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# **Utilization of the Allen Gene Expression Atlas to gain further insight into glucocorticoid physiology in the adult mouse brain**

Konstantinos Kalafatakis<sup>1,2,\*</sup>, Nikolaos Giannakeas<sup>1</sup>, Stafford L. Lightman<sup>2</sup>, Ioannis Charalampopoulos<sup>3</sup>, Georgina M. Russell<sup>2</sup>, Markos Tsipouras<sup>1</sup>, Alexandros Tzallas<sup>1</sup>

<sup>1</sup> Department of Informatics & Telecommunications, School of Informatics & Telecommunications, University of Ioannina, Arta, Greece

<sup>2</sup> Laboratories for Integrative Neuroscience and Endocrinology, Bristol Medical School, University of Bristol, Bristol, United Kingdom

<sup>3</sup> Department of Pharmacology, School of Medicine, University of Crete, Heraklion, Crete, Greece & Institute of Molecular Biology and Biotechnology, Foundation of Research and Technology Hellas, Heraklion, Crete, Greece.

\* corresponding author

Konstantinos Kalafatakis MD MSc PhD MAPS MRSB

e-mail: kgkalafatakis@gmail.com; kk13382@bristol.ac.uk

Department of Informatics & Telecommunications, School of Informatics &

Telecommunications, University of Ioannina, Kostakioi, GR-47100, Arta, Greece

## Abstract

Glucocorticoid neurodynamics are the most crucial determinant of the hormonal effects in the mammalian brain, and depend on multiple parallel receptor and enzymatic systems, responsible for effectively binding with the hormone (and mediating its downstream molecular effects) and altering the local glucocorticoid content (by adding, removing or degrading glucocorticoids), respectively. In this study, we combined different computational tools to extract, process and visualize the gene expression data of 25 genes across 96 regions of the adult C57Bl/6J mouse brain, implicated in glucocorticoid neurodynamics. These data derive from the anatomic gene expression atlas of the adult mouse brain of the Allen Institute for Brain Science, captured via the *in situ* hybridization technique. A careful interrogation of the datasets referring to these 25 genes of interest, based on a targeted, prior knowledge-driven approach, revealed useful pieces of information on spatial differences in the glucocorticoid-sensitive receptors, in the regional capacity for local glucocorticoid biosynthesis, excretion, conversion to other biologically active forms and degradation. These data support the importance of the corticolimbic system of the mammalian brain in mediating glucocorticoid effects, and particularly hippocampus, as well as the need for intensifying the research efforts on the hormonal role in sensory processing, executive control function, its interplay with brain-derived neurotrophic factor and the molecular basis for the regional susceptibility of the brain to states of prolonged high hormonal levels. Future work could expand this methodology by exploiting Allen Institute's databases from other species, introducing complex tools of data analysis and combined analysis of different sources of biological datasets.

**Keywords:** Glucocorticoid neurodynamics; adult mouse brain; Anatomic Gene Expression Atlas; Allen Brain Atlas-Driven Visualizations

**Abbreviations:** ABADV: Allen Brain Atlas-Driven Visualizations, AGE: absolute gene expression (intensity), AGEA: anatomic gene expression atlas, FSL: FMRI Software Library (University of Oxford), GC(s): glucocorticoid(s), GR(s): glucocorticoid receptor(s), ISH: *in situ* hybridization, MR(s): mineralocorticoid receptor(s), RGE: relative gene expression (intensity). Abbreviations of the brain regions can be found in Supplementary Table 2. Abbreviations of the names of the genes under investigation can be found in Supplementary Figure 2.

