

## SHORT COMMUNICATION

# Food consumption increases cell proliferation in the python brain

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## ABSTRACT

Pythons are model organisms for investigating physiological responses to food intake. While systemic growth in response to food consumption is well documented, what occurs in the brain is currently unexplored. In this study, male ball pythons (*Python regius*) were used to test the hypothesis that food consumption stimulates cell proliferation in the brain. We used 5-bromo-12'-deoxyuridine (BrdU) as a cell-birth marker to quantify and compare cell proliferation in the brain of fasted snakes and those at 2 and 6 days after a meal. Throughout the telencephalon, cell proliferation was significantly increased in the 6 day group, with no difference between the 2 day group and controls. Systemic postprandial plasticity occurs quickly after a meal is ingested, during the period of active digestion; however, the brain displays a surge of cell proliferation after most digestion and absorption is complete.

**KEY WORDS:** Feeding, Postprandial, *Python regius*, Telencephalon, Neurogenesis, BrdU, Snake

## INTRODUCTION

Organisms that feed infrequently provide researchers with powerful opportunities to study the physiological responses to feeding because such responses tend to be so much more dramatic than in frequently feeding organisms (Secor and Carey, 2016). Pythons exhibit an exceptionally high specific dynamic action (SDA), or postprandial increase in metabolic rate in order to process a meal (Houlihan, 1991; Jobling, 1994; McCue, 2006; Secor, 2009). To put this into perspective, the maximum increase in metabolic rate for humans during digestion is about 25% and in fishes is 136%, but for a python consuming a meal that is 25% of its body mass, this number is an extraordinary 687% (Westerterp, 2004; Secor, 2009). The python's dramatic SDA and the associated morphological changes of organs have made them model organisms for investigations of physiological responses to food intake. During periods of extended fasting, the gastrointestinal (GI) tract is relatively inactive, with reduced activity in key GI tract and accessory organs, including the pancreas, kidneys, intestine and liver (Cox and Secor, 2008; Lignot et al., 2005; Secor, 2003; Secor and Diamond, 1995; Secor et al., 2000; Starck and Beese, 2001). Pythons can ingest prey exceeding half of their own body mass (Secor, 2008), stimulating a massive, systemic upregulation of metabolic rate and digestive function within 24 h of the meal (Cox and Secor, 2008; Lignot et al., 2005; Secor and Diamond, 1995). To

support the dramatically increased demands, the cardiovascular system is also upregulated at this time, with up to 5-fold increases in ventilation rate, cardiac output and heart rate (Starck et al., 2004), and metabolic rate may increase as much as 44-fold to support the energetic demands of digestion, assimilation and organ growth (Secor, 2008; Secor and Diamond, 1997).

While physiological responses of various tissues to fasting and feeding have been well documented, the brain has received very little attention. When considering the brain as a whole, total brain volume remains constant during prolonged fasting in salmon (Soengas et al., 1996). In frequently feeding species like birds and mammals, the effects of feeding and food restriction on brain cell proliferation and survival vary. For example, in young chickens, food restriction decreases the number of newly born neurons in the hippocampus (Robertson et al., 2017). Dietary restriction increases cell proliferation but decreases new neuron survival in the dorsal dentate gyrus in young adult mice (Staples et al., 2017), and dietary restriction decreases new neuron survival in the dentate gyrus in young rats (Cardoso et al., 2016). However, in older adult mice, dietary restriction increases survival of new neurons in the dentate gyrus (Lee et al., 2000). Neurogenesis occurs beyond early development and into adulthood in all vertebrate classes, but at a much higher rate and is more widespread throughout the telencephalon in reptiles than in mammals (Barker et al., 2011; Bond et al., 2015; Font et al., 2001; Goffinet, 1983; González-Granero et al., 2011; Lopez-Garcia et al., 1988, 1990; Pérez-Cañellas and García-Verdugo, 1996). No studies have examined the effects of feeding on neural cell proliferation in reptiles; however, other factors are known to affect cell proliferation in lizards. For example, in psammomorphus lizards (*Psammomorphus algirus*), cold temperatures reduce cell proliferation and migration significantly throughout the telencephalon (Penafiel et al., 2001). Delgado-González et al. (2008) found that proliferation of cells in the olfactory regions in Gallot's lizard (*Gallotia galloti*) was higher during the spring and summer than during winter and autumn. Other studies found the highest rates of proliferation in the medial cortex of the brain, which has been associated with memory and spatial navigation (Pérez-Cañellas and García-Verdugo, 1996). In red-sided garter snakes (*Thamnophis sirtalis parietalis*), the number of proliferating cells is higher in the autumn than in the spring (Maine et al., 2014). Given that food directly contributes to generalized growth and development of tissues, it is likely that the act of feeding or the resultant absorbed nutrients could stimulate neurogenesis related to brain growth throughout life (Nieto-Estévez et al., 2016; Turkmen et al., 2017; van der Meeren et al., 2009).

