Updated neuronal numbers of the rat hippocampal formation: redesigning the hippocampal model

Jon I Arellano[1*] and Pasko Rakic[1,2*]

[1]Department of Neuroscience, Yale University, New Haven, CT, United States, [2]Kavli Institute for Neuroscience at Yale, Yale University, New Haven, CT, United States

* Corresponding authors: jon.arellano@yale.edu, pasko.rakic@yale.edu,

Keywords: hippocampus, neuron number, rat, stereology, entorhinal, subiculum

Abstract

The hippocampal formation is a functional entity that includes the hippocampus, subicular complex and the entorhinal cortex, and has an essential role in learning and memory, emotional processing and spatial coding. The well-defined structure of hippocampal fields and the segregation of the connections has made this structure a favorite candidate for functional models, that rely on fundamental information such the number of neurons populating the hippocampal fields. Existing models on the rat rely on neuronal populations obtained from single studies, so we aimed to obtain more representative estimates by analyzing all available data. We found 68 studies that overall contained 293 stereological estimates of neuronal numbers. The resulting averages for males showed 1,000,000 neurons for the granule cell layer (GCL); 50,000 for the hilus; 220,000 for CA3; 25,000 for CA2; 350,000 for CA1 and 280,000 for the Subiculum. Entorhinal cortex (EC) averages came from females: 100,000 neurons in layer II and 250,000 in layer III. Most of those estimates are significantly different from those traditionally used in hippocampal models (e.g.: 2-fold difference in EC layer II), revealing a new architecture of the rat hippocampal formation that might help build more realistic models of hippocampal connectivity and function. In addition, analysis of the data by age, sex or strain showed a trend of sexual dimorphism in the number of neurons in the GCL and EC, with higher neuronal number in males. Across strains, Wistar males showed consistently higher number of neurons than Sprague-Dawley. And, unexpectedly, adolescent animals typically showed lower numbers of neurons than adults. Although not statistically significant, those differences by sex, strain or age were consistent across fields, suggesting they might reflect actual biological features.

Introduction

The hippocampal formation plays a critical role in memory consolidation, emotional processing, and spatial navigation, making it a central subject of physiological and functional research. It comprises the hippocampus, subicular complex and entorhinal cortex, which are interconnected by strong, spatially segregated, unidirectional, and mostly bilateral connections. These characteristics make it an ideal candidate for connectivity and functional models, which require precise quantitative data on neuronal populations and synaptic connectivity.

A seminal review by (Amaral et al., 1990) provided a foundational quantitative description of hippocampal structure and connectivity in the rat, based on the limited studies available at the time, serving as a reference for numerous models (Treves and Rolls, 1992, 1994; Patton and McNaughton, 1995; Henze et al., 2002; Aimone et al., 2006, 2009; Leutgeb et al., 2007; Kempermann, 2011; Krueppel et al., 2011; Snyder and Cameron, 2012; Rolls, 2013; Newman and Hasselmo, 2014; Cole et al., 2020; Berdugo-Vega et al., 2023; Borzello et al., 2023; Vandael and Jonas, 2024). However, numerous subsequent studies have expanded upon these estimates, providing a more comprehensive and accurate picture of neuronal populations within the hippocampal formation.

To this end, we compiled stereological estimates of neuron numbers from 68 studies encompassing 293 data points across the granular cell layer (GCL) and hilus of the dentate gyrus (DG), fields CA3-CA1, the subicular complex including subiculum, pre-, para- and post-subiculum and layers II, III and V/VI of the medial and lateral entorhinal cortex. The dataset predominantly includes male subjects (68%) as is normal in hippocampal studies, with another 24% from females, and 8% mixed groups. Also, there was a clear bias towards young animals (up to 6 months of age) which represent 68% of the datapoints, with limited data from middle-aged or old animals, reflecting a historical youth bias in rodent research (Snyder, 2019; Arellano et al., 2024). Regarding strains, Wistar and Sprague-Dawley (SD) represented 83% of the estimates, with another 10% from Fischer 344 and the remaining 8% from Long Evans, Lewis and Holtzman. In summary, slightly more than half of the estimates come from Wistar or SD males up to 6 months of age.

A notable feature of the data is the large variability in neuron estimates, particularly in the GCL and CA fields. A major limitation is the scarcity of female data, typically excluded due to concerns over hormonal influences (Gould et al., 1990; Zhang et al., 2008a). Only the GCL contained enough females to obtain a reliable average, while in the EC most data come from females. For the rest of the fields, averages are based on males, and sex dimorphism, a feature consistently described in the rodent hippocampus (Madeira et al., 1992; Andrade et al., 2000; Nuñez et al., 2003b; Smith et al., 2008), remained difficult to assess.

The average in the GCL was 1,070,000 neurons for males, 950,000 for females, the and the average of both was 1,010,000. For the rest of the fields, averages were calculated for males, except in the EC, where most data came from females. Overall averages were 50,000 neurons for the hilus; 220,000 for CA3; 25,000 for CA2; 350,000 for CA1; 280,000 for the Subiculum, 100,000 neurons for the EC layer II and 250,000 in EC layer III. While the updated average for the GCL is very similar to the traditional estimate, the updated averages for the rest of fields (hilus, CA fields, subiculum and EC) differ significantly from those used traditionally as described by (Amaral et al., 1990), with values that reach 2-fold difference (e.g: EC layer II or the subiculum). Also, we provide separate estimates for CA3 and CA2, a differentiation described by (Amaral et al., 1990) that has been lost in most subsequent studies, where both fields are described together, although they have been reported to exhibit important differences in connectivity and function.

Comparisons between strains have shown heterogeneous results across studies (Boss et al., 1985, 1987; Seress, 1988). Our dataset showed that Wistar males consistently exhibited 9-24% more neurons than SD males except in the hilus, where the difference was reversed. Although those differences were not significant, likely due to the relatively small sample sizes and their large variability across studies, the relative consistency of the findings suggests adult Wistar males might have more neurons than SD males.

Several reports have shown that adolescent rats tend to exhibit less neurons than adults in CA fields (Madeira et al., 1992; Wakuda et al., 2008; Elibol-Can et al., 2014). While such differences are expected in the dentate gyrus, where neurogenesis is still significant during adolescence and young adulthood (Arellano and Rakic, 2024), it is surprising in the rest of the hippocampal fields, as neurons are generated largely pre- and perinatally and by the onset of adolescence, around 3 weeks of age (Arellano et al., 2024), the number of cells identifiable as neurons is expected to match adult levels. We found consistently lower neuronal counts in adolescents, though limited sample sizes prevent definitive conclusions.

Overall, the data presented here represent the most comprehensive collection of neuron number estimates for the rat hippocampal formation to date, and we hope will provide a valuable resource to update neuronal populations for hippocampal models, help define sex, strain and age differences, and facilitate comparative studies across species.

Methods

Field segregation and nomenclature

The hippocampal formation consists of three main structures: the hippocampus, the subicular complex and the entorhinal cortex. The hippocampus contains the dentate gyrus (DG) and the hippocampus proper also known as Ammon's horn or Cornu Ammonis (CA) fields CA1-CA3. The dentate gyrus has two cellular layers: the granular cell layer (GCL), composed of mostly granular cells, and the polymorphic layer or hilus, that consists of a narrow territory inside the dentate blades, between the GCL and CA3 (Maliković et al., 2022).

The demarcation of the territories of the CA fields requires some detailed explanations. Cajal described two regions in the Ammon's horn: regio inferior, populated by large pyramidal cells near the DG, and regio superior, composed of smaller pyramidal cells proximal to the subiculum (Ramon y Cajal, 1893). Later on, (Lorente de Nó, 1934) described 4 subfields: CA4, CA3, CA2 and CA1. Field CA4 corresponded to the pyramidal cells located inside the DG blades, (Amaral, 1978), but subsequent studies have found no differences in morphology or connectivity between CA4 and CA3 neurons and thus contemporary definitions consider CA4 as part of the CA3 field (Amaral et al., 2007; Maliković et al., 2022). Lorente de Nó separated the regio inferior, populated by large pyramidal cells into two subfields: CA3, located proximal to the DG and receiving mossy fiber input from granule cells and CA2, a small field bearing pyramidal cells with similar morphology to those in CA3 but lacking mossy terminal input from the DG. Also, he differentiated CA1 as populated by smaller pyramidal cells, resembling the regio superior of Cajal. However, subsequent studies on the hippocampus usually did not recognize CA2 as a separate field, and either overlooked it, or merged it with CA3, in a division that ultimately resembled that of Cajal (Ramon y Cajal, 1893; Schlessinger et al., 1978; Boss et al., 1987; Seress, 1988; Ishizuka et al., 1990). There were several reasons for merging both fields, as some authors considered CA2 as a small transitional field (e.g.: (Gaarskjaer, 1986; Woodson et al., 1989). Also, there were practical reasons, as the morphology of the pyramidal cells in CA2 is very similar to those in CA3 (Woodhams et al., 1993; Ishizuka et al., 1995) and it is difficult to separate them. Ultimately, it was reasoned that adding CA2 to field CA3 would not affect significantly the quantification of neurons in CA3, given the small size of CA2 (e.g.: (Boss et al., 1987). This practice of merging CA3 and CA2 has become standard, and we found only 4 studies reporting

on CA3, and 2 studies quantifying CA2. In rare cases, reports on CA3 do not specify if they are referring to CA3 or CA3 and CA2 combined, so we classified those estimates to the best of our knowledge based on the figures or descriptions provided by the authors. Regarding CA2, we found 2 quantifications, providing a rough estimate that lacks reliability. Despite this scarcity, it seems relevant to consider CA2 separately, as recent research has revealed its unique molecular profile and connectivity, setting CA2 clearly apart from their neighbors (Middleton and McHugh, 2020). Hopefully, this renewed interest will lead to more stereological descriptions of its neuronal population in the future.

Regarding the rest of the fields, the subicular complex is normally parcellated in three regions: presubiculum, subiculum and parasubiculum, although some studies (e.g.: (Amaral et al., 2024)) further differentiate the post-subiculum, corresponding to the posterior (temporal) presubiculum (Swanson and Cowan, 1977). The entorhinal cortex is divided in rodents in a medial domain (medial entorhinal cortex: MEC) and a lateral domain (lateral entorhinal cortex: LEC) (Witter et al., 2017).

Methodology

Many articles reported here were already part of a collection on the subject by the authors. The collection was expanded by comprehensive online searches, but many articles were found as references in other reports.

We included estimates from naïve animals and sham controls from experimental conditions as the variability did not differ in both populations. However, transgenic animals and controls subjected to treatments that could produce neuronal death, like large accumulated doses of BrdU (e.g.: Olesen et al., 2015; Alemu et al., 2019)) were excluded. For each estimate, we recorded strain, sex and age at sacrifice of the subjects. The definitions of life stages follow previous published data from our lab (Arellano et al., 2024). In cases where age was not reported and body weight was provided instead, we estimated age using growth charts for Sprague-Dawley (Brower et al., 2015) and Wistar rats (Xie and Zaidi, 2013).

Neuron counts are reported per hemisphere, as is standard in the literature. When studies reported the total number of neurons in both hemispheres (e.g.: (Amaral et al., 2024), values were halved. Most reports do not indicate if estimates are uni- or bilateral and in those cases, we have assumed data is per hemisphere. In a few cases, separate numbers for left and right hemisphere are provided (e.g.: (Baldwin et al., 1997; Lister et al., 2006)) and the average of both hemispheres was listed. Some studies reported data on mixed males and females that is included in tables to illustrate the overall distributions but have been excluded from further analysis given their low number and the potential for sex dimorphism in the number of neurons.

The original study by (Cassell, 1980) correspond to his PhD thesis and could not be retrieved. We added its data as reported by (Amaral et al., 1990). Animals were described as adults, and we assumed an age of 2.5 months, after a previous study by (Cassell and Brown, 1977) reported animals weighing 290-300 gr, equivalent in Wistar rats to about 11 weeks of age (Xie and Zaidi, 2013).

The mean is used to obtain average values of number of neurons in each field, but when possible, the median is also provided, as an alternative estimate of the average value that is less affected by outliers or skewed data. The variability of the estimates was estimated using the

coefficient of variation CV), corresponding to the standard deviation divided by the mean. Outliers were identified using the Tukey method (Tukey, 1977): values below quartile 1 (Q1) – (1.5 × interquartile range (IQR)) or above Q3 + (1.5 × IQR) were excluded from statistical analyses. Normality was verified with the Shapiro-Wilkins test, allowing comparisons with the Welch t-test, a variant of the Student's t-test that requires normal distribution but assumes unequal variances or uneven sample sizes. Statistical significance was set at $p<0.05$. The minimum number of estimates required for statistical analysis is 5. For completeness, averages obtained from less than 5 estimates are shown in tables, but they are italicized to indicate limited reliability.

Results

We have collected 68 studies documenting a total of 293 stereological estimates of number of neurons in the DG; CA fields, subicular complex and entorhinal cortex of rats, as detailed in **Table 1**. Studies used 6 strains of rats, although 83% of the estimates originate from Wistar (Wi; 45%) and Sprague-Dawley (SD; 38%). Other strains, including Fisher 344 (F344), Long Evans (LE), Holtzman (Ho), and Lewis (Lew) were analyzed more sparingly.

Regarding sex, two thirds (68%) of the estimates come from males while about one fourth (24%) are from females and the remaining 8% from mixed males and females. In terms of age, two thirds (67%) of the estimates come from adult animals: 38% from young adults, 16% from middle aged and 13% from old animals. Developing, infant and adolescent animals accounted for one third of the estimates (**Table 1**).

Strains	Studies (%)	Estimates (%)	Age	Studies (%)	Estimates (%)	Sex	Studies (%)	Estimates (%)
Wi	28 (41)	131 (45)	Infant	9 (13)	28 (10)	M	49 (72)	200 (68)
SD	31 (46)	112 (38)	Adol.	22 (32)	66 (23)	F	17 (25)	69 (24)
F344	6 (9)	28 (10)	Y. adult	35 (51)	113 (39)	M-F	7 (10)	24 (8)
LE	3 (4)	16 (5)	Mid age	17 (25)	48 (16)			
Lew	1 (1)	3 (1)	Old age	12 (18)	38 (13)			
Ho	2 (3)	3 (1)						
Total	71 (104)	293 (100)		95 (140)	293 (100)		73 (107)	293 (100)

Table 1. *Summary of the strain, sex and age of the animals in the collected studies. Total studies were 68, but the sum of percentages exceeds 100% because some studies analyzed animals at different ages, from two strains or for both male and females. Age intervales defined according to (Arellano et al., 2024): adolescence (Adol.) starts at 0.75 months; young adulthood (Y. adult) starts at 2 months; middle age (Mid. age) starts at 6 months and old age starts at 15 months. SD: Sprague-Dawley; Wi: Wistar; F344: Fisher 344; LE: Long-Evans; Lew: Lewis; Ho: Holtzman.*

The summary of the findings regarding updated neuronal populations is shown in Fig. 1. The next sections will analyze in detail the findings in each field of the hippocampal formation.

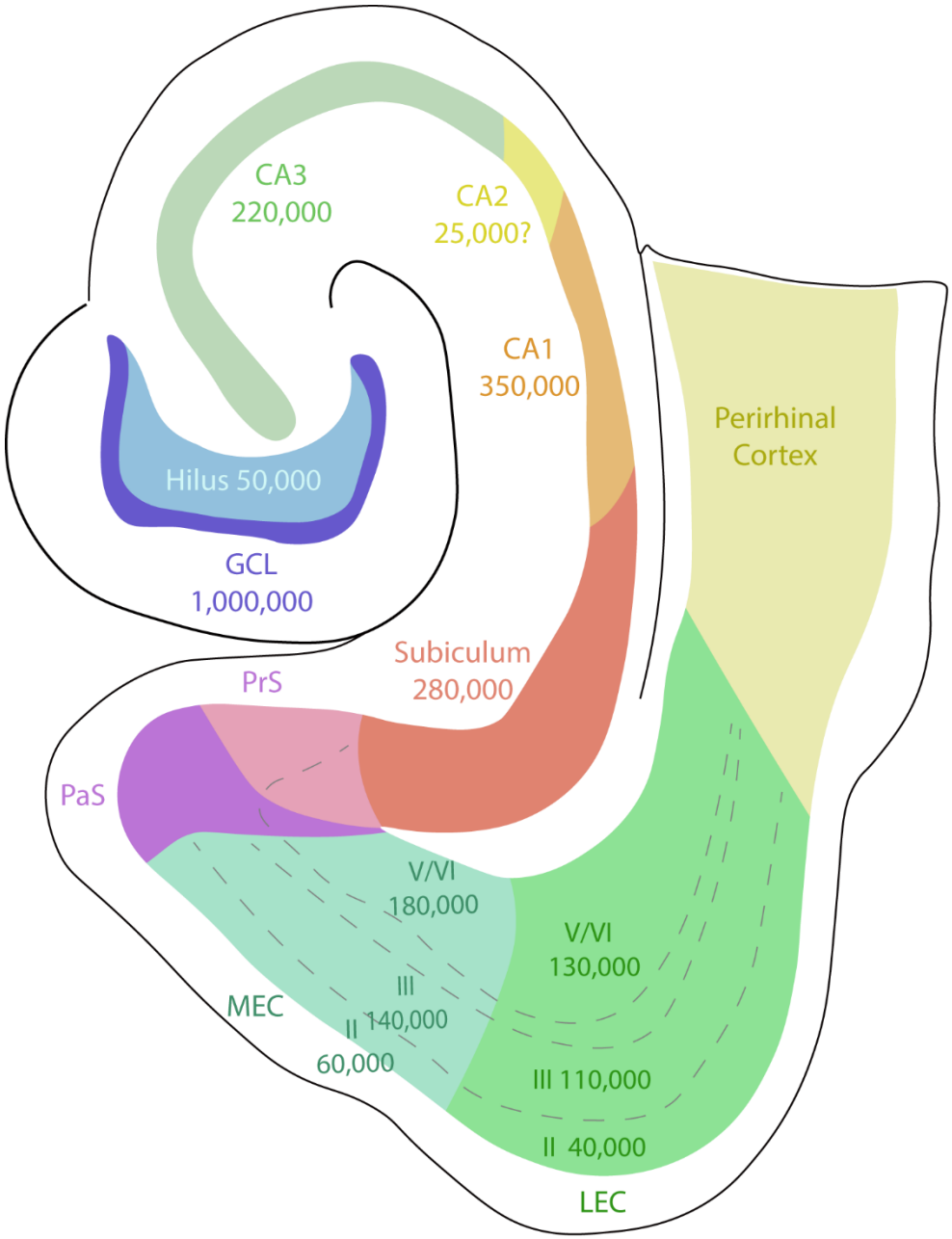

Figure 1. *Schematic drawing of the hippocampal formation of the rat (horizontal view) showing the average neuronal populations obtained in the present analysis. Data correspond to adult males except in the EC, where adolescent and females (adult and adolescent) are averaged (see text). Presubiculum (PrS) and parasubiculum (PaS) are not annotated given the potentially inaccurate averages (see text). Modified from (Cappaert et al., 2015)*

Granular cell layer

The granular cell layer (GCL) was the most frequently studied region, with 45 reports providing 75 estimates of granule cell numbers (**Table 2)**. Six of those estimates were from mixed

males and females; 16 from females, and 52 on males. Data involved 6 strains, although more than 90% of estimates originate from Wistar or SD rats. Also, almost 60% of the estimates (n=44) come from adult animals, with the remaining ~40% providing information on developing animals, up to two months of age (**Table 2**).

