Causes and consequences of seasonal changes in the braincase and brain size of the common shrew *Sorex araneus*

Doctoral thesis for obtaining the

academic degree Doctor of

Natural Sciences

submitted by

Lázaro Tapia, Javier

at the

Universität
Konstanz

Faculty of Sciences

Department of Biology

Konstanz, 2017

Konstanzer Online-Publikations-System (KOPS)
URL: http://nbn-resolving.de/urn:nbn:de:bsz:352-2-1c23h6q3ifzjd2

Date of the oral examination: 15th March 2018

1. Reviewer: Prof. Dr. Manfred Gahr

2. Reviewer: Prof. Dr. Martin Wikelski

Contents

Summary

Non-migratory animals living in harsh seasonal environments have developed various coping strategies involving seasonal changes in anatomy, physiology and behavior. One of the most outstanding but poorly investigated cases of anatomical seasonal change is the process undergone by several species of red-toothed shrews (subfamily Soricinae), known as Dehnel's Phenomenon. Extreme reversible seasonal phenotypic variation of skull size, spine length and body mass was first described in the common shrew (*Sorex araneus).* Offspring are born in late spring/early summer and quickly reach a first size peak at dispersal. Then braincase height – the most frequently used proxy for skull size in the research of this phenomenon – decreases in autumn, reaching a 17–26% smaller minimum in winter. In spring, coinciding with sexual maturation, braincase height increases again by 12–18%, reaching a second peak in the shrews' second summer. The few surviving post-reproductive individuals then begin a second decline, but die before their second winter. Most organs also decrease in mass toward winter and regrow in early spring. This includes the brain with a 10-26% winter mass decline and 9-16% spring regrowth. These size reductions are hypothesized to reduce energy requirements during winter, with regrowth in spring allowing full exploitation of the only reproductive period in the shrews' life.

In my thesis I attempted to answer a series of open questions on Dehnel's Phenomenon. I studied a population of *S. araneus* in South Germany. I explored different aspects of the seasonal differences in both skull and brain which involve detailed descriptions of the overall and structural anatomical changes at different levels as well as the consequences of the phenomenon in behavior.

First, I wanted to monitor the changes in braincase size at the individual level. To date this shrinkage and regrowth of skull was only described with *post mortem*

measurements of extracted bones and organs, i.e. one measurement per animal. Most authors agree that these data reflect changes within an individual despite collection at population level and are not a result of seasonal selection against large individuals. To exclude the possibility of Dehnel's Phenomenon being enhanced or even caused by seasonal size biased selection, repeated measures at an individual level were needed. Using time series of x-ray images I showed for first time that in anticipation of winter individual free-ranging shrews shrink their braincases by an average -15.3% (individual maximum -20.1%). Thereafter, during winter and spring, shrews then re-grow their braincases by +9.3% (individual maximum +13.2 %). My results confirm that this variation is thus not caused or enhanced by seasonal size dependent mortality, but reflects profound individual changes in bone anatomy.

Second, I attempted to investigate the seasonal changes in brain structure at the regional and cellular levels. I described in detail the volumetric differences between seasons and sexes in particular brain regions, revealing that different regions varied in the magnitude of change. For some regions males and females showed different patterns. Also, I attempted to study the changes in neuron morphology. I observed a general decrease in soma size and total dendrite volume in the caudoputamen and anterior cingulate cortex. This neuronal retraction can partially explain the overall tissue shrinkage in winter which is not sufficient to explain the entire seasonal process, but this represents a first step to understand the underlying drastic transformations in brain tissue.

Last, I assessed the changes on skull and brain size at the population level from postmortem samples. Also I wanted to investigate the possible cognitive consequences of the seasonal brain size change. The average decrease in braincase height from July juveniles to February subadults was -12% and the regrowth to adults (June -August) was +13%. I reported a 21% loss of brain mass in winter and 17% re-increase in spring. Additionally, I observed that these changes in turn correlated to the animals' cognitive performance. Search paths of smaller-brained winter individuals in a spatial learning task are longer compared to those of large brained summer juveniles, and spring adults, whose brains have regrown. This implies a trade-off between energetic limitations and cognitive advantages as an adaptation to seasonally fluctuating resources.

In conclusion, I attempted to enlighten some of the factors that cause the seasonal changes in the skull and the brain size of the common shrew, as well as its implications in behavior within an ecological-evolutionary framework. This study system can help us to better understand the complex processes that shape the development and evolution of the mammalian skull and brain. But the study of this phenomenon also has great implications in medical research, particularly in investigations related to degenerative processes of bone and brain tissues.

Zusammenfassung

Sesshafte Tiere haben verschiedene Strategien entwickelt um mit extremen Umweltbedingungen umgehen zu können. Dazu gehören unter anderem saisonale Veränderungen der Anatomie, Physiologie sowie im Verhalten. Einer der außergewöhnlichsten, aber wenig erforschten Fälle von saisonalen, anatomischen Veränderungen ist das Dehnel's Phänomen, das bei verschiedenen Arten von Rotzahnspitzmäusen (Unterfamilie *Soricinae*) beobachtet werden kann. Es umfasst extreme reversible saisonale phänotypische Veränderungen des Schädels, der Länger der Wirbelsäule und des Körpergewichtes und wurde erstmals bei der Waldspitzmaus (*Sorex araneus*) beschrieben. Die Jungen kommen Ende Frühjahr/Anfang Sommer zur Welt und erreichen schnell ihr Größenmaximum, bevor sie aus dem Nest flüchten. Die Schädelhöhe – das in der Untersuchung dieses Phänomens am gängigsten genutzte Maß für Schädelgröße – nimmt vom Herbst zum Winter um 17-26% ab. Im Frühjahr, wenn auch die Geschlechtsreife einsetzt, nimmt die Schädelhöhe wieder 12-18% zu und erreicht im zweiten Sommer einen zweiten Höhepunkt. Nachdem sich die Waldspitzmaus fortgepflanzt hat beginnt bei den wenigen überlebenden Tieren eine zweite Abnahme. Diese sterben in der Regel aber vor dem zweiten Winter. Die Mehrheit der Organe schrumpft von Herbst zu Winter und wird im Frühjahr wieder größer. Dazu gehört auch das Gehirn, das im Winter 10-26% an Masse verliert und im Frühjahr wieder 9-16% wächst. Es wird angenommen, dass diese Reduktion der Größe dazu dient im Winter Energie zu sparen. Das Wiederwachsen im Frühjahr erlaubt der Spitzmaus dann ihre ganze Energie in ihrer einzigen Reproduktionsphase zu investieren.

In meiner Dissertation versuchte ich eine Reihe von offenen Fragen zu Dehnel's Phänomen zu beantworten. Dazu untersuchte ich eine Population von Waldspitzmäusen in Süddeutschland. Ich beleuchtete verschiedene Aspekte der saisonalen Unterschiede

am Schädel sowie im Gehirn. Dies beinhaltet detaillierte Beschreibungen sowohl von der Gesamtveränderung und strukturellen und anatomischen Veränderungen auf verschiedenen Ebenen als auch die Konsequenzen des Phänomens auf das Verhalten.

Zu Beginn quantifizierte ich sowohl saisonale Größenveränderungen von Hirnregionen als auch Veränderungen auf zellulärer Ebene. In dieser Arbeit beschreibe ich detailliert, dass das Volumen verschiedener Hirnregionen saisonal und bei Männchen und Weibchen unterschiedlich stark variiert. Desweiter untersuchte ich saisonale Formveränderungen bei Neuronen. Ich fand eine kontinuierliche Abnahme des Somas und dendritischen Volumens im Caudoputamen und im anterioren Cingulum. Dieser Verkürzung von Neuronen kann teilweise zur Verringerung des Gesamtgewebes im Winter beitragen, erklärt aber nicht den gesamten saisonalen Prozess der Größenveränderung. Dieses Ergebnis ist aber ein erster Schritt, der zum Verständnis der grundlegenden Mechanismen dieser außerordentlichen Veränderung im Hirngewebe beiträgt.

Zum Schluss wollte ich die Veränderungen am Schädel an einzelnen Individuen nachverfolgen. Bisher konnte das Schrumpfen und Wachsen nur anhand von Messungen toter Tiere beschrieben werden, was bedeutet, dass es nur einen Messwert pro Tier gibt. Die meisten Autoren gehen davon aus, dass diese Daten nicht das Ergebnis saisonaler Selektion sind, sondern, dass Veränderungen in einzelnen Individuen wiedergespiegelt werden. Um die Möglichkeit auszuschließen, dass das Dehnel’s Phänomen von saisonal beeinflusster Größenselektion verstärkt oder sogar verursacht wird, müssen wiederholt dasselbe Individuum gemessen werden. Mit Hilfe von wiederholten Röntgenaufnahmen des gleichen Tieres, konnte ich zum ersten mal zeigen, dass der Schädel bei wildlebenden Spitzmäusen zu Beginn des Winters um durchschnittlich -15.3% (Maximalwert -20.1%) schrumpft. Anschließend vergrößert sich der Schädel im Frühjahr wieder um +9.3%. Diese Ergebnisse bestätigen, dass die Veränderungen nicht durch größenabhängige Mortalität beeinflusst oder verursacht werden, sondern fundierte individuelle Veränderungen der Knochenanatomie darstellen.

Als nächstes wollte ich Größenveränderungen am Schädel und Gehirn auf Populationsebene überprüfen, sowie daraus folgende mögliche kognitive Konsequenzen testen. Durchschnittlich war der Schädel von subadulten Wintertieren im Februar -12% flacher im Vergleich zu juvenilen Sommertieren im Juli. Die Zunahme der Schädelhöhe von subadulten Wintertieren zu adulten Sommertieren im Juni und August lag bei +13%. Die Hirnmasse nahm zum Winter um -21% ab und im Frühjahr wieder um +17% zu. In diesem Zuge fand ich heraus, dass die Größenveränderung im Gehirn direkt mit Veränderungen der kognitiven Fähigkeit zusammenhängt. Ein Verhaltenstest, der räumliches Lernvermögen wiederspiegelt, zeigte, dass der zurückgelegte Weg bei Tieren mit kleinerem Hirnvolumen im Winter länger ist verglichen zu juvenilen und adulten Sommertieren mit größeren Gehirnen. Das bedeutet, dass es zwischen Energieverbrauch und kognitiven Fähigkeiten als Anpassung an saisonale Veränderungen, zu einem Kompromiss kommen muss.

Zusammenfassend konnte ich einige Faktoren offenlegen, die die saisonalen Veränderungen im Schädel und Gehirn der Waldspitzmaus verursachen, sowie die Auswirkungen im Verhalten unter einem ökologisch-evolutionären Rahmen darstellen. Dieses Model kann uns helfen die komplexen Prozesse der Entwicklung und Evolution von Schädel und Gehirn in Säugetieren besser zu verstehen. Zusätzlich kann die genauere Untersuchung dieses Phänomens auch große Auswirkungen auf die medizinische Forschung haben. Speziell Forschungen an degenerativen Prozesse von Knochen und Hirngewebe könnten hiermit unterstützt werden.

General introduction

The study of the development and evolution of the brain approached from an eco-physiological standpoint can help to elucidate the underlying selective pressures, i.e., how the environment shapes the size and structure of the brain. More specifically, this perspective may also help us to better understand the link between brain anatomy and how different individuals or species cope with changes in their environment, by means of more or less plastic behavioral responses. Environmental changes across seasons provide a good framework to make predictions on possible anatomical and behavioral adaptations. Seasonal changes in brain macro- and microarchitecture have been studied in birds (Barnea and Nottebohm, 1996; Brenowitz et al., 1991; Nottebohm, 1981; Smith et al., 1997; Tramontin et al., 1998) and some mammals (Barker et al., 2003; Lavenex et al., 2000a; Popov and Bocharova, 1992; Popov et al., 1992). These kinds of studies have provided compelling insights on how environmental factors can determine brain size, brain structure and behavior.