To understand more about postprandial brain plasticity in reptiles, we investigated the effects of food consumption on brain cell proliferation in ball pythons, *Python regius* (Shaw 1802). We separated pythons into several treatment groups (2 days post-feeding, 6 days post-feeding and fasted control) to test the hypothesis that food consumption affects brain cell proliferation and to characterize the time course of the response. If postprandial neural cell proliferation follows similar patterns to systemic

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upregulation during digestion, we would expect to see an increase in dividing cells during the time when active digestion is occurring. However, delayed neural cell proliferation (e.g. occurring after the meal has been digested and absorbed) could mean that brain growth is stimulated by positive energy balance following assimilation of a large quantity of nutrients. By quantifying cell proliferation in distinct brain regions following feeding, we also assessed whether postprandial neuroplasticity is a generalized phenomenon or is restricted to specific functional regions of the brain.

## MATERIALS AND METHODS

### Animals and experimental design

Twenty-one juvenile male *P. regius* were purchased from Reptile City in Honey Grove, TX, USA. We chose juveniles to match our methods with the majority of studies in pythons examining digestive physiology, which have used juveniles as study animals (Andrew et al., 2015; Cox and Secor, 2007; Helmstetter et al., 2009; Lignot et al., 2005; Secor and Diamond, 1997, 2000; Secor et al., 2000, 2012; Wang et al., 2002). Furthermore, neurogenesis during the juvenile stages may have long-lasting effects on brain function, which makes this phase an important time frame to study (Lee et al., 2014; Nieto-Estévez et al., 2016).

Upon arrival of the snakes (4 March 2016), we measured their body mass (mean±s.e.m.: 286.3±16.8 g, range 190.7–430.2 g) and snout–vent length (mean±s.e.m.: 63.93±1.37 cm, range: 53.5–77 cm). Snakes were kept in individual, clear, newspaper-lined 54-quart (~61 l) containers with an 8 in-long (~20 cm) piece of lengthwise-cut polyvinyl chloride piping (6 in diameter, ~15 cm) as a hide-box. A heating wire was placed under one end of each container to provide thermal gradients. Lights were turned on at 07:00 h and turned off at 17:00 h; however, there was also ambient light from a window, which provided natural light before and after the lights in the room were on (increasing from approximately 11.5 h light:12.5 h dark in March to 14 h light:10 h dark in May). Each python was given water *ad libitum* and fed every 2 weeks (live mice, 10% of body mass) during a month-long acclimation period before starting the experiment. After the acclimation period, all snakes were fasted for 30 days. All procedures were approved by the California Polytechnic State University Institutional Animal Care and Use Committee (protocol #1625).