DG

Reference	Strain	Age (months)	Sex	# neurons (millions)
Maier and West, 2001	SD	0.33	M-F	0.47
Wang and Gondré-Lewis, 2013	SD	0.5	M-F	1.02
Schlessinger et al., 1975	Ho	0.75	M-F	0.63
Seress and Pokorny 1981	Wi	1	M-F	0.64
Seress, 1988	LE	4	M-F	0.66
Seress, 1988	SD	4	M-F	0.89
Merrill et al., 2003	F344	5	F	1.22
Merrill et al., 2003†	F344	24	F	1.16
Nunez and McCarthy 2003	SD	0.25	F	0.22
Nunez et al., 2003	SD	0.25	F	0.17
Smith et al., 2008	SD	0.3	F	0.33
Nunez et al., 2003b	SD	0.75	F	0.58
Madeira et al., 1988	SD	1	F	0.88
Boss et al., 1985	SD	1	F	1.03
Smith et al., 2008	SD	2.25	F	0.56
Boss et al., 1985	SD	4	F	0.99
Madeira et al., 1991	SD	6	F	0.82
Boss et al., 1985	SD	12	F	1.04
Boss et al., 1985	Wi	1	F	0.71
Mulders et al., 1997	Wi	1	F	1.2
Boss et al., 1985	Wi	4	F	1.02
Boss et al., 1985	Wi	12	F	0.81
Bonthius et al., 2001	Lew	3	M	1.37
West et al., 1988	*F344*	*12*	*M*	*outlier 2.06*
Rapp and Gallagher 1996	LE	6	M	1.15
Rapp and Gallagher 1996	LE	28	M	1.22
Nunez et al., 2003	SD	0.25	M	0.23
Nunez and McCarthy 2003	SD	0.25	M	0.24
Smith et al., 2008	SD	0.3	M	0.36
Nunez et al., 2003b	SD	0.75	M	0.67
Madeira et al., 1988	SD	1	M	1.19
Wakuda et al., 2008	SD	1.5	M	0.79
Thind et al., 2010	SD	1.5	M	1.1
Smith et al., 2008	SD	2.25	M	0.65

Keles et al., 2019	SD	2	M	1.01
McCullers et al., 2002	SD	2	M	0.76
Grady et al., 2003	SD	3	M	1.51
Wakuda et al., 2008	SD	3	M	0.77
Dalby et al., 1998	SD	3	M	0.98
Czeh et al., 2013$	SD	3	M	0.94
Fitting et al., 2010	SD	5.25	M	1.17
Madeira et al., 1991	SD	6	M	1
Fitting et al., 2008	SD	7.5	M	0.97
Elibol-Can et al., 2014	Wi	0.03	M	0.19
Elibol-Can et al., 2014	Wi	0.33	M	0.5
Heine et al., 2004	Wi	0.5	M	0.43
Miki et al., 2005	Wi	0.5	M	0.44
Miki et al., 2005	Wi	1	M	0.52
West 1990	Wi	1	M	1.08
Aslan et al., 2006	*Wi*	*1*	*M*	*outlier 1.77*
Sousa et al., 1998b	Wi	1	M	0.95
Bayer et al, 1982	Wi	1	M	0.89
Elibol-Can et al., 2014	Wi	1	M	0.78
West et al., 1991	Wi	1	M	1.2
Heine et al., 2004	Wi	1.5	M	0.84
Hosseini-Sharifabad & Nyengaard 2007	Wi	2	M	1.08
Elibol-Can et al., 2014	Wi	2	M	1.04
Gaarskjaer (1978) ‡	Wi	2.3	M	0.99
Gaarskjaer (1978) ‡	Wi	2.3	M	1.19
Rasmussen et al., 1996	Wi	2.5	M	1.55
Aslan et al., 2006	Wi	3	M	1.08
Cardoso et al., 2008	Wi	3	M	1.13
Bayer et al, 1982	Wi	4	M	0.99
Xavier et al., 1999	Wi	4.5	M	0.85
Lukoyanov et al., 2004	Wi	5	M	1.15
Sousa et al., 1998a, b, 1999	Wi	6	M	1.2
Cardoso et al., 2011	Wi	6	M	1.16
Bayer et al, 1982	Wi	6.5	M	1.11
Heine et al., 2004	Wi	12	M	0.78
Bayer et al, 1982	Wi	12	M	1.28
West and Andersen, 1980	*Wi*	*12*	*M*	*outlier 2.17*
Lukoyanov et al., 1999,2000	Wi	16	M	1.31
Sousa et al., 1998a	Wi	24	M	1.16

Heine et al., 2004	Wi	24	M	0.74
Rasmussen et al., 1996	*Wi*	*24*	*M*	*outlier 1.86*
Adult male average (CV; n)				1.074 (0.20; 31)
Adult female average (CV; n)				0.953 (0.22; 8)
Adult male median				1.08
Adult female median				1.005

Table 2*. Number of neurons in the GCL (in millions). Data is ordered by sex, strain and age. Data include coefficient of variation (CV) and the number of estimates (n). Tukey fences (0.48-1.65) detected 4 outliers (highlighted in italics).*
†*Merrill et al., 2003 used cognitively impaired and unimpaired 24-month-old animals that rendered similar results. The average of both groups is reported here.*
‡*Gaarskjaer 1978 used two correction methods for stereological quantification*

Table 2 shows a wide range of estimates, as expected that granule cell (GC) neurogenesis occurs mostly postnatally. However, variability is high, particularly in developing and young adults, with almost 3-fold difference between estimates. Tukey fences (Tukey, 1977) were 0.48-1.65, revealing 4 outliers (1.86-2.17 million) that were excluded from further analysis (**Table 2**). Male average without outliers was 1.07 million with an almost identical median of 1.08, suggesting a symmetric distribution of the data. Female average was about 10% lower, 0.95 million with a slightly higher median of 1.005, suggesting a slight asymmetry toward lower values.

Graphical representation of the data (without outliers; **Fig 2**) shows an exponential increase in neuron number early postnatally that grows at much lower pace after age 2-3 months, reflecting granule cell neurogenesis that occurs mostly after birth, with a peak around the end of the first postnatal week (Schlessinger et al., 1975; Bayer, 1980a). This explosive phase is followed by a sharp decrease during adolescence to continue at low levels during adulthood (Arellano and Rakic, 2024). In this regard, adolescent (0.75-2 months of age) males showed 15% less neurons than adult (p=0.022; one-way Welsch student-t test) as expected since neurogenesis is significant in the GCL at that age. Female adolescents showed a more moderate 8% difference with adults, that was not significant (p=0.3) given the low number of estimates. By strains, adolescent Wistar males have 19% less neurons than adults, a significant difference (p=0.038; one-tailed Welch's t-test), while adolescent SD males showed a maller 4% difference with adults, but their average might not be reliable (n=4) (**Table 3**).

Modelling adult neurogenesis shows that about 12% of granule cells are added during adulthood in rats, between age 2 months and 21 months (Arellano and Rakic, 2024). If there is no cell replacement, it would imply that the population of granule cells would increase about 12% between young and old individuals. Since we obtained regression curves for the GCL population, we could test their predictions regarding the number of neurons added to the GCL across adulthood. The regression curves considering data from all strains and sexes, data only from males; or data only from Wistar males, all predict net increases higher than 300,000 GCs during adulthood (2-21 months). Because there is large variability in the data from infants that might derail the regression, it can be argued that restricting the analysis to adult males could provide a more accurate regresion. However, the distribution of adult datapoints still presents large variability at young ages (2-5 months) and as a result, the regression is virtually non-existent (R2=0.004), and, the equation (y =

0.0171ln(x) + 1.0466) predicts an increase of 40,000 neurons across adulthood (2-21 months), corresponding to a 3.72% of the 1.08 million GCs in adult males, a much lower percentage than the expected ~12% (Arellano and Rakic, 2024). Thus, it seems that the large variability described at young ages (0-5 months) does not allow obtaining reliable equations to describe the dynamic of the population during adulthood.

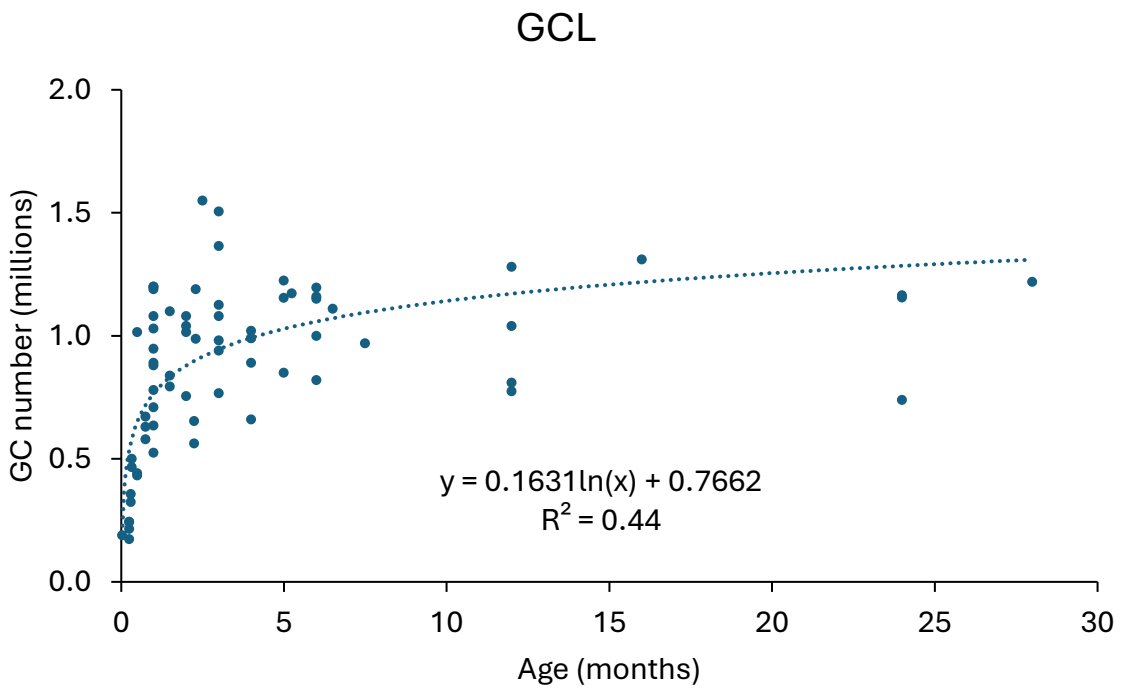

***Figure 2**. Estimates of the number of GCs across age. Note the explosive early increase reflecting postnatal neurogenesis of GCs. Most datapoints are concentrated in developmental stages and young adulthood, with fewer estimates at middle and old ages. Large variability of the estimates is a characteristic across all ages. Regression was moderate to a logarithmic curve (R2=0.44), but fitness was poor across adulthood (see text).*

Strain differences

Previous studies have shown strain differences in the number of GCs (Boss et al., 1985; Seress, 1988) (**Table 2**). In our sample, we focus the analysis between adult Wistar and SD males, as there were only scarce data on other strains or in females to establish meaningful comparisons (**Table 3**). The distributions for Wistar and SD males across age is shown in **Fig 3**. The main feature in both is still the large variability of the data and the concentration of datapoints in developing animals and young adults, with few data on middle aged and old animals from Wistar and virtually no data from SD. Both distributions overlap loosely, fitting logarithmic regression curves but only with moderate fitness due to the large dispersion of the data at any age. Wistar males had 11% more GCs than SD, (p=0.18; two-tailed Welch's t-test), a difference likely related to the virtual lack of middle- and old-age datapoints in SD, stages in which GC numbers are typically higher. Cloud representation of the data for adult males and females shows that both Wistar and SD males have

similar clusters around 1 million GCs (**Fig 3**). Considering the overall variability of the data, no reliable averages can be obtained from female data or from minority strains LE, Lewis and F344.

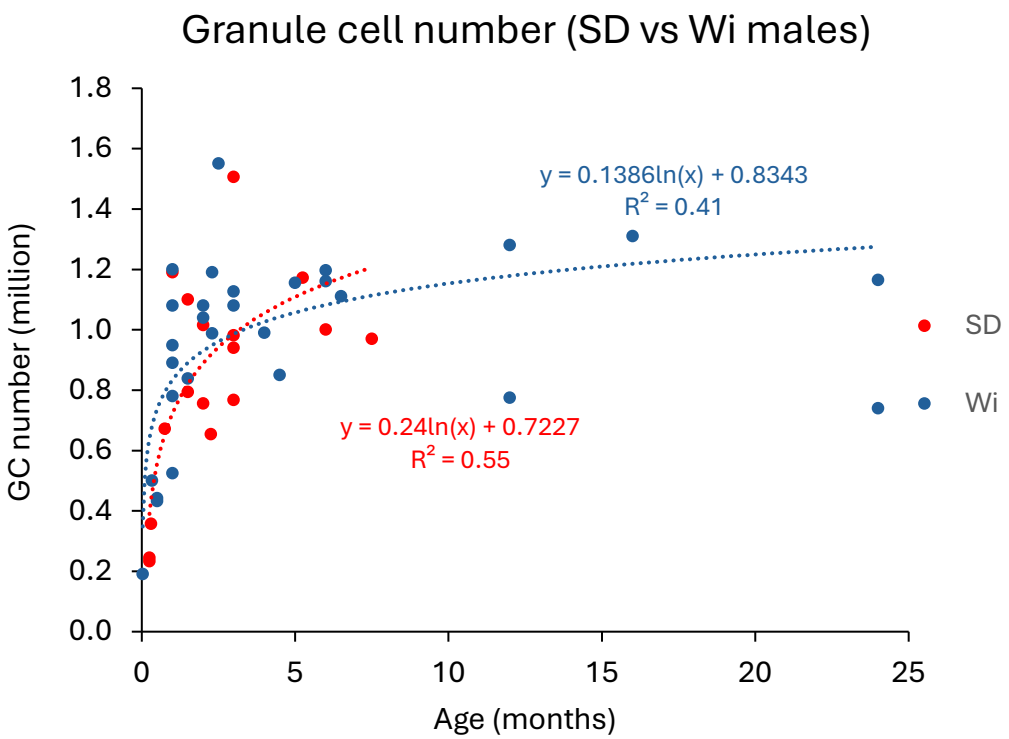

Figure 3*: Granule cell number estimates from SD and Wistar males across age. The main feature is still the large variability of the data at any age. Note the virtual absence of middle aged or old adult datapoints for SD males. Both distributions show moderate fit to logarithmic curves, although the lack of aged animals in SD strain questions the predictive value of the equation. M: male; SD: Sprague-Dawley; Wi: Wistar.*

	Males			Females
Age	SD (CV, n)	Wistar (CV, n)	Total (CV, n)	Total (CV, n)
Adolescent	*0.94 (0.26, 4)*	0.89 (0.24, 7)	0.91 (0.24, 11)	0.88 (0.28, 5)
Adult	0.98 (0.25, 10)	1.10 (0.18, 18)	1.07 (0.20, 31)	0.95 (0.24, 8)

Table 3*: Comparison of GC numbers in SD and Wistar males and in total males and females for both adolescent and adult animals. Average for adolescent SD males might not be reliable (n=4) and is highlighted in italics. Total males and females include all strains; SD: Sprague-Dawley; CV: coefficient of variation; n: number of estimates.*

Overall, the data collected here does not support significant differences between SD and Wistar strains, in line with the inconsistent results provided by studies focused on that question (Boss et al., 1985; Seress, 1988).

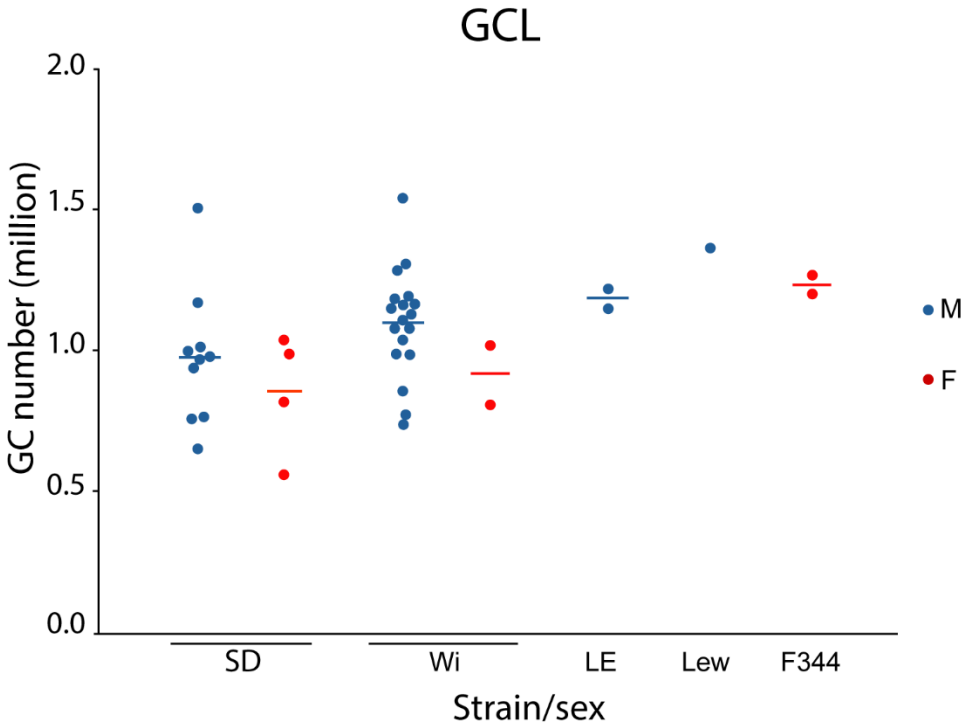

***Figure 4**. Cloud representation of adult males and females by strain. Averages are represented by horizontal lines. Most values in all distributions orbit around ~1 million GCs, with higher estimates from the few LE, Lewis and F344 data. Outliers are not represented. GC: granule cells; LE: Long Evans; Lew: Lewis; F344: Fisher 344.*

Sex differences

Previous studies have noted differences in the number of granule cells between sexes in SD rats, with (Madeira et al., 1988, 1991; Nuñez et al., 2003a, 2003b), supported also by data from (Smith et al., 2008). Overall, those studies showed that the DG of females exhibits 9-26% less GCs than the male DG both during early development in the first postnatal month (Madeira et al., 1988; Nuñez and McCarthy, 2003; Nuñez et al., 2003b; Smith et al., 2008) or in adult animals (Madeira et al., 1991; Smith et al., 2008) (**Table 2**). Comparison between males and females in our dataset is illustrated in **Fig. 4, 5 and Table 3**. Both distributions are fitted to logarithmic curves with moderate to low fitness, as expected from the large variability of the data. However, the shape and asymptotic level of both curves show a slight difference, and adult females exhibit 11% lower average neuron number (0.953 million) than adult males (1.074 million), a difference that was however not significant (p=0.09; one-tailed Welch's t-test) (**Table 3).**

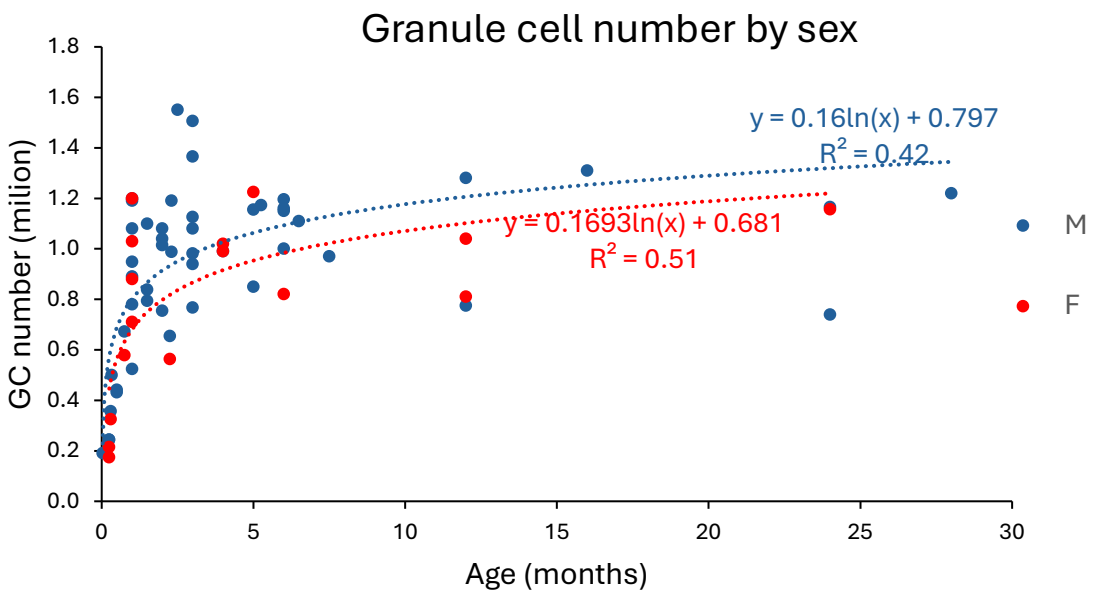

Figure 5*: Best fit logarithmic regression curves of the number of granule cells across age for males and females (all strains) shows moderate fitness and a small displacement of the curves, suggesting an overall small difference in the number of GCs in both sexes. M: male; F: female.*

Sexual dimorphism has been shown in both Wistar and SD rats (Madeira et al., 1988, 1991; Roof and Havens, 1992; Nuñez et al., 2003a, 2003b; Smith et al., 2008), however we could not confirm those findings, as there were too few female estimates for each strain (n=2-4) to obtain reliable information.

The data collected here show differences of ~10% between Wistar and SD strains (males) and between overall males and females, but they are not significant, as the samples are small and the variability high. While the inter-strain difference shows inconsistent results across studies, the sex difference has been extensively and consistently reported, suggesting it might hint to a biological feature. The average of the male and female averages is 1.005 million GCs, in good agreement with the traditional 1 million value used in many descriptions and models of the rat hippocampus.

