

Adult hippocampal neurogenesis is age dependent and differently modulated in male and female wild house mice

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Abstract

Adult hippocampal neurogenesis (AHN) is often studied with respect to age and physiological neuromodulators in laboratory rodents. Here, we analyzed modulatory effects of age as well as steroid hormones and endocannabinoids (eCBs) in hair on AHN in a population of wild, free-ranging house mice exposed to naturally varying environmental conditions. AHN was assessed in 73 male and female house mice (*Mus musculus domesticus*) of known age using design-based stereology in brain sections immunostained for Ki-67-positive proliferating cells and Doublecortin (DCX)-positive young neurons. Age emerged as the strongest predictor of AHN, with exponential decline across individuals aged 2 to 26 months, similar to the decline reported for C57BL/6J lab mice. In both sexes, AHN further decreased with increasing levels of both the stress hormone corticosterone and the sex hormones testosterone and progesterone. Higher eCB concentrations (2-arachidonoylglycerol, 2-AG, and anandamide, AEA) were positively associated with AHN in both sexes. However, when all five neuromodulators were analyzed together, separately for each sex and corrected for age, most of these effects were no longer significant, while sex differences became evident. In females, age was the dominant predictor of AHN, followed by testosterone and progesterone. In males, by contrast, 2-AG emerged as most important predictor, followed by age and progesterone. Our study on wild house mice from a free-ranging population revealed that an integrative approach allows new insights into sex-specific modulation of age-dependent adult hippocampal neurogenesis.

1 Introduction

Adult hippocampal neurogenesis (AHN) refers to the lifelong generation of new neurons in the adult brain, from the proliferation of neural stem and progenitor cells in the dentate gyrus of the hippocampus to their maturation and integration into existing circuits (Altman and Das, 1965; Kempermann, 2022). Rather than simply replacing lost neurons, AHN seems to help the brain in its extraordinary plasticity to adapt to new environmental conditions throughout life and allow behavioral flexibility (Amrein et al., 2011; Kempermann, 2016). So far, AHN has been documented in at least 110 mammalian species (Amrein, 2015; Terreros-Roncal et al., 2023). In humans, neurogenesis clearly exists in childhood. Adult human neurogenesis also occurs, but at much lower levels than in rodents (Disouky et al., 2026).

Functionally, AHN has been shown to support learning, memory, emotional regulation, and mental health. It is central for cognitive flexibility, for example by adjusting memories to a new context and encoding new information, especially spatial and episodic memory (Kempermann, 2016, 2022). The hippocampus further contributes to regulation of the hypothalamic–pituitary–adrenal (HPA) axis, with the consequence that AHN plays a role in stress and mood. Chronic stress is one factor that reduces AHN, and reduced neurogenesis is itself linked to a prolonged stress response with longer secretion of glucocorticoid (stress) hormones, and to decreased ability to cope with stress (Schoenfeld and Gould, 2012). Neuromodulators of AHN thus include glucocorticoids (cortisol in most mammals, corticosterone in rodents) and other steroid hormones (Galea et al., 2013). Testosterone is suggested to protect newly generated neurons, increasing AHN in males (Duarte-Guterman et al., 2019; Spritzer and Galea, 2007).

The role of progesterone on AHN is less established. Acute administration of progesterone is neuroprotective predicting greater survival of young immature neurons and thus higher AHN (Guennoun, 2020). Progesterone therefore has attracted considerable attention in clinical research as a potential treatment for Alzheimer's disease and acute brain injuries (Montes et al., 2019; Singh et al., 2024). Prolonged progesterone administration in lab animals, in contrast, seems to have no effect (Chan et al., 2014; Wan et al., 2021), while AHN is reduced during late gestation, when progesterone increases (Rolls et al., 2008). Recent evidence further suggests a central role of endocannabinoids (eCB) in AHN. Anandamide (AEA) indirectly enhances AHN through its buffering effect of stress reactions, and 2-arachidonoylglycerol (2-AG) has a direct effect through activation of the CB1 receptor that enhances neuron proliferation and differentiation in the hippocampus (reviewed in Oddi et al., 2023).

The model of choice for most neuroscience studies on AHN are laboratory murine rodents while motivated by the hope of applying knowledge of neurogenesis to improve regenerative medicine. Several studies revealed a general decrease in neurogenesis with increasing age (Ben Abdallah et al., 2010; Kase et al., 2020; Snyder, 2019). Such studies often use mice and rats of a relatively young age. According to a recent overview, most studies on adult neurogenesis used 2–5 month-old animals (Arellano et al., 2024), restricting knowledge about neurogenesis in more mature or old animals. In wild rodents, the regulation of AHN has been studied in adults of unknown age, where age was estimated by morphological correlates (Cavegn et al., 2013; Epp et al., 2009; Klaus et al., 2012; Wojtowicz, 2011). In addition, stress hormones are usually collected through blood samples, which is a challenging method under field conditions as sample collection induces stress (Carlitz et al., 2019).