An outstanding example of seasonal anatomical variation has been observed in some representatives of the shrew family Soricidae. In 1949-1950 the polish biologist August Dehnel described a process in different soricid species – the common shrew *Sorex araneus,* the pygmy shrew *Sorex minutus* and the water shrew *Neomys fodiens* – namely a change in the shape and dimensions of the skull, more specifically the braincase, along their life span (Dehnel, 1949a; Dehnel, 1950). The braincase capacity and external skull measurements of these animals, which are born in summer, first reach a maximum one month after they are born; then they decrease in autumn, reaching a minimum in winter. In the common shrew, in January-February the skull height is 18% smaller than in summer. In the following spring, when shrews reach sexual maturity and along the reproductive period, the braincase re-grows over 12%, not quite reaching the juvenile size. After this second summer peak the braincase undergo a second autumn decline before the end of their lifespan (Dehnel, 1949b; Dehnel, 1950). Nowadays this is known as the Dehnel phenomenon or Dehnel effect and several studies have investigated it in some more detail. The variation in braincase capacity is linked with a proportional change in brain mass (Bielak and Pucek, 1960). This process is accompanied by seasonal size changes of different organs, such as the liver, heart, kidneys and spleen (Pucek, 1965a). Even the spine is shortened in winter, resulting in a decrease of the total body length (Hyvarinen, 1969). The sum of these results in a 25-45% reduction of the whole body in the winter, depending on the geographic location (Churchfield, 1981; Hyvarinen, 1969; Pucek, 1965a). Furthermore, the seasonal shrinkage and regrowth of the skull is not restricted to soricids, but it has been also reported in some mustelids (Buchalczyk and Ruprecht, 1977; Dechmann et al., 2017; Kruska, 1993; LaPoint et al., 2017; Schmidt, 1992; Weiler, 1992).

All descriptions of seasonal changes of brain and skull have been performed on postmortem individuals. This implies that the differences in size between seasons are inferred as average differences at the population level, and not as actual changes in each individual. This difference could thus reflect a change in the population structure. For example, if a size biased mortality during autumn can favor the survival of the smallest individuals towards winter, as has been observed in other small mammals such as voles (Zub et al., 2014) and weasels (Szafrańska et al., 2013). Also, such differences might be enhanced by a cohort turnover, as in the short-tailed shrew (Dapson, 1968). To confirm whether Dehnel's phenomenon is actually an individual change, we monitored individual shrews and measured their skulls across seasons. In Chapter 1I performed a mark-recapture study along one year, and I used X-ray images to collect *in vivo* measurements of skulls. This approach allowed me to monitor each individual across seasons and quantify the within individual changes in the braincase. In parallel I collected the same data from the same population from post-mortem extracted skulls at the same study site (Chapter 3) and showed that Dehnels Phenomenon indeed occurs in individuals and is not enhanced by size-biased selection. This latter dataset also allowed me to verify the correlated change in brain size.

Under the assumption that the morphological changes occur within individuals, different authors have suggested adaptive explanations for the seasonal changes described above. Red-toothed shrews are strictly homeothermic animals, which do not hibernate, and must maintain an exceptionally high metabolic rate year round (Taylor, 1998). During winter they suffer a reduction in the quality and quantity of available prey, incurring a critical resource limitation for these animals (Churchfield et al., 2012). A simple proportional decrease in overall body size during winter would provide an advantage, by reducing the absolute metabolic demand, and consequently the prey consumption rate (Gliwicz and Taylor, 2002; McNab, 2010; Taylor et al., 2013). Furthermore, the intensity of the seasonal changes varies geographically, and the pattern, at least in Europe, is more intense in northern and eastern populations than in southern and western ones (Churchfield, 1990; Pucek, 1963; Pucek, 1970; Pucek and Markov, 1964). These differences have been attributed to climate and ecological factors, as populations inhabiting areas with more severe winters would suffer higher limitations in prey availability during this period, so a more pronounced decrease in body size would incur an advantage. Therefore, this classic hypothesis proposes that the Dehnel Phenomenon is an adaptive strategy for overwintering in terms of energetics and resource availability (Mezhzherin, 1964; Pucek, 1970). However, this hypothesis does not explain all the morphological changes across seasons, particularly in the brain. It has been observed that different brain regions change differently along the year, some of them – especially the neocortex and the hippocampus - undergoing large and reversible size transformation, while other ones only change size in one direction or even remain stable – such as the cerebellum or the olfactory bulbs (Yaskin, 1994). To assess the changes in brain structure (Chapter 3) I performed histological analyses on brains from the three main seasonal size stages, large summer juveniles, size-decreased winter subadults and re-grown spring/summer adults. First, based on serial sectioning and Nissl stain I made precise 3D reconstructions of main brain regions. From these reconstructions I quantified the changes in the different regions and their contribution to the overall change. Also, I was able to describe sexual differences in each brain region. I found the most drastic shrinkage and regrowth in the midbrain and in the neocortex. The last one however showed a pronounced winter decline, but it was not followed by a regrowth in spring. The hippocampus also changed seasonally, but not as intensively as in the previous Russian study (Yaskin, 1994). This difference suggests not only a geographical variation in the intensity of Dehnel's Phenomenon, but also difference in the structural seasonal changes in the brain.

The mechanisms that lead to the drastic transformation in the brain tissue of shrews are still unknown. During ordinary ontogeny in most mammals brain shows a logarithmic growth until reaching a plateau in adulthood (Dobbing and Sands, 1979). This usual increase in size is attributed to a combination of different factors: increase in cell numbers; increase in white matter; proliferation of the synapses, which implies the development of dendritic spines and synaptic buttons; and increase in the size and complexity of dendritic trees. In exceptional cases, in which brain regions change seasonally, some of these processes were found to be reversed, leading to a regression in brain tissue. For example, hippocampal dendritic trees shrink during hibernation in ground squirrels (Popov et al., 1992). In *Sorex* shrews, Bartkowska et al. (2008) concluded that changes in cell numbers do not contribute to the massive seasonal changes in brain size. Pucek, (1965b) observed that changes in water and fat contents contribute to some extent, but do not provide an exhaustive explanation. These two studies are the only attempts to clarify the underlying causes of the tissue shrinkage and regrowth at the cellular level in shrews. We thus used a different approach based on the assessment of changes in the extent and complexity of dendritic arbors to shed light on this question. In Chapter 2 I attempted to elucidate the underlying cellular changes that cause the seasonal differences in volume by quantifying differences in neuron size and morphology. Based on silver impregnated sections, I traced individual neurons in the three stages. I measured the seasonal differences in soma size, dendrite length and volume, and spine number and density in three brain regions. I observed a summer to winter decline in neuronal soma size in the caudoputamen and in the somatosensory and anterior cingulate cortices, as well as in the basal dendritic volume in the anterior cingulate cortex. However, these changes alone are not sufficient to account for the total winter volumetric decline, so there must be other mechanisms of change involved that should be explored in future studies.

The large seasonal changes in brain size and structure suggest possible changes in brain functionality. The changes in the size of brain structures might be driven by different cognitive demands along the life cycle of the shrews. It has been proposed that the seasonal changes in the size of the hippocampus could be positively correlated with the variation in home range size (Yaskin, 2005). Shrews expand their home ranges from winter to spring and summer, specially males (Churchfield, 1990; Rychlik, 1998). Thus, shrews may be able to survive with a smaller hippocampus and thus should show lower spatial cognitive skills in winter; but the larger hippocampus in spring should be correlated with improved spatial capacities in the second spring and summer, especially in adult males. However, to date the possible consequences of Dehnel phenomenon on cognition remains unexplored. In my thesis I tested differences in spatial cognition between seasons. For this purpose in Chapter 3 I tested shrews from the three stages – summer juvenile; winter subadult; spring adult – in a spatial learning task. Spatial ability was measured as the path length needed to find a food item hidden in the arena, using a visual cue as reference, along repeated trials. I observed that the small-brained winter subadults used average larger search paths than the big-brained summer juveniles and regrown adults.

Chapter 1

Profound reversible seasonal changes of individual skull size in a mammal

Postnatal size changes in most vertebrates are unidirectional and finite once the individual reaches full size (West et al., 2001). In rare cases, changes of body length may occur in response to harsh environmental conditions. Such reactionary changes are distinct from seasonal, often anticipatory morphological changes, such as the reversible size change of some adult bird brains (Nottebohm, 1981). A unique pattern of profound anatomical change has been described for the body, skull and brain size of red-toothed shrews and some mustelids (Bielak and Pucek, 1960; Dechmann et al., 2017; Dehnel, 1949a). The seasonal 20% decrease and 15% re-growth of the most common proxy, braincase height, were documented at population level from extracted skulls post-mortem. Quantifying intra-individual change had so far been methodologically prohibitive. Here, we followed the intra-individual change in skull size and body mass throughout the full cycle in wild recaptured shrews (*Sorex araneus*). Using X-ray images we showed that individuals decreased the size of their braincases in anticipation of winter by an average -15.3%. Braincases then partially regrew in spring by 9.3%. Body mass decreased by 17.6% and then dramatically increased by 83% in spring. Thus, we demonstrate that the dramatic changes incurred by Dehnel's Phenomenon occur in the individual's bone and other tissues.

We trapped *S. araneus* in Möggingen Germany (longitude 8.994, latitude 47.766) between June 2014 and October 2015 with wooden live-traps (PPUH A. Marcinkiewicz, Rajgród, Poland) baited with mealworms and checked at two-hour intervals. We anesthetized animals in an induction chamber (Surgivet; oxygen flow rate 1 l/min, 5% Isoflurane) connected to a Titus System (Dräger, Lübeck). The anesthetized individual was placed into a form-fitting foam bed to ensure a standardized body position. We then took both ventral and lateral X-ray images of the skull in a Faxitron MX 20 cabinet (26 kV, 6 sec) using an OPG Imaging Plate (Gendex) and a scanner (DenOptix /Gendex) (Fig. A.1). While under anesthesia and before release at the place of capture, we individually marked shrews with subcutaneous passive integrated transponders (UNO PICO, 7x1.5mm). We measured body mass to the nearest 0.01g with an electronic scale. Animals were X-rayed once per month at most, except for individuals used for determination of technical measurement error (see Supplemental Experimental Procedures for details). All handling and sampling methods were approved by the Regierungspräsidium Freiburg, Baden-Württemberg (35-9185.81/G-11/21, 35-9185.81/G-14/28).

On the X-ray images we measured braincase height (BCH), skull length (SKL) and braincase weight (BCW) (Fig. A.1). We size corrected all skull measurements by dividing by the non-changing maxillary tooth row length (Fig. A.2): i.e., BCH_{cor}, SKL_{cor}, BCW_{cor}. We checked for non-linearity in the overall seasonal patterns for BCH_{cor}, SKL_{cor}, BCW_{cor}, tooth row and body mass using generalized additive mixed models (GAMM). In addition, we tested for significance of within-subject change of skull measures with paired t-tests (see Supplemental Experimental Procedures for details).

Our data show that all three skull measurements changed reversibly in individuals. BCH_{cor} decreased in shrews caught repeatedly in summer and then again in winter (paired t-test, d.f. = 28, $t = 10.39$, $P < 0.001$; Fig 1.1). BCH_{cor} of individuals we then recaptured after sexual maturation the following spring had partially regrown (paired t-test, d.f. = 13, $t = 2.60$, $P < 0.05$; Fig. 1B). The few surviving post-reproductive individuals then began a second decline, but sample size for this second pre-mortal decline was not sufficient for analyses. Size changes were not sexually dimorphic (see Supplemental Experimental procedures).