Hilus

The hilus corresponds to the polymorphic layer of the DG and is populated by excitatory mossy cells and GABAergic interneurons. Data on females was scarce and heterogeneous, with 4 estimates from three different strains (F344, Wistar and SD) with estimates ranging from 45,000 to 63,000, suggesting the average of 52,000 neurons might not be reliable (**Table 4, 5**). Restricting the analysis to males, the hilus showed an overall average of 49,000 neurons in adult males, with a clear outlier of 115,000 neurons (Xavier et al., 1999), far above the upper Tukey fence of ~71,000 for adults). The median was very close, 50,000 suggesting symmetrical distribution of data and adequate outlier detection. Analysis by strains shows adult Wistar males having 7% less neurons

than SD males (P=0.31; two-tailed Welch's t-test; **Table 5**), a reversed trend that in other fields, were Wistar males consistently have more neurons than SD males. Fisher 344 males (n=3) showed an average of 49,000 neurons (CV=0.04), a value in between the other two strains (**Fig 7**).

Hilus

Reference	Strain	Age (months)	Sex	# neurons
Seress, 1988	CFY	4	M-F	31,000
Seress, 1988	LE	4	M-F	34,000
Mulders et al., 1997	Wi	1	F	53,000
Ramsden et al., 2003	SD	4	F	63,200
Merrill et al., 2003	F344	5	F	47,000
Merrill et al., 2003†	F344	24	F	45,000
Hattiangady et al., 2005	F344	4	M	49,452
Hattiangady et al., 2005	F344	12	M	50,522
Hattiangady et al., 2005	F344	24	M	46,777
Thind et al., 2010	SD	1.5	M	47,300
Buckmaster and Jongen-Relo, 1999	SD	1.75	M	47,000
Keles et al., 2019	SD	2	M	37,060
Lister et al., 2005	SD	3	M	52,425
Lister et al., 2006‡	SD	3	M	49,838
Dalby et al., 1998	SD	3	M	58,500
Czeh et al., 2013	SD	3	M	60,000
Jiao and Nadler, 2007	SD	4.5	M	37,580
Fitting et al., 2010	SD	5.25	M	52,495
Buckmaster and Dudek, 1997	SD	6	M	41,093
Fitting et al., 2008	SD	7.5	M	51,672
Tuunanen and Pitkanen 2000	SD	8.5	M	53,306
Grady et al., 2003	SD	3	M	65,420
Miki et al., 2005	Wi	0.5	M	38,100
West 1990	Wi	1	M	49,100
West et al., 1991	Wi	1	M	53,000
Miki et al., 2005	Wi	1	M	35,200
Sousa et al., 1998b	Wi	1	M	44,019
Gaarskjaer (1978)	Wi	2.3	M	42,500
Rasmussen et al., 1996	Wi	2.5	M	40,000
Cardoso et al., 2008	Wi	3	M	55,065
Lukoyanov et al., 2004	Wi	5	M	43,872
Xavier et al., 1999	*Wi*	*5*	*M*	*outlier 115,000*
Sousa et al., 1998a,b, 1999	Wi	6	M	46,326
Cardoso et al., 2011	Wi	6	M	55,241

Maia et al., 2014	Wi	8	M	49,323
Rasmussen et al., 1996	Wi	24	M	50,000
Sousa et al., 1998a	Wi	24	M	44,730
Adult male average (CV, n)				49,269 (0.15, 23)
Adult female average (CV, n)				*51,733 (0.19, 3)*
Adult male median				*50,000*

Table 4*. Number of neurons in the hilus.* Data is ordered by sex, strain and age. *Averages include coefficient of variance (CV) and the number of estimates (n). Average for females is not reliable (n=3) and is highlighted in italics. Tukey fences for adults were (26,687-70,685) identifying 1 outlier (Xabier et al., 1999) highlighted in italics.*
†Merrill et al., 2003 used cognitively impaired and unimpaired 21-month-old animals that rendered similar results, so the average of both groups is reported here.
‡Lister et al., 2006 reported data for left and right hemispheres. The average is listed here.

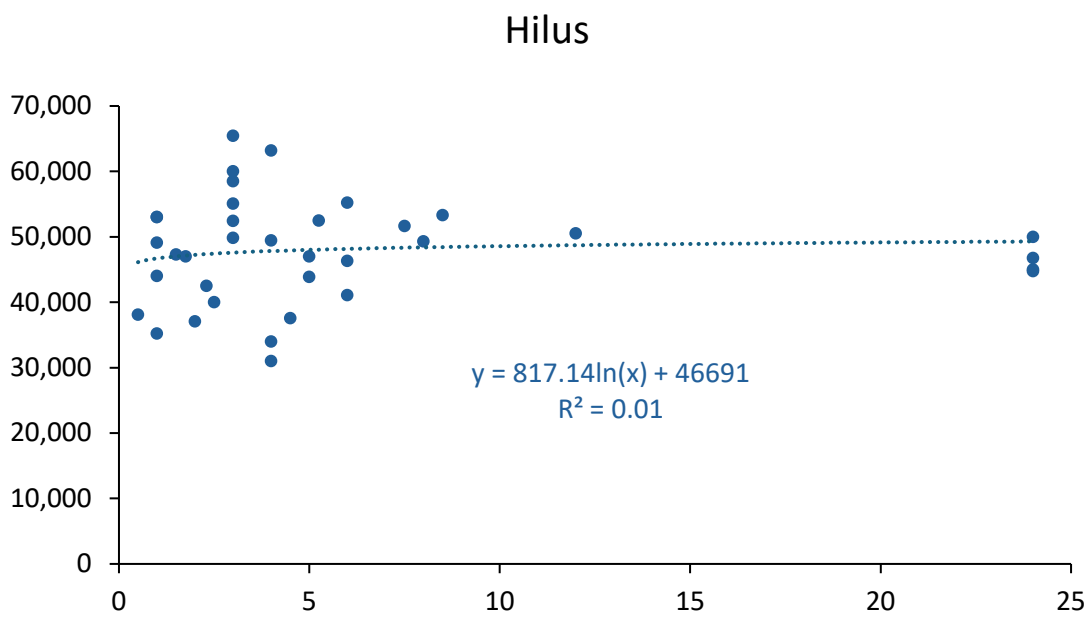

Figure 6. *Number of neurons in the hilus across age. Note the large dispersion of the data at young ages and the scarce number of datapoints in middle aged and old animals.*

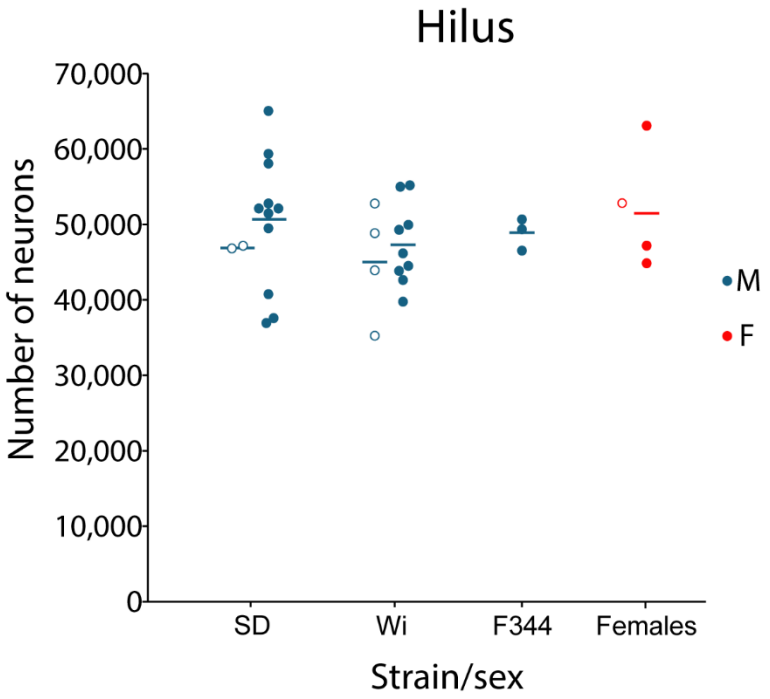

***Figure 7**. Number of neurons in the hilus by strain and sex. Circles represent adolescent values and filled dots represent adults. Averages are represented by horizontal lines. For simplicity, female data is pooled from 3 strains.*

Contrary to granule cells that exhibit protracted postnatal neurogenesis, neurons in the rest of hippocampal fields are generated largely pre- and perinatally, reaching a peak around the end of the first postnatal week and then pruning to reach adult numbers around P15 (Bayer, 1980b, 1980a; Bandeira et al., 2009). As differentiation proceeds during those postnatal weeks, it seems reasonable to assume that by the onset of adolescence, at around 3 weeks of age (Arellano et al., 2024), the number of cells identifiable as neurons would match adult levels. However, previous data have shown that 1-month-old rats consistently exhibit less neurons than adult animals (Madeira et al., 1992; Wakuda et al., 2008; Elibol-Can et al., 2014). In our dataset, the average for adolescent males in the hilus was 46,000, about 7% less than in adults (p=0.14; one-tailed Welch's t-test). Comparison by strain showed similar small differences, although they are not reliable given the small number of adolescent estimates **(Table 5).**

	Males			Females
	SD (CV, n)	Wistar (CV, n)	Total (CV, n)	Total (CV, n)
Adolescent	*47,150 (0.004; 2)*	*45,330 (0.17; 4)*	45,937 (0.13; 6)	*53,000 (1)*
Adult	50,854 (0.18; 11)	47,451 (0.11; 9)	49,269 (0.15; 23)	*51,733 (0.19, 3)*

***Table 5**: Average number of hilar neurons in adolescent and adult Wistar and SD males, and in all adult male and females. Averages for adolescent SD and Wistar males and for total females are not reliable (low n) and are highlighted in italics. Total adult males and females include all strains. SD: Sprague-Dawley; CV: coefficient of variation; n: number of estimates.*

Overall, both the average and the median suggest the hilus has 50,000 neurons in males, while there are not enough female datapoints to reach a reliable average. Regarding strains, contrary to other fields, Wistar shows slightly lower (7%) average neurons compared to SD, a difference that is not statistically significant.

CA3

Field CA3 has been the focus of many studies, as is one of the components of the tri-synaptic circuit in the hippocampus. However, few studies (n=6) have quantified the number of neurons exclusively in CA3, and the majority (n=30) count the number of neurons of CA3 and CA2 combined (for detailed explanation see section "Field segregation and nomenclature" in the material and methods). A few reports did not clearly specify if their definition of CA3 includes CA2 or not, so we have sorted them to the best of our knowledge based on the figures and descriptions provided by the authors, into **tables 6** (CA3) and **table 7** (CA2 and CA3).

CA3

Reference	Strain	Age (months)	Sex	# neurons
Wang and Gondré-Lewis, 2013†	SD	0.5	M-F	205,000
Smith et al., 2008	SD	0.3	F	157,000
Smith et al., 2008	SD	2.25	F	364,000
Smith et al., 2008	SD	0.3	M	146,000
Wakuda et al., 2008	SD	1.5	M	177,000
Smith et al., 2008	SD	2.25	M	368,000
Baldwin et al., 1997‡	SD	2.5	M	152,571
Cassell et al., 1980§	Wi	2.5	M	143,000
Czeh et al., 2013	SD	3	M	260,000
Wakuda et al., 2008	SD	3	M	186,000
Average adult males (CV, n)				222,314 (0.42, 5)
CA3 as 89% of CA3/CA2 (adult males)				218,257

Table 6*. Number of neurons in field CA3. Data is ordered by sex, strain and age. CA3 estimate as a fraction CA3/CA2 is also listed (see CA3/CA2 and CA2 sections).* SD: Sprague-Dawley; CV: coefficient of variation.
†Wang and Gondré-Lewis, 2013 data represents half the number of neurons per brain.
‡*Baldwin et al., 1997 data correspond to the average of left and right hemispheres.*
§(Cassell, 1980) *data from* (Amaral et al., 1990)*; age extrapolated from* (Cassell and Brown, 1977).

The 6 studies on CA3 alone provided 10 estimates, all but one in SD rats. The scarcity of data for females (n=2) or adolescents (n=1) precludes obtaining reliable averages, with only one estimate for adult females showing a relatively high figure of 364,000 neurons. Regarding males, the 5 estimates corresponding to young adults show an average of 222,000 neurons with large dispersion of the data (CV~0.4).

CA3-CA2

Most studies on CA3 actually include CA2, resulting in a combined field CA3-CA2. We found 33 studies that produced 45 estimates of CA3/CA2, but only 7 estimates were from females and only 2 of those from adult females with an average of 244,000 neurons (**Table 7**). There are 4 additional estimates using mixed sexes, with 2 adult values showing low figures of 170-184,000 neurons.

CA2-CA3

Reference	Strain	Age (months)	Sex	# neurons
Maier and West, 2001	SD	0.33	M-F	293,000
Schlessinger et al., 1978	Ho	0.75	M-F	150,000
Seress, 1988	LE	4	M-F	184,000
Seress, 1988	SD	4	M-F	170,000
Nuñez et al., 2003b	SD	0.75	F	273,000
Boss et al., 1987	SD	1	F	330,000
Madeira et al., 1992	SD	1	F	161,000
Ramsden et al., 2003	SD	4	F	298,000
Madeira et al., 1992	SD	6	F	189,000
Boss et al., 1987	Wi	1	F	210,000
Mulders et al., 1997	Wi	1	F	250,000
Rapp and Gallagher 1996	LE	6	M	228,000
Rapp and Gallagher 1996	LE	28	M	225,000
Bonthius et al., 2001	Lew	3	M	300,000
Nuñez et al., 2003b	SD	0.75	M	335,000
Madeira et al., 1992	SD	1	M	168,000
Grady et al., 2003	SD	2	M	262,200
McCullers et al., 2002	SD	2	M	108,686
Keles et al., 2019	SD	2	M	143,512
Lister et al., 2005	SD	3	M	181,570
Lister et al., 2006†	SD	3	M	177,633
Fitting et al., 2010	SD	5.25	M	416,498
Madeira et al., 1992	SD	6	M	192,000
Fitting et al., 2008	SD	7.5	M	252,504
Elibol-Can et al., 2014	Wi	0.03	M	150,000
Elibol-Can et al., 2014	Wi	0.3	M	260,000
Miki et al., 2005	Wi	0.5	M	169,300
Miki et al., 2005	Wi	1	M	149,600
Elibol-Can et al., 2014	Wi	1	M	290,000
West 1990	Wi	1	M	202,000
West et al., 1991	Wi	1	M	250,000

Sousa et al., 1998b	Wi	1	M	244,068
Elibol-Can et al., 2014	Wi	2	M	330,000
Hosseini-Sharifabad & Nyengaard 2007	Wi	2	M	190,000
Gaarskjaer (1978)	Wi	2.3	M	190,000
Rasmussen et al., 1996	Wi	2.5	M	360,000
Cassell et al., 1980‡	Wi	2.5	M	157,000
Xavier et al., 1999	Wi	4.5	M	361,000
Lukoyanov et al., 2004	Wi	5	M	225,769
Sousa et al., 1998a, b, 1999	Wi	6	M	242,418
Cardoso et al., 2011	Wi	6	M	238,055
Maia et al., 2014	Wi	8	M	248,881
Lukoyanov et al., 1999,2000	Wi	16	M	267,000
Rasmussen et al., 1996	Wi	24	M	350,000
Sousa et al., 1998a	Wi	24	M	243,797
Adult male average (CV, n)				245,480 (0.31, 24)
Adult male median				240,000
Adult female average (CV, n)				*243,500 (0.32, 2)*

Table 7*. Number of neurons in field CA3-CA2. Data is ordered by sex, strain and age. The female average is based in only 2 estimates and is highlighted in italics to indicate is not reliable. Tukey fences for adult animals were (42,500-426,500); no outliers were found.*
†*Lister et al., 2006 reported data for left and right hemispheres. The average is listed here.*
‡(Cassell, 1980) *data from* (Amaral et al., 1990)*; age extrapolated from* (Cassell and Brown, 1977).

Number of neurons across age

Graphical representation of the data shows large variability at early ages (**Fig. 8**), precluding regression (R2=0.05). The variability is not explained by the effect of sex or strains, as the same lack of regressions is present when segregating the data according to those variables (data not shown).

Adolescent males showed similar neuronal numbers than adults, with only 5% lower estimate (p=0.34; one-tailed Welch's t-test)**.** Adolescent females (n=4) averaged 238,000 neurons (CV=0.3), similar to the adult female average of 240,000 (n=2), although those averages are not reliable given the low number of estimates (**Table 8)**.

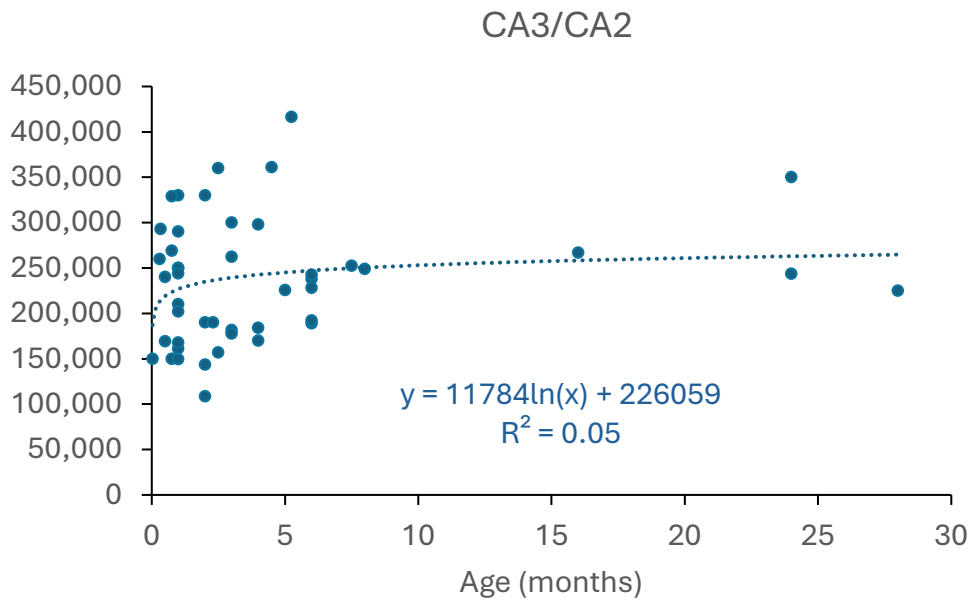

Figure 8 *Distribution of all CA3-CA2 estimates across age. The data show large variability particularly at early ages and has no datapoints across middle age. The large variance precludes obtaining regression (R2=0.05).*

Comparisons by strain

When comparisons are established by strains, adult Wistar males showed 17% more neurons than SD males, a difference that was however not significant (p=0.27; two tailed Welch's t-test) (**Table 8).** Adolescent Wistar males showed 13% less neurons than adults (p=0.14; one-tailed Welch's t-test), while no reliable average could be obtained for adolescent SD males (**Table 8**). Other strains included Long Evans (n=2) and Lewis (n=1) with estimates of 227,000 and 300,000 neurons, also unreliable given their low number of datapoints (**Fig. 9)**.