**Author contributions:**

Konstantinos Kalafatakis – contributed to study design, data collection/ processing, data analysis, data visualisation and paper writing

Nikolaos Giannakeas – contributed to data collection/ processing, data visualisation and revised the manuscript

Stafford L. Lightman – contributed to study design and wrote the paper

Ioannis Charalampopoulos – contributed to study design and wrote the paper

Georgina M. Russell – contributed to data analysis and revised the manuscript

Markos Tsipouras – contributed to data collection/ processing and revised the manuscript

Alexandros Tzallas – contributed to study design, data analysis and paper writing

## Introduction

The anatomic gene expression atlas (AGEA) of the adult mouse brain by the Allen Institute for Brain Science [1] is an online, publicly accessible transcriptome-based atlas of the adult C57Bl/6J mouse brain, depicting the spatial registration of the expression intensity of 4376 mouse genes into 51533, 200  $\mu\text{m}$ -diameter cubic voxels of the mouse brain, based on the extensive *in situ* hybridization (ISH) dataset of the Institute. These pieces of information could be proven very useful in delineating the genomic basis of the mouse brain's anatomy, forming reasonable transcriptome-derived hypotheses on brain function, as well as gaining further insight into the underlying biochemistry of various brain regions, which may also have functional implications.

Glucocorticoids (GCs) constitute a very crucial class of steroid hormones, regulating a vast number of neurological processes under baseline and stressful conditions. They are secreted from the adrenal glands and rapidly reach the brain, acting on their two target receptors, the glucocorticoid (GRs) and mineralocorticoid receptors (MRs). The hormonal neurodynamics constitute a very important aspect of GC regulatory capacity in the brain, because the nuclear and membranous variants of GRs and MRs show a preferable affinity for binding with natural GCs at different hormonal concentrations, and thus dynamic GC oscillations define which combination of GC-sensitive receptors will be activated, producing temporo-spatially different cellular effects [2]. The main parameters regulating GC neurodynamics are (i) the complex circadian (and underlying pulsatile) rhythm by which they are secreted from the adrenal glands, and secondarily (ii) the capability of neural/ glial cells to locally synthesize and metabolize GCs, (iii) the capability of neural/ glial cells to pump out GCs from their microenvironment, (iv) the differential expression of the cellular machinery responsible for interacting with GCs, and finally (v) the local existence of other molecules which interact with GCs, either directly or indirectly at a functional level [3].

This report aims at (i) comparing the data derived from the mouse AGEA with current knowledge based on other sources of evidence on GC neurodynamics, (ii) identify new

domains of potential scientific interest on the topic for future preclinical research, and (iii) propose a simplified methodology for performing a targeted, prior knowledge-driven interrogation of the massive AGEA. Future work could expand this methodology by exploiting Allen Institute's databases from other species, introducing complex tools of data analysis and combined analysis of different sources of biological datasets.

## **Materials & Methods**

### Gene identification based on current knowledge

The methodological pipeline is summarized in Supplementary Figure 1. We identified 25 genes contained in the AGEA, whose expression was considered to be involved in four major domains related to GC physiology; these genes either (i) characterise the GC-synthesizing capacity of the cells, or (ii) lead to attenuated GC stimulation (due to GC deactivation or excretion from cells/-tissues, or transfer of GC precursors to other biosynthetic pathways), or (iii) characterise the sensitivity of the cells/-tissues to GC stimulation, or finally (iv) express other stimuli that synergize with GC stimulation. Thirteen genes have been included in the first category (Tspo, Tspo2, STaR, Scp2, Cyp11a<sub>1</sub>, Hsd3b<sub>1,2,4-7</sub>, Cyp21a<sub>1</sub> and Hsd11b<sub>1</sub>), six genes in the second category (Cyp17a<sub>1</sub>, Hsd11b<sub>2</sub>, Srd5a<sub>1-3</sub> and Abcb1a), four in the third category (Nr3c1, Nr3c2, Fkbp4, Fkbp5) and two in the fourth category (Bdnf, Ntrk2). More details on the abbreviations and the functional significance of these genes for GC physiology can be found in Supplementary Figure 2 [4–14].