Pythons were placed into three groups ( $n=7$  each) in a stratified random manner, and mass within each group was not significantly different ( $P=0.77$ ). With a sample size of 7 per group, there is approximately 80% power to detect a 2.2 multiplicative change in overall cell density across treatments. Following the 30 day fast, one group was fed a large meal (live mice, 20% of body mass) and then 2 days (D2) later the snakes were euthanized and brain tissue was collected. A second treatment group was similarly fed 6 days (D6) before tissue collection and a control group (C) was fasted for the duration of the experiment before tissue collection. Brains from all the groups were collected on the same day. We chose these time points to examine cell proliferation during and after active digestion (D2 and D6, respectively). In previous studies on pythons that were fed similar-sized meals, the oxygen consumption, organ mass (intestines, stomach, liver, pancreas, heart, lungs, kidneys), digestive enzymes, regulatory peptides, cardiac output and heart rate were found to all substantially increase at 2 days post-feeding, but decreased by 6 days post-feeding (Cox and Secor, 2008; Lignot et al., 2005; Secor et al., 1994, 2001, 2002; Secor and Diamond, 1995; Starck and Beese, 2001; Wang et al., 2002). The day before brain tissue collection (see below), all pythons were given an intraperitoneal injection of 100 mg kg<sup>-1</sup> of 5-bromo-2'-

deoxyuridine (BrdU; item BP25085; Fisher BioReagents, Fair Lawn, NJ, USA) diluted to a concentration of 20 mg ml<sup>-1</sup> in reptile Ringer's solution to label proliferating cells (Holding et al., 2012).

### Tissue preparation

Twenty-four hours after BrdU injection, snakes were injected with ketamine (50 mg kg<sup>-1</sup>) then deeply anesthetized by isoflurane inhalation. After each snake was non-responsive to inversion or a tail pinch, they were transcardially perfused with a wash solution [0.9% NaCl, 0.1% NaNO<sub>2</sub>, 0.1 mol l<sup>-1</sup> phosphate buffer (PB)] followed by 4% paraformaldehyde in 0.1 mol l<sup>-1</sup> PB. Brains were then extracted and immersed in 4% paraformaldehyde for 24 h, followed by another 24 h in 0.1 mol l<sup>-1</sup> PB solution. Each brain was then embedded in gelatin overnight to form blocks around the brain tissue, then placed again in 4% paraformaldehyde overnight. Gelatin-embedded brains were placed in a 30% sucrose solution in 0.1 mol l<sup>-1</sup> PB and kept at 4°C until each block sank. These blocks were frozen in dry ice and stored at -80°C until sectioning. Brains were cut on a cryostat (Bright OTF-5000) into 8 series of 36 µm-thick coronal sections. The first series was directly mounted onto slides (Fisherbrand Double Frosted Microscope Slides treated with Vectabond, Vector Laboratories, Burlingame, CA, USA), hydrated with mounting solution to flatten the sections on the slides, and allowed to dry overnight. These sections were then stained with Cresyl Violet after drying. All other sections were stored in cryoprotectant (20% glycerin in 0.1 mol l<sup>-1</sup> PB) at -20°C until immunohistochemical processing.

### BrdU immunohistochemistry

New cell proliferation was examined using immunohistochemistry for BrdU as in Holding et al. (2012). Every eighth section through the olfactory bulbs and telencephalon (adjacent sections from the Cresyl Violet-stained sections) were used. Free-floating sections were washed with phosphate-buffered saline (PBS) three times for 5 min each, followed by incubation in 4 mol l<sup>-1</sup> HCl for 15 min to initiate DNA denaturation. Following this, all sections were rinsed once for 5 min in PBS before being added to a 3.8% sodium borate wash for 10 min. Sections were rinsed in PBS three times for 10 min each, then immediately placed in a blocking solution composed of 5% normal horse serum (S-2000, Vector Laboratories) and 0.5% hydrogen peroxide in PBS with 0.3% Triton-X 100 (PBST) for 60 min on a rotating shaker table. Immediately following blocking, sections were incubated in primary antibody (mouse anti-BrdU, clone: Bu20a, cat. no.: M0744, Dako Laboratories, Santa Clara, CA, USA) at a dilution of 1:1000 in PBST for 24 h on a rotating shaker table. After this period, sections were rinsed in PBST three times for 5 min each and then incubated in 1:100 secondary antibody (biotinylated horse anti-mouse antibody, cat. no.: BA-2000, Vector Laboratories) for 60 min on a rotating shaker table. Sections were rinsed in PBST three times for 5 min each before being incubated in avidin–biotin–peroxidase solution (Elite ABC kit, cat. no.: PK6100, Vector Laboratories) for 1 h on a rotating shaker table. Sections were then washed in PBST three times for 5 min each, and then were immersed in a chromogen and hydrogen peroxide solution in PBS (cat. no.: SK-4700, Vector Laboratories) for 4 min to visualize primary antibody binding. Following this staining, all sections were washed in PBS two times for 5 min each and were mounted onto slides and coverslipped after drying for 24 h. Negative control tests were also carried out which consisted of excluding the primary antibody and the antigen retrieval step and blocking staining with excess BrdU. All negative controls had no staining.