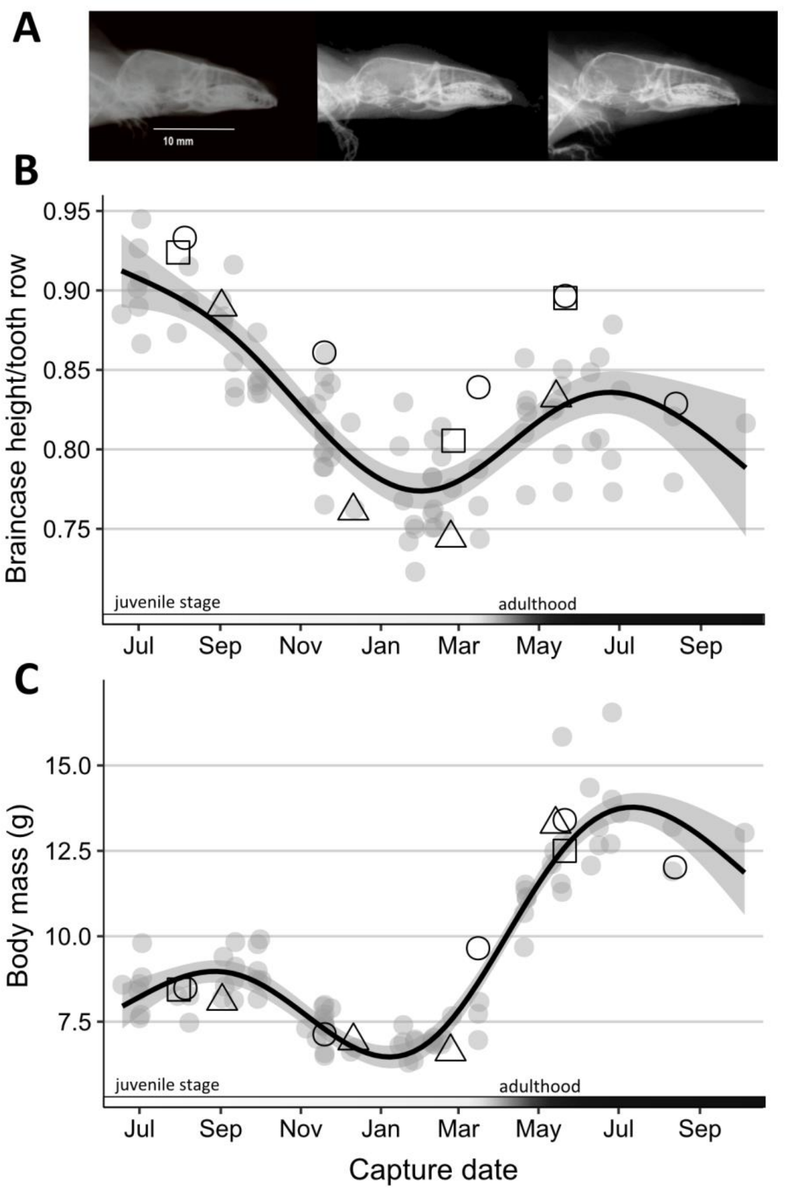

Figure 1.1. Individual changes in braincase height and body mass. (**A**) X-ray images of one individual as a juvenile in July (left), size-decreased subadult in February (center), and re-grown adult in May (right). White scale bar is 10 mm. (**B**) Repeated $\mathrm{BCH}_{\mathrm{cor}}$ from X-ray images of all recaptures (grey dots). Solid line and shaded area represent fitted values and standard error of the model (GAMM, n = 95 observations, N = 37 individuals, e.d.f. (smooth term) = 3.906, $P < 0.001$). Symbols highlight three individuals with time between first and last capture > 8 months. Individual in Fig. 1.1A corresponds to the open squares. The shaded bar along the x-axis indicates progression from juveniles (white portion of the bar) to adults (black). **C)** Individual changes in body mass from recaptures. Solid line and shaded area represent fitted values and standard error of the model (GAMM, n = 89 observations, N = 37 individuals, e.d.f. (smooth term) = 3.974, $P < 0.001$).

All 12 individuals we caught during all three stages (summer juveniles, winter subadults, and spring/summer adults) exhibited the full phenotypic cycle – first summer peak, winter decrease, and spring regrowth. Mean decrease and later increase in BCH_{cor} as assessed by X-rays were -15.4% and +9.1%, respectively. The greatest changes within a single individual we recaptured during all stages were -16.3 and +11.9% (September to February, and February to May). The greatest changes in individuals caught only during two of the stages were -20.1% and +13.2%. These are conservative assessments, because it is unlikely that any individual was captured at the exact time of size extremes. The other metrics followed the same pattern, but to a lesser extent (Fig. A.2; SKL_{cor}: GAMM, $n = 87$, e.d.f. = 3.784, P(smooth t.) < 0.001; BCW_{cor}: GAMM, $n = 83$, e.d.f. = 3.598, P(smooth t.) < 0.01). We also found a reversible change in individual body mass (Fig. 1.1C). On average, shrews decreased their body mass by 17.6% from July to February; but then they showed a dramatic increase during spring (83.4% from February to May). These results also match a previous study (Taylor et al., 2013) in northeastern Poland which reported an average body mass decrease of 20.9% from summer to winter in recaptured animals.

We thus document the greatest postnatal reversible individual change in mammalian skull size along with mass known to date, matching previously published patterns from post-mortem skulls (Dechmann et al., 2017; Pucek, 1970). Relative or absolute BCH of recaptured and non-recaptured first-summer individuals was the same (t-test: d.f. = 59, $t = 0.226$, $P > 0.5$). Thus, a possible size-biased seasonal selection or a cohort turnover did not enhance the seasonal patterns we observed.

The morphological changes that we document are part of an extraordinary adaptive process that enables individuals to morph from a larger summer phenotype to winter phenotype by reducing the size of the body. The winter reduction in the body size of the shrew including the braincase and the correlated change in brain mass (Bielak and Pucek, 1960) are hypothesized to be an over-wintering strategy, in alternative to hibernation or migration (Pucek, 1970). An average decrease in body mass of 19% leads to a winter reduction of 18.2 % in shrews' absolute resting metabolic rate (Taylor et al., 2013), which means animals may partially compensate for a higher energy budget due to colder temperatures. This might also reduce the absolute food requirements. Thus, reducing size may improve the probability for survival of the high-metabolic shrews (Rychlik, 1998). The partial regrowth into the adult phenotype in spring may then increase competitiveness during their only reproductive period when both sexes expand and aggressively defend their territories (Rychlik, 1998). A recent study showing similar patterns in post-mortem skulls of two weasel species indicates that this pattern may be taxonomically more important than we currently know(Dechmann et al., 2017; LaPoint et al., 2017).

The changes in skeletal size in the common shrew we describe illustrate how environmental forces shape the evolution of the mammalian body, skeleton and organs, including the skull and brain and offers opportunities for further exploration and study. Our results show the extraordinary extent to which the postnatal mammalian skeleton can maintain flexibility if the proper genetic programs are activated. This opens new and important avenues in understanding how certain degenerative processes can potentially be reversed in the skeleton and other tissues.

Acknowledgments

We thank Marion Muturi for assistance in the field and laboratory, and Teague O’Mara, Maude Baldwin, Alexander Steinbrecht, Anneke van Heteren and Sharon Swartz for comments on early versions of this manuscript. This project was funded by the Max Planck Society through the Max Planck - Poland Biodiversity Initiative. We declare no competing interests.

Chapter 2

Profound seasonal changes in brain size and architecture in the common shrew

Abstract

The seasonal changes in brain size of some shrews represent the most drastic reversible transformation in the mammalian central nervous system known to date. Brain mass decreases 10-26% from summer to winter and regrows 9-16% in spring, but the underlying structural changes at the cellular level are not yet understood. Here we describe the volumetric differences in brain structures between seasons and sexes of the common shrew (Sorex araneus) in detail, confirming that changes in different brain regions vary in the magnitude of change. Notably, shrews show a decrease in hypothalamus, thalamus, and hippocampal volume and later regrowth in spring, whereas neocortex and striatum volumes decrease in winter and do not recover in size. For some regions, males and females showed different patterns of seasonal change from each other. We also analyzed the underlying changes in neuron morphology. We observed a general decrease in soma size and total dendrite volume in the caudoputamen and anterior cingulate cortex. This neuronal retraction may partially explain the overall tissue shrinkage in winter. While not sufficient to explain the entire seasonal process, it represents a first step towards understanding the mechanisms beneath this remarkable phenomenon.

Introduction

An animal’s brain structure and size arise from developmental mechanisms that are shaped by socioecological adaptations and life history (Striedter 2005). The brain functions related to behavior and cognitive processes, in particular, provide individuals with the capacity to adapt to environmental changes over the lifespan. But maintenance and function of brain tissue require large amounts of energy, using up a substantial proportion of individuals' metabolic budget (Aiello and Wheeler 1995; Laughlin et al. 1998; Niven and Laughlin 2008). Therefore, the actual size of an animal’s brain and each of its constituent parts are likely the result of a tradeoff between the advantages of higher computational capacity and the costs of energetic demands. However, the physiological and cellular mechanisms that lead to an optimal neural structure remain unclear (Bullmore and Sporns 2012).

Of particular interest for understanding these adaptive and energetic tradeoffs are species exhibiting large variation of brain size between individuals or over the lifespan. Habitat seasonality provides predictable fluctuations in the environment and resource availability, which impact energy budget allocation. Consequently, seasonal changes in physiology and behavior of animals are often observed (but see Bolhuis and Macphail 2001). The best known examples of seasonal brain plasticity are found in some songbirds, where the size of song control nuclei change in anticipation to the breeding season (Nottebohm 1981; Brenowitz et al. 1991; Smith et al. 1997; Tramontin et al. 1998). For example, in canaries the volume of the HVC increases by 50% from fall to spring, and the robust nucleus of the arcopallium (RA) increases by 43%, leading to a change in overall brain mass of 13% (Nottebohm 1981). In food hoarding black-capped chickadees the hippocampus decreases by 17% during the non-hoarding season, when the spatial cognitive demands of food caching are reduced (Krebs et al. 1989; Barnea and Nottebohm 1994, 1996; Smulders et al. 1995; Bartkowska et al. 2008). The volumetric changes in the avian HVC and hippocampus have been associated with changes in cell numbers (Tramontin et al. 1998; Smulders et al. 2000); but changes in the RA are based on neuron size and spacing, as well as an increase in dendritic trees (Smith et al. 1997; Tramontin and Brenowitz 2000). Similar processes can also be found in food-hoarding mammals. The males of Richardson's ground squirrel store food before hibernation, and hippocampal size increases by 15% during that period (Burger et al. 2013). Similarly, hippocampus volume increases during the caching period in gray squirrels (Lavenex et al. 2000b) and chipmunks (Barker et al. 2003). In contrast, fluctuations in hippocampal volume of mammals do not appear to be correlated with changes in total cell numbers (Lavenex et al. 2000a; Barker et al. 2003, 2005). Notably, during hibernation ground squirrels exhibit a reversible decrease in hippocampal dendrite arbors, as well as in the number and size of dendritic spines (Popov and Bocharova 1992; Popov et al. 1992), suggesting that dendritic and synaptic plasticity are important mechanisms underlying the volumetric reorganization. Seasonal brain changes in other mammals, including humans (Hofman and Swaab 1992), are restricted to microstructure and biochemistry of hypothalamic (Hofman and Swaab 2002) and hippocampal regions (Magariños et al. 2006; Workman et al. 2009).