### *A priori* model for data interpretation (Supplementary Figure 3)

Gene expression belonging to the third category (as specified above) determines the tissue sensitivity to GCs; the higher the relative Nr3c2 (MR) expression the more sensitive the brain region to low GC levels. Moreover, the higher the relative Nr3c1 (GR) expression the more sensitive the brain region to high GC levels [15]. On another note, recent evidence indicates that availability between co-chaperones Fkbp4 and Fkbp5 is an important determinant of the

genomic, GR-dependent GC effects; normally, after hormone binding to GR, Fkbp5 (bound to the latter) is exchanged against Fkbp4, which allows the recruitment of dynein, consequently mediating the nuclear translocation of the complex, advancing GR-dependent transcriptional activity. The more Fkbp5 is present after GR activation by the hormone over Fkbp4, the more of the GR-containing complexes do not exchange co-chaperones, thus holding more of the receptors in a state with less affinity for GCs and decreasing the amount of GR translocating to the nucleus [16]. Various Fkbp5 polymorphisms in man, leading to an upregulation of its protein expression, have been associated with GR resistance and neuropsychiatric disease.

In relation to the genes belonging to the first category we aim at: (i) comparing the ISH data between the mitochondrial transmembrane proteins, once thought to contribute to cholesterol transportation; (ii) investigating whether there is a predominant Hsd3b isoform in the mouse brain [17]; (iii) identifying which brain regions are characterized by a high endogenous GC biosynthetic capacity, i.e. which brain regions are characterized by a combined adequate relative expression of STaR, Cyp11a<sub>1</sub>, Hsd3b, Cyp21a<sub>1</sub> and Hsd11b<sub>1</sub>. Questions under investigation for the genes belonging to the second category include: (i) which brain regions possess a high capacity for excreting steroids (i.e. express in high relative amounts Abcb1a); (ii) which brain regions are GC-resistant (i.e. highly express Hsd11b<sub>2</sub>); (iii) in which brain regions high steroidogenic capacity of androgens (i.e. highly express Cyp17a<sub>1</sub>) could antagonize local GC biosynthesis; (iv) which brain regions have the capacity to modify GC stimulation (i.e. highly express Srd5a isoforms). Lastly, it will be tested whether there is a correlation between GR expression and BDNF and/or TrkB expression across the brain of the adult mouse; regions which highly co-express GR, TrkB (and/or BDNF) will be specified. All these questions refer to baseline, resting conditions of the healthy adult mouse brain.

#### Technical aspects of the Allen Institute's ISH experiments where the data derive from

All ISH experiments, where the gene expression data derive from, have been conducted in adult male mice (*mus musculus*) of the C57BL/6J inbred strain, aged 56 weeks. Antisense RNA probes have been used on brain sections cut in the sagittal plane. More details on the technical

aspects of the ISH experiments can be found in [18]. All 25 genes, included in this report, have been studied under these settings in at least one experiment. In two cases (Tspo and Ntrk2) two identical experiments per gene have been conducted; the data we report derive from the average between the two experiments per gene. The 27 experiments (raw data) included in this report can be found in <http://mouse.brain-map.org/>. Experiment IDs can be found in Supplementary Table 1.

### Brain segmentation and data collection

Data collection was based on the Allen Brain Atlas-Driven Visualizations (ABADV), which is a publicly accessible web-based tool created to retrieve and visualize expression energy data from the mouse AGEA across multiple genes and brain structures [19]. The spatial segmentation of the mouse brain was performed according to Allen Institute's taxonomic system and based on ABADV's capabilities to provide gene expression energy data for all 25 genes under investigation by the 27 experiments mentioned above. The grey matter of the mouse brain was segmented into ninety-eight regions (Supplementary Table 2), and for ninety-six of them ABADV was able to retrieve genomic data for all genes and experiments under investigation. ABADV was systematically not able to provide data for 2 brain regions (Edinger-Westphal nucleus and trochlear nucleus).

### Data visualisation

We've used sections of the Allen Mouse Brain volumetric atlas 2012 and the masks corresponding to the 96 brain regions in NIFTI file format, downloaded from <https://scalablebrainatlas.incf.org/mouse/ABA12>. Image processing and visualization has been performed by FSL (FMRI Software Library, which is a comprehensive library of analysis and visualisation tools for brain imaging data) [22]. Fslview, a 2D/3D brain volume viewer, has been used for image inspection, and fslutils to process the masks corresponding to each of the brain regions with the available genomic data.