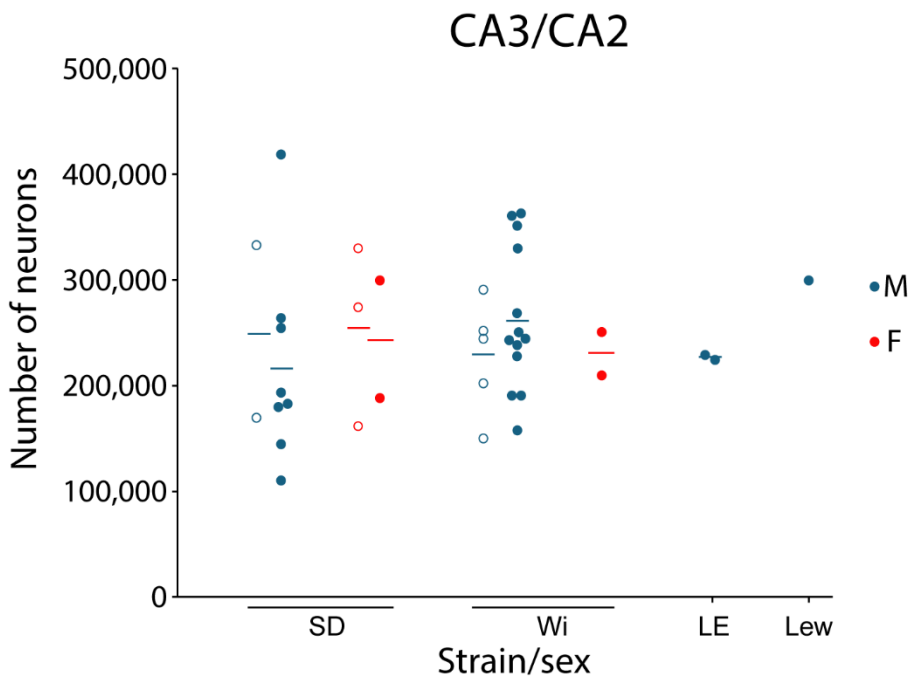

Figure 9. *Cloud graph showing the estimates of number of neurons in CA3/CA2 per strain and sex. Circles represent adolescent values and filled dots represent adults. Averages are represented by horizontal lines. The very high value (416,000; Fitting et al., 2010) for SD males was not identified as an outlier (see text). Note that female information is scarce and only available for SD and Wi strains.*

	Males			Females
Age	SD (CV, n)	Wistar (CV, n)	Total (CV, n)	Total (CV, n)
Adol.	*248,500 (0.46, 2)*	227,134 (0.24, 5)	233,238 (0.28, 7)	244,000 (0.26, 5)
Adult	216,825 (0.44, 8)	261,840 (0.26, 13)	245,480 (0.31, 24)	*243,500 (0.32, 2)*

Table 8: *Average of CA3-CA2 neurons per strain in adolescent and adult males. Adolescent SD and adult female data are not reliable (n=2) and are highlighted in italics. Total adult males include all strains. SD: Sprague-Dawley; CV: coefficient of variation; n: number of estimates.*

Comparisons by Sex

We can only obtain reliable estimates of the population of CA3-CA2 for adult males, with an average of 245,000 neurons, similar to the median of 240,000, a minor difference likely due to a high value of 416,000 neurons in SD males (**Fig 9**). There are only 2 estimates for adult females, making their average unreliable. However their average of 244,000 is virtually identical to that of adolescent females (n=5) and adult males, suggesting there might not be significant differences between sexes in adults, in line with previous studies (Madeira et al., 1992; Smith et al., 2008) (**Table 6**).

Number of neurons in CA3 alone

The neuronal population of CA3 alone in males can be estimated by subtracting the population of CA2 from the total of CA3/CA2. As described in the CA2 section, CA2 might represent ~11% of CA3-CA2, implying CA3 alone is about 89% of CA3-CA2, or 218,000 neurons (89% of 245,000) (**Table 7, 8)**. That value is consistent with the average of 222,000 obtained from CA3 alone (**Table 6)**, and thus it seems reasonable to assume that CA3 alone might have ~220,000 neurons.

CA2

As indicated above, almost all studies describe pooled data for fields CA2 and CA3. However, we found two studies that quantified CA3 and CA2 separately. Both show very different results, with a 3-fold difference in their estimate for CA2 **(Table 9)**.

CA2				
Reference	**Strain**	**Age (months)**	**Sex**	**N**
Wang and Gondre-Lewis, 2013†	SD	0.5	M-F	35,000
Cassell 1980‡	Wi	2.5	M	12,390
Average				23,695

Table 9*. Estimates of number of neurons in CA2.*
†(Wang and Gondré-Lewis, 2013) value correspond to half the originally reported data that corresponded to the whole brain.
‡(Cassell, 1980) *data from* (Amaral et al., 1990)*; age extrapolated from* (Cassell and Brown, 1977).

These two studies also reported that CA3 exhibited 143,000 (Cassell, 1980; Amaral et al., 1990) and 205,000 neurons (Wang and Gondré-Lewis, 2013) to a total of 157,000 and 240,000 for CA3-CA2, respectively. Thus, in addition to the large differences in the CA2 estimates, there was a 30% difference in their estimates of CA3/CA2. One way to overcome such differences in absolute number of neurons is normalize the estimates as fractions of the total CA2/CA3 population. Still those fractions vary 2-fold, from 7.9% (Cassell, 1980) to 14.6% (Wang and Gondré-Lewis, 2013), suggesting that both studies segregated differently CA2 and CA3 fields, a likely possibility considering that neurons look similar in cellular staining in both fields (see nomenclature section in material and methods). If we average those two proportions we obtain that CA2 represents 11% of the CA3/CA2 neuronal population, or 27,000 neurons (11% of 245,000), that is in range with the average of 24,000 obtained from the absolute estimates by (Cassell, 1980) and (Wang and Gondré-Lewis, 2013) (**Table 9).**

Thus, until more data about the neuronal population of CA2 are available, it seems reasonable to assume that CA2 might have ~25,000 neurons, or about 11% of the combined population of CA3-CA2.

CA1

We found 34 studies documenting 52 estimates of number of neurons in CA1, but only 9 estimates were obtained in females, and only 3 of those were from adult animals, with a wide range

from 180,000 to 312,000 neurons, producing a relativeley low average of 232,000 neurons (**Table 11**). Another 7 estimates came from mixed male and female animals, with 4 adult estimates averaging 335,000 neurons. Regarding males, we found 36 estimates mostly from Wistar (n=16) and SD (n=15) animals (**Table 10**). There was one study (Czéh et al., 2013) reporting CA1-CA2 together, with a total of 370,000 neurons. If we assume CA2 has about 25,000 neurons (see CA2 section), the resulting estimate for CA1 is 345,000 neurons, in good agreement with the overall average.

CA1

Reference	Strain	Age (months)	Sex	N
Schlessinger et al., 1978	Ho	0.75	M-F	320,000
Seress 1988	LE	4	M-F	262,000
Maier and West 2001	SD	0.33	M-F	239,000
Wang and Gondré-Lewis, 2013†	SD	0.5	M-F	340,000
Seress, 1988	SD	4	M-F	244,000
Heggland et al., 2015	Wi	6	M-F	470,616
Heggland et al., 2015	Wi	18	M-F	362,637
Smith et al., 2008	SD	0.3	F	141,000
Nunez et al., 2003b	*SD*	*0.75*	*F*	*outlier 705,000*
Boss et al., 1987	SD	1	F	420,000
Madeira et al., 1992	SD	1	F	255,000
Smith et al., 2008	SD	2.25	F	180,000
Madeira et al., 1992	SD	6	F	302,000
Boss et al., 1987	Wi	1	F	320,000
Mulders et al., 1997	Wi	1	F	382,000
Cassell 1980‡	Wi	2.5	F	213,000
Rapp and Gallagher 1996	LE	6	M	378,000
Rapp and Gallagher 1996	LE	28	M	403,000
Bonthius et al., 2001	Lew	3	M	427,000
Smith et al., 2008	SD	0.3	M	177,000
Nunez et al., 2003b	*SD*	*0.75*	*M*	*outlier 734,000*
Madeira et al., 1992	SD	1	M	335,000
Wakuda et al., 2008	SD	1.5	M	282,874
Grady et al., 2003	SD	2	M	387,800
Keles et al., 2019	SD	2	M	197,736
McCullers et al., 2002	SD	2	M	309,000
Smith et al., 2008	SD	2.25	M	238,000
Wakuda et al., 2008	SD	3	M	320,371
Lister et al., 2005	SD	3	M	330,998
Lister et al., 2006§	SD	3	M	305,370
Czeh et al., 2013¶	SD	3	M	345,000

Fitting et al., 2010	SD	5.25	M	262,181
Madeira et al., 1992	SD	6	M	362,000
Fitting et al., 2008	SD	7.5	M	233,042
Elibol-Can et al., 2014	Wi	0.03	M	160,000
Elibol-Can et al., 2014	Wi	0.3	M	260,000
Miki et al., 2005	Wi	0.5	M	176,800
West et al., 1991	Wi	1	M	380,000
West 1990	Wi	1	M	361,000
Miki et al., 2005	Wi	1	M	152,700
Elibol-Can et al., 2014	Wi	1	M	290,000
Sousa et al., 1998b	Wi	1	M	398,519
Elibol-Can et al., 2014	Wi	2	M	350,000
Rasmussen et al., 1996	Wi	2.5	M	570,000
Xavier et al., 1999	Wi	4.5	M	247,000
Lukoyanov et al., 2004	Wi	5	M	352,540
Sousa et al., 1998a, b, 1999	Wi	6	M	425,069
Cardoso et al., 2011	Wi	6	M	358,513
Maia et al., 2014	Wi	8	M	348,615
Lukoyanov et al., 1999,2000	Wi	16	M	420,000
Sousa et al., 1998a	Wi	24	M	396,482
Rasmussen et al., 1996	*Wi*	*24*	*M*	*Outlier 670,000*
Adult male average (CV, n)				346,422 (0.23, 23)
Adult male median				351,000

Table 10. *Estimates of number of neurons in CA1. Data is ordered by sex, strain and age. Most data come from SD and Wistar males. Tukey fences for adult animals were (60,227-598,205) detecting 3 outliers (highlighted in italics). CV: coefficient of variation; n: number of datapoints).*
†(Wang and Gondré-Lewis, 2013) value correspond to half the originally reported data that corresponded to the whole brain.
‡(Cassell, 1980) *data from* (Amaral et al., 1990)*; age extrapolated from* (Cassell and Brown, 1977).
§Lister et al., 2006 reported data for left and right hemispheres. The average is listed here.
¶Czeh et al., 2013 reported data on fields CA1/CA2 combined. CA1 value estimated after subtracting number of neurons of CA2 (see text).

Number of neurons across age

Graphical representation of the data across age shows very large variability at early ages, lack of datapoints across middle age (7-15 months; **Fig. 10**) and more homogeneous estimates from old animals. Regression of the data shows poor fit to a logarithmic function (R2=0.22). As in CA2-CA3, the large variability of the data across time cannot be explained by the effect of sex or strains, and similar poor regressions were found when segregating the data according to those variables (data not shown).

Adolescent males had 9% less neurons than adults (p=0.2; one-way Welch's test), with this difference raising to 18% in Wistar (p=0.12; one-way Welch's test). The low number of adolescent estimates prevented comparisons for SD males or females (**Table 11**). Regarding aging, our data in middle aged and old animals is scarce and does not allow reliable comparisons.

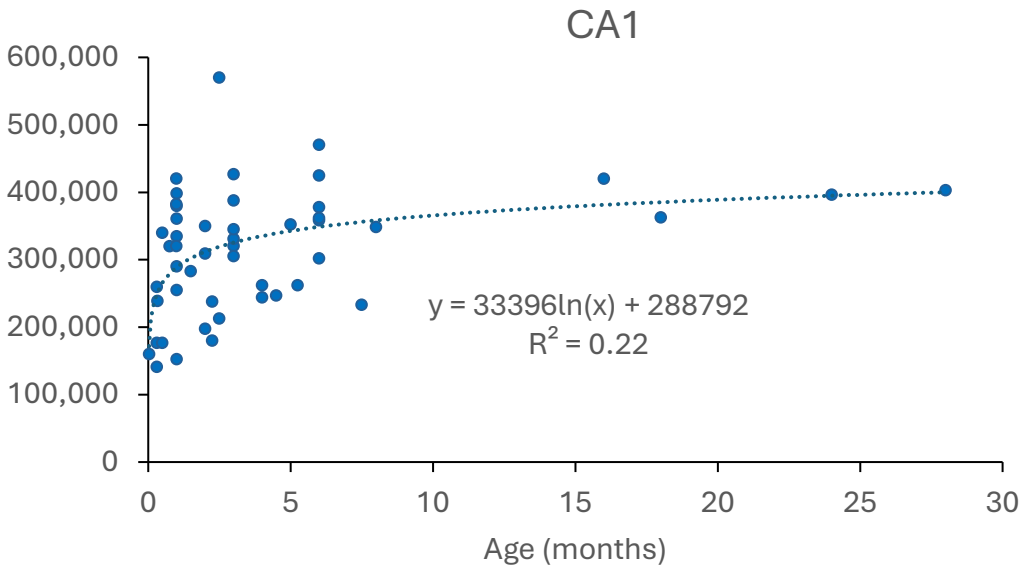

***Figure 10**. Number of neurons in CA1. As in the other fields, there is very large variability at early ages, and there are no values for middle aged animals, while values for old anmals are quite homogeneous. Overall, the data fit loosely a logarithmic function (R2=0.2). Three very high values (over 670,000) were detected as outliers and are not represented here (see **Table 10**).*

Comparisons by Sex and strain

The low number of estimates for females do not allow obtaining reliable averages. Anecdotally, the average for adolescent females is virtually identical to the average of adult males, much closer than the average of adult females (**Table 11).**

When adult animals are compared by strains, adult SD males showed 22% less neurons than Wistar, (**Table 11** and **Fig. 11**), a difference that was not significant (p=0.09). Other strains included Long Evans (n=2) with very similar average to Wistar (390,000) and Lewis, with a single, high value of 427,000 neurons.

	Males			Females
Age	SD (CV, n)	Wistar (CV, n)	Total (CV, n)	Total (CV, n)
Adol.	*308,937 (0.12, 2)*	316,444 (0.32; 5)	314,229 (0.27; 7)	*344,250 (0.21, 4)*
Adult	299,227 (0.20; 11)	385,358 (0.23; 9)	346,422 (0.23; 23)	*231,667 (0.27, 3)*

***Table 11:** Average of CA1 neurons per strain in adolescent and adult males and females. Adolescent SD male and adolescent and adult female data are not reliable (n=2-4) and are highlighted in italics.*

Total adult males include all strains. SD: Sprague-Dawley; CV: coefficient of variation; n: number of estimates.

Overall, if we consider all strains, from both the average and the median, it can be concluded that CA1 might exhibit about 350,000 neurons in males, while there is no reliable data for females. Once again, as in other fields, adolescents exhibit slightly lower number of neurons, although the difference is not significant.

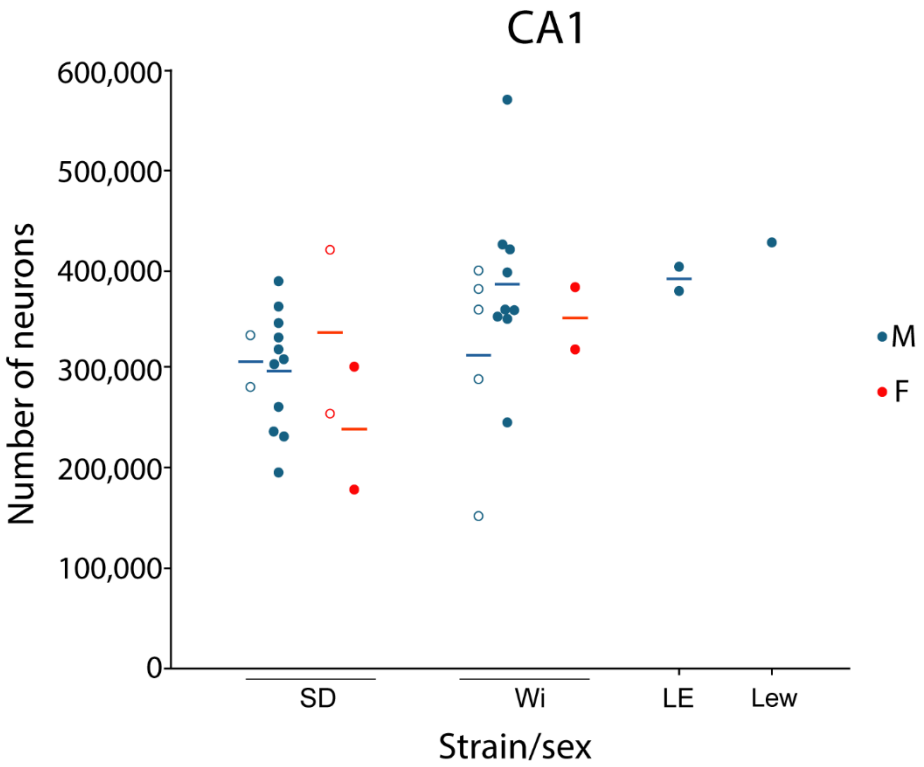

***Figure 11**. Number of neurons in CA1 by strain and sex. Circles represent adolescent values and filled dots represent adults. Averages are represented by horizontal lines. Note the larve variability of Wistar males compared to SD males*.

Subicular complex

We collected a total of 12 studies reporting 23 estimates on the subicular complex fields, mostly on the subiculum (13 estimatess; **table 12**), while 3 studies reported on the postsubiculum, presubiculum, and parasubiculum (**table 13**).

Subiculum

Most estimates in the subiculum orbit around 300,000 neurons regardless of age, sex or strain, with a few discordant estimates (3 values below 200,000 and a high one close to 400,000 neurons), including a particularly low value of 128,000 neurons (Cassell, 1980) that was however not detected as an outlier (Tukey fences: 22,079-492,883) (**Fig. 12**). Adult males show an average of 277,000 neurons, and a slightly higher median of 290,000 neurons, while there are insufficient data

to obtain reliable averages for females or adolescents. Comparisons by strain show large differences (30%) but are also not reliable given the low number of estimates (**Table 13** and **Fig. 12**).

Subiculum

Reference	Strain	Age (months)	Sex	N
Heggland et al., 2015	Wi	18	M+F	389,650
Mulders et al., 1997	Wi	1	F	300,200
Cassell 1980†	Wi	2.5	F	128,000
Andrade et al., 2000	Wi	6	F	296,000
Lister et al., 2005	SD	3	M	198,630
Lister et al., 2006‡	SD	3	M	197,500
Fitting et al., 2010	SD	5.25	M	312,252
Fitting et al., 2008	SD	7.5	M	260,148
West 1990	Wi	1	M	303,000
Rasmussen et al., 1996	Wi	2.5	M	300,000
Andrade et al., 2000	Wi	6	M	350,000
Cardoso et al., 2011	Wi	6	M	316,331
Rasmussen et al., 1996	Wi	24	M	280,000
Adult male average (CV, n)				276,858 (0.20, 7)
Adult male median				290,000

***Table 12**. Number or neurons in the rat subiculum. No outliers were detected (Tukey fences for adults: 22,079-492,883).*
†(Cassell, 1980) *data from* (Amaral et al., 1990)*; age extrapolated from* (Cassell and Brown, 1977)*.*
‡Lister et al., 2006 reported data for left and right hemispheres. The average is listed here.

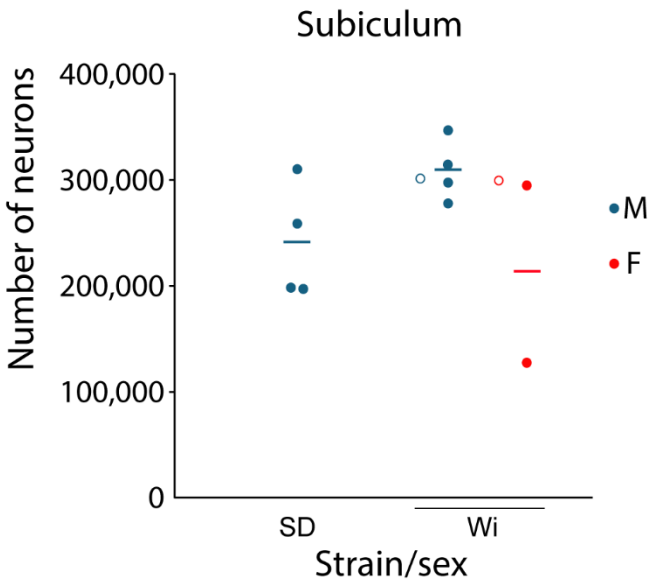

Figure 12. *Neuron number in the subiculum per strain and sex. Circles represent adolescent values and filled dots represent adults. Averages are represented by horizontal lines. Note the large variability in female data.*

	Males			Females
Age	SD (CV, n)	Wistar (CV, n)	Total (CV, n)	Total (CV, n)
Adult	*242,133 (0.22; 4)*	*311,583 (0.10; 4)*	276,858 (0.20; 8)	*212,000 (0.56, 2)*

Table 13: *Average of subicular neurons per strain adult males and females. Data per strain and for females are shown for completeness but are not reliable (n=2-4) and are highlighted in italics. Total adult males include all strains. SD: Sprague-Dawley; CV: coefficient of variation; n: number of estimates.*

In summary, there is limited number of datapoints for the subiculum, but consensus from the average and the median suggests that a good estimate of the population in males can be ~280,000 neurons.

Presubiculum, Parasubiculum and Postsubiculum

We found 3 studies reporting the number of neurons in the pre-, para- and postsubiculum (**Table 14**). The latter field is not commonly differentiated, and corresponds to the posterior presubiculum (Swanson and Cowan, 1977). The 3 studies obtained quite different estimates, maybe related to the fact that all three used rats of different age, sex and strain, although the differences were large enough to suggest that other factors such as differences in the histological definition of the fields or methodological criteria might be contributors. Because of the heterogeneity of the subjects and the results, no averages were estimated, as they do not seem to be meaningful or representative.