### Measurement and cell density

The production of new brain cells in reptiles occurs primarily at the ventricular zone (VZ) along the ventricle walls in the telencephalon, and the new cells migrate away from the VZ toward different regions of the telencephalon (Alvarez-Buylla et al., 1990; Lopez-Garcia et al., 1988, 1990). Study regions in the brain were defined based on the work of Halpern (1980) and Smeets (1988) and determined by direct observation of Cresyl Violet-stained sections under a microscope (Leica DM750). All Cresyl Violet-stained sections for each python were photographed with a dissecting microscope (Leica EZ4HD) at 16× magnification. These digital images were used to determine and delineate the study regions of the brain, which included the accessory olfactory bulbs and olfactory bulbs making up the olfactory region (AOB/OB), the retrobulbar regions (RB), the cortex, the dorsal ventricular ridge and nucleus sphericus (DVR/NS) and the septal nucleus (SN) (Fig. 1). The DVR and NS were combined and analyzed together, as was done in Maine et al. (2014), because we quantified cell proliferation only, not migration, so cells born along the ventral side of the ventricle could migrate into either the DVR or NS. The lateral, medial and dorsal regions of the cortex were also grouped and analyzed together (Maine et al., 2014). We did not quantify BrdU-immunoreactive (ir) cells along the third ventricle in the diencephalon because there were few to no immunoreactive cells along the third ventricles.

All anti-BrdU-stained sections were observed under a microscope by a treatment-blind observer. Cell counts were carried out in the predetermined regions along the ventricle at the ependymal surface of both the right and left hemispheres of each section per brain to obtain a total cell count in each predetermined region. A cell was considered ependymal if it was within 50 μm of a ventricle (Almli and Wilczynski, 2009; Maine et al., 2014; Fig. 1). As expected, labeled cells in the parenchyma were rare because we injected BrdU 1 day before tissue preparation and proliferating cells (born along the ventricles) would not have had time to migrate into the parenchyma (Lopez-Garcia et al., 1988, 1990). Total cell counts were calculated for each region as well as for each brain per individual. All anti-BrdU-stained sections were photographed using a dissecting microscope (Leica EZ4HD) at 16× magnification and saved as digital images. The length of the ventricle in millimeters in each region was measured using ImageJ. To obtain cell density (cells mm<sup>-1</sup>), we identified the number of BrdU-positive cells found in each region along the length of the ventricle in that region. Overall cell density per brain was calculated using the same method,

by summing all of the cell counts for all brain regions and the entire length of the ventricles from all brain regions.

