



Commentary

Do food-caching chickadees grow their hippocampus every autumn when they need to cache food and then shrink it for the rest of the year?



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ARTICLE INFO

Article history:

Received 21 March 2022
 Initial acceptance 19 April 2022
 Final acceptance 11 May 2022
 Available online 5 July 2022
 MS. number: AS-22-00150R

Keywords:

brain
 food caching
 food-caching bird
 hippocampus
 seasonal variation
 spatial cognition

Understanding brain plasticity as it relates to environmental variation is one of the big issues in behavioural neuroscience and neuroecology. It is common, especially in popular literature to report that food-caching birds such as chickadees expand their hippocampus volume, which is involved in spatial learning and memory, by adding more neurons during the time when they are intensely caching food (autumn) and then reduce the hippocampus size and the number of hippocampal neurons during the rest of the year when they either do not cache food (summer) or cache much less (winter, spring). If true, this would be a highly adaptive mechanism allowing food-caching birds to change their memory capacity associated with seasonal variation in memories of food caches.

It is unfortunate that this claim is so persistent when the hard evidence is not that simple – while there are some data showing that food-caching black-capped chickadees, *Poecile atricapillus*, and willow tits, *Poecile montanus*, sampled during the month of peak caching indeed had larger hippocampus and more hippocampal neurons compared to other individuals sacrificed during other periods (Lange et al., 2022; Smulders, Casto et al., 2000; Smulders et al., 1995; Smulders, Shiflett et al., 2000), there is a large body

of work suggesting that these data may either be associated with potential sampling bias rather than with the actual biological processes or, alternatively, the seasonal patterns shown in these studies may be unrelated to variation in food caching but rather related to something else (reviewed in Croston et al., 2015; Pravosudov & Roth, 2013; Pravosudov et al., 2015). Comparison with noncaching great tits, *Parus major*, in Lange et al. (2022) also suggests either a potential sampling bias combined with random sampling errors due to small sample size or a seasonal pattern unrelated to food caching as great tits sampled at different seasons showed mostly unexplained, but significant, variation both in hippocampal volume and in the total number of hippocampal neurons, including a peak in autumn. The key detail here is that great tits do not cache food, and therefore, the somewhat similar seasonal patterns in both food-caching willow tits and noncaching great tits cannot be explained by seasonal variation in food caching. Such similarities between these two species suggests either a sampling bias or a yet unexplainable seasonal pattern unrelated to food caching in both species.

There are extensive data that do not support the claim of seasonal variation in hippocampus volume being associated with variation in food caching, especially when it comes to the total number of hippocampal neurons. First, there were studies including several attempts to replicate results from Smulders, Casto

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et al. (2000), Smulders et al. (1995) and Smulders, Shiflett et al. (2000) using the same focal species, the black-capped chickadee, which all failed to detect seasonal differences in hippocampal morphology associated with food caching (Barnea & Nottebohm, 1994; Hoshoooley et al., 2005; Hoshoooley & Sherry, 2004, 2007). These studies used slightly different methods and, in particular, had birds in captivity for a short period before the sacrifice. Lange et al. (2022) argued that a captive environment can result in reduction in hippocampal volume, which is well supported by available data (LaDage et al., 2009; Phillmore et al., 2022; Roth et al., 2012; Smulders, Casto et al., 2000; Tarr et al., 2009), and therefore, these studies could not have possibly detected seasonal differences even if they existed in wild birds. What Lange et al. (2022) do not discuss is that captivity has no detectable effects on the number of hippocampal neurons in birds brought into captivity, even for relatively long periods of time (Freas et al., 2013; LaDage et al., 2009; Roth et al., 2012). These findings argue against the conclusions of Lange et al. (2022) and suggest that the studies using a brief captivity period before sampling should have detected significant seasonal variation in the number of neurons if it were present – yet they detected no such variation (Barnea & Nottebohm, 1994; Hoshoooley et al., 2005; Hoshoooley & Sherry, 2004, 2007).