Presubiculum II/III	**Strain**	**Age (months)**	**Sex**	**N**
Mulders et al., 1997	Wi	1	F	334,000
Amaral et al., 2024†	SD	3	M	122,794
Cardoso et al., 2011	Wi	6	M	249,266
Parasubiculum II/III				
Mulders et al., 1997	Wi	1	F	150,000
Amaral et al., 2024†	SD	3	M	96,188
Cardoso et al., 2011	Wi	6	M	135,362
Pre- and parasubiculum V/VI				
Mulders et al., 1997	Wi	1	F	218,000
Amaral et al., 2024†	SD	3	M	87,231
Postsubiculum				

Amaral et al., 2024†	SD	3	M	139,050
Cardoso et al., 2011	Wi	6	M	190,200

Table 14. *Number of neurons in pre-para- and post-subiculum. The three studies used rats of different age, sex and strain, and produced largely different results, and therefore no averages were calculated.*
†Amaral et al., 2024 reported data for both hemispheres combined; listed data is half the original value.

Entorhinal cortex

The entorhinal cortex (EC) is the main interface of communication between the neocortex and the hippocampus. In murine rodents, the EC is divided in two areas: the medial entorhinal cortex (MEC) and the lateral entorhinal cortex (LEC). In addition, the entorhinal cortex presents 6 layers, although its main feature is that layer IV is basically acellular, populated only with sparse neurons from layer III and V, and is called lamina dissecans, as it separates upper layers II and III from lower layers V and VI (Witter et al., 2017). We will present the data by layers and separated for MEC and LEC. Interestingly, contrary to other fields, most data on the EC comes from females.

EC layer II

Six studies provided 9 estimates of number of neurons in EC layer II. Some studies differentiated layers and other segregated fields MEC and LEC. Animals were mostly females, from 2 strains and ages 1-21 months old (**Table 15**).

MEC layer II	**Strain**	**Age (months)**	**Sex**	**N**
Gatome et al., 2010	Wi	1	F	57,900
Mulders et al., 1997	Wi	1	F	66,000
Merril et al., 2001	F344	5	F	59,448
Merril et al., 2001†	F344	21	F	63,759
Kumar and Buckmaster 2006‡	S-D	2	M	107,900
Average females (CV)				61,777 (0.06)

LEC layer II	**Strain**	**Age (months)**	**Sex**	**N**
Mulders et al., 1997	Wi	1	F	46,000
Merril et al., 2001	F344	5	F	36,192
Merril et al., 2001†	F344	21	F	42,712
Average females (CV)				41,635 (0.12)

EC Layer II (MEC+LEC)	**Strain**	**Age (months)**	**Sex**	**N**
Mulders et al., 1997	Wi	1	F	112,000
Peterson et al., 1994	F 344	2+	F	103,000

Peterson et al., 1996	F 344	2+	F	97,690
Merrill et al., 2001	F344	5	F	95,640
Merrill et al., 2001†	F344	21	F	106,470
Cardoso et al., 2011	Wi	6	M	131,665
Cardoso et al., 2008	Wi	3	M	119,264
Average females (CV)				102,960 (0.06)
Average males (CV)				125,465 (0.07)

Table 15. *Number of neurons in MEC and LEC Layer II and total EC layer II. Female data are very consistent across studies, with one estimate 75% higher from males in MEC. In contrast, male data for total layer II shows 18% more neurons.*
†Merrill et al., 2001 reported data for impaired and unimpaired 21-month-old animals. The average is listed here.
‡Kumar and Buckmaster 2007 estimate included layer I and II.

EC layer III

The same studies provided data for EC layer III as detailed in **Table 16**

MEC layer III	**Strain**	**Age (mo)**	**Sex**	**# Neurons**
Mulders et al., 1997	Wi	1	F	128,000
Merrill et al., 2001	F344	5	F	152,881
Merrill et al., 2001†	F344	21	F	141,523
Kumar and Buckmaster 2006	SD	2	M	110,100
Average females (CV)				140,801 (0.09)

LEC layer III	**Strain**	**Age (mo)**	**Sex**	**# Neurons**
Mulders et al., 1997	Wi	1	F	119,000
Merrill et al., 2001	F344	5	F	105,269
Merrill et al., 2001†	F344	21	F	108,118
Average females (CV)				110,796 (0.07)

EC layer III	**Strain**	**Age (mo)**	**Sex**	**# Neurons**
Mulders et al., 1997	Wi	1	F	247,000
Merrill et al., 2001	F344	5	F	258,150
Merrill et al., 2001†	F344	21	F	249,640
Cardoso et al., 2011	Wi	6	M	294,655
Cardoso et al., 2008	Wi	3	M	290,872
Average females (CV)				246,792 (0.02)
Average males (CV)				292,764 (0.01)

*T**able 16.** Number of neurons in MEC and LEC Layer III and total EC layer III. As in layer II, the female estimates are very consistent across studies.*
†Merrill et al., 2001 reported data for impaired and unimpaired 21-month-old animals. The average is listed here.

The estimates from upper layers of the entorhinal are exceptional as they come mostly from females. Also, those female estimates are unexpectedly consistent across studies (CV ~0.1), regardless of strain or age. Furthermore, the estimate for the entire LII is also very similar to estimates from other studies (Peterson et al., 1994, 1996)(**Table 15**). Such consistency suggests that those averages might be close to the actual biological values for females, although the limited number of datapoints does not allow obtaining definitive conclusions. Regarding males, we found only one study focused in MEC using a different strain (SD) and showing 75% more neurons in layers I and II and 22% less neurons in Layer III than in females (Kumar and Buckmaster, 2006) (**Tables 15 and 16**). Those differences might be due to sex dimorphism, strain differences, methodology factors like differential segregation of MEC and LEC fields, or a combination of them. (Amaral et al., 2024) also studied adult SD males, reporting averages for upper and deeper layers of MEC that are about 35% and 22% higher than our calculated estimate for females However, their results in LEC showed similar or lower number of neurons in upper and deeper layers, respectively (**Table 15-17**), suggesting sex differences might be restricted to MEC, or the differences are due to differential segregation of both areas. In this regard, (Cardoso et al., 2008, 2011) studied males providing estimates for layers II and III without differentiating MEC and LEC, that were 19% and 17% higher (respectively) than the female values **(Table 15-16),** suggesting that there might be a sex difference in number of neurons that might be masked or exaggerated by differential demarcation of MEC and LEC in different studies. Nonetheless, the low number of estimates from both sexes precludes obtaining reliable conclusions.

EC Layer V/VI

Regarding EC deep layers, we found 4 studies reporting data on MEC and LEC. All estimates combined layer V and VI, and originated from 3 different strains, included both sexes and different ages from as shown in **table 16**.

MEC layer V/VI	**Strain**	**Age (mo)**	**Sex**	**# Neurons**
Merrill et al., 2001	F344	5	F	183,849
Merrill et al., 2001†	F344	21	F	175,887
Mulders et al., 1997	Wi	1	F	185,000
Kumar and Buckmaster 2006	SD	2	M	241,400
Amaral et al., 2024‡	SD	3	M	203,513
Average females (CV)				181,579 (0.14)
Average males (CV)				222,456 (0.12)

LEC layer V/VI	**Strain**	**Age (mo)**	**Sex**	**# Neurons**
Merrill et al., 2001	F344	5	F	125,291
Merrill et al., 2001†	F344	21	F	124,064
Mulders et al., 1997	Wi	1	F	144,000

Amaral et al., 2024‡	SD	3	M	118,000
Average females (CV)				131,118 (0.09)

EC layer V/VI	Strain	Age (mo)	Sex	# Neurons
Merrill et al., 2001	F344	5	F	309,140
Merrill et al., 2001†	F344	21	F	299,951
Mulders et al., 1997	Wi	1	F	329,000
Cardoso et al., 2011	Wi	6	M	347,907
Cardoso et al., 2008	Wi	3	M	339,870
Amaral et al., 2024‡	SD	3	M	334,631
Average females (CV)				312,697 (0.05)
Average males (CV)				340,803 (0.02)

Table 17. *Number of neurons in MEC and LEC Layers V and VI and total EC layer V/VI. As in other layers of the EC, estimates are quite consistent across studies.*
†Merrill et al., 2001 reported data for impaired and unimpaired 21-month-old animals. The average is listed here.
‡Amaral et al., 2024 reported data for both hemispheres combined; listed data is half the original value.

Estimates of neuron number in deep layers of the EC showed consistency in female estimates, and more divergent estimates in males, particularly the estimates of MEC and LEC. When the whole EC is considered, the data is homogenous in both sexes, suggesting once again that the delineation of MEC and LEC might not be consistent across studies. Both sexes show similar averages, but males exhibit a 10% higher estimate. The limited number of datapoints and the strain differences do not allow extracting definitive conclusions, but it seems reasonable to conclude that males might have 10 to 20% more neurons in the EC than females.

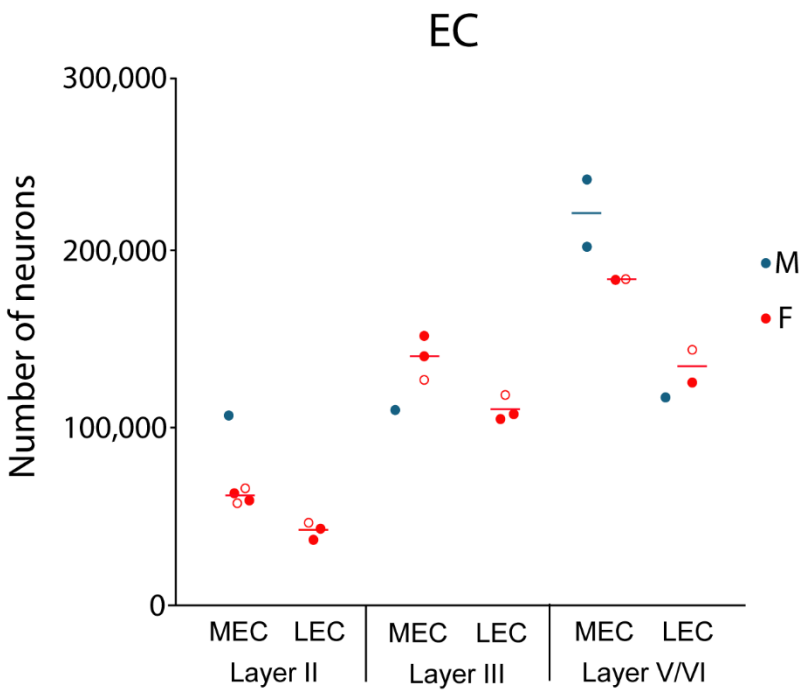

***Figure 13**. Number of neurons per layer in the EC by domain and sex. Circles represent adolescent animals while circles represent adults. Averages are represented by horizontal lines. Note the striking consistency of female estimates, particularly in upper layers.*

As mentioned before, there was a surprising homogeneity in the estimates by layers coming from female animals. In addition, comparison of the number of neurons per layer and per entorhinal subarea (MEC/LEC) shows very consistent patterns (**Table 18**). In both MEC and LEC, layer II contains 15% of the neurons, layer III has 38% and deep layers V/VI 47%, indicating that the number of neurons is split almost evenly between upper and lower layers. The same consistency is observed in the number of neurons in each layer between MEC and LEC. MEC layers consistently take about 60% of the neurons, and LEC layers the remaining 40% (**Table 18**). This type of analysis was not possible for males, but similar consistency should be expected.

	Number of neurons			**% of all layers**			**% of all areas**		
Layer	**MEC**	**LEC**	**EC**	**MEC**	**LEC**	**EC**	**MEC**	**LEC**	**EC**
II	61,777	41,635	103,412	16	15	15	60	40	100
III	140,801	110,796	251,597	37	39	38	56	44	100
V/VI	181,579	131,118	312,697	47	46	47	58	42	100
total	384,157	283,549	667,706	100	100	100	58	42	100

***Table 18**. Number of neurons per layer in the EC of female rats (from tables 12-14).*

When considering all layers of MEC, LEC and the whole EC, we can compare data from 7 studies, including both sexes, 4 strains and ages 1-27-month-old. Even with such subject variability, the estimates are not very divergent, with 5-15% differences. Contrary to the results by layers, females show higher number of neurons in MEC, but lower in LEC and whole EC than males, once again suggesting that there are not enough datapoints to obtain reliable conclusions regarding sex differences (**Table 19 and Fig 14**).

Reference	Strain	Age	Sex	MEC	LEC	EC
Heggland et al., 2015	Wi	18	M-F	507,650	348,777	856,427
Mulders et al., 1997	Wi	1	F	379,000	309,000	688,000
Merrill et al., 2001	F344	5	F	396,178	266,752	662,930
Merrill et al., 2001†	F344	21	F	381,168	278,064	659,232
Kumar and Buckmaster 2006	SD	2	M	459,400		
Rapp et al., 2002	LE	6	M	264,416	375,458	641,176
Rapp et al., 2002	LE	27	M	256,752	377,289	638,235
Amaral et al., 2024‡	SD	3	M	474,989	264,800	739,789
Cardoso et al., 2008	Wi	3	M	-	-	750,006
Cardoso et al., 2011	Wi	6	M	-	-	774,227
Average females (CV)				385,449 (0.02)	284,605 (0.08)	670,054 (0.02)
Average males (CV)				363,889 (0.33)	339,182 (0.19)	708,687 (0.09)
Average all (CV)				389,944 (0.22)	317,163 (0.17)	707,502 (0.07)

Table 19. *Number of neurons in MEC, LEC and EC from different studies. Note the heterogeneity of sex, strain and age in the animals used.*
†Merrill et al., 2001 reported data for impaired and unimpaired 21-month-old animals. The average is listed here.
‡Amaral et al., 2024 reported data for both hemispheres combined; listed data is half the original value.

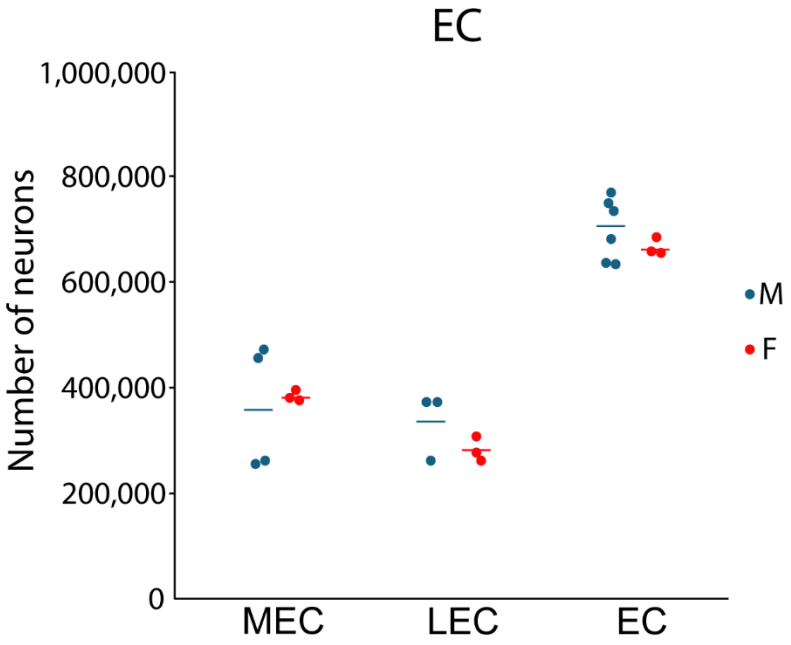

Figure 14. *Number of neurons in MEC, LEC and total EC from studies detailed in* ***Table 19****. Averages are represented by horizontal lines. Female estimates are surprisingly homogeneous across fields.*

Overall, the data from the entorhinal cortex shows a surprising pattern of consistency in the female data across studies, even when originated from animals of different strain and age. This might indeed mean there is consistency across age, sex and strains, or maybe differences due to those factors are canceling each other producing artificial homogeneity, or simply it might a coincidence given the small sample of datasets. Adding males introduces more variability, particularly regarding MEC and LEC estimates, suggesting the delineation of those areas might be different across studies producing heterogeneous results. However, when considering the whole EC by layers, males exhibit consistently 10-20% more neurons than females. Of particular interest are the differences in layer II, as this layer is the main source of projections to outer molecular layer of the DG. Males exhibit 18% more neurons than females in this layer, slightly over the 11% higher number of GCs in the DG in males, that might indicate that the ratio of connectivity between those two populations might be maintained in both sexes.

Regarding age, data across ages from adolescent to old adults are homogeneous, and therefore, contrary to other fields, we have included all age data to obtain averages, to maximize the number of datapoints. Summary data for the EC is presented in table 19, with females and males showing similar averages, but with inverted trends between MEC and LEC. Overall, all datasets are quite consistent showing between 670,000 (females) and 709,000 (males) neurons in the EC, a 6% difference.

Summary of the data

The estimated average of the number of neurons in each field of the hippocampal formation is provided in **table 20**. Estimates for males and females are the average of all strains and are rounded for clarity and simplicity. Reliable estimates for females are provided for the GCL and EC, while estimates in parenthesis represent probable averages based on few datapoints including animals 1 month or older.

		Adult males		Adult females	
		Average	95% CI	Average	95% CI
DG	GCL	1,070,000	995,000-1,152,000	950,000	775,000-1,131,000
	hilus	50,000	46,000-52,000	-	-
CA	CA3	220,000	106000-338,000	-	-
	CA2	*~25,000*	*~12,000-35,000*	-	-
	CA3-CA2	245,000	213,000-278,000	-	-
	CA1	350,000	311,000-381,000	-	-
SUB	Sub	280,000	231,000-323,000	-	-
MEC	II	-	-	60,000	56,000-68,000
	III	-	-	140,000	110,000-172,000
	V/VI	-	-	180,000	169,000-194,000
LEC	II	-	-	40,000	29,000-54,000
	III	-	-	110,000	93,000-129,000
	V/VI	-	-	130,000	103,000-159,000

Table 20. *Summary of the data for all fields. Averages for males and females and 95% confidence intervals (CI). CA2 estimate is highlighted in italics as is based in only 2 datapoints. CA2 95% CI is not informative and has been replaced by the range of estimates; Averages are rounded for simplicity and clarity and might not correspond to the midpoint of CIs.*

Regarding differences by strains, a summary of the averages of Wistar and SD adult males is provided in **table 21** except for EC, that did not have enough datapoints for these strains. Wistar exhibits about 10-20% more neurons than SD in all fields analyzed except in the hilus, where SD showed more neurons than Wistar. However, those differences were not statistically significant.

	SD	CV	n	Wistar	CV	n	Diff	Sign.
GCL	980,000	0.25	10	1,100,000	0.18	18	11%	p=0.18
hilus	50,854	0.18	11	47,451	0.11	9	-7%	0=0.31
CA3/CA2	216,825	0.44	8	261,840	0.26	13	17%	p=0.27
CA1	299,227	0.20	11	385,358	0.23	9	22%	p=0.09
Sub	*242,133*	*0.23*	*4*	*311,583*	*0.10*	*4*	22%	-

Table 21. *Summary of the differences in the number of neurons between adult Wistar and SD males across fields. Differences are calculated as (1-(SD/Wi))*100. Statistical comparisons were performed with two-tailed Welch's t-test. The average for Wistar males in the subiculum is highlighted in italics as originates from only 4 estimates, producing a likely unreliable average and precluding statistical comparisons.*

Discussion

The hippocampal formation is essential for learning and memory, and its characteristic architecture, with distinct fields interconnected through unidirectional, spatially segregated projections, makes it an optimal candidate for models relating structure and function. A starting point of those models is the quantitative definition of the neuronal populations involved. The seminal report of (Amaral et al., 1990) provided one of the first comprehensive accounts of neuronal populations in the hippocampal formation and their connectivity, and quickly became the reference for most subsequent descriptions or models of the hippocampal formation, e.g.: (Treves and Rolls, 1992, 1994; Patton and McNaughton, 1995; Henze et al., 2002; Aimone et al., 2006, 2009; Leutgeb et al., 2007; Kempermann, 2011; Krueppel et al., 2011; Snyder and Cameron, 2012; Rolls, 2013; Newman and Hasselmo, 2014; Cole et al., 2020; Berdugo-Vega et al., 2023; Borzello et al., 2023; Vandael and Jonas, 2024). Since the data in that publication were based on the few available studies at the time, we have aimed to update those estimates with a comprehensive search of available studies. We reason that an update is useful to build more precise models of connectivity required for more precise inferences on function. Also, a large dataset might provide insights on age, strain or sex differences in neuronal populations that are relevant to make informed choices of experimental animals.