### Data normalisation and analysis

The values corresponding to the gene expression intensity per gene of interest and brain region have been normalised on the basis of the whole-brain mean expression intensity of the corresponding gene (relative gene expression, RGE). Brain regions with  $RGE > 1.50$  for a given gene will be considered as expressing this gene in high relative quantities, while brain regions with  $RGE < 0.50$  or  $0.10$  for a given gene will be considered as expressing this gene in low or extremely low relative quantities, respectively. For ratios on the expression intensities between two genes, the absolute gene expression intensities (AGE) as well as RGEs have been co-evaluated. For the six Hsd3b isoforms and the two enzymatically active Hsd5a isoforms, the expression intensities of the corresponding genes have been attributed one RGE per type of enzyme and brain region by dividing the sum of the AGEs per brain region to the sum of the whole-brain mean expression intensities of the corresponding genes. A measure of the probability of GC biosynthetic capacity per brain region was calculated by multiplying the RGEs of the genes of the five relevant enzymes (STaR, Cyp11a<sub>1</sub>, Hsd3b, Cyp21a<sub>1</sub> and Hsd11b<sub>1</sub>). For correlation analysis, normality in the distribution of data (Shapiro–Wilk test) has been used, and Spearman's rank-order correlation was preferred due to the non-normality in the distribution of the relevant data.

## **Results**

### General overview of the raw data retrieved by ABADV

Figure 1 presents an overview of the raw data retrieved by ABADV in the form of a heatmap. Some genes, like Fkbp4, Fkbp5 and Ntrk2 are expressed in larger quantities across the whole brain, while others, like Hsd3b<sub>2</sub>, Hsd11b<sub>2</sub>, Srd5a<sub>2</sub> show the opposite trend. Perirhinal regions of the cerebral cortex and regions of the middle, medial midbrain (like IF, RL, CLI and DR) have a low expression of most genes under investigation. Furthermore, cerebral and cerebellar cortex, including hippocampus, collectively, express higher quantities of the study

genes, while midbrain expresses lower quantities. An overview of the whole-brain grey matter mean expression intensity of all 25 genes of interest can be found in Supplementary Figure 4.

### Nr3c1 and Nr3c2 spatially divide the mouse brain into regions of differential GC functional capacity

The genomic expression of GC-sensitive receptors varies among different brain regions (Supplementary Figure 5); brain areas showing low RGEs ( $< 0.50$ ) for both, GRs and MRs are anterior parts of the frontal cortex (VISC and OT), PERI, most parts of the subcortical nuclei (STRd, PALd, PALv, PALc, FS), including BLA and TRS. Similarly, most hypothalamic (including PVR and LZ), midbrain nuclei (including SCm, SCs, IC and PAG), and the pons. Among these brain regions, six exhibit particularly low RGEs ( $< 0.10$ ) for both genes; these are MS, IF, PPN, SAG, RR and RN. On the contrary, cortical areas showing high RGEs ( $> 1.50$ ) for both, GRs and MRs are AUD, VIS, SS, PTLp and ACA. The same applies for the major hippocampal areas (DG, CA, FC). IG highly expresses MRs, while TEa, ENT, ECT, RSP, SUB, TR, SUB, PRE, thalamus, LA and ME highly express GRs (Figure 2).

Co-evaluation of the AGEs and RGEs of Fkbp4 and Fkbp5 across the mouse brain have identified 5 primary (OT, FRP, PERI, FC, STRd) and 11 secondary regions (MOB, GU, VISC, CLA, PAA, TR, CB, DG, CA, FS and LT), where Fkbp5 transcriptional output exceeds the corresponding Fkbp4 in absolute (AGE ratio  $> 1.00$ ) and relative terms (RGE ratio  $> 1.50$ ) (primary regions) or in relative terms only (secondary regions) under baseline conditions (Supplementary Figure 5). These areas, therefore, could be particularly susceptible in (inherent/genetic or acquired) conditions leading to a (further) increase in Fkbp5 levels and the Fkbp5:Fkbp4 ratio, resulting to GR resistance. It is worth noting that all primary and most secondary regions belong to the corticolimbic system, whose dysregulation is key in neuropsychiatric disease. Moreover, it is also worth mentioning that four of these brain regions are highly GC-sensitive, expressing high levels of GRs, and belong to either the hippocampal formation (FC, DG, CA) or to the amygdalo-cortical network (TR), whose dysfunction lies in the core of mood and cognitive disorders.