Studying wild animals is important as it can offer insight into how natural processes shape adult neurogenesis, instead of recent artificial selection and standardized conditions, as in domesticated and laboratory rodents. To understand age-dependent AHN and its interplay with individual steroid and eCB signaling in wildlife therefore requires sampling animals of known age covering adult life. Here, we analyze AHN in wild house mice (*Mus musculus domesticus*) from a large free-ranging population in Switzerland studied since 2002, where individuals were followed from birth until death or dispersal (König et al., 2015). On a single day we collected individuals of both sexes of varying age (ranging between 2 and 26 months) and sampled their brains and hair. AHN was assessed using design-based stereology in series of brain sections immunostained for Ki67-positive proliferating cells and Doublecortin (DCX)-positive young neurons. For comparison, we also assessed granule cells, reflecting mature neurons. We combined this with analysis of hair steroid hormone concentrations (the stress hormone corticosterone and the sex hormones testosterone and progesterone) and hair eCBs (2-AG and AEA). Previous work on the study population has revealed that steroids measured in hair reflect hormone secretion over the past 2–3 months, mirroring reproductive behavior as well as social and environmental conditions experienced naturally (Carlitz et al., 2019, 2022).

We tested the following hypotheses on AHN in the free-ranging study population. We expected, first, a decrease in AHN with increasing age in wild house mice of both sexes, but no decrease in hippocampal granule cells. We then asked if the age-related pattern of AHN differs from that of C57BL/6J lab mice,

using previous data (Ben Abdallah et al., 2010). Second, we predicted modulating effects of steroid hormones accumulated in hair. Higher corticosterone should be associated with decreased AHN in both sexes and higher testosterone with increased AHN at least in males. In females, we expected increased progesterone to negatively affect AHN. We further explored the effect of previous reproductive history in females, measured by their number of embryos implanted throughout life, which positively correlates with corticosterone and progesterone (Carlitz et al., 2019). Third, for eCBs measured in hair we expected increased levels of 2-AG and AEA to positively modify AHN.

Taken together, this integrative study on wild house mice exposed to naturally varying ecological and social conditions over many generations will shed light on the functional role of steroid hormones and eCBs on age-dependent adult hippocampal neurogenesis.

2 Methods

2.1 Study population

The wild study animals originated from a population of free-ranging house mice (*Mus musculus domesticus*) in a barn near Zurich, Switzerland. The population lives under conditions typical for commensal house mice in Central Europe and has been studied continuously since 2002. The inside of the barn (72 m²) is structured with low plastic walls in four sections. Nevertheless, mice can access all parts of the building and leave or enter as they wish through holes, cracks in the wall or under the roof. Smaller plastic walls, bricks, and wooden elements allow for cover. Food and water are provided three times a week and are freely accessible in all sections. Predators smaller than mice cannot enter the building, nevertheless, cats, foxes and owls are present in the surroundings. Reproduction (birth of litters) is closely monitored and has been recorded in the population every month of a year, although breeding peaks during spring and summer (March to August), and is lowest in winter (December to February; Ferrari et al. 2019; Evans et al. 2021).

To facilitate documentation of the population's demography and reproductive behavior, the barn is equipped with 40 artificial nest boxes (15cm in diameter, lids can be opened for inspection), where mice sleep and rest during their inactive periods and which females prefer for rearing litters (Harrison et al., 2018). During regular nest checks (at least every 12 days) we search in all nest boxes for new litters, estimate the age of the pups and record their sex and body weight. When pups are predicted to be 13 days old, we go back to their nest and collect small tissue samples from their ears for later genetic analysis. We further catch the entire population every 6–8 weeks. Mice that reached a body weight of at least 17.5g are tagged for life-long identification (with subcutaneously injected RFID tags, Trovan ID-100A) and have another ear sample taken. By matching genotypes from this sample to genotypes from a pup (of known age) we determine the date of birth of each tagged individual. For further details on data collection and methods used in this long-term project, see König and Lindholm (2012) and König et al. (2015).

2.2 Study animals for AHN analyses

On December 2nd, 2020, we collected 73 adult individuals from the population (32 males and 41 females; see Supplementary_Table_1 for details). At the end of October 2020, the population had consisted of more than 460 adults (at least 160 males and 290 females). Based on our information on the tagged adult mice, we chose individuals from different sections and nest boxes in the barn, covering an equal number of males and females and large variation in age. Nevertheless, some opportunistic sampling had to be done because several pre-selected individuals evaded detection on that day.