### Data analysis

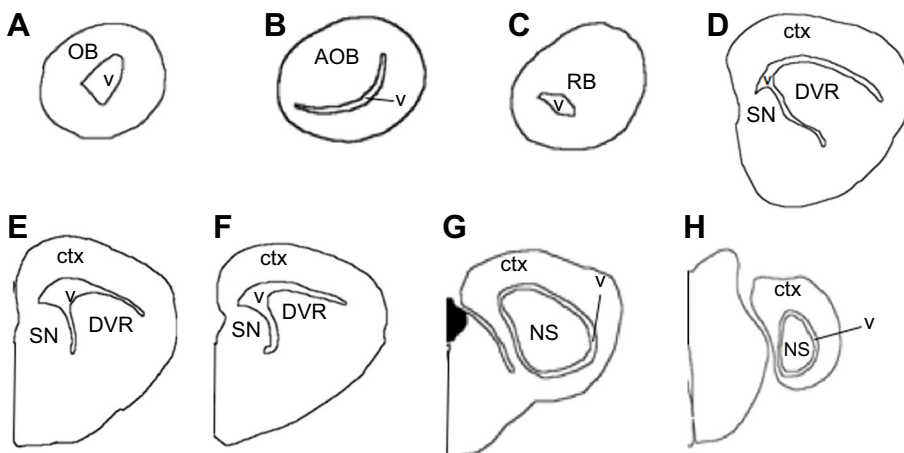
All data were analyzed using JMP Pro software (SAS Institute Inc., version 12.2.0). Cell densities were log-transformed to meet the normality assumption. An ANOVA was performed to examine overall, brain-wide total new cell density by treatment group with Tukey–Kramer HSD tests to determine which treatment groups were different. We used a one-way multivariate analysis of variance (MANOVA) to determine whether there were treatment effects on new cell densities in all brain regions simultaneously. When the MANOVA yielded a significant main effect, we performed separate univariate analysis of variance (ANOVA) and Tukey–Kramer HSD to separately compare treatment effects on new cell densities in each predetermined study region. We also compared regional brain density profiles across the treatment groups using profile analysis. Non-parallel profiles would indicate that the treatment effect on new cell density differs by brain region. When the MANOVA indicated a lack of parallelism in the profiles, we examined pairwise profile segments across treatments to identify which specific pairs of brain regions responded differently to treatments.

One python brain in group D2 showed inconclusive BrdU staining and was excluded from all analyses, leaving this group with a sample size of 6. There was also one python from the control group that had a portion of the olfactory bulb damaged during sectioning, causing several tissue sections to be unusable for further staining. Therefore, we did not include the olfactory bulb cell density of that snake in our analysis.

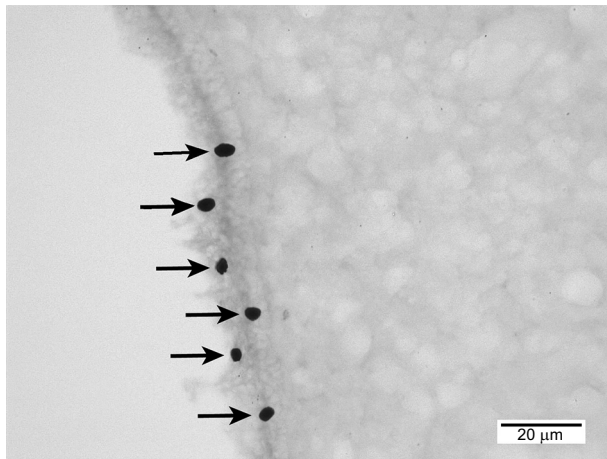
## RESULTS AND DISCUSSION

### Treatment effects on new cell density

BrdU-ir cells were found along the VZ, as expected, in every brain region of interest through the telencephalon (Fig. 2). Treatment had a significant effect on brain-wide total new cell density ( $F_{2,17}=37.06$ ,  $P<0.0001$ ; Fig. 3A). Total new cell density in group D6 was significantly higher than that in both groups C and D2 ( $P<0.0001$ ), and there was no significant difference between groups C and D2 ( $P=0.942$ ). Pythons exhibit postprandial systemic plasticity and metabolic upregulation immediately after ingesting a meal, with increases in digestive and cardiovascular organ mass and function (Ott and Secor, 2007; Secor et al., 2000). However, in our study, the D2 group, which corresponds to the time at which digestive and cardiovascular organs show extreme hyperplasia and/



**Fig. 1. Schematic drawings showing coronal hemi-sections from the rostral portion of the brain through the telencephalon of *Python regius*.** Sections A–H depict the subdivisions used for cell counts, from rostral to caudal through the brain. BrdU-labeled nuclei were found and counted along the ventricles, labeled 'v', within each section above. AOB, accessory olfactory bulb; ctx, cortex; DVR/NS, dorsal ventricular ridge/nucleus sphericus; OB, olfactory bulb; RB, retrobulbar; SB, septal nucleus. Sections A–H were outlined from stained images in GNU Image Manipulation Program (GIMP; free software, version 2.8.18).