We have collected 293 estimates of the number of neurons in the main hippocampal fields, subiculum and upper and lower layers of the EC, plus some additional estimates of other subicular

complex fields like pre- para- and post-subiculum. A main feature of the data is the large variability of the estimates across studies, especially in CA3/CA2 (CV=0.26-0.44); GCL (0.18-0.25), CA1 (CV=0.20-0.23) and subiculum (CV=0.2). More homogeneous data were found in the hilus (CV=0.11-0.18), and especially in the EC, where females showed unusual homogeneity of estimates (CV=0.03-0.09). In this regard, large variability is expected during early postnatal development, when neurons are not fully differentiated, and their identification may differ across studies. However, it was observed across all ages, even across adult animals of the same sex and strain, as is the case for adult SD males in CA3/CA3 (n=8) that showed a CV of 0.44**.** It could be speculated that there is a correlation between the variability of the estimates and neuronal density, such that fields with high neuronal density (GCL, CA fields) present more variability while fields with more disperse neurons (hilus, subiculum, EC) have more homogeneous estimates. Intuitively, high neuronal density makes more difficult resolving individual neurons for accurate quantification, but its actual contribution to the observed variability cannot be warranted.

Updated vs traditional averages

As mentioned above, most models and descriptions of the hippocampal formation (Treves and Rolls, 1992, 1994; Henze et al., 2002; Aimone et al., 2006, 2009; Leutgeb et al., 2007; Kempermann, 2011; Krueppel et al., 2011; Rolls, 2013; Newman and Hasselmo, 2014; Berdugo-Vega et al., 2023; Borzello et al., 2023; Vandael and Jonas, 2024) have adopted the populations described by (Amaral et al., 1990) for the tri-synaptic pathway: 200,000 neurons in EC layer II; 1,000,000 neurons in the GCL (Boss et al., 1985); 330,000 in CA3/CA2 (Boss et al., 1987) and 420,000 for CA1 (Boss et al., 1987). No data were available at the time for the EC, so their estimate of 200,000 neurons for EC layer II is a rough calculation based on the product of the areal density of neurons in EC layer II and the surface area of the EC, originally reported with a question mark to emphasize the approximate value of that estimate (see fig 1 in (Amaral et al., 1990)). They also described 30,000 in the hilus (Seress, 1988), about 12,000 neurons in CA2 and 128,000 in the subiculum based on (Cassell, 1980), but those estimates have not been used in subsequent models.

The results of our meta-analysis show an updated average for the GCL that is virtually identical to the traditional value of 1 million neurons. However, there are marked differences in the rest of populations. Our average of EC layer II describes half the number of neurons, while our updated average of CA3/CA2 and CA1 are 26% and 19% lower than the averages reported by (Boss et al., 1987). The difference in EC layer II is reasonable considering the rough methodology used to obtain the original estimate. However, the differences in CA fields are a little more perplexing, as the original estimates from (Boss et al., 1987) were obtained in 1 month-old SD females. Our meta-analysis show that adolescence age, female sex and SD strain are all factors that contribute to obtain lower number of neurons compared to adult age, male sex and Wistar strain (see next sections). Therefore, one would expect that those adolescent SD females would exhibit significantly lower neuronal populations than our average of adult males that are enriched in Wistar males, while the opposite is true. In fact, (Boss et al., 1987) described much larger neuronal populations in CA3/CA2 and CA1 (30-55% larger) in adolescent SD females than Wistar, a result that is contrary to the trends observed in our meta-analysis, showing consistently larger neuronal populations in Wistar compared to SD (see strain differences). Indeed, the estimates provided by (Boss et al., 1987) for CA3/CA2 and CA1 are clearly outside the 95% confidence interval we obtained for both fields. There is no clear explanation for that divergence, and we can only speculate that the large interindividual and inter-cohort differences in neuronal numbers that are common in this type of studies, plus the variability introduced by diverse methodologies used by different labs might contribute to produce

quite divergent results as shown across our meta-analysis, and as is discussed later in the *Variability, accuracy, and reliability of the data* section. But in conclusion, it seems that the data from adolescent SD females from (Boss et al., 1987) might not be the most representative of the population of neurons in CA fields of the adult rat, and can be updated to more balanced estimates of 245,000 neurons for CA3/CA2 and 350,000 for CA1, based on more than 20 estimates. Also, our analysis indicates that CA3 might have 220,000 neurons and CA2 ~25,000, the latter based on limited data from 2 studies.

While most models have relied in the above describe data, there are also others that have used partially different sources:

A model of the connectivity of the DG (Patton and McNaughton, 1995) used the 200,000 neuronal estimate for EC layer II and 1,000,000 for the GCL based on (Amaral et al., 1990), but updated the hilar number to 50,000 neurons based on (West et al., 1991), a value that is virtually identical to the one we found in our meta-analysis.

Another model by (O'Reilly and McClelland, 1994) also used the 200,000 estimate for EC layer II and 1,000,000 for GCL, although they listed 850,000 GCs for their actual model. They described CA3 as having 160,000 neurons (Squire et al., 1989) likely after (Cassell, 1980), and CA1 as having 250,000 neurons, citing (Boss et al., 1987), although they probably meant (West et al., 1991). In addition to the difference in EC layer II already discussed, their values of CA3/CA2 and CA1 would represent 35% and 27% less neurons that the ones obtained in our analysis, and much lower percentages compared with the estimates of (Boss et al., 1987) commonly used in most models.

An additional model from the Snyder lab (Snyder and Cameron, 2012; Cole et al., 2020), described 112,000 and 247,000 neurons for EC layer II and III respectively after (Mulders et al., 1997); 1,200,000 neurons for the GCL and 250,000 for CA3/CA2, based on (West et al., 1991). This study is much closer to our results as the value for EC layers and CA3/CA2 are very similar to our estimates, while their GCL population is about 20% higher than our (male and female combined) average.

In summary, models of the hippocampal formation have used neuronal populations that are typically based on single studies for each field. That implies that subjective factors in the choice of the study of reference might largely determine the properties of the hippocampal network, and might produce vastly different results between models, as the quantitative relations between fields change dramatically. In this regard, it is hard to compare a model with 1 million GCs, 160,000 CA3/CA2 neurons and 250,000 CA1 pyramidal cells with another with the same number of GCs but double number of CA3 neurons (330,000) and almost 70% more CA1 neurons (250,000). Thus, to build more robust and accurate models, it seems reasonable to standardize neuronal populations, and the average of all available studies after removing outliers, seems the most reasonable option.

Sex Differences

Studies on the hippocampus have traditionally favored the use of males to avoid the influence of cycling hormonal changes present in females, as the hippocampus is very responsive to hormonal fluctuations, sometimes in a specific manner (Gould et al., 1990; Zhang et al., 2008a). Indeed, this might be the reason behind the sexual dimorphism described in this structure: the male

hippocampus is larger, heavier and contains more neurons than the female hippocampus (Madeira et al., 1992; Andrade et al., 2000; Nuñez et al., 2003b). The number of GCs has been reported to be higher in males in several strains of rats (Madeira et al., 1988, 1991; Nuñez et al., 2003a, 2003b; Smith et al., 2008) and studies in mice have shown that both sexes exhibit similar initial population of GCs early postnatally, that start differentiating during early adolescence (P20-P27) by increased cell death in females (Wimer et al., 1988).

In our dataset, we recorded a consistent difference in the GCL, showing a 10% difference between female (0.95 million) and male (1.06 million) GCs (all strains), although that difference was not statistically significant (p=0.10) given the small samples and large variability of the data. In this line, differences (~15%) were also observed when males and females are compared by strain (Wistar and SD), although the number of female datapoints is very low (n=2-4) and therefore those averages might not be reliable. Overall, this trend is consistent with other reports showing sexual dimorphism in the number of GCs in the dentate gyrus of the rat. This difference might be present in all strains, in agreement with the dimorphism in brain and body weight described in this species (Kakolewski et al., 1968; Goodrick, 1980; Madeira et al., 1988, 1991; Nuñez et al., 2003a, 2003b). Sex related differences have been also observed in mice (Wimer et al., 1988; Wimer and Wimer, 1989) and in guinea pigs (Severi et al., 2005), indicating this might be a general feature in rodents, and maybe in other species.

Regarding other fields, different studies have reported males having 17-24% more neurons than females in CA1 and subiculum across different ages, from P10 to 6 months of age in both SD and Wistar animals (Madeira et al., 1992; Andrade et al., 2000; Smith et al., 2008). However, available data from CA3/CA2 show differences between males and females during development in 3 week-old rats (Nuñez et al., 2003a, 2003b). Also, (Smith et al., 2008) reported that SD females showed 7% more neurons than males at P9, but this difference disappeared in young adults (P68), consistent with data from (Madeira et al., 1992) describing similar averages in adolescent and adult SD males and females, suggesting sex differences in CA3/CA2 might subside in adulthood. Data on the EC is scarce, but quite homogeneous across studies, and generally shows larger number of neurons (6-24%) in males than in females. Such differences are not totally consistent across MEC and LEC areas, a finding that might be related to differences in the delineation of those areas in different studies.

Given these potential sex differences in most fields, it would be desirable to obtain homogeneous data from males, the sex of choice of most studies on the hippocampal formation. In that regard, the scarce data on males in the EC is an obstacle to get such homogeneous dataset, and we have incorporated female data, that is also surprisingly homogeneous across studies, conferring it some seal of reliability, although such convergence might be just serendipitous, considering the low number of datapoints (n=4).

Strain differences

The studies collected here used animals from 6 strains, but strain comparisons were only meaningful between SD and Wistar strains, and were limited to males, given the scarce data on females. Wistar males had 11-22% more neurons in most fields analyzed, with a minimum in the GCL (11%) and a maximum in CA1 (22%), while the hilus showed an inverse relation, with Wistar having 7% less neurons than SD. No reliable comparison was possible in the subiculum or EC. It is

important to emphasize that in small samples as the ones analyzed here, a few high or low estimates that do not qualify as outliers might shift the average producing differences that might not be real. However, the relative consistency of the differences between Wistar and SD suggests there might be an actual biological difference between them, with Wistar males exhibiting 10-20% more neurons in most fields.

In this regard, some studies have focused in the comparison between strains, and for example, (Seress, 1988) analyzed LE versus CFY (a variety of SD) rats and showed that SD had ~30% more neurons in GCL, but ~7-10% less in hilus, CA2-CA3 and CA1, suggesting that potential differences across strains might not be consistent across fields, as our data show. However, the groups analyzed by (Seress, 1988) contained mixed sexes and therefore the strain effect might be confounded with sex differences as described in the previous section. (Boss et al., 1985, 1987) compared Wistar and SD females and found large differences between them, as discussed early in the section *Updated vs traditional averages.* Their comparison in the GCL at 1, 4 and 12 months of age, showed that SD females had 45% and 28% more granule cells than Wistar at 1 and 12 months of age, but similar numbers at 4 months, while in CA3/CA2 Wistar had ~36% less neurons ($p<0.05$) and 24% less in CA1 ($p>0.05$). These differences are substantial and show higher neuron number in SD than in Wistar, an inverse trend to that in our dataset. Arguably, these studies are expected to produce more reliable comparisons, as both strains are quantified with the same methodology by the same researchers. However, a point of contention is the results in the GCL, that are inconsistent across ages. The most likely explanation for these inconsistencies may be the large interindividual variability in the number of neurons in each strain, that might produce large differences in the same or separate experiments (see section *Variability, accuracy, and reliability of the data*).

Age differences

Exploring neuronal numbers across age suggests two main questions: first, at what age hippocampal fields reach their adult complement of neurons, and second, if neuron numbers change with aging. To answer these questions, separate analysis must be made for the GCL and the rest of hippocampal fields, given their different neurogenic dynamics.

About the first question regarding when hippocampal fields reach their adult complement of neurons, the GCL shows protracted neurogenesis that reaches a peak at the end of the first postnatal week (Schlessinger et al., 1975; Bayer, 1980a) to subsequently decrease sharply during infancy and adolescence to reach low levels during adulthood (Arellano and Rakic, 2024). This continuous neurogenic environment complicates defining an adult number of neurons as a stable population across time. But modelling the process of neurogenesis reveals that at the onset of adulthood, by 2 months of age, neurogenesis generates ~3.4% of the GCL population, a percentage that will decline following a power function to reach much lower levels afterwards (~1% by age 6 months) (Arellano and Rakic, 2024), suggesting that the population will not change significantly after the onset of adulthood. Indeed, the same model and other studies have predicted that during the entire adulthood period between 2-21 months of age (Arellano et al., 2024), only ~8-12% new neurons are produced in the rat (Ninkovic et al., 2007; Imayoshi et al., 2008; Lazic, 2012; Pilz et al., 2018; Arellano and Rakic, 2024). So, if there is no cell replacement, it would imply that during the adulthood period, the population of granule cells can be considered stable within a 12% margin, that is about the same variation expected due to strain or sex differences, and much lower than interindividual differences that can reach almost 2-fold, as discussed in the section "Variability, accuracy, and reliability of the data" below.

Comparisons in our dataset of the number of GCs in adolescent (0.75-2-months-old, (Arellano et al., 2024) and adults showed that, as expected, adolescents had about 7-18% less neurons than adults (excluding SD males, that had fewer than 5 datapoints). In this regard, available studies providing data on adolescent and adults show discrepant results, with Wistar male and females having about 20% less neurons than adults (Bayer et al., 1982; Boss et al., 1985), but adolescent Wistar males or SD females having the same or more than adults (Bayer et al., 1982; Heine et al., 2004). These divergent results suggests once again that variability in the quantification between groups is very high, obscuring the expected age differences, manifesting the lack of resolution of comparative studies.

Regarding the stability of the population of GCs across adulthood, our dataset does not allow a reliable analysis given the scarce data on middle aged and old animals and their large variability (~1.6-fold variation between estimates at 12 and 24 months of age). As described in the results, the regression equations seem quite inaccurate, predicting more than 300,000 GCs are added to the GCL along adulthood (2-21 months (Arellano et al., 2024)). Those numbers are very high, representing 33-38% of the GC population, raising questions about their accuracy. In this line, the early study by (Bayer et al., 1982) quantified the number of GCs at 1, 4, 6.5 and 12 months of age, reporting a steady increase from 890,000 at 1 month to 1.28 at 12 months, in line with the increase described above, and interpreted as the product of adult neurogenesis (Bayer et al., 1982; Crespo et al., 1986; Kempermann, 2011). However, with exception of Rasmussen et al., 1996, that reported a 20% increase between 2.5 and 24 months, all subsequent studies have not replicated those increases, showing stable or decreased numbers of neurons in older animals (Boss et al., 1985; Madeira et al., 1988, 1991; Rapp and Gallagher, 1996; Sousa et al., 1998a; Merrill et al., 2003; Heine et al., 2004; Aslan et al., 2006). Also, available estimates of adult neurogenesis across adulthood based on empirical data describe 8-12% of the population in the rat (Ninkovic et al., 2007; Imayoshi et al., 2008; Lazic, 2012; Pilz et al., 2018; Arellano and Rakic, 2024), far from the 30-40% predicted by the regression mode. Furthermore, as extensively discussed elsewhere (Arellano and Rakic, 2024), such large increase in number of neurons imply the presence of approximately similar numbers of differentiating neurons expressing specific markers such as DCX or PSANCAM, in contrast with the much more modest numbers that have been described (Rao et al., 2005, 2006; Zhang et al., 2008b; Epp et al., 2009; Rennie et al., 2009).

It can be argued that restricting the data to adult males could provide a more accurate estimate. However, the distribution of adult datapoints still presents large variability at young ages (2-5 months) and as a result, the regression is virtually non-existent ($R2=0.004$), and, not surprisingly, the resulting equation describes an increase of 40,000 neurons across adulthood (2-21 months), corresponding to less than 4% of the 1.08 million GCs in adult males, a much lower percentage than the expected ~12% (Arellano and Rakic, 2024). Thus, it seems that the large variability described at young ages (0-5 months) does not allow obtaining reliable equations to describe the dynamic of the population during adulthood.

Regarding the rest of hippocampal fields, they exhibit mostly embryonic neurogenesis that tappers during the first postnatal week (Bayer and Altman, 1974; Bayer, 1980a) producing a population peak around age 1 week, followed by neuronal pruning that leads to adult neuronal numbers by age 2 weeks (Bandeira et al., 2009). Hippocampal neurons differentiate along the first postnatal weeks and thus by the time animals reach post-puberal adolescence at around 1 month of age (Arellano et al., 2024), it is expected that neurons occupy their adult positions and show

adult-like soma morphology that would allow their quantification at similar rates than in adults. However, studies quantifying the number of neurons in CA fields of 1-month-old and adult animals have shown consistently that younger animals exhibit less neurons (8-17% less) than adults, applying to both males and females (Madeira et al., 1992; Wakuda et al., 2008; Elibol-Can et al., 2014). Comparing averages of adolescent and adult animals in our sample identifies a similar trend, with adolescent animals showing 5-20% less neurons than adults. Those differences were not statistically significant (except in the GCL, as developmental neurogenesis proceeds at that age) as the number of datapoints were typically low and very variable for adolescents. However, the consistency of the trend across fields, strains and sexes, and the similar differences reported by the studies mentioned above suggest that indeed the differences might be reflecting a biological feature. It does not necessarily mean that there are less neurons in 1-month old animals but may indicate that a population of late migrating or late differentiating neurons might escape quantification in those fields, creating a mismatch between adolescent and adult neuronal numbers.

In this regard, it would be advantageous if adolescent and adult estimates could be pooled, as that would increase the n to obtain more robust averages and establish more reliable comparisons. Given the differences found in the hilus and CA fields, we refrained from adding adolescents to the adult data, and we described the average number of neurons based only on adults. However, in the subiculum and EC there was much more homogeneity, without marked differences between adolescent and adults, or across sexes or strains (a feature that could be an artifact of the low number of samples). Given the low number of estimates in the EC, we took advantage of the large homogeneity of the female estimates across age or strains, and pooled adolescent and adults to increase the number of estimates.

The second question regarding age-related loss of neurons in these fields could not be studied in our dataset, as, in addition to the large variability, only 13% of the datapoints corresponded to old animals, with typically only 1 or 2 estimates per field. Also, there is large variability across life stages, and for example, in field CA3/CA2 the range of values in young adults (2-6 months; (Arellano et al., 2024)) is almost 4-fold (108,000-416,000, conditions that do not allow reliable comparisons. However, some of the studies collected here have made comparisons across ages, showing no differences in the number of neurons in the hilus, CA2/CA3 or CA1 between young and old adults (Rapp and Gallagher, 1996; Rasmussen et al., 1996; Hattiangady et al., 2005). Only one study reported a ~30% decrease in CA1 between 6 and 18 months, a difference that was however, not statistically significant (Heggland et al., 2015). Thus, it seems that hippocampal populations are essentially stable across adulthood.

Rostro-caudal differences

Most studies report the overall number of neurons of the hippocampal fields, but the distribution of those neurons has been reported to change across the septo-temporal axis of the hippocampus. Traditionally, It has been considered that there are more GCs at septal levels than at temporal levels in adult Wistar males, and an inverse gradient is present for CA3, with more neurons temporally than septally (Gaarskjaer, 1978; Amaral et al., 2007) have been emphasized, that showed. However, (Wang and Gondré-Lewis, 2013) described similar number of neurons in CA3, but about double number of GCs and about triple number of CA1 pyramidal cells in the temporal half than in the septal half of the hippocampus in P14 mixed male and female SD rats. It is not clear if those different gradients might represent strain or age differences or might be due to

methodological differences, but those mixed results suggest more studies are needed to clarify those potential gradients.

Variability, accuracy, and reliability of the data

Across the manuscript, we have repeatedly emphasized the large variability of the datapoints except in the entorhinal cortex of females, that shows surprising coherency across studies. Such variability is particularly evident when data are plotted across age, as developing animals and young adults show very large dispersion that does not follow the expected exponential curve. Intuitively, the robust methodology behind stereological studies is expected to provide homogeneous results, although we have found large range of estimates (sometimes almost 4-fold) and several outliers. Potential methodological differences might include different sampling methods, criteria to divide hippocampal fields or to identify neurons to be counted, criteria to exclude other cells, and overall stereological schemes. But beyond methodological factors, maybe the most relevant contributor is biological variability. A good illustration of this comes from the data of (Ben Abdallah et al., 2010) showing that same sex littermates can show almost 2-fold differences in the number of neurons (~480,000 to ~730,000 for male littermates; Fig 9 in (Ben Abdallah et al., 2010) even if the analysis is performed with the same methodology, at the same time, by the same experienced researcher (Dr. L. Slomianka, personal communication). Since we are collecting averages, interindividual and inter-cohort differences are to certain extent buffered. However, large variability was present between averages in different studies, even when using animals of the same sex and strain and of similar age.