Once a study animal was caught, we recorded its sex and body weight. Afterwards it was euthanized, its brain was dissected, weighed, and fixed. To be able to later analyze potential effects of processing time of brain extraction on AHN, we recorded the time in minutes between catching and euthanizing an individual (time in captivity), and between death and brain dissection (post-mortem delay). We further shaved hair from the animal's lower back (10-15mg of hair) for later hormone analysis. For females, placental scars and developing embryos were counted in the uterus, representing their total number of embryos implanted throughout life (reproductive history; Carlitz et al. 2019).

2.3 Brain cell analysis

Dissected brains were immersion fixed in 4% PFA containing 15% picric acid for 24h. Brain hemispheres were separated. Left hemispheres were HEMA embedded, and series of coronal sections (20µm thick) were used to estimate total hippocampal granule cell numbers. Right hemispheres were embedded in a gelatin matrix and frozen, and series of coronal sections were used for immunohistochemistry to allow counting Ki67-positive proliferating cells and DCX-positive cells. For detailed description of the methods used, see Supplementary_Methods: Hippocampal cell analysis, which further gives examples for brain sections with Ki67- and DCX-immunostaining of wild house mice aged 2, 12 and 26 months.

2.4 Steroid hormone and endocannabinoid (eCB) analysis from hair

Hair samples were shaved from the lower back of an animal and kept in individual paper envelopes at room temperature until analysis. Hair preparation followed an established protocol and included washing with isopropanol and metabolite extraction from 5 ± 0.5 mg of non-pulverized hair with methanol for 18h (Carlitz et al., 2019). Steroid hormones (corticosterone, testosterone, progesterone) and eCBs (AEA and 1-AG as proxy for 2-AG) were quantified via liquid chromatography–tandem mass spectrometry (LC–MS/MS; Gao et al. 2016, 2021).

2-AG is one of the key regulators of cell proliferation, also functionally present in the hair follicle's keratinizing cells (Smith and Satino, 2021). Nevertheless, 2-AG cannot be measured in hair because it undergoes spontaneous non-enzymatic isomerization to the more stable isomer 1-AG. This conversion increases with time, temperature, and under neutral to alkaline conditions. 1-AG seems to have no biological function in peripheral blood circulation or in the hair follicle (Dócs et al., 2017). Consequently, 1-AG concentrations measured in the hair shaft likely reflect former 2-AG concentrations in the

peripheral blood vessels, the hair follicle, or the keratinizing cells. Throughout the manuscript, we therefore refer to the eCB 2-AG, although technically 1-AG had been measured.

2.5 Statistics

All analyses were performed using R (version 4.4.3; 2025-02-28; R Core Team 2023). Count data of Ki67- and DCX-positive cells followed a negative binomial distribution, whereas age approached normality after log-transformation.

We checked whether the number of hippocampal granule cells varied depending on age and sex. In addition, we tested whether the number of Ki67- and DCX-positive cells were affected by the time in captivity prior to death or by the time between death and brain extraction by fitting negative binomial generalized linear models using the `glm.nb` function in the MASS package (Venables and Ripley, 2002).

To test age-related AHN in our study animals (hypothesis 1), we investigated whether the number of Ki67- and DCX-positive cells was affected by sex and age, while controlling for brain weight and body weight. According to distribution patterns of the two AHN markers, we fitted a negative binomial generalized linear model (`glm.nb`, MASS, Venables and Ripley 2002).

To compare age-dependent AHN of wild house mice with that of lab mice, we extracted data on Ki67- and DCX-positive cell counts and age for C57BL/6J mice from Ben Abdallah et al. (2010) using WebPlotDigitizer v4.8 (apps.automeris.io/wpd4; accessed 2026-03-05). Ki67 and DCX cell counts were then modelled for wild and lab mice as a function of age and origin. We did not include sex in the models, since this information is not provided by Ben Abdallah et al. (2010).

To analyze the modulating effects of steroid hormones (hypothesis 2) and of endocannabinoids (eCBs; hypothesis 3) on AHN, we proceeded in three steps. (i) We considered each metabolite separately together with sex against Ki67- or DCX-positive cells (negative binomial generalized linear models; `glm.nb`, MASS). (ii) We combined all five metabolites (corticosterone, testosterone, progesterone, 2-AG and AEA) in separate models for each AHN marker. Here, we used separate models for sex, since sex hormone profiles across age differ between males and females (Carlitz et al., 2019). (iii) We tested combined effects of age and all five metabolites on AHN in separate models for each AHN marker and sex. Further, for females we tested for an effect of the number of embryo implantations in the uterus (scars and currently developing embryos) on AHN.

Finally, we tested how age was predicted by steroids and eCBs in both sexes using linear models with log-transformed age as response variable. We did so to facilitate comparison with the above models in which steroids and eCBs predicted AHN markers.

3 Results

3.1 Effects of handling and processing of brains on AHN in wild house mice

Animals spent 10 to 99 minutes in cages prior to euthanasia (time in captivity). The post-mortem delay, defined as the interval between death and brain extraction, ranged from 18 to 72 minutes (Supplementary_Table_1). Study males were two to ten months old, females three to 26 months and by coincidence, older females tended to spend less time in captivity prior to euthanasia ($r=-0.21$, $p = 0.08$). Ki67 and DCX cell counts were not significantly predicted by either time in captivity or post-mortem delay, when controlling for age and sex.