The strongest evidence against the claim of seasonal variation in the number of hippocampal neurons comes from our own common garden study using food-caching black-capped chickadees in which we collected nestlings from the wild and hand-reared and maintained them for a year in uniform laboratory conditions (Roth et al., 2012). The main goal of that experiment was to establish population level differences in hippocampal volume and the number of hippocampal neurons in wild chickadees from different environments. This study found that differences between populations in both hippocampal volume and number of neurons across sampling locations persisted in the laboratory-reared birds despite the same environment and experiences (Roth et al., 2012). However, the strongest evidence against seasonal variation in the number of hippocampal neurons comes from comparing the hippocampal morphology of birds that have spent their entire lives in captivity, which by nature are extremely experience-poor environments, to that of wild birds collected at the peak of food caching (September) (Roth et al., 2012). These wild birds unquestionably had vastly different experiences associated with food caching and were sacrificed right after capture, similar to the birds in Lange et al.'s (2022) study. If the hypothesis of seasonal variation in hippocampus size due to changes in the number of neurons associated with variation in food-caching intensity is correct, these birds should have had the largest hippocampal volume and the largest number of neurons at that time. Strikingly, there were no significant differences in the total number of hippocampal neurons between wild birds collected at the peak of seasonal food caching and birds that had never seen their natural environment and cached orders of magnitude less in captive conditions (sampled in February; Roth et al., 2012). On the other hand, hippocampal volume in hand-reared birds was indeed smaller compared to the wild-sampled birds, supporting the idea that a captive environment results in hippocampal volume reduction, but no changes in the number of hippocampal neurons. These data were based on a much larger sample size than those in other studies (24 wild birds and 24 hand-reared birds). So how is it biologically possible that variation in the number of caches under natural conditions (which are enriched and diverse compared to any captive environment) results in significant changes in both the hippocampus volume and the total number of neurons, but chickadees that spent their entire life in experience-poor captivity had the same number of hippocampus neurons as wild birds during the peak of food caching?

Additional data on food-caching mountain chickadees, *Poecile gambeli*, also strongly contradicts the idea that variation in food-caching experiences can result in differences in the number of hippocampal neurons – birds that were sampled directly in the wild at the peak of autumn food caching had similar numbers of neurons as chickadees collected at the same locations but which had spent 5 months in captivity and were sampled in spring (Freas et al., 2013). In my opinion, these two studies comparing birds sampled in the wild at the peak of caching to birds that spent either their entire life or a considerable amount of time in captivity prior to being sampled in spring argue that caching experience cannot affect the number of hippocampal neurons.

Our data are consistent with much of the mammalian literature suggesting that environmental conditions and experience can quickly affect hippocampal volume via changes in dendritic branching, which can occur very rapidly (Roth et al., 2012, 2017). At the same time, there is not much evidence that animals can quickly add (rather than replace – e.g. Barnea & Nottebohm, 1994) many neurons to the existing hippocampal circuits when conditions change. Previous work on hippocampal neurogenesis showed that it takes 6 weeks or longer from the time the neuron progenitor is born to the time it becomes a functional neuron incorporated in the existing neural circuits (in chickadees, only 32% of cells labelled with cell division marker BrdU expressed a mature neuron phenotype (NeuN expression) following 6 weeks after cell division; Hoshoooley & Sherry, 2004). Barnea and Nottebohm (1994) also reported that a peak in the number of surviving hippocampal neurons measured 6 weeks after marking these cells with BrdU in October in black-capped chickadees, which means that the peak was actually detected in November when some but not all of these neurons became functional (Hoshoooley & Sherry, 2004), which is far past the food-caching peak (Barnea & Pravosudov, 2011; Pravosudov, 2006). Barnea and Nottebohm (1994) specifically discussed neuronal replacement via neurogenesis rather than increasing the number of neurons.

So, if willow tits had significantly more neurons in September, they had to start producing these neuron progenitors in late July – early August, and they should be detectable in the count of neurons in August. Yet Lange et al. (2022) reported much fewer neurons in August. In contrast, Hoshoooley and Sherry (2004, 2007) detected no significant seasonal variation in hippocampal neuron recruitment rates in both food-caching black-capped chickadees and non-caching house sparrows, *Passer domesticus*, while Hoshoooley et al. (2005) detected the highest neuronal recruitment rates in January – which is not consistent with the idea that the highest neuron recruitment rates should be associated with a peak in food caching. It is possible that these results were affected by captivity – as captivity in wild-caught birds was reported to reduce the population of new hippocampal neurons (LaDage et al., 2010; Phillmore et al., 2022; but see Tarr et al., 2009, for showing no effect of captivity). However, even under a captive environment, neurogenesis rates may reflect underlying differences. For example, the differences between food-caching chickadees and noncaching house sparrows were still present despite potential captive effects, and differences in memory use and in the quality of captive environments were also shown to affect adult neurogenesis rates in captivity (LaDage et al., 2010; Phillmore et al., 2022).