### Cortical regions and hippocampus exhibit the strongest local GC biosynthetic capacity

Almost half of all brain regions investigated have extremely low to no probability for local GC biosynthetic activity since the RGE of at least one of the crucial enzymes or enzyme groups involved in the pipeline of cholesterol transportation and GC biosynthesis (StAR, Cyp11a<sub>1</sub>, Hsd3b, Cyp21a<sub>1</sub>, Hsd11b<sub>1</sub>) lie between 0-0.07 (Supplementary Figure 6), where in most cases the genomic expression of at least 1-2 more of the enzymatic groups is usually also below 0.50. These 43 regions belong to almost the entire hypothalamus and most of midbrain regions, thalamus, NDB, PALd, most amygdalar areas and a few cortical regions (PERI, AUD and TR). The enzyme whose expression is (nearly) missing in almost all of these parts of the brain is Cyp21a<sub>1</sub>. Another 23 brain regions also exhibit a very low probability for locally producing GCs. In these regions (the remaining hypothalamic, midbrain and the remaining regions of the cortical subplate, pons, the remaining subcortical nuclei, most retrohippocampal regions and few cortical sites like PAA, ILA or DP) two to four of the crucial enzyme groups exhibit RGEs below 0.50 and typically between 0.15-0.35. These trends particularly involve Cyp11a<sub>1</sub>, Cyp21a<sub>1</sub> and Hsd11b<sub>1</sub>.

Finally, 30 brain regions (cerebellar and most regions of the cerebral cortex, hippocampus and to a lesser degree ACB, sAMY and medulla) have better probability for local GC biosynthetic capacity, as (i) none of the five crucial enzymes involved in the process is expressed in lower RGE than 0.10, (ii) in all cases at least 4 of these enzymes have an RGE > 0.50, and (iii) in almost all cases at least 2 of those enzymes have RGEs exceeding 1.00. From these regions, FC of the hippocampus shows an impressive expression of these enzymes involved in steroidogenesis, with high RGEs for all of them (StAR = 7.33, Cyp11a<sub>1</sub> = 1.50, Hsd3b = 2.41, Cyp21a<sub>1</sub> = 2.39 and Hsd11b<sub>1</sub> = 1.79). Other regions with notable potential local GC capacity include the whole hippocampal formation, cortical areas (ORB, ECT, ENT, RSP, SS) and olfactory areas (MOB, AON) (Figure 3). It is worth noting that 13 of the 30 brain regions, which express in appreciable amounts the enzymes responsible for local GC biosynthesis, are regions which also express high levels of either MRs or GRs or both. FC

(increased steroidogenic capacity, increased GR and MR levels), ECT (increased steroidogenic capacity, increased GR levels but almost absent MR levels), ENT and RSP (increased steroidogenic capacity, increased GR levels) are the most notable of them.

On another note, there is a reasonable thought that other steroidogenic pathways (mediated for instance by Cyp17a<sub>1</sub> and leading to the production of dehydroepiandrosterone or androstenedione) may antagonize local GC biosynthesis (Supplementary Figure 6). Comparison of both, AGEs and RGEs of Cyp21a<sub>1</sub> and Cyp17a<sub>1</sub> leads to the assumption that in only one region (MO) from those potentially possessing local GC biosynthetic capacity, GC precursors could be preferentially redirected to other steroid biosynthetic pathways (for instance towards androgen production); in that area, Cyp21a<sub>1</sub> expression is significantly lower compared to Cyp17a<sub>1</sub> expression in both absolute (AGE ratio < 0.5) and relative terms (RGE ratio < 0.5). In all other similar cases, the corresponding brain regions have a very low to no probability for local GC biosynthesis anyway.