3.2 Effect of age on AHN, with covariates sex, body weight, and brain weight (hypothesis 1)

The total number of granule cells in the hippocampus of wild house mice was not significantly influenced by age and sex (both $p = 0.9$; model not shown). Age, however, was the strongest predictor of numbers of Ki67- and DCX-positive cells in both sexes, as expected (hypothesis1; model outputs in Table_1; see Figure_1 for values of male and female wild mice). Age alone explained 68% of the variance in Ki67 and 78% in DCX cell counts (numbers extracted from a single predictor model with age, model not shown).

Table_1

In addition to age, body weight was negatively associated with Ki67 and DCX cell counts and positively associated with brain weight relative to age and body weight (Table_1, Supplementary_Figure_1), suggesting increased AHN in heavier relative brains. Although sex did not predict AHN beyond these three factors, females had higher absolute brain weight than males, as well as higher relative brain weight corrected for body weight (Supplementary_Figure_2).

AHN activity in lab mice from the inbred strain C57BL/6J and in wild house mice of at least 60 days old showed a similar exponential decline with increasing age. Lab mice younger than 100 days had significantly higher Ki67 and DCX cell counts than same aged wild mice (Figure_1; Table_2). Nevertheless, the most extreme DCX values of young wild mice were within the range of lab mice, while Ki67 of lab mice were markedly higher (Figure_1)

Figure_1

Table_2

3.3 Neuromodulatory effects of steroids and endocannabinoids on AHN (hypotheses 2 and 3)

Neuromodulatory effects of steroids and endocannabinoids (eCBs) on AHN were assessed at three levels (see 2.5; each modulator separately, all five combined, and all combined including age). Variance

inflation factors indicated acceptable collinearity among steroids and eCBs in both sexes (maximum: VIF_2-AG < 3). Including age did not substantially increase collinearity in females (VIF_AEA = 3.1; VIF_2-AG = 2.8) but led to moderately higher collinearity in males (maximum VIF_age = 4.4; VIF_2-AG = 3.1), suggesting that age was more closely associated with neuromodulators in males than in females.

3.3.1 Steroid hormones

Corticosterone. Increasing concentrations of corticosterone alone were associated with lower AHN (Ki67 and DCX) in both sexes, as predicted (hypothesis 2). At similar corticosterone levels, males had higher AHN than females (Figure_2A; Supplementary_Table_2A). In addition, males reached a given corticosterone level at a younger age than females (Figure_2B; Supplementary_Table_2B). Both findings point to sex-specific regulation of corticosterone and/or AHN regulation.

Corticosterone, however, did not predict Ki67 or DCX cell counts when tested in combination with the other neuromodulators in either sex, suggesting a limited role of long-term corticosterone on AHN in wild house mice (Figure_2C; Supplementary_Table_2C).

Figure_2

Testosterone. When analyzed alone, the results for testosterone were similar to those for corticosterone and contradicted our hypothesis 2. In both sexes, higher testosterone was associated with lower AHN. At similar testosterone concentrations, males had higher AHN than females (Figure_2A; Supplementary_Table_2A). Also, males reached a given testosterone level at a younger age than females (Figure_2B; Supplementary_Table_2B). Both findings point to sex-specific regulation of testosterone and/or AHN regulation.

When tested with the other neuromodulators, testosterone remained significantly negatively associated with AHN in both sexes (albeit not for DCX in males). When adding age, testosterone only remained predictive for DCX in females (Figure_2C; Supplementary_Table_2C).

Progesterone. Although expected only in females, increasing progesterone concentrations when tested alone were associated with significantly lower AHN (Ki67 and DCX) in both sexes (Figure_2A; Supplementary_Table_2A). In parallel, progesterone increased with age in both sexes at similar rates (Figure_2B; Supplementary_Table_2B).

The association between progesterone and AHN remained significant in both sexes when tested in combination with the other neuromodulators (except for female Ki67; Figure_2C; Supplementary_Table_2C). When also controlling for age, progesterone remained a significant negative predictor for Ki67-positive cells in males and for DCX-positive cells in females (Figure_2C; Supplementary_Table_2C).

