10.1037/0735-7044.116.5.884

Bannerman, D. M., Rawlins, J. N., McHugh, S. B., Deacon, R. M., Yee, B. K., Bast, T., ... Feldon, J. (2004). Regional dissociations within the hippocampus—memory and anxiety. Neuroscience and Biobehavioral Reviews, 28(3), 273–283. https://doi.org/10.1016/j.neubiorev.2004.03.004

Bannerman, D. M., Sprengel, R., Sanderson, D. J., McHugh, S. B., Rawlins, J. N., Monyer, H., & Seeburg, P. H. (2014). Hippocampal synaptic plasticity, spatial memory and anxiety. Nature Reviews. Neuroscience, 15(3), 181–192. https://doi.org/10.1038/nrn3677

Barbas, H., & Blatt, G. J. (1995). Topographically specific hippocampal projections target functionally distinct prefrontal areas in the rhesus monkey. Hippocampus, 5(6), 511–533. https://doi.org/10.1002/hip.450050604

Bast, T., Pezze, M., & McGarrity, S. (2017). Cognitive deficits caused by prefrontal cortical and hippocampal neural disinhibition. British Journal of Pharmacology, 174(19), 3211–3225. https://doi.org/10.1111/bph.13850

Bast, T., Wilson, I. A., Witter, M. P., & Morris, R. G. (2009). From rapid evolutions to functional homologues of the mammalian hippocampus? Neuroscience and Biobehavioral Reviews, 24(4), 465–484. https://doi.org/10.1016/j.neuroscience.2009.03.068

Cembrowski, M. S., Wang, L., Sugino, K., Shields, B. C., & Spruston, N. (2016). Hipposeq: A comprehensive RNA-seq database of gene expression in hippocampal principal neurons. eLife, 5, e14997. https://doi.org/10.7554/eLife.14997

Colombo, M., & Broadbent, N. (2000). Is the avian hippocampus a functional homologue of the mammalian hippocampus? Neuroscience and Biobehavioral Reviews, 24(4), 465–484. https://doi.org/10.1016/s0149-7634(00)00166-6

Cremer, C. M., Lubke, J. H., Palomero-Gallagher, N., & Zilles, K. (2011). Laminar distribution of neurotransmitter receptors in different reeler mouse brain regions. Brain Structure & Function, 216(3), 201–218. https://doi.org/10.1007/s00429-011-0303-3

Cremer, C. M., Palomero-Gallagher, N., Bidmon, H. J., Schleicher, A., Speckmann, E. J., & Zilles, K. (2009). Pentylenetetrazole-induced seizures affect binding site densities for GABA, glutamate and adenosine receptors in the rat brain. Neuroscience, 163(1), 490–499. https://doi.org/10.1016/j.neuroscience.2009.03.068

Cembrowski, M. S., & Spruston, N. (2019). Heterogeneity within classical cell types is the rule: Lessons from hippocampal pyramidal neurons. Nature Reviews. Neuroscience, 20(4), 193–204. https://doi.org/10.1038/s41586-019-0125-5

Dahl, D., & Sarvey, J. M. (1989). Norepinephrine induces pathway-specific long-lasting potentiation and depression in the hippocampal dentate gyrus. Proceedings of the National Academy of Sciences of the United States of America, 86(12), 4776–4780. https://doi.org/10.1073/pnas.86.12.4776

Dong, H. W. (2008). The Allen reference atlas: A digital color brain atlas of the mouse brain. Hoboken, NJ, US: John Wiley & Sons Inc.

Dong, H. W., Swanson, L. W., Chen, L., Fanselow, M. S., & Toga, A. W. (2009). Genomic-anatomic evidence for distinct functional domains in hippocampal field CA1. Proceedings of the National Academy of Sciences of the United States of America, 106(28), 11794–11799. https://doi.org/10.1073/pnas.0812608106

Dubovsky, V., & Manahan-Vaughan, D. (2019). Distinct time-course of alterations of groups I and II metabotropic glutamate receptor and GABAergic receptor expression along the dorsoventral hippocampal axis in an animal model of psychosis. Frontiers in Behavioral Neuroscience, 13, 98. https://doi.org/10.3389/fnbeh.2019.00098

Duman, R. S., Sanacora, G., & Krystal, J. H. (2019). Altered connectivity in depression: GABA and glutamate neurotransmitter deficits and reversals by novel treatments. Neuron, 102(1), 75–90. https://doi.org/10.1016/j.neuron.2019.03.013

Edelmann, E., & Lessmann, V. (2018). Dopaminergic innervation and modulation of hippocampal networks. Cell and Tissue Research, 373(3), 711–727. https://doi.org/10.1007/s00441-018-2800-7

Fusco, M. S., & Dong, H. W. (2010). Are the dorsal and ventral hippocampus functionally distinct structures? Neuron, 65(1), 7–19. https://doi.org/10.1016/j.neuron.2009.11.031