An important question is whether captivity affects hippocampal neurogenesis directly via stress caused by captivity, as most studies bring wild-caught birds into captivity, or whether such captivity effects are indeed mainly driven by reduction in memory and space use. At least one study suggests that it is the former and not the latter as black-capped chickadees reared from the nests and maintained in a captive environment for the rest of their lives had similar adult hippocampal neurogenesis rates as their counterparts

sampled directly in the wild during the peak of autumn food caching (Roth et al., 2012). Hand-reared birds were unlikely to perceive captive environments as stressful compared to wild birds brought into captivity. Similar to the total number of hippocampal neurons, neurogenesis rates measured by DCX labelling were similar between wild birds sampled at the peak of food caching and hand-reared birds sampled in spring. Clearly these data are also not consistent with the idea that hippocampal neurogenesis rates vary based on seasonal differences in food-caching intensity.

Overall, much of the existing data are inconsistent with the seasonal variation hypothesis associated specifically with seasonal variation in food caching, especially regarding the number of neurons. It is more plausible that hippocampus volume can change rather rapidly via dendritic connections in response to variation in environmental conditions (see Roth et al., 2017), but the overall evidence argues against the idea that seasonal variation in food-caching experiences can affect the total number of hippocampal neurons.

So why have some previous studies reported seasonal variation in hippocampus volume and neuron numbers? Each of these studies has relied on sampling small numbers of different birds at different seasons instead of investigating hippocampal changes within the same individuals (Lange et al., 2022; Smulders, Casto et al., 2000; Smulders et al., 1995; Smulders, Shiflett et al., 2000), meaning that sampling biases cannot be excluded. There is large individual variation in caching rates, and caching rates appear significantly repeatable across time in chickadees (Sonnenberg et al., 2022), so small sample sizes may suffer from sampling biases associated with nonrandom sampling due to methods commonly used to collect wild birds. Ideally, collecting methods should allow random sampling of a representative population at any given season, but this is hard to achieve with wild unmarked birds. For example, Smulders et al. (1995) trapped chickadees using feeders during the winter and using playback during the summer while Lange et al. (2022) used playback and decoys in all seasons. These methods can be potentially problematic because not all individuals are equally attracted to feeders or playbacks and different individuals may be more attracted to feeders or playback during different seasons. Moreover, an individual's condition, which may change throughout the winter, also likely affects how a bird visits feeders or responds to playback. As a result, it is possible and likely that some phenotypes may be either under-represented or over-represented during some sampling periods, resulting in nonrandom biased sampling across different seasons. Such biases can be potentially reduced by larger sample sizes but would likely be exacerbated by small sample sizes when sampling involves just a few individuals that came to the feeders/playbacks first.

While measuring changes in the number of neurons in the same individuals may not be possible, changes in brain (i.e. hippocampal) volume may be recorded using MRI methods in live birds. For example, volumes of some avian vocal control brain regions vary seasonally – such seasonal variation was first documented by sampling different individuals at different seasons, but later was confirmed within the same individuals using MRI (Van der Linden et al., 2004, 2009). Of course, although it is possible that the differences among individuals sampled at various time periods in the aforementioned studies may be real, larger sample sizes are needed to provide convincing evidence. This evidence is critical, as other data do not support the claim of seasonal variation in hippocampus volume being associated with variation in food caching, especially when it comes to the total number of hippocampal neurons.

So, what is next? It seems that more work is needed – either using much larger sample sizes or by sampling live birds using MRI. Lange et al. (2022) argued that all laboratory work is insufficient to

detect seasonal changes because even the best laboratory conditions are a far cry from a natural environment. However, this argument is not supported by evidence showing that even long-term captive environments, while affecting hippocampal volume, do not affect the number of neurons (Freas et al., 2013; LaDage et al., 2009; Roth et al., 2012). In the meantime, we should be careful with the claim that food-caching birds can expand their hippocampus by adding more neurons when they cache the most – there is just too much contradictory evidence.

Author Contribution Statement

I am a single author on this manuscript and I wrote it.

Acknowledgments

I thank Ben Sonnenberg, Carrie Branch and Joe Welklin for constructive and critical comments on this commentary. I was funded by the U.S. National Science Foundation (Grants IOS1856181 and IOS2119824). Two anonymous referees provided constructive comments that significantly improved the paper.

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