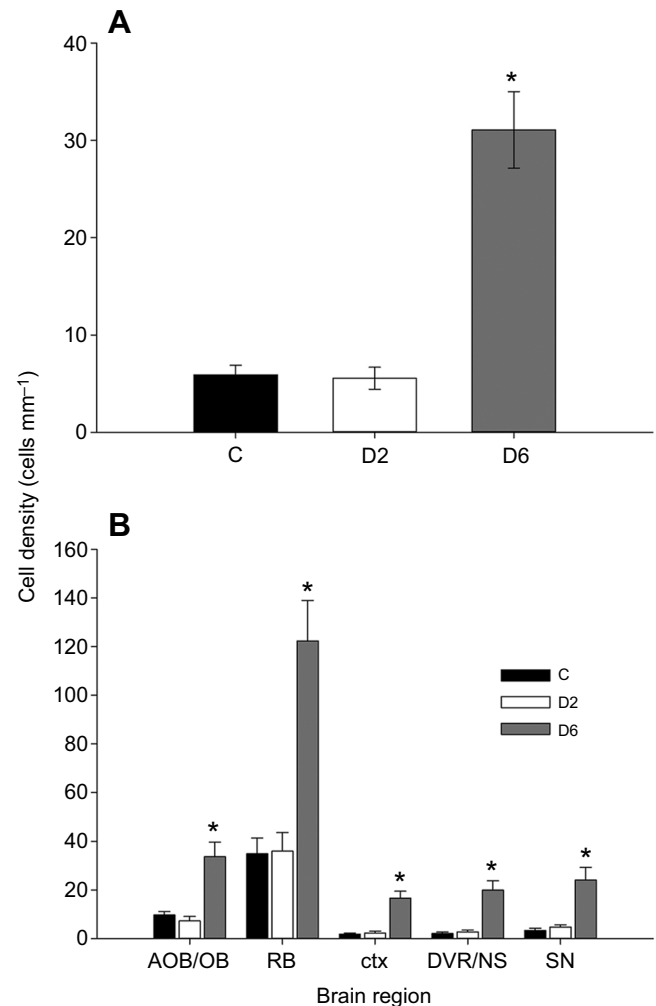


**Fig. 2. Image of BrdU-stained nuclei in a brain tissue section of *Python regius*.** The arrows indicate the BrdU-stained nuclei along the ventricle. Scale bar: 20  $\mu\text{m}$ . Cells were considered to be within the ventricular zone if they were within 50  $\mu\text{m}$  of the ventricle.

or hypertrophy, did not show increased cell proliferative activity compared with the fasted controls. In contrast, a massive increase in cell proliferation was observed in the D6 group, which was measured at the time when most of the digestion and absorption should be complete.

The difference in the time course of the response of GI and cardiovascular organs compared with that of the brain may be related to the functional roles of these organs during feeding. During a python's SDA response, when significant postprandial metabolic regulation is necessary, brain cell proliferation is low or similar to baseline levels. It is possible that brain cell proliferation may not increase at this point because most of the python's energy is focused toward the immediate need: the energetically expensive process of digesting and absorbing a large meal, in which both body reserves and nutrients from the ingested meal are used to 'pay' for the metabolic costs of feeding (Secor and Diamond, 1995; Starck et al., 2004). Visceral organs of pythons respond to feeding by upregulating many genes related to metabolic processes, cell growth and proliferation, and protective responses to oxidative stress (Andrew et al., 2015, 2017; Duan et al., 2017), in keeping with the fact that proliferation of cells in these organs occurs during the SDA window. Proliferative activity in the brain does occur during this time period, but it is not extensive until 6 days after feeding. This likely reflects the fact that new brain cells are not needed for the immediate processes of digestion and absorption, but after these processes are complete, energy can be used for growth or maintenance of other systems, including the brain.