To maximize accuracy, we searched for outliers using Tukey fences (Tukey, 1977). Interestingly, we only found outliers in the high end of the data, suggesting the method might be less sensitive in the low end, as some very low values (less than 50% of the average) were not detected as outliers. Beyond those extreme values, simple inspection of the estimates across fields inevitably leads to the notion that some studies seem to be producing under- or overestimates. For example, the results of Seress 1988 represent 70-80% of the average for adult males in all fields reported (GCL, CA3-CA2 and CA1) a point that has been raised before (Jabès et al., 2010). Similarly, the results of (Cassell, 1980) represent 50-60% of the average of adult males in each field (CA3-CA2, CA1, Subiculum). On the high side, the study by (Rasmussen et al., 1996) provide estimates that are similar to the average in some fields (CA3-CA2, hilus, subiculum) but their estimates of DG and CA1 are 55-70% higher than the average, and others considered outliers (up to 86% higher than the average).

The expectation is that, after eliminating outliers, the resulting average will balance low and high values producing a reliable estimate of the average population. However, given the small number of studies available, the balance between those potentially under- and overestimating studies is not warranted. In that regard, we have calculated the median to compare with the mean, a source of valuable information on the symmetry of the data after removing outliers. The median obtained from fields with low number of datapoints (CA2, CA3, Subiculum) was far from the average and was not used. But we found basically matching mean and median in the rest of fields, even in the dataset for GCL neurons in females, that has only 8 datapoints. The matching between mean and median suggests the distributions obtained are symmetrical and the average is reliable. Nonetheless, still some of the data seem to be “off” (like the value of 416,000 neurons for CA3/CA2),

but there is no objective criterium to select or discard studies beyond the outlier test. Thus, no further effort was conducted to "clean" the data beyond removing outliers in each field.

In the collected data, variability is slightly higher in the GCL and CA fields, and lower in the hilus, subiculum and EC. It is tempting to speculate that a possible reason for that difference might be the high neuronal density of GCL and CA fields, that might make difficult the identification and quantification of neurons compared to the hilus, subiculum and EC, fields that exhibit much lower neuronal density.

Optimally, estimates of number of neurons in each field should be obtained for each sex and strain separately. However, the scarce data for females in most fields and the lack of enough datapoints for each strain, including the most populated, Wistar and SD, compelled us to compromise and obtain the average number of neurons from all males, regardless of strain. This might be an artifactual value, diluting differences between strains, but is a good overall estimate of the number of neurons in a generic male rat for modeling studies, as detailed data on projections and synaptic connectivity has been reported also across different strains. Exceptionally, we estimated the population of the EC based on female data as, contrary to the rest of the fields, most studies were performed on females. Also, we exceptionally included adolescent data in the adult pool in the EC, as all female datapoints exhibited an unusual congruency across studies that supports their accuracy, although more datapoints would be useful to confirm that the converging estimates are accurate and are not the product of mere coincidence.

Conclusion

As part of a larger effort to review and update the model of connectivity of the hippocampal formation, we present here a comprehensive collection of studies reporting the number of neurons in the fields of the hippocampal formation. The main feature of the available data is the large variation of the estimates, that makes difficult to compare data across age, strains or sex. This fact was reflected in the lack of statistical significance of most of those comparisons, even if rendering differences of 20-30%. However, some of the differences are consistent across fields, like the 10-20% larger neuronal population in Wistar compared with SD in the GCL, CA fields and subiculum, suggesting they might be reflecting a biological feature. Also, surprisingly, quantification of adolescent animals tends to reveal lower number of neurons than in adults. Although the difference was not significant, there was a consistent 5-20% difference that suggest it might reach significance with larger samples. This is surprising as by the onset of adolescent the full complement of hippocampal neurons (except in the DG) is expected to be in place and functional. Regarding sex differences, sexual dimorphism has been reported in the hippocampus of rodents, with larger and more populated fields in males. However, female data is poorly represented in the collected data, as males are the traditional subject of hippocampal studies to avoid potential effects of hormonal cycles. Thus, no reliable comparison could be established between males and females, except in the GCL, where males showed 11% more GCs than females, a difference that was however not significant. Also, few but relatively homogeneous data from males and females in the EC suggest also the presence of 10-20% more neurons in males.

Comparison of our results with neuronal populations in existing hippocampal models shows marked differences in most fields that reach 2-fold differences, as existing models have obtained their data from individual studies that might not be representative. We propose that more reliable

values can be obtained by averaging all data available, that produces the most representative and "least inaccurate" measure available. We hope these estimates will be useful to improve comparisons across species and to build more accurate models of hippocampal function.

Refs from Tables:

(Schlessinger et al., 1975, 1978; Gaarskjaer, 1978; Cassell, 1980; West and Andersen, 1980; Seress and Pokorny, 1981; Bayer et al., 1982; Boss et al., 1985, 1987; Madeira et al., 1988, 1991, 1992; Seress, 1988; West et al., 1988, 1991; West, 1990; Peterson et al., 1994, 1996; Rapp and Gallagher, 1996; Rasmussen et al., 1996; Baldwin et al., 1997; Buckmaster and Dudek, 1997; Mulders et al., 1997; Dalby et al., 1998; Sousa et al., 1998b, 1998a, 1999; Buckmaster and Jongen-Rêlo, 1999; Lukoyanov et al., 1999, 2000, 2004; Xavier et al., 1999; Andrade et al., 2000; Tuunanen and Pitkänen, 2000; Bonthius et al., 2001; Maier and West, 2001; Merrill et al., 2001, 2003; McCullers et al., 2002; Rapp et al., 2002; Grady et al., 2003; Nuñez and McCarthy, 2003; Nuñez et al., 2003b, 2003a; Ramsden et al., 2003; Heine et al., 2004; Hattiangady et al., 2005; Lister et al., 2005, 2006; Miki et al., 2005; Aslan et al., 2006; Kumar and Buckmaster, 2006; Hosseini-Sharifabad and Nyengaard, 2007; Jiao and Nadler, 2007; Cardoso et al., 2008, 2011; Fitting et al., 2008, 2010; Smith et al., 2008; Wakuda et al., 2008; Gatome et al., 2010; Thind et al., 2010; Czéh et al., 2013; Wang and Gondré-Lewis, 2013; Elibol-Can et al., 2014; Maia et al., 2014; Heggland et al., 2015; Keleş et al., 2019; Amaral et al., 2024)

References

Aimone, J. B., Wiles, J., and Gage, F. H. (2006). Potential role for adult neurogenesis in the encoding of time in new memories. *Nat Neurosci* 9, 723–727. doi: 10.1038/nn1707

Aimone, J. B., Wiles, J., and Gage, F. H. (2009). Computational influence of adult neurogenesis on memory encoding. *Neuron* 61, 187–202. doi: 10.1016/j.neuron.2008.11.026

Alemu, J. L., Elberling, F., Azam, B., Pakkenberg, B., and Olesen, M. V. (2019). Electroconvulsive treatment prevents chronic restraint stress-induced atrophy of the hippocampal formation-A stereological study. *Brain Behav* 9, e01195. doi: 10.1002/brb3.1195

Amaral, A. C., Lister, J. P., Rueckemann, J. W., Wojnarowicz, M. W., McGaughy, J. A., Mokler, D. J., et al. (2024). Prenatal protein malnutrition decreases neuron numbers in the parahippocampal region but not prefrontal cortex in adult rats. *Nutr Neurosci*, 1–14. doi: 10.1080/1028415X.2024.2371256

Amaral, D. G. (1978). A Golgi study of cell types in the hilar region of the hippocampus in the rat. *J Comp Neurol* 182, 851–914. doi: 10.1002/cne.901820508

Amaral, D. G., Ishizuka, N., and Claiborne, B. (1990). Neurons, numbers and the hippocampal network. *Prog Brain Res* 83, 1–11. doi: 10.1016/s0079-6123(08)61237-6

Amaral, D. G., Scharfman, H. E., and Lavenex, P. (2007). The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). *Prog Brain Res* 163, 3–22. doi: 10.1016/S0079-6123(07)63001-5

Andrade, J. P., Madeira, M. D., and Paula-Barbosa, M. M. (2000). Sexual dimorphism in the subiculum of the rat hippocampal formation. *Brain Research* 875, 125–137. doi: 10.1016/S0006-8993(00)02605-6

Arellano, J. I., Duque, A., and Rakic, P. (2024). A coming-of-age story: adult neurogenesis or adolescent neurogenesis in rodents? *Front. Neurosci.* 18. doi: 10.3389/fnins.2024.1383728

Arellano, J. I., and Rakic, P. (2024). Modelling adult neurogenesis in the aging rodent hippocampus: a midlife crisis. *Front Neurosci* 18, 1416460. doi: 10.3389/fnins.2024.1416460

Aslan, H., Songur, A., Tunc, A. T., Ozen, O. A., Bas, O., Yagmurca, M., et al. (2006). Effects of formaldehyde exposure on granule cell number and volume of dentate gyrus: A histopathological and stereological study. *Brain Research* 1122, 191–200. doi: 10.1016/j.brainres.2006.09.005

Baldwin, S. A., Gibson, T., Callihan, C. T., Sullivan, P. G., Palmer, E., and Scheff, S. W. (1997). Neuronal cell loss in the CA3 subfield of the hippocampus following cortical contusion utilizing the optical disector method for cell counting. *J Neurotrauma* 14, 385–398. doi: 10.1089/neu.1997.14.385

Bandeira, F., Lent, R., and Herculano-Houzel, S. (2009). Changing numbers of neuronal and non-neuronal cells underlie postnatal brain growth in the rat. *Proc Natl Acad Sci U S A* 106, 14108–14113. doi: 10.1073/pnas.0804650106

Bayer, S. A. (1980a). Development of the hippocampal region in the rat. I. Neurogenesis examined with 3H-thymidine autoradiography. *J Comp Neurol* 190, 87–114. doi: 10.1002/cne.901900107

Bayer, S. A. (1980b). Development of the hippocampal region in the rat. II. Morphogenesis during embryonic and early postnatal life. *J Comp Neurol* 190, 115–134. doi: 10.1002/cne.901900108

Bayer, S. A., and Altman, J. (1974). Hippocampal development in the rat: cytogenesis and morphogenesis examined with autoradiography and low-level X-irradiation. *J Comp Neurol* 158, 55–79. doi: 10.1002/cne.901580105

Bayer, S. A., Yackel, J. W., and Puri, P. S. (1982). Neurons in the rat dentate gyrus granular layer substantially increase during juvenile and adult life. *Science* 216, 890–892. doi: 10.1126/science.7079742

Ben Abdallah, N. M.-B., Slomianka, L., Vyssotski, A. L., and Lipp, H.-P. (2010). Early age-related changes in adult hippocampal neurogenesis in C57 mice. *Neurobiol Aging* 31, 151–161. doi: 10.1016/j.neurobiolaging.2008.03.002

Berdugo-Vega, G., Dhingra, S., and Calegari, F. (2023). Sharpening the blades of the dentate gyrus: how adult-born neurons differentially modulate diverse aspects of hippocampal learning and memory. *The EMBO Journal* 42, e113524. doi: 10.15252/embj.2023113524

Bonthius, D. J., Woodhouse, J., Bonthius, N. E., Taggard, D. A., and Lothman, E. W. (2001). Reduced seizure threshold and hippocampal cell loss in rats exposed to alcohol during the brain growth spurt. *Alcohol Clin Exp Res* 25, 70–82.

Borzello, M., Ramirez, S., Treves, A., Lee, I., Scharfman, H., Stark, C., et al. (2023). Assessments of dentate gyrus function: discoveries and debates. *Nat. Rev. Neurosci.* 24, 502–517. doi: 10.1038/s41583-023-00710-z

Boss, B. D., Peterson, G. M., and Cowan, W. M. (1985). On the number of neurons in the dentate gyrus of the rat. *Brain Res* 338, 144–150. doi: 10.1016/0006-8993(85)90257-4

Boss, B. D., Turlejski, K., Stanfield, B. B., and Cowan, W. M. (1987). On the numbers of neurons in fields CA1 and CA3 of the hippocampus of Sprague-Dawley and Wistar rats. *Brain Res* 406, 280–287. doi: 10.1016/0006-8993(87)90793-1

Brower, M., Grace, M., Kotz, C. M., and Koya, V. (2015). Comparative analysis of growth characteristics of Sprague Dawley rats obtained from different sources. *Lab Anim Res* 31, 166–173. doi: 10.5625/lar.2015.31.4.166

Buckmaster, P. S., and Dudek, F. E. (1997). Neuron loss, granule cell axon reorganization, and functional changes in the dentate gyrus of epileptic kainate-treated rats. *J Comp Neurol* 385, 385–404.

Buckmaster, P. S., and Jongen-Rêlo, A. L. (1999). Highly specific neuron loss preserves lateral inhibitory circuits in the dentate gyrus of kainate-induced epileptic rats. *J Neurosci* 19, 9519–9529. doi: 10.1523/JNEUROSCI.19-21-09519.1999

Cappaert, N. L. M., van Strien, N. M., and Witter, M. P. (2015). *Hippocampal formation*. Amsterdam4thElsevier Academic. doi: 10.1016/B978-0-12-374245-2.00020-6

Cardoso, A., Assunção, M., Andrade, J. P., Pereira, P. A., Madeira, M. D., Paula-Barbosa, M. M., et al. (2008). Loss of synapses in the entorhinal-dentate gyrus pathway following repeated induction of electroshock seizures in the rat. *Journal of Neuroscience Research* 86, 71–83. doi: 10.1002/jnr.21474

Cardoso, A., Lukoyanova, E. A., Madeira, M. D., and Lukoyanov, N. V. (2011). Seizure-induced structural and functional changes in the rat hippocampal formation: Comparison between brief seizures and status epilepticus. *Behavioural Brain Research* 225, 538–546. doi: 10.1016/j.bbr.2011.07.057

Cassell, M. D. (1980). The Numbers of Cells in the Stratum Pyramidal of the Rat and Human Hippocampal Formation. Bristol, UK: University of Bristol.

Cassell, M. D., and Brown, M. W. (1977). Cell counts for the stratum pyramidale of the hippocampus of the rat. *Life Sciences* 21, 1187–1191. doi: 10.1016/0024-3205(77)90119-9

Cole, J. D., Espinueva, D. F., Seib, D. R., Ash, A. M., Cooke, M. B., Cahill, S. P., et al. (2020). Adult-Born Hippocampal Neurons Undergo Extended Development and Are Morphologically Distinct from Neonatally-Born Neurons. *J Neurosci* 40, 5740–5756. doi: 10.1523/JNEUROSCI.1665-19.2020

Crespo, D., Stanfield, B. B., and Cowan, W. M. (1986). Evidence that late-generated granule cells do not simply replace earlier formed neurons in the rat dentate gyrus. *Exp Brain Res* 62, 541–548. doi: 10.1007/BF00236032

Czéh, B., Ábrahám, H., Tahtakran, S., Houser, C., and Seress, L. (2013). Number and regional distribution of GAD65 mRNA-expressing interneurons in the rat hippocampal formation. *Acta Biologica Hungarica* 64, 395–413. doi: 10.1556/abiol.64.2013.4.1

Dalby, N. O., West, M., and Finsen, B. (1998). Hilar somatostatin-mRNA containing neurons are preserved after perforant path kindling in the rat. *Neurosci Lett* 255, 45–48. doi: 10.1016/s0304-3940(98)00707-1

Elibol-Can, B., Kilic, E., Yuruker, S., and Jakubowska-Dogru, E. (2014). Investigation into the effects of prenatal alcohol exposure on postnatal spine development and expression of synaptophysin and PSD95 in rat hippocampus. *Int J Dev Neurosci* 33, 106–114. doi: 10.1016/j.ijdevneu.2013.12.003

Epp, J. R., Barker, J. M., and Galea, L. A. M. (2009). Running wild: neurogenesis in the hippocampus across the lifespan in wild and laboratory-bred Norway rats. *Hippocampus* 19, 1040–1049. doi: 10.1002/hipo.20546

Fitting, S., Booze, R. M., Hasselrot, U., and Mactutus, C. F. (2008). Differential Long-Term Neurotoxicity of HIV-1 Proteins in the Rat Hippocampal Formation: A Design-Based Stereological Study. *Hippocampus* 18, 135–147. doi: 10.1002/hipo.20376

Fitting, S., Booze, R. M., Hasselrot, U., and Mactutus, C. F. (2010). Dose-dependent long-term effects of Tat in the rat hippocampal formation: a design-based stereological study. *Hippocampus* 20, 469–480. doi: 10.1002/hipo.20648

Gaarskjaer, F. B. (1978). Organization of the mossy fiber system of the rat studied in extended hippocampi. I. Terminal area related to number of granule and pyramidal cells. *Journal of Comparative Neurology* 178, 49–71. doi: 10.1002/cne.901780104

Gaarskjaer, F. B. (1986). The organization and development of the hippocampal mossy fiber system. *Brain Res* 396, 335–357. doi: 10.1016/0165-0173(86)90004-4

Gatome, C. W., Slomianka, L., Lipp, H. P., and Amrein, I. (2010). Number estimates of neuronal phenotypes in layer II of the medial entorhinal cortex of rat and mouse. *Neuroscience* 170, 156–165. doi: 10.1016/j.neuroscience.2010.06.048

Goodrick, C. L. (1980). Effects of long-term voluntary wheel exercise on male and female Wistar rats. I. Longevity, body weight, and metabolic rate. *Gerontology* 26, 22–33. doi: 10.1159/000212390

Gould, E., Woolley, C. S., Frankfurt, M., and McEwen, B. S. (1990). Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. *J. Neurosci.* 10, 1286–1291. doi: 10.1523/JNEUROSCI.10-04-01286.1990

Grady, M. S., Charleston, J. S., Maris, D., Witgen, B. M., and Lifshitz, J. (2003). Neuronal and glial cell number in the hippocampus after experimental traumatic brain injury: analysis by stereological estimation. *J Neurotrauma* 20, 929–941. doi: 10.1089/089771503770195786

Hattiangady, B., Rao, M. S., Shetty, G. A., and Shetty, A. K. (2005). Brain-derived neurotrophic factor, phosphorylated cyclic AMP response element binding protein and neuropeptide Y decline as early as middle age in the dentate gyrus and CA1 and CA3 subfields of the hippocampus. *Experimental Neurology* 195, 353–371. doi: 10.1016/j.expneurol.2005.05.014

Heggland, I., Storkaas, I. S., Soligard, H. T., Kobro-Flatmoen, A., and Witter, M. P. (2015). Stereological estimation of neuron number and plaque load in the hippocampal region of a transgenic rat model of Alzheimer's disease. *Eur J Neurosci* 41, 1245–1262. doi: 10.1111/ejn.12876

Heine, V. M., Maslam, S., Joëls, M., and Lucassen, P. J. (2004). Prominent decline of newborn cell proliferation, differentiation, and apoptosis in the aging dentate gyrus, in absence of an age-related hypothalamus-pituitary-adrenal axis activation. *Neurobiol Aging* 25, 361–375. doi: 10.1016/S0197-4580(03)00090-3

Henze, D. A., Wittner, L., and Buzsáki, G. (2002). Single granule cells reliably discharge targets in the hippocampal CA3 network in vivo. *Nat Neurosci* 5, 790–795. doi: 10.1038/nn887

Hosseini-Sharifabad, M., and Nyengaard, J. R. (2007). Design-based estimation of neuronal number and individual neuronal volume in the rat hippocampus. *J Neurosci Methods* 162, 206–214. doi: 10.1016/j.jneumeth.2007.01.009

Imayoshi, I., Sakamoto, M., Ohtsuka, T., Takao, K., Miyakawa, T., Yamaguchi, M., et al. (2008). Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. *Nat Neurosci* 11, 1153–1161. doi: 10.1038/nn.2185

Ishizuka, N., Cowan, W. M., and Amaral, D. G. (1995). A quantitative analysis of the dendritic organization of pyramidal cells in the rat hippocampus. *Journal of Comparative Neurology* 362, 17–45. doi: 10.1002/cne.903620103

Ishizuka, N., Weber, J., and Amaral, D. G. (1990). Organization of intrahippocampal projections originating from CA3 pyramidal cells in the rat. *J Comp Neurol* 295, 580–623. doi: 10.1002/cne.902950407

Jabès, A., Lavenex, P. B., Amaral, D. G., and Lavenex, P. (2010). Quantitative analysis of postnatal neurogenesis and neuron number in the macaque monkey dentate gyrus. *Eur J Neurosci* 31, 273–285. doi: 10.1111/j.1460-9568.2009.07061.x

Jiao, Y., and Nadler, J. V. (2007). Stereological analysis of GluR2-immunoreactive hilar neurons in the pilocarpine model of temporal lobe epilepsy: correlation of cell loss with mossy fiber sprouting. *Exp Neurol* 205, 569–582. doi: 10.1016/j.expneurol.2007.03.025

Kakolewski, J. W., Cox, V. C., and Valenstein, E. S. (1968). Sex differences in body-weight change following gonadectomy of rats. *Psychol Rep* 22, 547–554. doi: 10.2466/pr0.1968.22.2.547

Keleş, A. İ., Nyengaard, J. R., and Odacı, E. (2019). Changes in pyramidal and granular neuron numbers in the rat hippocampus 7 days after exposure to a continuous 900-MHz electromagnetic field during early and mid-adolescence. *J Chem Neuroanat* 101, 101681. doi: 10.1016/j.jchemneu.2019.101681

Kempermann, G. (2011). *Adult Neurogenesis*., 2nd edition. New York: Oxford University Press.