#### GC excretion, deactivation and 5 $\alpha$ -reduction mechanisms could shield certain brain regions from the stimulatory input of GCs

Compared to the other genes included in this study, P-glycoprotein's (Abcb1a) genomic expression varies the least among different brain regions. Only twelve of them show an RGE higher than 1.50 and another ten show an RGE lower than 0.50 (Supplementary Figure 7). None of the regions with a low P-glycoprotein expression are highly sensitive to GC effects. On the contrary, there are two brain regions, expressing high levels of GRs, which also express high levels of P-glycoprotein: LA and RSP. In these regions, as well as the rest showing increased expression of P-glycoprotein (like various olfactory areas, PL, ILA, ORB, PAR, NDB or MS), the effects of the hormonal signaling output could be attenuated, especially when it comes to synthetic GCs with high GR potency like dexamethasone.

Almost half of the brain regions studied (mainly subcortical regions; striatal, amygdalar, hypothalamic and the midbrain) express very low levels of Hsd11b<sub>2</sub> (RGE ranging from 0 to 0.13), i.e. seem to possess a very low GC deactivation capacity (Supplementary Figure 7).

Nevertheless, most of these brain regions are anyway characterised by a moderate to low GC sensitivity, as they express generally low levels of GRs and MRs. Four regions, though, belonging to this category are sites of high GR (ME) or MR expression (IG) or both (FC, ACA), further highlighting the sensitivity of these regions to GC effects. On the contrary, 10 brain regions are characterised by high expression of Hsd11b<sub>2</sub> (RGE ranging from 2.50 to 8.75); eight of them have anyway moderate sensitivity to the hormonal effects based on the genomic expression of MRs and GRs, i.e. the presence of Hsd11b<sub>2</sub> in high abundance further desensitizes them, particularly against low GC levels (various olfactory areas, ILA, ORB, POST, LT, SCs). SUB and TR, expressing GRs in high abundance, are characterised by a relatively high GC deactivation capacity as well.

Finally, some brain regions show increased expression intensities (RGE > 1.50) of the two active 5 $\alpha$ -reductase isoenzymes (Supplementary Figure 7). Among those regions are olfactory areas, ILA, ORB, RSP, FRP, AI, parts of the hippocampal formation (FC, IG), a few striatal and midbrain regions. Lastly, it is worth noting that ME, which expresses very low Hsd11b<sub>2</sub> levels, also lacks expression of the active 5 $\alpha$ -reductases.

#### BDNF and GC molecular pathways seem to be highly correlated

Analysis on the spatial pattern of the genomic expression intensities between GR (Nr3c1), TrkB (Ntrk2) and BDNF (Bdnf) across the mouse brain revealed a significant degree of correlation [Nr3c1-Ntrk2;  $r(96) = 0.511$ ,  $p < 0.01$ , Nr3c1-Bdnf;  $r(96) = 0.560$ ,  $p < 0.01$ , TrkB-Bdnf;  $r(96) = 0.596$ ,  $p < 0.01$ ], further supporting other sources of evidence indicating that GC and BDNF effects are calibrated, and that GCs can interfere with the TrkB-dependent downstream pathways in a GR-dependent manner (Figure 4).

## **Discussion**

The massive AGEA is a very powerful source of data, the utilisation of which could not only support the proper design of hypothesis-driven translational research, but also provide pieces

of information that actively advance the experimental process or increase its quality. The two major limitations of the AGEA data are (i) their static nature, related to the experimental settings under which they were captured, and (ii) the fact that they are not necessarily a reflection of cellular function, related to the technique of *in situ* hybridization. They give us an idea of the expression intensity of genes across the brain, for a given moment, under baseline experimental conditions. Nevertheless, a targeted, prior knowledge-driven interrogation of the massive AGEA can strengthen or weaken some of our views on brain physiology (of the corresponding species) and reveal new domains of potential neuroscientific interest for future translational research.

In the case of GC neurodynamics in mice, it is important to identify the brain regions most susceptible to the hormonal systematic effects, as well as those possessing the potential to locally alter these dynamics (by either producing steroids *de novo* or removing steroids from their microenvironment or enzymatically converting them to biologically inactive molecules or metabolites with attenuated stimulatory capacity) (Supplementary Table 3). Such knowledge would enhance our efforts to properly model (i) the neural networks affected by the complex, circadian and ultradian GC biorhythm, under baseline conditions, and the GC stimulation during stress responses and (ii) the parts of the central nervous system most susceptible to adverse events from the prolonged use of high levels of synthetic GCs. Finally, we could better appreciate the involvement of certain brain regions to the neuropathology of disorders characterised by GC-dependent dysfunction in relevant experimental models of disease.