Since progesterone in females correlates with reproductive history, we investigated the effect of the number of embryos implanted throughout life (*in utero* scars and developing embryos). An increasing

number of embryo implantations was associated with decreasing AHN (fewer Ki67- and DCX-positive cells). DCX and Ki67 cell counts were more closely associated with the number of embryos implanted (40% and 53% explained variance) than with progesterone (21% and 38% explained variance; Supplementary_Table_3), suggesting that reproductive history affected AHN beyond progesterone signaling. Testing AHN as a function of progesterone and implantations showed that the number of DCX-positive cells was significantly negatively associated with both predictors. While the standardized effect size was twice as high for the number of embryo implantations as for progesterone, this model explained 59% of variance of female DCX (Supplementary_Table_3). Ki67, in contrast, was only affected by the number of implantations. Embryo implantations were highly correlated with age (65% explained variance) such that effects of age on Ki67 and DCX covered all effects of implantations (Supplementary_Table_3).

3.3.2 Endocannabinoids

Brain and hair samples were collected during early December, when breeding activity in the population was low (only one female was pregnant among the selected study animals). We nevertheless suggest that our findings of AHN being associated with progesterone concentrations and number of implantations are representative for the entire year since house mice are not seasonal breeders (Bronson, 1979).

2-AG and AEA. In accordance with our prediction (hypothesis 3), increasing 2-AG and increasing AEA, when analyzed alone, correlated significantly with increasing AHN (Ki67 and DCX cell counts) in both sexes (Figure_2B; Supplementary_Table_2B). 2-AG showed the strongest associations with Ki67- and DCX- positive cells compared to all other neuromodulators, explaining 45% of DCX variance as compared to 34% explained variance by progesterone or AEA (Supplementary_Table_2B). In contrast to the steroid hormones, the endocannabinoids 2-AG and AEA decreased with age at similar rates in both sexes (Figure_2B; Supplementary_Table_2B).

When tested in combination with other neuromodulators, 2-AG remained significant only for DCX in males even when controlling for age (Figure_2C; Supplementary_Table_2C). The effect was not exclusively driven by the young males with highest AHN as male DCX was still significantly predicted by 2-AG in a model that excluded young individuals of less than 100 days of age (model not shown). Effects of AEA were masked by other neuromodulators and age, such that AEA did not significantly predict AHN in combined models in either sex (Figure_2C; Supplementary_Table_2C).

4 Discussion

Adult hippocampal neurogenesis (AHN) in wild house mice from a free-ranging population was assessed using design-based stereology in brain sections immunostained for Ki-67-positive proliferating cells and Doublecortin (DCX)-positive young neurons. Age emerged as the strongest predictor of AHN, showing an exponential decline across individuals aged 2 to 26 months.

When examining individual steroid hormones measured in hair, we found in both sexes that AHN decreased with increasing levels of the primary rodent stress hormone corticosterone, as expected, but also with increasing testosterone, contrary to expectation. Higher progesterone levels were also associated with reduced AHN in both sexes. Endocannabinoid concentrations in hair (2-arachidonoylglycerol, 2-AG, and anandamide, AEA) showed the anticipated pattern, with higher concentrations predicting increased AHN.

However, when all five neuromodulators were analyzed together and age was accounted for, most of these effects were no longer significant, while some sex differences became evident. In females, age was the dominant predictor of AHN, followed by testosterone and progesterone. In males, by contrast, 2-AG emerged as most important predictor, followed by age and progesterone.

4.1 Predominant effect of age on adult hippocampal neurogenesis with body and brain weight as modulators

Age was the strongest and most consistent predictor of AHN in wild house mice of both sexes, whereas hippocampal granule cell numbers remained stable across age. Our brain sampling protocol did not influence the number of proliferating or young neurons, as AHN was unaffected by either the duration of captivity (i.e., the time between capture and euthanasia) or the post-mortem delay (i.e., the time between death and brain dissection).

Both proliferating cells and young neurons declined exponentially, with age alone explaining more than three-quarters of the observed variance. This finding is consistent with previous experimental studies on rodents (Arellano et al., 2024; Snyder et al., 2009). Nonetheless, AHN in wild mice did not cease entirely; instead, neurogenesis persisted at a low level throughout late adulthood and remained detectable even in individuals older than two years.

The age-dependent decline in AHN observed in wild house mice mirrors patterns reported in C57BL/6J (Ben Abdallah et al., 2010). However, C57BL/6J mice exhibited substantially higher numbers of proliferating and young neurons than wild mice at ages below three to four months, in line with earlier reports of elevated cell proliferation rates in this inbred strain). In older individuals, AHN levels converged between wild and C57BL/6J mice.

Age is commonly used as an indicator of physical and reproductive development, as is body weight. In the study population, adult house mice increase body weight with age (Ferrari et al., 2019; Gerber et al., 2021), and we found that AHN – a measure of neuronal plasticity – declined with higher body weight. Specifically, AHN was lower in males and females of above average body weight relative to their age, suggesting that higher body weight itself covaries with reduced neurogenesis. Wild females exhibited higher absolute and relative brain weights (adjusted for body weight) than males (Figure_2), in contrast to recent findings in lab rodents (Guma et al., 2024; Spring et al., 2007). Moreover, for a given age and body weight, individuals with higher brain weights had higher levels of AHN. Taken together, these

findings suggest that heavier brains may mitigate the negative effects of increasing age and body weight on neurogenesis.