To learn more about the structural basis of adaptive brain size variability, we investigated the most extreme known case of individual seasonal variation in mammalian brain size and architecture. The brains of some species of red-toothed shrews (Sorex spp.) decrease in mass from summer to winter by 20% or more, followed by regrowth of ca. 15% (i.e. Dehnel's Phenomenon; (Bielak and Pucek 1960; Pucek 1965a; Yaskin 1994; Bartkowska et al. 2008)). These changes are accompanied by correlated variation in braincase size (Dehnel 1949; Serafinski 1955; Caboń 1956; Bielak and Pucek 1960; Taylor et al. 2013; Lázaro et al. 2017), as well as the size of internal organs including the spleen and liver (Pucek 1965b), and the length of the spine (Hyvarinen 1969). The change in overall brain size dramatically affects brain architecture across seasons; in a Russian population of shrews, neocortex and hippocampus show the most profound winter decrease compared to other brain regions, while other regions remain stable in size or grow in the spring (Yaskin 1994). In parallel, cognitive skills also exhibit seasonal variability, with small-brained winter shrews showing lower spatial learning skills than both large-brained summer juveniles and spring adults (Lázaro et al. 2018). This result is congruent with winter decrease in the hippocampus and neocortex, as these regions process information on spatial cognition, cue sensitivity and memory. Similar to food-storing birds and ground squirrels, the changes in shrew brain and behavior have been attributed to different space use across the seasons (Lázaro et al. 2018). Individual territories are smaller during winter, but then expand during the breeding season in spring and summer (Stockley and Searle, 1998; Yaskin, 2005).

In this study we examined neuroanatomical reorganization underlying seasonal changes in brain size of a red-toothed shrew species, the common shrew (Sorex araneus). We first confirmed that overall brain size changes at our study site in southern Germany and measured the volumes of brain regions over the one-year lifespan from individuals collected at this location. This was important as seasonal changes may vary in their extent based on the severity of local conditions and previous studies were carried out at higher latitudes (Pucek 1970). We therefore predicted a less pronounced seasonal change in our study population than in the northern populations. In addition, we analyzed the magnitude of change in each brain region. Since these differences might be driven by the changes in cognitive demands along the seasons, we expected to find a more intense winter decrease in regions with diminished functions during that period such as hippocampal and cortical areas. Furthermore, we expected that if overall brain size regrowth in spring is driven by territory expansion in preparation for reproduction, then sex differences would be evident, especially in the hippocampus as the expansion of territories in spring is more intense in males than in females (Stockley et al. 1994; Rychlik 1998; Stockley and Searle 1998; Yaskin 2005). Also, an important determinant for the differences between regional changes might be their differential flexibility. Ontogenetic timing can be a predictor of evolvability and plasticity (Finlay and Darlington 1995; Clancy et al. 2001), thus we expected latter developed regions such as neo-, rhinal and piriform cortices to undergo more drastic changes. However, if the seasonal change is driven by a purely energetic constraint in winter, we then predict to observe the least drastic winter decline in the least costly regions, i.e. thalamic regions, minimizing energy demands during the period of resource scarcity.

We assessed further whether the mechanisms of size variation were identifiable at the cellular level. A previous study found no evidence for changes in cell numbers in the hippocampus of shrews over the lifespan (Bartkowska et al. 2008). Thus, we predicted that size changes would be driven by differences in neuron morphology. To test this, we traced Golgi impregnated neurons in several selected brain regions. At the cellular level we expected to observe changes in the soma and/or dendritic morphology of seasonally changing regions that correlate with the magnitude of the change in the specific brain region.

This study makes an important contribution to understanding the link between brain size and the underlying anatomical structures in this unique mammalian species where such pronounced brain size variability occurs predictably within individuals over their lifespan.

Methods

Trapping and processing of specimens. Trapping took place monthly between August 2013 and October 2015 in Möggingen, Germany (longitude 8.994, latitude 47.766). Shrews were trapped with wooden live-traps (PPUH A. Marcinkiewicz, Rajgród, Poland) baited with mealworms and checked at two-hour intervals. Once caught we brought the shrews to the laboratory, where we perfused them transcardially with phosphate buffered saline (PBS) followed by freshly prepared 4% formaldehyde in PBS under deep isoflurane anasthesia. We immediately extracted the brains from the skull, separated the hemispheres, and weighed them to the nearest 0.001g before postfixation for two weeks in 4% buffered paraformaldehyde. We then transferred the tissue to PBS/0.1% sodium azide at 4^{o}C for long term storage. The right hemisphere was used to reconstruct brain region volumes; the left hemisphere was used for Golgi staining and neuron morphology analyses.

Age and sex determination. Based on the time of the year and the degree of gonadal development (Churchfield 1990), we classified individuals into three age groups: summer juvenile (sexually immature, from June-September); winter subadult (sexually immature, from December-March); and spring-summer adult (sexually mature, from May-August). Because *S. araneus* has a maximum life span of ~18 months, there is no generation overlap of mature adults. During the very brief period of overlap between summer juveniles and adults, they can be easily distinguished by the degree of development of the gonads (Churchfield 1990).

To determine the sex of immature individuals (all individuals until the spring following the year of birth) we used a PCR-based gonosomal sexing method (C. Roos, DPZ Göttingen, unpublished). DNA was extracted from tail tip samples using standard DNAeasy kits (Qiagen, GmbH, Hilden).

Calculation of brain region volumes. We quantified the volume of brain structure from 10 individuals (5 males and 5 females) of each age group (N = 30). Before sectioning, the left hemisphere was immersed in a series of 10, 20 and 30% sucrose in PBS for cryoprotection. We cut the tissue on a freezing sliding microtome (Reichert-Jung Hn-40) to obtain 30µm-thick coronal sections. We mounted every fifth section on slides and stained theem with 0.5% cresyl violet (Fig. 2.1, 2.2). We examined the following brain regions: olfactory bulb, neocortex, rhinal and piriform cortices, caudoputamen, amygdala, nucleus accumbens, thalamus, hypothalamus, hippocampus, dentate gyrus, CA1, CA2, CA3, subiculum and cerebellum and the total hemisphere. We located and defined these brain regions based on cytoarchitectural descriptions from insectivores (Catania et al. 1999; Catania 2000; Naumann et al. 2012). As a reference we also used a mouse brain atlas (Paxinos and Franklin 2013). We used an Olympus BX51 microscope under an Olympus UIS2 Plan N 2x (NA = 0.02) dry objective interfaced with a Neurolucida software system (MBF Bioscience, Williston, VT, USA) to outline each brain region (Fig. 2.2). The system utilized a MicroFire Digital CCD 2 Megapixel camera (Optronics, Goleta, CA, USA), and a HP Z27i monitor with 2560x1440 resolution. The Cavalieri principle was used to calculate the volume of each region from the sum of brain region areas measured in each section multiplied by the interval distance and section thickness. Volumes were automatically calculated in the software extension Neurolucida Explorer.

A correction factor was used for each individual to account for the shrinkage occurring during the histological processing of the tissue (de Sousa et al. 2010). The correction factor for each brain was calculated as the quotient between the freshly extracted hemisphere volume and the final volume of that whole hemisphere derived from the measurement of outlined slides. The fresh hemisphere volume was calculated by dividing the fresh hemisphere mass by the specific gravity of brain tissue (1.036 g/cm3; Stephan 1960). Each brain-specific correction factor was then applied to the brain region volumes for that specimen.

The final volume obtained for each brain region as well as whole hemispheres were size corrected dividing by the upper tooth row, a metric which is stable across seasons (Lázaro et al. 2017). All tracings were done blind by a single observer (MM).

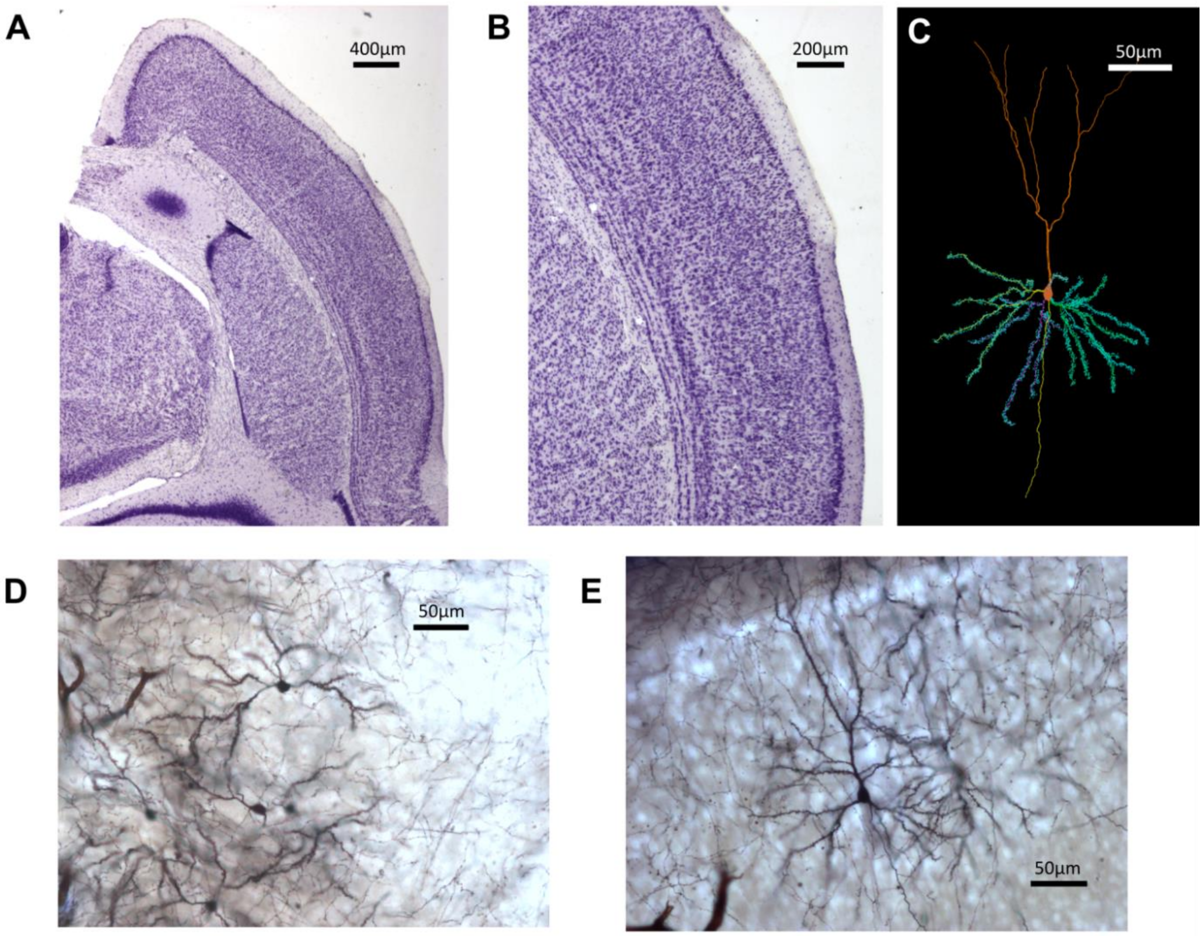

Figure 2.1. Examples of histological sections and neuron tracings. A, Nissl-stained section of a shrew brain hemisphere showing a dorsal medial area. **B,** detail of a Nissl-stained section depicting a portion of neocortex. **C,** neuron tracing as depicted by Neurolucida. **D,** medium spiny neurons in the caudoputamen stained with the Golgi technique. **E,** pyramidal neurons in the neocortex.