In terms of mechanism, any number of neuronal, hormonal or energetic mediators may stimulate cell proliferation in the python brain. Stimulatory mechanisms have been identified for several python tissues, including the presence of nutrients causing enterocyte proliferation and growth (Helmstetter et al., 2009) and specific fatty acids stimulating cardiac myocyte hypertrophy (Riquelme et al., 2011). Multiple hormonal systems regulate responses to feeding and fasting (Secor and Carey, 2016). Upregulation of hormones involved in growth regulation, such as insulin-like growth factor 1 (IGF-1), may play a role in stimulating postprandial neural cell proliferation, as it does in mammals (Mairet-Coello et al., 2009; Otaegi et al., 2006; Vogel, 2013; Yuan et al., 2015). Recently, in mice, hypothalamic proopiomelanocortin neurons, which decrease in activity during fasting, were shown to



**Fig. 3. Effects of treatment on new cell density.** (A) Mean ( $\pm$ s.e.m.) overall density of BrdU-immunoreactive (ir) cells in the telencephalon in snakes that were fasted (C, control;  $n=7$ ) or were 2 days (D2;  $n=6$ ) or 6 days (D6;  $n=7$ ) post-feeding. Snakes in the D6 group had significantly more BrdU-ir cells than those in the D2 or C group ( $*P<0.0001$ ). (B) Mean ( $\pm$ s.e.m.) density of BrdU-ir cells in all five brain regions for each treatment group. In each region, snakes in the D6 group had significantly more BrdU-ir cells than those in the D2 or C group ( $*P<0.001$ ). AOB/OB, accessory olfactory bulb/olfactory bulb; RB, retrobulbar region; ctx, cortex; DVR/NS, dorsal ventricular ridge/nucleus sphericus; SN, septal nuclei.

directly innervate specific neurogenic regions of the sub-ventricular zone and increase proliferation after feeding (Paul et al., 2017). Also in rodents, the peripheral hormone ghrelin, which typically is increased during the post-absorptive state, increases neural cell proliferation and new neuron survival (Moon et al., 2009; Zhao et al., 2014). More research is needed to better understand the signals that may be up-regulated after feeding or during fasting and their possible roles in regulating neural cell proliferation and neurogenesis, especially in reptiles.

We used a one-way MANOVA to investigate differential treatment effects among brain regions, which indicated that treatment had a significant effect on cell proliferation ( $F_{10,24}=4.43$ ,  $P=0.0013$ ). Individual one-way ANOVA for each brain region demonstrated that treatment had a significant effect on the mean new cell density (AOB/OB:  $F_{2,16}=15.38$ ,  $P=0.0002$ ; RB:  $F_{2,17}=11.09$ ,  $P=0.0008$ ; cortex:  $F_{2,17}=15.95$ ,  $P=0.0001$ ; DVR/NS:  $F_{2,17}=24.28$ ,  $P<0.0001$ ; SN:  $F_{2,17}=17.90$ ,  $P<0.0001$ ; Fig. 3B). In

every brain region analyzed, the mean new cell density in the D6 group was significantly higher than that in both the D2 and C groups ( $P \leq 0.0013$ ), and there was no significant difference between the D2 and C groups ( $P \geq 0.187$ ). Profile analysis demonstrated non-parallelism in the profiles across treatments ( $F_{8,26} = 4.43$ ;  $P = 0.043$ ), indicating differential treatment effects in the brain regions studied. However, the *post hoc* profile comparisons on the differential effect of feeding among brain regions were not significant after applying a Bonferroni correction ( $\alpha = 0.005$ ,  $P \geq 0.019$ ). Together, our results indicate that there may be a trend for some regions (cortex, DVR/NS, SN) to be affected more by feeding than others (AOB/OB, RB; Fig. 3B). For example, the cortex region in the D6 group had 8.3 times more BrdU-ir cells than that in the control group, but in the AOB/OB region, the D6 group had only 2.5 times more BrdU-ir cells than the control group. The functional significance of this is unclear but could be related to differences in brain regions that are involved with regulating feeding behaviors. Differential regulation of neural cell proliferation can occur in mice, where fasting decreased proliferation of distinct pools of neural stem cells in the sub-ventricular zone (Paul et al., 2017).