4.2 Modulating effects of steroid hormones and endocannabinoids on adult hippocampal neurogenesis

Each neuromodulator showed similar effects on AHN in both sexes when analyzed individually and without accounting for age. Increasing concentrations of steroid hormones (corticosterone, progesterone, testosterone) were associated with reduced neurogenesis, whereas higher levels of endocannabinoids (eCBs, here 2-AG and AEA) predicted higher AHN (Figure_2A). However, when all metabolites were analyzed jointly while controlling for age, only few proved to have an additional and significant effect on neurogenesis.

First, corticosterone emerged as the least important predictor of AHN in both sexes, although some males had relatively high concentrations (2.5 times the median). This finding was unexpected, given previous experimental studies (Hill et al., 2008). We do not think that this discrepancy is due to methodological differences, as corticosterone levels measured in hair and blood serum are well correlated (Koren et al., 2019; Yu et al., 2015). In C57BL/6 mice, experimental stress such as repeated restraint and forced swimming have been shown to reduce exploratory behavior, increase anxiety, and decrease AHN, although none of these effects were observed in wild-derived house mice (Kurilova et al., 2023). This suggests that wild house mice may be more resilient to stress or, alternatively, that the stress levels experienced by our study animals were less severe than in experimental setups. Overall, glucocorticoids may not be strong drivers of neurogenesis in house mice under natural conditions.

Second, regarding the sex hormones, testosterone in wild house mice did not exhibit neuroprotective effects in the hippocampus of either sex, contrary to our expectations. Previous experimental studies have shown that testosterone enhances the survival of newly generated neurons, particularly in males (Duarte-Guterman et al., 2019; Spritzer and Galea, 2007; Wainwright and Galea, 2013). Progesterone, too, was negatively associated with AHN. We had expected such an effect in females, consistent with patterns observed during reproductive states characterized by chronically elevated progesterone, such as pregnancy, when cell proliferation is reduced (Rolls et al., 2008; Singh et al., 2024). Progesterone levels increase in females with the number of embryo implantations (Carlitz et al., 2019). In our study, implantation number had a stronger negative effect on AHN than progesterone itself. Because implantation number increased with age, the effect, however, was no longer detectable when analyzed together with age.

When jointly analyzing all neuromodulators and accounting for age, the negative association between testosterone or progesterone and AHN remained significant for young DCX cells in females. In males, progesterone remained significantly predictive for the number of Ki67 cells, but the effect of testosterone disappeared.

Third, increasing endocannabinoid concentrations had positive effects on AHN, as predicted, and 2-AG emerged as the strongest single correlate among the five tested neuromodulators in both sexes. However, when analyzed jointly with other neuromodulators and age, we observed neuroprotective effects in males only. 2-AG was most important for male AHN by its positive effect on young DCX cells. AEA had been described to buffer the negative glucocorticoid effects (Oddi et al., 2023). However, this eCB had no overall effect as a neuromodulator of AHN when accounting for age. The latter might not be surprising, given that glucocorticoids seem not be important mediators of AHN in our study population.

4.3 Integrated analysis of steroid and endocannabinoids suggests sex differences in the modulation of adult hippocampal neurogenesis

We did not measure acute circulating levels of steroid hormones and endocannabinoids. Instead, we analyzed their accumulation in hair over a broader time window of two to three months. Under natural conditions, these integrated measures are expected to capture integrated physiological variation. From an evolutionary perspective, such longer states of physiological modulators are likely to be more informative to understand the process of neuron integration that allows brain plasticity. Therefore, relationships between steroid hormones as well as endocannabinoids accumulated in hair and AHN might be better interpreted as components of broader physiological processes rather than direct causal drivers.

Although overall AHN levels did not differ between sexes, some underlying physiological interplay appear to be sex-specific. For similar concentrations of testosterone and corticosterone, males had higher AHN than female wild house mice, suggesting sex-specific sensitivity, and both hormones also showed sex-specific age-trajectories, with a stronger age-related increase of both hormones in males than in females. Patterns for progesterone and the eCBs were similar between the sexes. Nevertheless, when modulatory effects were analyzed for all five neuromodulators together while correcting for age, significance patterns were sex-specific.

Concentrations of sex hormones in hair – accumulated over several months – reflect physiological reproductive development and reproductive history. Testosterone, for example, increases with body weight and number of sperm in wild males and, as mentioned before, progesterone increases with the number of embryo implantations in wild females (Carlitz et al., 2019). The observed sex-specific patterns beyond effects of age suggest that individual differences in developmental physiology are more important predictors for female AHN than for male AHN. Breeding imposes higher current and future reproductive costs on females than on males. Females may thus be under higher selective pressure to trade-off brain development against reproductive investment, explaining the pronounced negative effect of progesterone on AHN.