Krueppel, R., Remy, S., and Beck, H. (2011). Dendritic integration in hippocampal dentate granule cells. *Neuron* 71, 512–528. doi: 10.1016/j.neuron.2011.05.043

Kumar, S. S., and Buckmaster, P. S. (2006). Hyperexcitability, interneurons, and loss of GABAergic synapses in entorhinal cortex in a model of temporal lobe epilepsy. *J Neurosci* 26, 4613–4623. doi: 10.1523/JNEUROSCI.0064-06.2006

Lazic, S. E. (2012). Modeling hippocampal neurogenesis across the lifespan in seven species. *Neurobiol Aging* 33, 1664–1671. doi: 10.1016/j.neurobiolaging.2011.03.008

Leutgeb, J. K., Leutgeb, S., Moser, M.-B., and Moser, E. I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science* 315, 961–966. doi: 10.1126/science.1135801

Lister, J. P., Blatt, G. J., DeBassio, W. A., Kemper, T. L., Tonkiss, J., Galler, J. R., et al. (2005). Effect of prenatal protein malnutrition on numbers of neurons in the principal cell layers of the adult rat hippocampal formation. *Hippocampus* 15, 393–403. doi: 10.1002/hipo.20065

Lister, J. P., Tonkiss, J., Blatt, G. J., Kemper, T. L., DeBassio, W. A., Galler, J. R., et al. (2006). Asymmetry of neuron numbers in the hippocampal formation of prenatally malnourished and normally nourished rats: a stereological investigation. *Hippocampus* 16, 946–958. doi: 10.1002/hipo.20221

Lorente de Nó, R. (1934). *Studies on the structure of the cerebral cortex. II, Continuation of the study of the ammonic system*. Leipzig: Johann Ambrosius Barth.

Lukoyanov, N. V., Brandão, F., Cadete-Leite, A., Madeira, M. D., and Paula-Barbosa, M. M. (2000). Synaptic reorganization in the hippocampal formation of alcohol-fed rats may compensate for functional deficits related to neuronal loss. *Alcohol* 20, 139–148. doi: 10.1016/s0741-8329(99)00069-5

Lukoyanov, N. V., Madeira, M. D., and Paula-Barbosa, M. M. (1999). Behavioral and neuroanatomical consequences of chronic ethanol intake and withdrawal. *Physiol Behav* 66, 337–346. doi: 10.1016/s0031-9384(98)00301-1

Lukoyanov, N. V., Sá, M. J., Madeira, M. D., and Paula-Barbosa, M. M. (2004). Selective loss of hilar neurons and impairment of initial learning in rats after repeated administration of electroconvulsive shock seizures. *Exp Brain Res* 154, 192–200. doi: 10.1007/s00221-003-1658-3

Madeira, M. D., Cadete-Leite, A., Andrade, J. P., and Paula-Barbosa, M. M. (1991). Effects of hypothyroidism upon the granular layer of the dentate gyrus in male and female adult rats: a morphometric study. *J Comp Neurol* 314, 171–186. doi: 10.1002/cne.903140116

Madeira, M. D., Paula-Barbosa, M., Cadete-Leite, A., and Tavares, M. A. (1988). Unbiased estimate of hippocampal granule cell numbers in hypothyroid and in sex-age-matched control rats. *J Hirnforsch* 29, 643–650.

Madeira, M. D., Sousa, N., Lima-Andrade, M. T., Calheiros, F., Cadete-Leite, A., and Paula-Barbosa, M. M. (1992). Selective vulnerability of the hippocampal pyramidal neurons to hypothyroidism in male and female rats. *J Comp Neurol* 322, 501–518. doi: 10.1002/cne.903220405

Maia, G. H., Quesado, J. L., Soares, J. I., do Carmo, J. M., Andrade, P. A., Andrade, J. P., et al. (2014). Loss of hippocampal neurons after kainate treatment correlates with behavioral deficits. *PLoS One* 9, e84722. doi: 10.1371/journal.pone.0084722

Maier, S. E., and West, J. R. (2001). Regional differences in cell loss associated with binge-like alcohol exposure during the first two trimesters equivalent in the rat. *Alcohol* 23, 49–57. doi: 10.1016/s0741-8329(00)00133-6

Maliković, J., Amrein, I., Vinciguerra, L., Lalošević, D., Wolfer, D. P., and Slomianka, L. (2022). Cell numbers in the reflected blade of CA3 and their relation to other hippocampal principal cell populations across seven species. *Front Neuroanat* 16, 1070035. doi: 10.3389/fnana.2022.1070035

McCullers, D. L., Sullivan, P. G., Scheff, S. W., and Herman, J. P. (2002). Mifepristone protects CA1 hippocampal neurons following traumatic brain injury in rat. *Neuroscience* 109, 219–230. doi: 10.1016/s0306-4522(01)00477-8

Merrill, D. A., Chiba, A. A., and Tuszynski, M. H. (2001). Conservation of neuronal number and size in the entorhinal cortex of behaviorally characterized aged rats. *Journal of Comparative Neurology* 438, 445–456. doi: 10.1002/cne.1327

Merrill, D. A., Karim, R., Darraq, M., Chiba, A. A., and Tuszynski, M. H. (2003). Hippocampal cell genesis does not correlate with spatial learning ability in aged rats. *J Comp Neurol* 459, 201–207. doi: 10.1002/cne.10616

Middleton, S. J., and McHugh, T. J. (2020). CA2: A Highly Connected Intrahippocampal Relay. *Annu Rev Neurosci* 43, 55–72. doi: 10.1146/annurev-neuro-080719-100343

Miki, T., Satriotomo, I., Li, H.-P., Matsumoto, Y., Gu, H., Yokoyama, T., et al. (2005). Application of the physical disector to the central nervous system: estimation of the total number of neurons in subdivisions of the rat hippocampus. *Anat Sci Int* 80, 153–162. doi: 10.1111/j.1447-073x.2005.00121.x

Mulders, W. H., West, M. J., and Slomianka, L. (1997). Neuron numbers in the presubiculum, parasubiculum, and entorhinal area of the rat. *J Comp Neurol* 385, 83–94.

Newman, E. L., and Hasselmo, M. E. (2014). CA3 sees the big picture while dentate gyrus splits hairs. *Neuron* 81, 226–228. doi: 10.1016/j.neuron.2014.01.004

Ninkovic, J., Mori, T., and Götz, M. (2007). Distinct modes of neuron addition in adult mouse neurogenesis. *J Neurosci* 27, 10906–10911. doi: 10.1523/JNEUROSCI.2572-07.2007

Nuñez, J. L., Alt, J. J., and McCarthy, M. M. (2003a). A new model for prenatal brain damage. I. GABAA receptor activation induces cell death in developing rat hippocampus. *Exp Neurol* 181, 258–269. doi: 10.3201/eid0906.030118

Nuñez, J. L., Alt, J. J., and McCarthy, M. M. (2003b). A novel model for prenatal brain damage. II. Long-term deficits in hippocampal cell number and hippocampal-dependent behavior following neonatal GABAA receptor activation. *Exp Neurol* 181, 270–280. doi: 10.3201/eid0906.020377

Nuñez, J. L., and McCarthy, M. M. (2003). Estradiol exacerbates hippocampal damage in a model of preterm infant brain injury. *Endocrinology* 144, 2350–2359. doi: 10.1210/en.2002-220840

Olesen, M. V., Wörtwein, G., and Pakkenberg, B. (2015). Electroconvulsive stimulation, but not chronic restraint stress, causes structural alterations in adult rat hippocampus--a stereological study. *Hippocampus* 25, 72–80. doi: 10.1002/hipo.22351

O'Reilly, R. C., and McClelland, J. L. (1994). Hippocampal conjunctive encoding, storage, and recall: Avoiding a trade-off. *Hippocampus* 4, 661–682. doi: 10.1002/hipo.450040605

Patton, P. E., and McNaughton, B. (1995). Connection matrix of the hippocampal formation: I. The dentate gyrus. *Hippocampus* 5, 245–286. doi: 10.1002/hipo.450050402

Peterson, D. A., Lucidi-Phillipi, C. A., Eagle, K. L., and Gage, F. H. (1994). Perforant path damage results in progressive neuronal death and somal atrophy in layer II of entorhinal cortex and functional impairment with increasing postdamage age. *J Neurosci* 14, 6872–6885. doi: 10.1523/JNEUROSCI.14-11-06872.1994

Peterson, D. A., Lucidi-Phillipi, C. A., Murphy, D. P., Ray, J., and Gage, F. H. (1996). Fibroblast growth factor-2 protects entorhinal layer II glutamatergic neurons from axotomy-induced death. *J Neurosci* 16, 886–898. doi: 10.1523/JNEUROSCI.16-03-00886.1996

Pilz, G.-A., Bottes, S., Betizeau, M., Jörg, D. J., Carta, S., April, S., et al. (2018). Live imaging of neurogenesis in the adult mouse hippocampus. *Science* 359, 658–662. doi: 10.1126/science.aao5056

Ramon y Cajal, S. (1893). Estructura del asta de Ammon y fascia dentata. *Ann. Soc. Esp. Hist. Nat.* 22.

Ramsden, M., Berchtold, N. C., Patrick Kesslak, J., Cotman, C. W., and Pike, C. J. (2003). Exercise increases the vulnerability of rat hippocampal neurons to kainate lesion. *Brain Res* 971, 239–244. doi: 10.1016/s0006-8993(03)02365-5

Rao, M. S., Hattiangady, B., Abdel-Rahman, A., Stanley, D. P., and Shetty, A. K. (2005). Newly born cells in the ageing dentate gyrus display normal migration, survival and neuronal fate choice but endure retarded early maturation. *Eur J Neurosci* 21, 464–476. doi: 10.1111/j.1460-9568.2005.03853.x

Rao, M. S., Hattiangady, B., and Shetty, A. K. (2006). The window and mechanisms of major age-related decline in the production of new neurons within the dentate gyrus of the hippocampus. *Aging Cell* 5, 545–558. doi: 10.1111/j.1474-9726.2006.00243.x

Rapp, P. R., Deroche, P. S., Mao, Y., and Burwell, R. D. (2002). Neuron number in the parahippocampal region is preserved in aged rats with spatial learning deficits. *Cereb Cortex* 12, 1171–1179. doi: 10.1093/cercor/12.11.1171

Rapp, P. R., and Gallagher, M. (1996). Preserved neuron number in the hippocampus of aged rats with spatial learning deficits. *Proc Natl Acad Sci U S A* 93, 9926–9930. doi: 10.1073/pnas.93.18.9926

Rasmussen, T., Schliemann, T., Sørensen, J. C., Zimmer, J., and West, M. J. (1996). Memory impaired aged rats: no loss of principal hippocampal and subicular neurons. *Neurobiol Aging* 17, 143–147. doi: 10.1016/0197-4580(95)02032-2

Rennie, K., De Butte, M., and Pappas, B. A. (2009). Melatonin promotes neurogenesis in dentate gyrus in the pinealectomized rat. *J Pineal Res* 47, 313–317. doi: 10.1111/j.1600-079X.2009.00716.x

Rolls, E. T. (2013). The mechanisms for pattern completion and pattern separation in the hippocampus. *Front Syst Neurosci* 7, 74. doi: 10.3389/fnsys.2013.00074

Roof, R. L., and Havens, M. D. (1992). Testosterone improves maze performance and induces development of a male hippocampus in females. *Brain Res* 572, 310–313. doi: 10.1016/0006-8993(92)90491-q

Schlessinger, A. R., Cowan, W. M., and Gottlieb, D. I. (1975). An autoradiographic study of the time of origin and the pattern of granule cell migration in the dentate gyrus of the rat. *J Comp Neurol* 159, 149–175. doi: 10.1002/cne.901590202

Schlessinger, A. R., Cowan, W. M., and Swanson, L. W. (1978). The time of origin of neurons in Ammon's horn and the associated retrohippocampal fields. *Anat Embryol (Berl)* 154, 153–173. doi: 10.1007/BF00304660

Seress, L. (1988). Interspecies comparison of the hippocampal formation shows increased emphasis on the regio superior in the Ammon's horn of the human brain. *J Hirnforsch* 29, 335–340.

Seress, L., and Pokorny, J. (1981). Structure of the granular layer of the rat dentate gyrus. A light microscopic and Golgi study. *J Anat* 133, 181–195.

Severi, S., Guidi, S., Ciani, E., and Bartesaghi, R. (2005). Sex differences in the stereological parameters of the hippocampal dentate gyrus of the guinea-pig before puberty. *Neuroscience* 132, 375–387. doi: 10.1016/j.neuroscience.2004.12.015

Smith, A. M., Pappalardo, D., and Chen, W.-J. A. (2008). Estimation of neuronal numbers in rat hippocampus following neonatal amphetamine exposure: A stereology study. *Neurotoxicology and Teratology* 30, 495–502. doi: 10.1016/j.ntt.2008.05.001

Snyder, J. S. (2019). Recalibrating the Relevance of Adult Neurogenesis. *Trends Neurosci* 42, 164–178. doi: 10.1016/j.tins.2018.12.001

Snyder, J. S., and Cameron, H. A. (2012). Could adult hippocampal neurogenesis be relevant for human behavior? *Behav Brain Res* 227, 384–390. doi: 10.1016/j.bbr.2011.06.024

Sousa, N., Almeida, O. F., Holsboer, F., Paula-Barbosa, M. M., and Madeira, M. D. (1998a). Maintenance of hippocampal cell numbers in young and aged rats submitted to chronic unpredictable stress. Comparison with the effects of corticosterone treatment. *Stress* 2, 237–249. doi: 10.3109/10253899809167288

Sousa, N., Madeira, M. D., and Paula-Barbosa, M. M. (1998b). Effects of corticosterone treatment and rehabilitation on the hippocampal formation of neonatal and adult rats. An unbiased stereological study. *Brain Res* 794, 199–210. doi: 10.1016/s0006-8993(98)00218-2

Sousa, N., Paula-Barbosa, M. M., and Almeida, O. F. (1999). Ligand and subfield specificity of corticoid-induced neuronal loss in the rat hippocampal formation. *Neuroscience* 89, 1079–1087. doi: 10.1016/s0306-4522(98)00311-x

Squire, L. R., Shimamura, A. P., and Amaral, D. G. (1989). "Memory and the hippocampus," in *Neural models of plasticity: Experimental and theoretical approaches*, (San Diego, CA, US: Academic Press), 208–239. doi: 10.1016/B978-0-12-148955-7.50016-3

Swanson, L. W., and Cowan, W. M. (1977). An autoradiographic study of the organization of the efferet connections of the hippocampal formation in the rat. *Journal of Comparative Neurology* 172, 49–84. doi: 10.1002/cne.901720104

Thind, K. K., Yamawaki, R., Phanwar, I., Zhang, G., Wen, X., and Buckmaster, P. S. (2010). Initial loss but later excess of GABAergic synapses with dentate granule cells in a rat model of temporal lobe epilepsy. *J Comp Neurol* 518, 647–667. doi: 10.1002/cne.22235

Treves, A., and Rolls, E. T. (1992). Computational constraints suggest the need for two distinct input systems to the hippocampal CA3 network. *Hippocampus* 2, 189–199. doi: 10.1002/hipo.450020209

Treves, A., and Rolls, E. T. (1994). Computational analysis of the role of the hippocampus in memory. *Hippocampus* 4, 374–391. doi: 10.1002/hipo.450040319

Tukey, J. W. (1977). *Exploratory Data Analysis*. Reading, MA: Addison-Wesley.

Tuunanen, J., and Pitkänen, A. (2000). Do seizures cause neuronal damage in rat amygdala kindling? *Epilepsy Research* 39, 171–176. doi: 10.1016/S0920-1211(99)00123-0

Vandael, D., and Jonas, P. (2024). Structure, biophysics, and circuit function of a "giant" cortical presynaptic terminal. *Science* 383, eadg6757. doi: 10.1126/science.adg6757

Wakuda, T., Matsuzaki, H., Suzuki, K., Iwata, Y., Shinmura, C., Suda, S., et al. (2008). Perinatal asphyxia reduces dentate granule cells and exacerbates methamphetamine-induced hyperlocomotion in adulthood. *PLoS One* 3, e3648. doi: 10.1371/journal.pone.0003648

Wang, H., and Gondré-Lewis, M. C. (2013). Prenatal nicotine and maternal deprivation stress de-regulate the development of CA1, CA3, and dentate gyrus neurons in hippocampus of infant rats. *PLoS One* 8, e65517. doi: 10.1371/journal.pone.0065517

West, M. J. (1990). Stereological studies of the hippocampus: a comparison of the hippocampal subdivisions of diverse species including hedgehogs, laboratory rodents, wild mice and men. *Prog Brain Res* 83, 13–36. doi: 10.1016/s0079-6123(08)61238-8

West, M. J., and Andersen, A. H. (1980). An allometric study of the area dentata in the rat and mouse. *Brain Res* 2, 317–348. doi: 10.1016/0165-0173(80)90012-0

West, M. J., Coleman, P. D., and Flood, D. G. (1988). Estimating the number of granule cells in the dentate gyrus with the disector. *Brain Res* 448, 167–172. doi: 10.1016/0006-8993(88)91114-6

West, M. J., Slomianka, L., and Gundersen, H. J. (1991). Unbiased stereological estimation of the total number of neurons in thesubdivisions of the rat hippocampus using the optical fractionator. *Anat Rec* 231, 482–497. doi: 10.1002/ar.1092310411

Wimer, C. C., and Wimer, R. E. (1989). On the sources of strain and sex differences in granule cell number in the dentate area of house mice. *Brain Res Dev Brain Res* 48, 167–176. doi: 10.1016/0165-3806(89)90073-4

Wimer, R. E., Wimer, C. C., and Alameddine, L. (1988). On the development of strain and sex differences in granule cell number in the area dentata of house mice. *Brain Res* 470, 191–197. doi: 10.1016/0165-3806(88)90237-4

Witter, M. P., Doan, T. P., Jacobsen, B., Nilssen, E. S., and Ohara, S. (2017). Architecture of the Entorhinal Cortex A Review of Entorhinal Anatomy in Rodents with Some Comparative Notes. *Front Syst Neurosci* 11, 46. doi: 10.3389/fnsys.2017.00046

Woodhams, P. L., Celio, M. R., Ulfig, N., and Witter, M. P. (1993). Morphological and functional correlates of borders in the entorhinal cortex and hippocampus. *Hippocampus* 3, 303–311. doi: 10.1002/hipo.1993.4500030733

Woodson, W., Nitecka, L., and Ben-Ari, Y. (1989). Organization of the GABAergic system in the rat hippocampal formation: a quantitative immunocytochemical study. *J Comp Neurol* 280, 254–271. doi: 10.1002/cne.902800207

Xavier, G. F., Oliveira-Filho, F. J., and Santos, A. M. (1999). Dentate gyrus-selective colchicine lesion and disruption of performance in spatial tasks: difficulties in “place strategy” because of a lack of flexibility in the use of environmental cues? *Hippocampus* 9, 668–681. doi: 10.1002/(SICI)1098-1063(1999)9:6<668::AID-HIPO8>3.0.CO;2-9

Xie, T., and Zaidi, H. (2013). Age-dependent small-animal internal radiation dosimetry. *Mol Imaging* 12, 364–375.

Zhang, J.-M., Konkle, A. T. M., Zup, S. L., and McCarthy, M. M. (2008a). Impact of sex and hormones on new cells in the developing rat hippocampus: a novel source of sex dimorphism? *Eur J Neurosci* 27, 791–800. doi: 10.1111/j.1460-9568.2008.06073.x

Zhang, W.-J., Tan, Y.-F., Yue, J. T. Y., Vranic, M., and Wojtowicz, J. M. (2008b). Impairment of hippocampal neurogenesis in streptozotocin-treated diabetic rats. *Acta Neurol Scand* 117, 205–210. doi: 10.1111/j.1600-0404.2007.00928.x