The data from the mouse AGEA indicate that (i) there are specific brain regions (whole hippocampal and certain cortical regions) where MRs, contrary to their general trend, are expressed in comparable quantities to GRs, (ii) Hsd3b<sub>2</sub> isoform is expressed in very low intensities across the brain (probably with the notable exception of the orbitofrontal cortex), (iii) most parts of the hippocampal formation and related areas are particularly GC-sensitive (DG, CA, IG, FC, PRE, SUB, ENT and RSP), (iv) the most GC-sensitive cortical regions relate primarily to the processing of salient stimuli (ACA, AUD, VIS, ECT, TEa), but also to executive control functions (SS, PTLp, MO), (v) the most GC-sensitive subcortical regions are thalami,

LA (with the TR) and ME, (vi) although Fkbp4 is generally expressed in much higher quantities compared to Fkbp5 (possibly ensuring the proper mediation of GR-dependent genomic effects after GC stimulation), there are brain regions where Fkbp4 predominance is not so evident (or even there is Fkbp5 predominance), and these brain regions might be most susceptible in conditions leading to GR resistance, (vii) the mouse brain is equipped with the enzymatic machinery to support local GC biosynthesis, especially in certain regions like FC, (viii) some biochemical processes (like the  $11\beta$ -dehydrogenation or the 5 $\alpha$ -reduction or the molecular excretion) could change the local hormonal neurodynamics in a brain region-specific manner, and (ix) the expression profiles of GRs, BDNF and TrkB are highly correlated, indicating common physiological processes and a strong interplay between the hormonal microenvironment and the neurotrophin regulation for the detailed control of behavioral tasks.

These data are in accordance with a substantial amount of work conducted over the last two decades, indicating that GCs (i) affect amygdalar responses mainly via GR-dependent mechanisms (regulating behaviour and memory processes in response to stressful events and emotionally arousing cues) [20,21], and (ii) show an impressive plasticity of effects in hippocampus (from gene expression and synaptic plasticity to regulation of cell survivability, neurogenesis and oligodendrogenesis) mediated via both, MRs and GRs [22–24]. The fact that two brain regions might differentially respond to the same stimulus, due to differences in the content of the various types of target receptors translating the signal to downstream pathways, is illustrated in the following example: high GC levels induce an AMPA receptor-dependent form of long-term potentiation [25], which is only short-lasting in hippocampus (characterised by high levels of GRs and especially MRs) but long-lasting in amygdala (characterised by moderate to high levels of GRs but low to undetectable levels of MRs) [26].

The data presented in this study, though, raise a number of topics for future experimental work, which have not been adequately explored so far. For instance, the importance of GCs on modulating sensory processing and executive control functioning should be investigated given the abundancy of GC-sensitive receptors in related cortical regions. Moreover, it should be also explored whether there are FKBP-related molecular

mechanisms underlying a brain-region specific development of GR resistance, and potential clinical implications leading to neuropsychopathology. From a pharmacological point of view, systematic attempts should be made to model whether the bioavailability or drug potency of synthetic corticosteroids is significantly modified in a brain-region specific manner by enzymes excreting or degrading the compounds or modifying their chemical form. In addition, the research efforts on the functional interplay between GCs and BDNF should be intensified, especially in the context of brain regions like the ACA, cortical areas of sensory processing and hippocampus, where GR and BDNF expression levels are particularly high. It is of special note that these specific brain regions consist the main substrates for the execution and regulation of high-order behaviors, like memory, cognition and stress responses, where GCs and BDNF are well-established key molecules to mediate such cellular and behavioral actions.