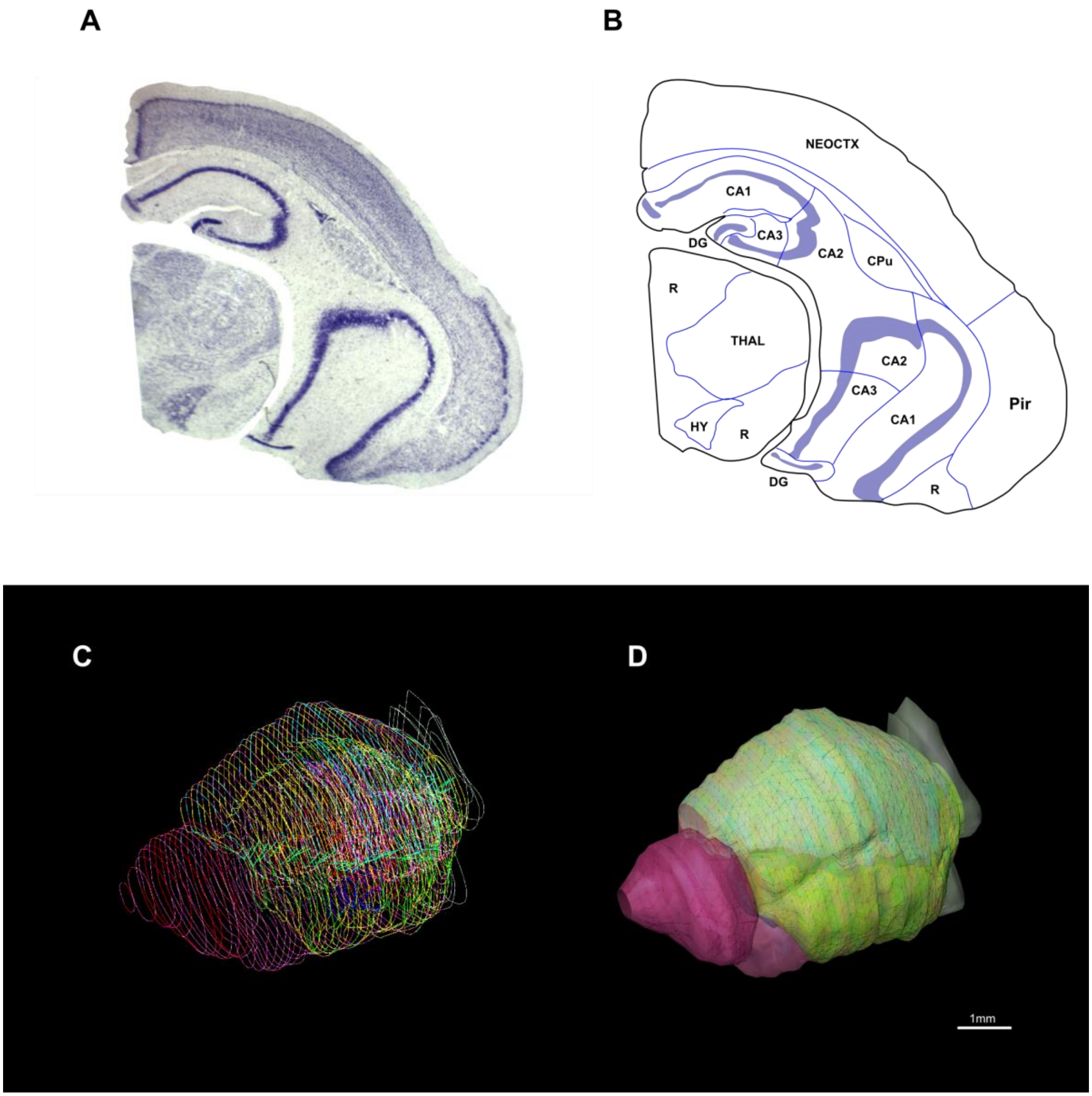

Figure 2.2. A, Coronal section of a hemisphere stained with Nissl. **B,** Outlines of the brain regions traced on a Nissl-stained section using Neurolucida software. NEOCTX: neocortex; Pir: piriform cortex; CPu: caudoputamen; DG: dentate gyrus; THAL: thalamus; HY: hypothalamus; R: rest of brain. **C,** stack of all section outlines of a hemisphere. **D,** 3D reconstruction of an hemisphere based on section outlines.

Neuron tracing and quantification. We used brains from five males of each of the three age groups (N = 15) to study neuron architecture. Right hemispheres were processed by a modified rapid Golgi technique (Scheibel and Scheibel 1978) (Fig. 2.1). After processing, they were cut coronally in two halves, and both tissue blocks were serially sectioned at 100μm with a Vibratome. We focused on three types of neurons: pyramidal neurons of layer III-IV in the anterior cingulate cortex (Fig. 2.3) and in the somatosensory cortex (Fig. 2.4); and medium spiny neurons of the caudoputamen (Fig. 2.5). The brain regions were identified based on cytoarchitectural criteria and using the Nissl-stained sections as reference. The Golgi technique only stains a limited number of neurons randomly, which allows visualizing separated cells and their processes (Scheibel and Scheibel 1978). We selected neurons that appeared fully impregnated, isolated from other stained neurons, with their soma centered within the section thickness and which had as complete dendritic trees as possible (Fig. 2.1).

We traced 25 neurons of from each brain region per age group, randomly chosen – within the above criteria – among the five individuals in each age group (total = 225 neurons, 75 neurons of each neuron type). To avoid any possible bias, all neuron-tracings were obtained by a single observer (JL) and were performed blind to the individual and age group. Neurons were quantified along x-, y-, and z-coordinates using the Neurolucida system (see above) under an Olympus UIS2 Plan N 100x (NA = 1.25) oil objective. For each neuron, the soma was traced in the widest two-dimensional point to obtain its cross-sectional area and the dendritic tree was traced accounting for dendritic diameter, marking all bifurcations and quantifying all visible spines, without determining spine type. Incomplete dendrites that were cut in the section edge were not followed into the adjacent section and were marked as incomplete endings. We quantified the following metrics: soma size (area), total dendritic length and volume, spine number and density. All metrics were automatically extracted in Neurolucida Explorer. For the cortical neurons only the basal dendrites were examined because often the apical dendrites were incomplete.

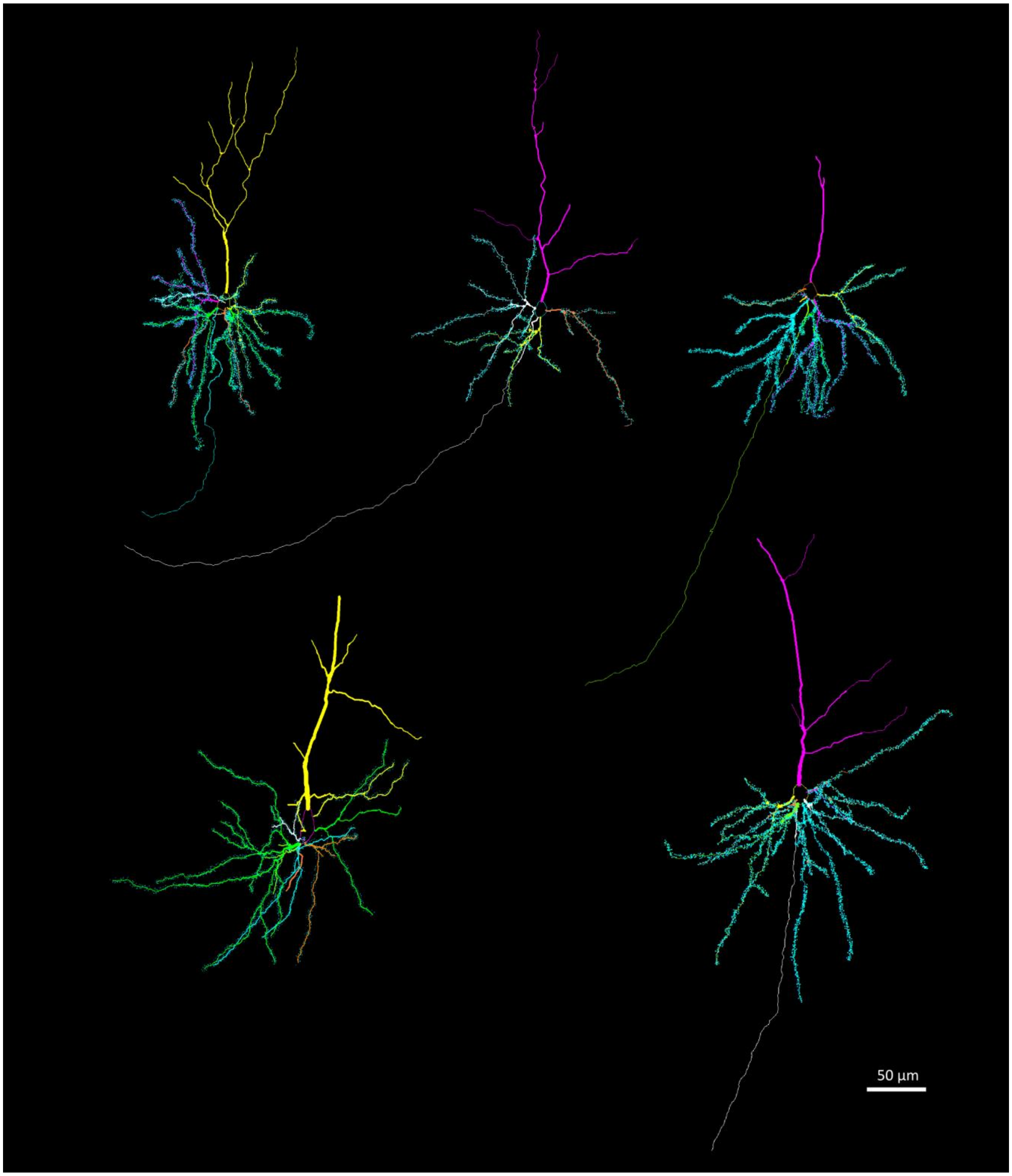

Figure 2.3. Examples of reconstructions of pyramidal neurons in the anterior cingulate cortex. Dendritic spines are indicated in the basal dendrites. Reconstructions were done in Neurolucida.