These results highlight the value of complementary approaches that integrate multiple neuromodulators and incorporate natural, variable environments, helping to build a more comprehensive understanding. We recommend that future investigations on AHN study the underlying mechanisms separately for both sexes.

Declarations

Ethics

Data were collected under the permit ZH098/19 from the Cantonal Veterinary Office, Kanton Zürich, Switzerland.

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Use of generative AI

During the preparation of this work, the authors used ChatGPT to improve language and readability.

Author contributions

Conceptualization: AKL, BK; Data curation: AKL, BK; Formal analysis: EC; Funding acquisition: AL, BK; Investigation: EC, AL, BK; Project administration: AL, BK; Visualization: EC; Writing – original draft: EC, AL, BK; Writing – review and editing: EC, AL, BK

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Tables

Table 1 and 2 are available in the Supplementary Files section.

Figures

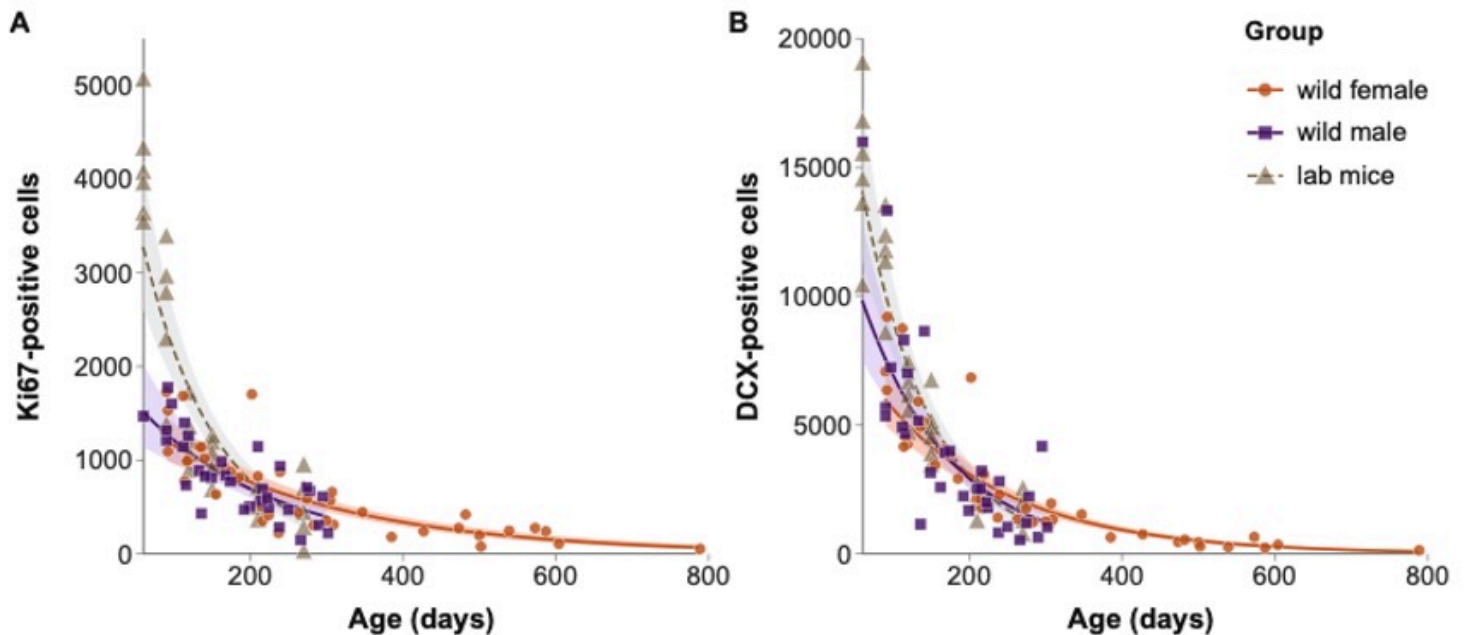


Figure 1

Adult hippocampal neurogenesis (AHN) measured as number of **A**: Ki67-positive cells and **B**: DCX-positive cells in the dentate gyrus as a function of age (days) and group. Grey triangles show C57BL/6J laboratory mice (pooled for both sexes, data from Ben Abdallah et al., 2010) while orange dots and purple squares show wild house mice (female and male) from a free-ranging population. The minimum age was 60 days for all groups.