### New cell density by region

New cell proliferation has been observed in all major subdivisions of the reptile telencephalon, with a substantially higher amount of neurogenesis documented in the main and accessory olfactory bulbs than in the rest of the brain (Font et al., 2001). Our results corroborate this pattern, with these regions having the highest new cell density in each treatment group. The retrobulbar region, which has also been called the rostral forebrain, or anterior olfactory nucleus in lizards, is directly linked to the olfactory bulb (Halpern, 1980). This area shows high rates of neurogenesis in lizards (Delgado-González et al., 2008; Shao et al., 2012) and snakes (Bales, 2014), and is one of the most significant regions for neurogenesis in reptiles studied so far (Font et al., 2001). New cells may proliferate to migrate toward the olfactory bulbs for future processing, a mechanism resembling the rostral migratory stream (RMS) in mammals (Font et al., 2001; Lois and Alvarez-Buylla, 1994; Pérez-Cañellas and García-Verdugo, 1996). However, while the RMS has been studied in mammals, its function is poorly documented in reptiles, and there are no cases in which food consumption or digestion has been considered in relation to it.

The SN, cortex and DVR/NS displayed the lowest new cell densities of the five brain regions investigated here. In contrast, Pérez-Cañellas and García-Verdugo (1996) found the highest rates of cell proliferation in the Moorish gecko (*Tarentola mauritanica*) occurred in the medial cortex, and Perez-Sanchez et al. (1989) found that the NS had the highest proliferative activity in the Iberian wall lizard (*Podarcis hispanica*). The olfactory bulbs were also quantified in these studies and were the next highest region of cell proliferation. Maine et al. (2014) found that the highest proliferative activity in *T. sirtalis parietalis* occurred in the SN, followed by the cortex, with the DVR and NS regions having the lowest proliferation (they did not quantify proliferation in the olfactory and retrobulbar regions).

The lack of a common pattern of cell proliferation throughout the brain among reptiles could be the result of several factors related to differences in life-history traits among different species or to differences in physiological state (i.e. fasted/fed, breeding/non-breeding, time of year). Even in mammals, there is evidence that regional differences in neurogenic capabilities may be species dependent (Bonfanti and Peretto, 2011). Comparative studies that examine reptile species with different ecological and physiological

characteristics may be important to give insight into the function and evolution of brain cell proliferation.

### Future directions and conclusions

This study demonstrates a stark increase in python brain cell proliferation over time after feeding, indicating that food consumption stimulates brain cell proliferation in pythons after digestion is complete. Given this, other studies on neurogenesis in reptiles should consider how food consumption may influence their results, especially if the animals are infrequent feeders. Future studies could continue to characterize the postprandial response of the brain by adding additional time points after food consumption to better refine our understanding of the time course of this effect on cell proliferation. Also, this study only quantified cell proliferation, and not migration and survival of the newly born cells; it would be beneficial to determine how feeding affects the course of the newly born brain cells and their differentiation into neurons. It would also be desirable to identify the cell type of the newly born cells as they migrate away from the ventricles. In lizards, most of the neural progenitor cells give rise to neurons, but that pattern differs in turtles, where there is more gliogenesis in addition to neurogenesis (González-Granero et al., 2011). The identification of newly born cells as neurons in snakes is currently challenging because antibodies against neuronal cell markers do not universally work in snakes (C.R.S., personal observation). Further research on species with different foraging modes would also help us understand neuroplasticity in an evolutionary context and provide comparative data regarding how energy balance and metabolism may affect neurogenesis.

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### Competing interests

The authors declare no competing or financial interests.

### Author contributions

Conceptualization: S.S.H., E.N.T., C.R.S.; Methodology: S.S.H., E.N.T., C.R.S.; Validation: S.S.H., C.R.S.; Formal analysis: S.S.H., A.A.S.; Investigation: S.S.H.; Resources: S.S.H., C.R.S.; Data curation: S.S.H., C.R.S.; Writing – original draft: S.S.H., A.A.S., E.N.T., C.R.S.; Writing – review and editing: S.S.H., A.A.S., E.N.T., C.R.S.; Visualization: S.S.H., C.R.S.; Supervision: A.A.S., C.R.S.; Project administration: S.S.H., C.R.S.; Funding acquisition: S.S.H.

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