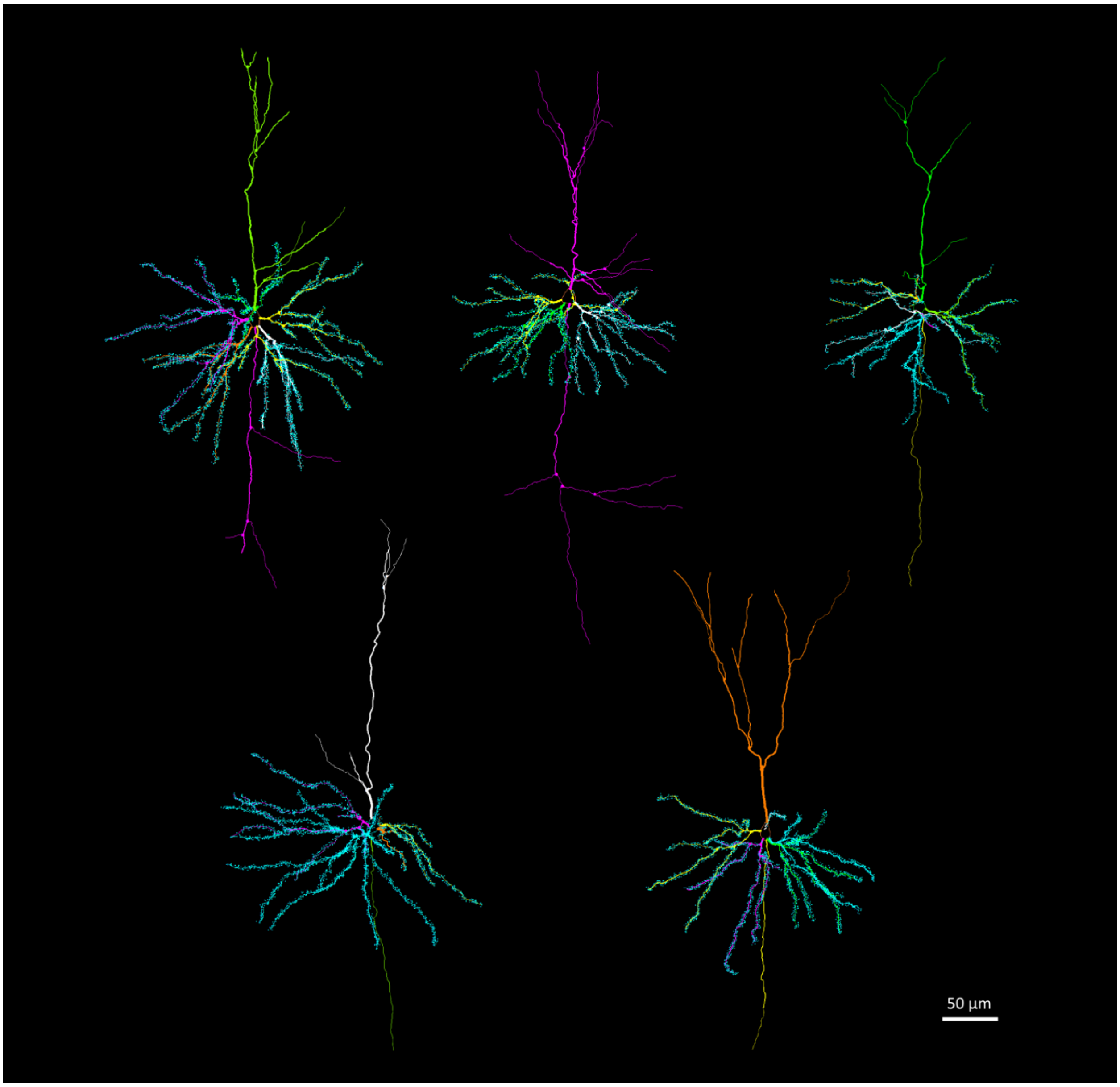

Figure 2.4. Examples of reconstructions of pyramidal neurons in the somatosensory cortex. Dendritic spines are indicated in the basal dendrites. Reconstructions were done in Neurolucida.

Data analyses. We analyzed the volumes of entire hemispheres using ANCOVA with age group, sex, and their interaction as explanatory variables. We used a linear mixed effects model to analyze the volumes of all brain regions, with age group, brain region and sex and their interactions as factors, and individual as random effect. To analyze the data from the neuron tracings we used a linear mixed-effects model for all metrics (soma size, dendritic length, dendritic volume, number of spines and spine density), which were used as dependent variables, age group as factor, and individual was included as random effect. We analyzed each region (anterior cingulate cortex, somatosensory cortex and caudoputamen) separately.

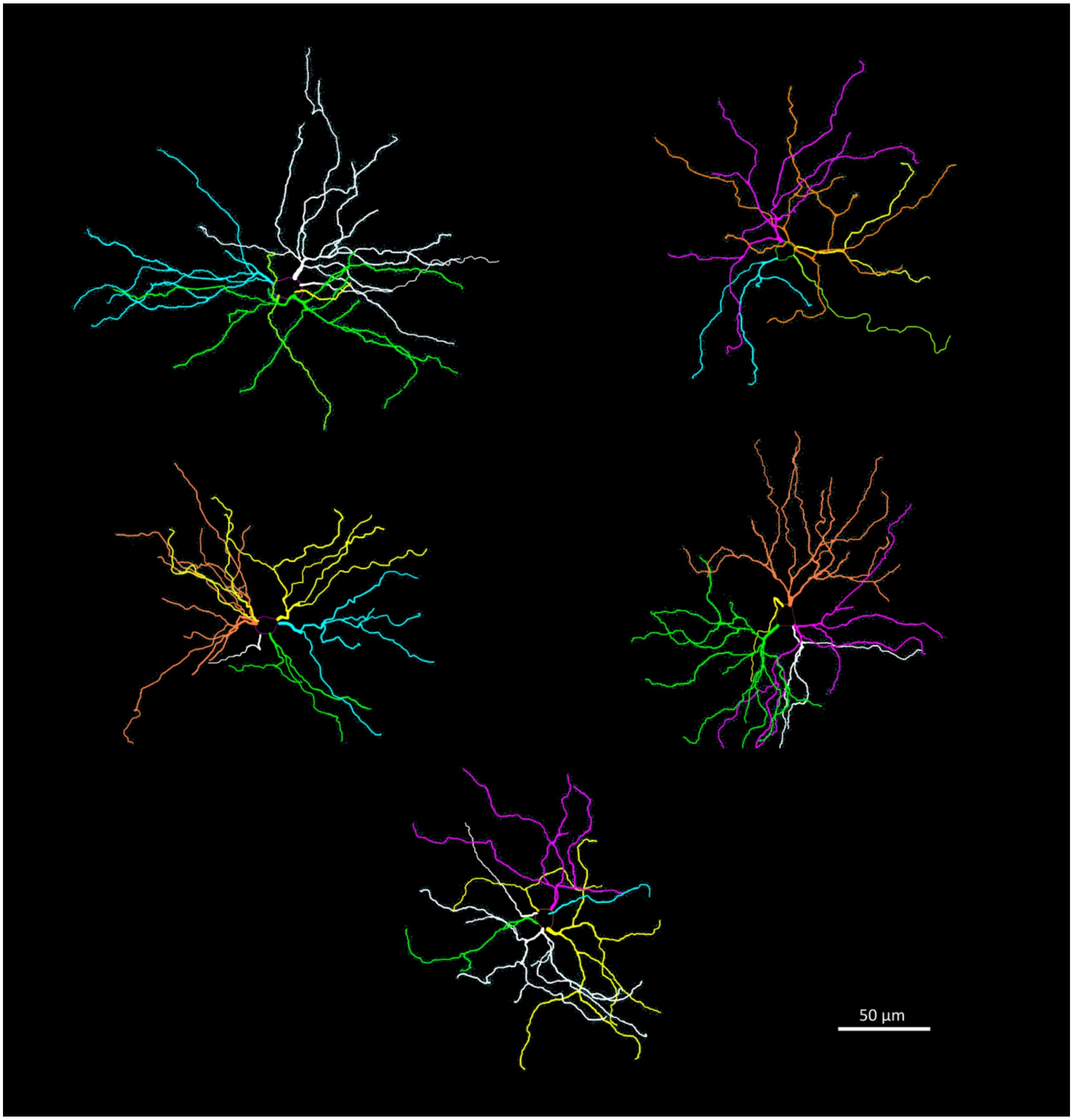

Figure 2.5. Examples of reconstructions of spiny neurons in the caudoputamen. Reconstructions were done in Neurolucida.

To quantify the differences between age groups, and between age groups and sexes, we estimated probabilities of the differences (P) and 95% Bayesian credible intervals (CrI). We used Monte Carlo simulations to obtain 20000 random values from the joint posterior distribution of the model parameters assuming flat prior distributions. We calculated 95% CrI as the 2.5% and 97.5% quantiles of the marginal posterior distributions of the parameters.

For the linear mixed models we used the function lmer from the R package lme4 (Bates et al. 2014). For Monte Carlo simulations we used the function sim from the R package arm (Gelman and Su 2015). All analyses were performed in R 3.3.1. (R Core Team 2015).

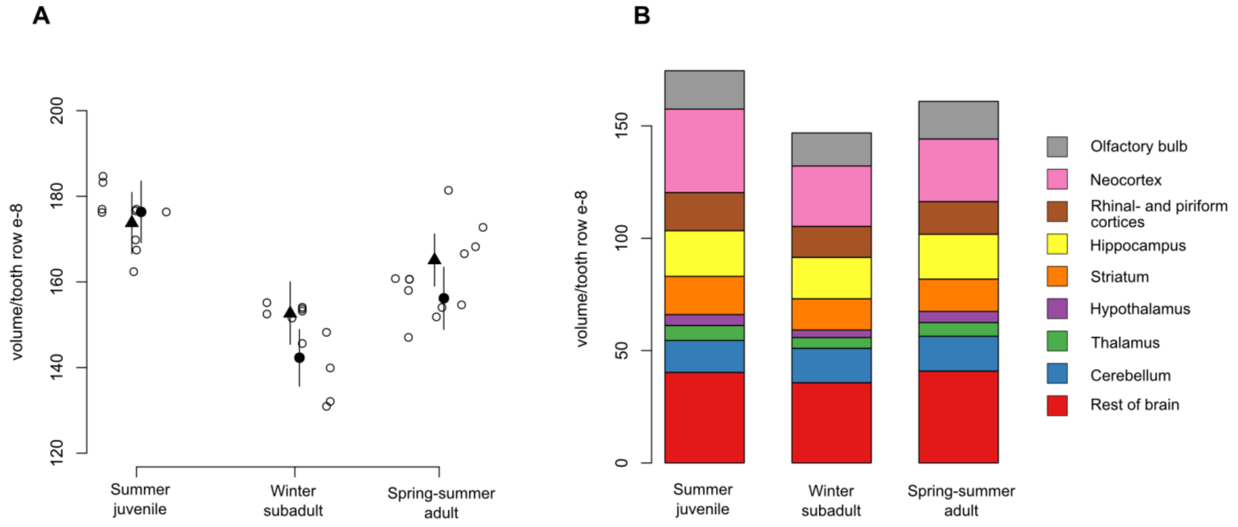

Figure 2.6. Seasonal changes in volume of brain and brain regions. A, volume of the entire brain hemisphere during the three age stages of the cycle, corrected by tooth row. Open circles represent individuals, triangles (males) and closed circles (females) are the means of each cycle stage, and bars are credible intervals. **B**, corrected volume for the entire brain and each brain region in the three stages. The area of each colored section represents the mean. See Table 2.1 for detailed results.

Results

Brain region volumes. Total hemisphere volumes decreased on average by 16.1% from summer juveniles to winter subadults, and increased again by 9.8% from winter subadults to spring-summer adults (Fig. 2.6A, see absolute values in Online Resource Table S1 and a comparative example in Fig. 7.7). The winter decrease was more pronounced in females than males, which led to females with smaller brains during winter. The spring regrowth was similar for both sexes and thus adult females' brains remained smaller than males among adults (Fig 2.2A, Table 2.1).