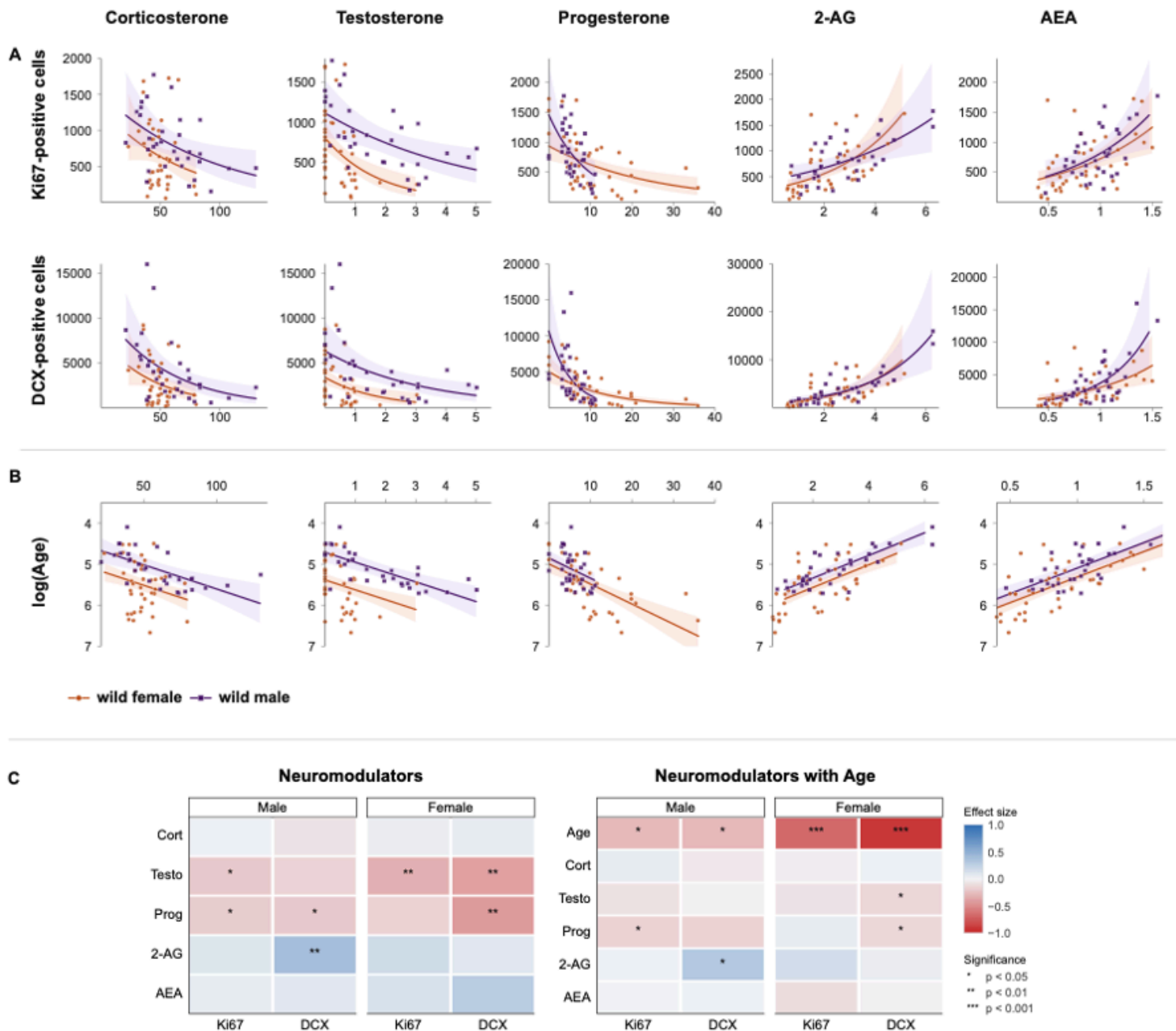


Figure 2

Relationship between neuromodulators (steroid hormones [pg/mg hair], endocannabinoids [ng/mg hair]), markers of adult hippocampal neurogenesis (number of Ki67- and DCX-positive cells), and age (days). **A**: Number of Ki67- and DCX-positive cells as a function of steroid hormone (corticosterone, testosterone, progesterone) and endocannabinoid (2-AG: 2-arachidonoylglycerol, AEA: anandamide) concentrations and sex. Data are shown separately for females (orange circles) and males (purple squares). Solid lines represent fitted regression curves with shaded 95% confidence intervals. **B**: Log-transformed age (days) as a function of steroid hormone and endocannabinoid concentrations and sex. To highlight how neuromodulators against age reveal similar patterns to neuromodulators against Ki67 and DCX, steroids and endocannabinoids (x-axis) are plotted as a function of age (y-axis) and the age is inverted. **C**:

Heatmaps summarizing standardized regression coefficients from models predicting for each sex the number of Ki67- or DCX-positive cells as a function of five neuromodulators (Cort: corticosterone, Testo: testosterone, Prog: progesterone, 2-AG: 2-arachidonoylglycerol, AEA: anandamide) excluding age (Neuromodulators) or including age (Neuromodulators with age). Colors indicate the direction and magnitude of associations. Asterisks denote statistical significance. For full model results, see Supplementary_Table_2A-C.

Supplementary Files

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