Finally, although we already have excellent data on the hormonal effects on the hippocampal formation, there is still a need to increase the spatial resolution of the research outputs; as the mouse AGEA data illustrate, GC-sensitive subregions of the hippocampal formation are not only characterised by an impressive variation in the content of MRs and GRs (high MRs-GRs in CA, DG and FC, predominance of GRs in PRE, SUM and RSP, predominance of MRs in IG, almost absence of MRs in ENT) but also by the capacity for *de novo* steroidogenesis (especially when it comes to FC). This implies that the hippocampal formation is very sensitive to the pattern of GC biorhythm, shows increased complexity when responding to that rhythm, and that it might be able to modify the presence of GCs in its microenvironment under certain conditions. All these issues need further experimental clarifications, especially in the context of physiological processes like synaptic plasticity, dendritic remodeling and adult neurogenesis, as well as in the context of neuropsychiatric disease related to GC imbalances affecting hippocampal function.

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### **Competing interests statement**

The authors declare no competing interests.

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### **Legend to Figure 1**

Heatmap of the absolute expression intensities of 25 genes across 96 grey matter regions of the adult mouse brain, as retrieved by Allen Brain Atlas-Visualisation tools. Abbreviations of brain regions have been adopted from the Allen Brain Atlas nomenclature (<http://mouse.brain-map.org/static/atlas>) and are also listed in Supplementary Table 1 of this paper. Abbreviations of the genes have been adopted from mouse genome informatics (<http://www.informatics.jax.org/>) and are also explained in the material and methods section of this paper. White color indicates zero expression intensity. The darker the red color, the higher the expression intensity of the corresponding gene in the corresponding brain region (maximum value 23.5). The left panel includes regions of the cerebral cortex, cerebellum, pons and medulla. The middle panel includes regions of the hippocampal formation, subcortical nuclei, amygdala and hypothalamus. The right panel includes midbrain areas.

### **Legend to Figure 2**

Four coronal heatmap-like sections of the mouse brain are illustrated (with the corresponding coronal level -Y- indicated in each slice, 0 being the most posterior and 527 the most anterior part of the mouse brain) around its 21 most glucocorticoid-sensitive regions, based on the expression of glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs). Abbreviations of these 21 regions are indicated in the Figure and their full name is explained below. For a detailed explanation on the whole spectrum of colors of the heatmap can be found in Supplementary Figure 5. Moreover, the nomenclature of all brain regions can be found in the coronal mouse Allen Brain Atlas (<http://mouse.brain-map.org/static/atlas>).

RGE: relative gene expression (of each brain region in comparison to the whole-brain grey matter mean gene expression)

ACA: anterior cingulate area, AUD: auditory cortex, CA: Ammon's horn, DG: dentate gyrus, ECT: ectorhinal cortex, ENT: entorhinal cortex, FC: fasciola cinerea, IG: induseum griseum, LA: lateral amygdala, ME: median eminence, MO: somatomotor cortex, PRE: presubiculum,

PTLp: posterior parietal association cortex, RSP: retrosplenial area, SS: somatosensory cortex, SUB: subiculum, TEa: temporal association cortex, TH: thalamus, TR: postpiriform transition area, RSP: retrosplenial cortex, VIS: visual cortex

### **Legend to Figure 3**

Nine sections of the coronal Nissl mouse Allen Brain Atlas (presented in a grey-scale color map as a NIFTI file format), overlain by a heatmap-like mask showing the probability (in arbitrary units) of local glucocorticoid biosynthetic capacity across the mouse brain, based on the relative expression intensity of the five enzymes involved in the process. The numbers in the Figure indicate the seven regions with the strongest possibility for local glucocorticoid biosynthetic capacity; (1) fasciola cinerea is by far the most prominent among them followed by (2) main olfactory bulb, (3) entorhinal cortex, (4) anterior olfactory nucleus, (5) retrosplenial area, (6) orbitofrontal cortex and (7) entorhinal cortex. More details can be found in Supplementary Figure 4. Moreover, the nomenclature of all brain regions can be found in the coronal mouse Allen Brain Atlas (<http://mouse.brain-map.org/static/atlas>).

### **Legend to Figure 4**

Spearman's rank-order correlation analyses between the relative gene expression intensities of brain-derived neurotrophic factor (Bdnf), its target receptor TrkB (Ntrk2), and glucocorticoid receptor (Nr3c1) across the 96 brain regions. The spatial pattern of the genomic expression intensities of all three genes in the mouse brain are strongly correlated.