When comparing brain region volumes among the age classes with Bayesian statistics, there was significant variation, with some regions undergoing more intense changes than others (Fig. 2.6B, 2.8, Table 2.1). The region showing the most intense changes in both directions was the hypothalamus (-31.6%/+47.8%, respectively), followed by the thalamus (-27.9%/+27.5%). Striatum volume decreased strongly from summer to winter by 18.2% but did not regrow in spring. This decrease was mainly explained by a decrease in the caudoputamen by 20.7%, but no statistically obvious changes in nucleus accumbens and amygdala (Fig. 2.9, Online Resource Table B.2). The hippocampus contributed to both winter decrease and spring regrowth in volume (-9.5%/+8.4%), although we found different patterns between hippocampal subregions (Fig. 2.10, Online Resource Table S3): CA2 (-15.8%/+24.2%) and dentate gyrus (-15.2%/+15.6%) displayed marked changes in volume, while the change in CA1 was less pronounced (-12.6%/+13.5%), and CA3 and subiculum did not change. Both sexes underwent a pronounced decline in CA1 from summer to winter, but only the CA1 of males regrew in spring by 18.4% leading to sexual dimorphism in adult CA1. The large neocortex showed one of the most intense proportional decreases (-27.7%) and thus the highest absolute change from summer to winter, although it did not regrow in spring. The rhinal and piriform cortices exhibited a profound winter decline (18.7%) and a clear but less intense spring regrowth (5.4%). We also found a seasonal, reversible (-13.7%/+13.6%), but sexually dimorphic, pattern in the olfactory bulb, with winter and spring females showing 13.7% and 14.9% lower volumes respectively. The cerebellum followed a different and more complex pattern. Cerebellar volume was sexually dimorphic in winter animals. Winter females had a 32.2% smaller cerebellum than males, but in spring the females' cerebellum increased and reached a similar size to males again (Fig. 2.8, Table 2.1). The volume of the remaining brain areas measured altogether ("Rest of brain" in Fig. 2.6, 2.8, Table 2.1) followed the pattern of summer-to-winter decrease and the winter-to-spring regrowth. The regrowth was more intense in males than in females, leading to sexual dimorphism in adults.

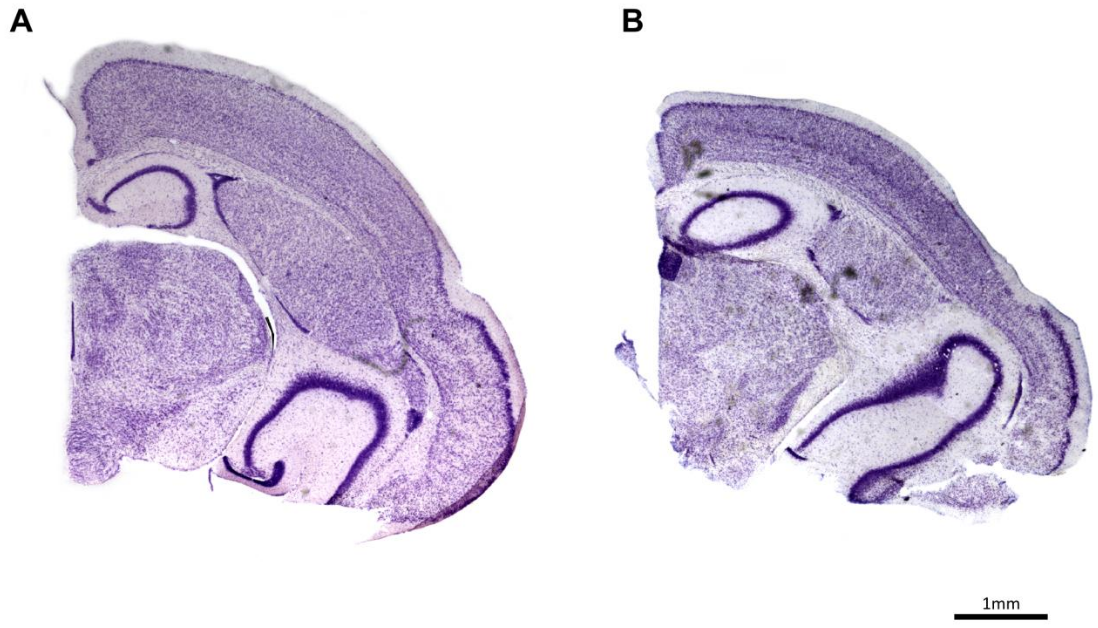

Figure 2.7. Exemplary brain coronal sections cut at a similar level in a summer juvenile (**A**) and a winter subadult (**B**) and depicted at the same scale.

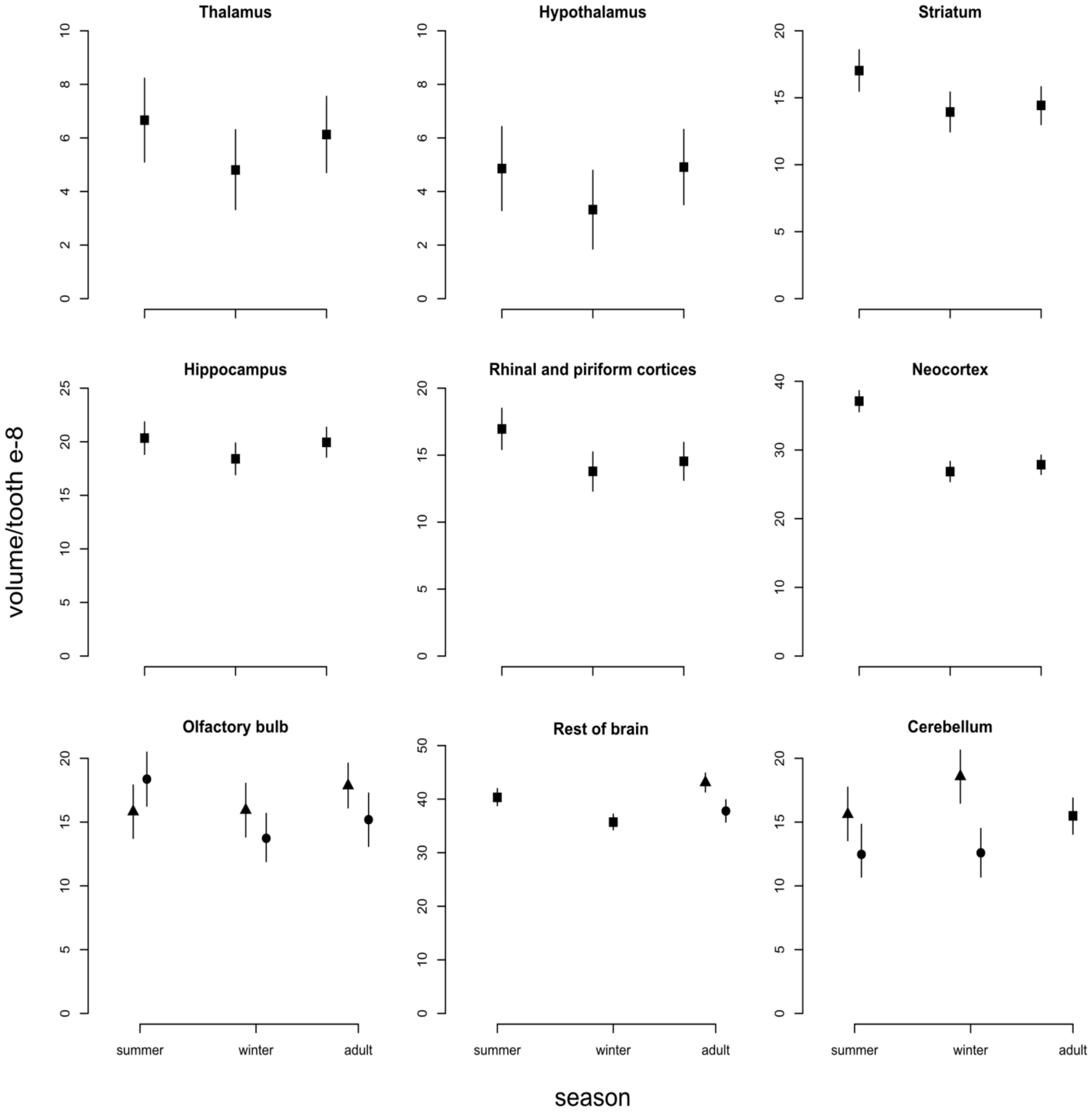

Figure 2.8. Seasonal changes in the volume of brain regions. Means at each stage and, whenever different, sex are represented by triangles (males) and closed circles (females). The mean of both sexes is depicted (squares) at the stages with no difference between sexes. Bars are credible intervals.

Table 2.1. Corrected volumes for all brain regions during the three age stages, mean differences between stages and probabilities (P) of the difference from summer juveniles to winter subadults (s-w) and from winter subadults to spring-summer adults (w-a) as calculated from the posterior distributions. The values of each sex are depicted only for the regions where differences were found. The values are volumes divided by tooth row (µm^3/ mm).

	Summer juvenile		Winter subadult		Spring-summer adult					
	Mean ± SD	95% CrI	Mean ± SD	95% CrI	Mean ± SD	95% CrI	Difference summer-winter	P(s-w)	Difference winter-adult	P(w-a)
Brain hemisphere	175.1 ± 6.8	169.6/180.6	147.0 ± 8.9	141.7/152.3	161.3 ± 9.6	156.4/166.4	-28.1 (-16.1%)	>0.99	14.3 (9.8%)	>0.99
males	173.8 ± 6.4	166.6/181.1	152.6 ± 2.7	145.3/159.8	165.1 ± 9.5	158.9/171.3	-21.2 (-12.8%)	>0.99	12.5 (8.2%)	>0.99
females	176.4 ± 7.7	169.1/183.6	142.3 ± 9.7	135.7/148.9	156.2 ± 7.7	149.0/163.3	-34.1 (-19.3%)	>0.99	13.9 (9.8%)	>0.99
Rest of brain	40.3 ± 3.7	38.8/41.9	35.7 ± 5.3	34.3/37.2	40.9 ± 3.6	39.5/42.3	-4.6 (-11.5%)	0.99	5.2 (14.5%)	>0.99
males	41.1 ± 3.2	38.9/43.1	36.1 ± 1.7	34.0/38.2	43.1 ± 2.1	41.3/44.9	-5.0 (-12.1%)	0.97	7.0 (19.4%)	>0.99
females	39.6 ± 4.5	37.5/41.8	35.4 ± 7.4	33.5/37.3	37.8 ± 3.0	35.7/39.9	-4.2 (-10.6%)	0.95	2.4 (6.8%)	0.83
Cerebellum	14.2 ± 3.5	12.7/15.8	15.3 ± 3.7	13.9/16.8	15.5 ± 3.0	14.1/16.9	1.1 (8.0%)	0.22	0.2 (1.1%)	0.55
males	15.6 ± 1.6	13.5/17.7	18.6 ± 2.3	16.5/20.7	14.7 ± 3.3	12.9/16.4	3.0 (19.0%)	0.05	-3.9 (-21.0%)	0.01
females	12.7 ± 4.4	10.7/14.8	12.6 ± 1.7	10.7/14.5	16.6 ± 2.3	14.5/18.7	-0.2 (-1.2%)	0.54	4.0 (31.9%)	0.99
Thalamus	6.7 ± 1.5	5.1/8.2	4.8 ± 0.7	3.3/6.3	6.1 ± 1.0	4.7/7.5	-1.9 (-27.9%)	>0.99	1.3 (27.5%)	>0.99
Hypothalamus	4.9 ± 1.8	3.3/6.4	3.3 ± 2.3	1.8 /4.8	4.9 ± 2.0	3.5/6.3	-1.5 (-31.6%)	>0.99	1.6 (47.8%)	>0.99
Striatum	17.0 ± 2.3	15.5/18.6	13.9 ± 1.2	12.5/15.4	14.4 ± 1.8	13.0/15.8	-3.1 (-18.2%)	>0.99	0.5 (3.5%)	0.74
Hippocampus	20.3 ± 1.8	18.8/21.9	18.4 ± 2.3	16.9/19.9	20.0 ± 2.0	18.5/21.4	-1.9 (-9.5%)	0.98	1.5 (8.4%)	0.96
Rhinal and piriform c.	17.0 ± 1.8	15.4/18.5	13.8 ± 0.9	12.3/15.3	14.5 ± 1.7	13.1/16.0	-3.2 (-18.7%)	>0.99	0.8 (5.4%)	0.88
Neocortex	37.1 ± 3.0	35.6/38.7	26.9 ± 1.8	25.4/28.3	27.9 ± 3.9	26.5/29.3	-10.3 (-27.7%)	>0.99	1.0 (3.7%)	0.78
Olfactory b.	17.1 ± 3.0	15.6/18.6	14.8 ± 1.4	13.3/16.2	16.8 ± 2.2	15.3/18.2	-2.3 (-13.7%)	0.99	2.0 (13.6%)	0.98
males	15.8 ± 2.4	13.7/17.9	15.9 ± 0.8	13.4/18.1	17.9 ± 2.1	16.1/19.6	0.1 (0.7%)	0.47	1.9 (12.1%)	0.95
females	18.4 ± 3.3	16.3/20.5	13.8 ± 1.1	11.9/15.7	15.2 ± 1.1	13.1/17.3	-4.6 (-25.1%)	>0.99	1.4 (10.4%)	0.88

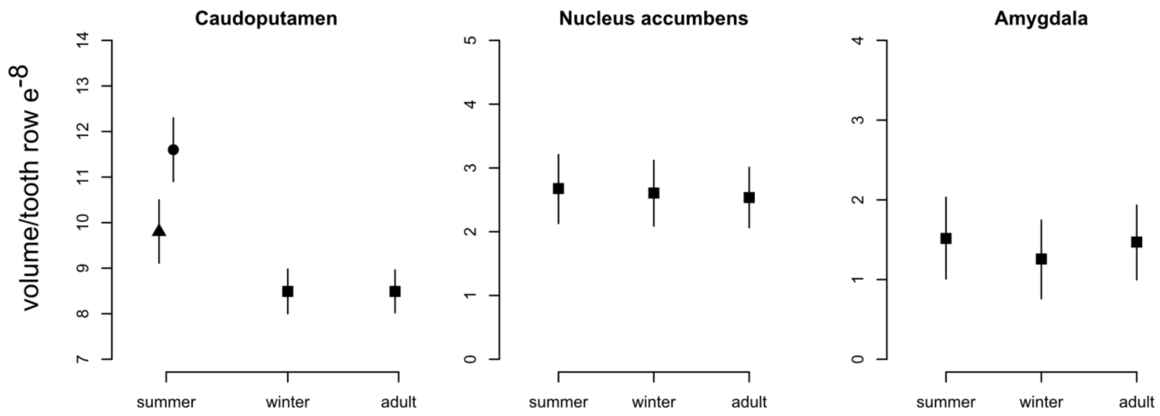

Figure 2.9. Seasonal changes in the volume of striatal subregions. Means at each stage and sex are represented by close circles (males) and triangles (females). The mean of both sexes is depicted (squares) at the stages with no difference between sexes. Bars are credible intervals.

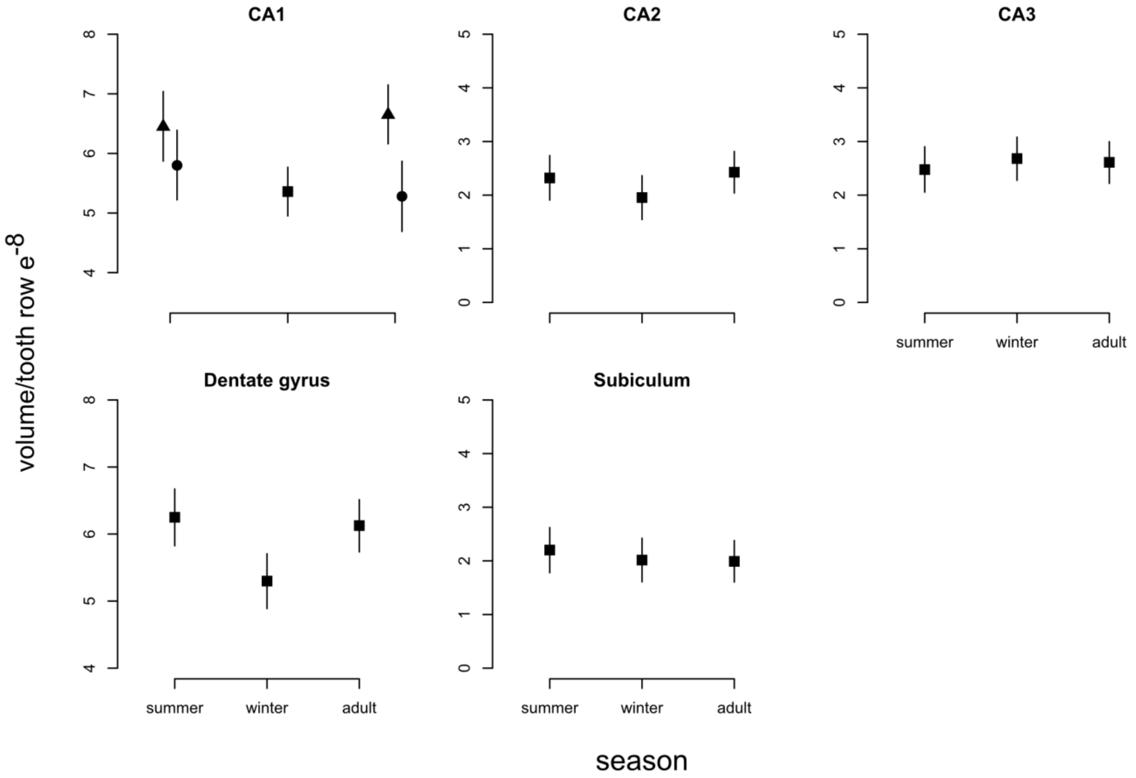

Figure 2.10. Seasonal changes in the volume of hippocampal subregions. Means at each stage and sex are represented by close circles (males) and triangles (females). The mean of both sexes is depicted (squares) at the stages with no difference between sexes. Bars are credible intervals.

Neuron tracing. Our results from the neuron tracings reveal patterns of variation between age groups in the three brain regions we examined (Fig.2.11, Table 2.2). In the caudoputamen we found a steady decline in all dendritic (21.8% in length and 32.9% in volume), soma (28.1%) and spine (37.1% in spine number and 18.4% in density) measures. This decline was more pronounced in the first phase (summer juvenile to winter subadult) than in the second phase (winter subadult to spring-summer adult) (Table 2). In contrast, the somatosensory cortex only showed a marked decline from summer to winter in soma size (17.7%) and in spine density (15.2%) from winter to adult. We also found a substantial decrease in soma size in the anterior cingulate cortex by 19.9%. In addition, in the anterior cingulate cortex there was a decrease from summer to winter in dendrite volume by 25.0%.

Discussion

Changes in volume of brain regions. Our results confirm the seasonal pattern of change in the volume of overall brain hemispheres of red-toothed shrews in Southern Germany that was previously reported from Russia and Poland (Bielak and Pucek 1960; Yaskin 1994). However, as expected, the pattern was expressed less strongly: the winter decline was 5.1% less pronounced than in north Poland (Pucek 1965b) and 10.2% less than in the Moscow region (Yaskin 1994). We found a decrease of 16.1% from summer juveniles to winter subadults and a subsequent increase by 9.8% in spring/summer adults. The decline in volume from juveniles to subadults happens in anticipation of winter and hence cannot be seen as an immediate reaction to temperature or food availability, but is more likely genetically encoded. When we analyzed the volume of each brain region separately, we observed that the different brain structures varied in the magnitude of change. Also, in some brain regions, seasonal changes varied between the sexes

In the mammalian brain we expect to find positive allometric correlations between overall brain size and each region caused by functional and/or developmental constraints (Finlay and Darlington 1995; Yopak et al. 2010; Charvet et al. 2011).

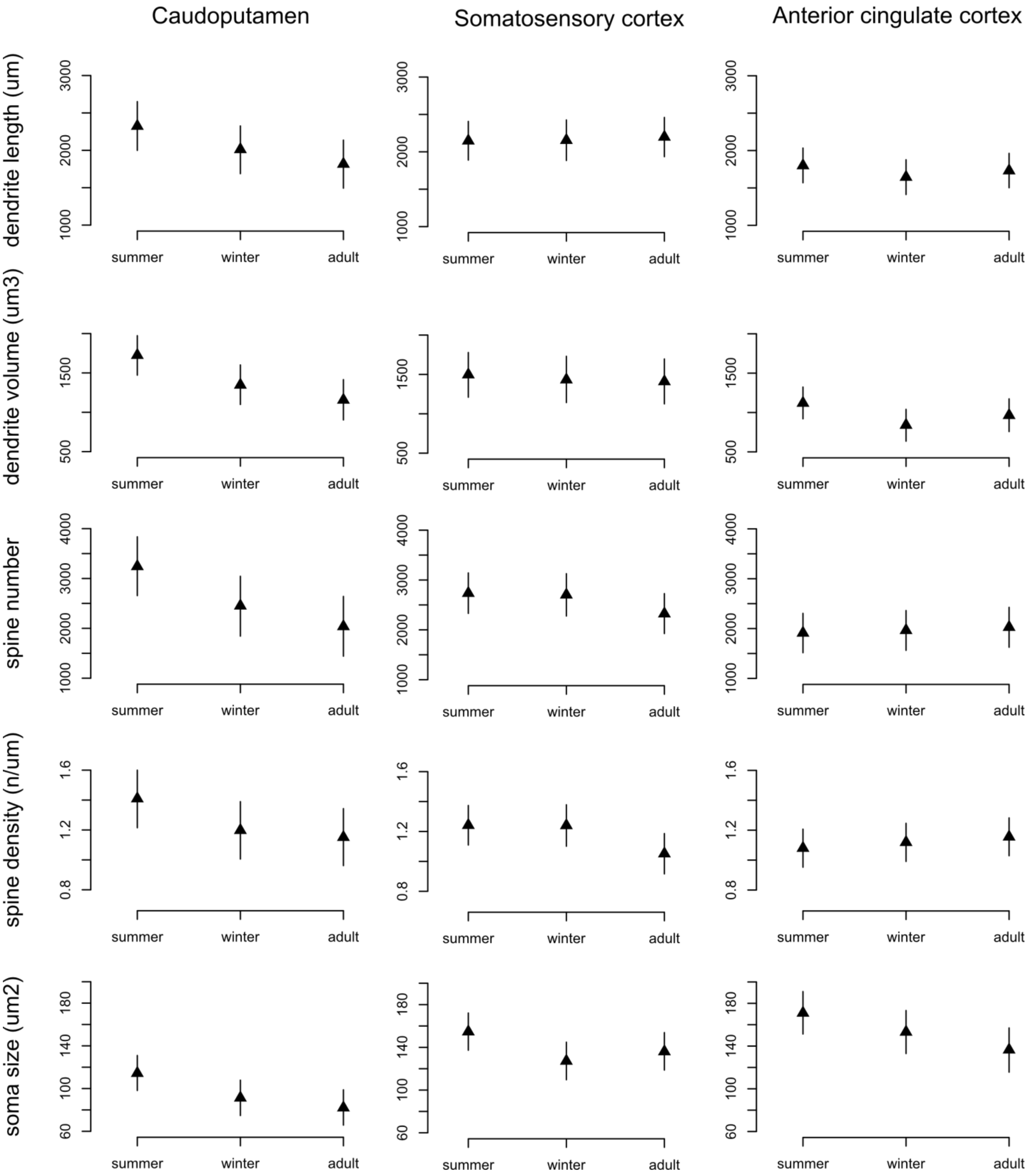

Figure 2.11. Seasonal changes in dendrite morphology and spine numbers. Graphs depict the results on the median spiny neurons of the caudoputamen (right column) and pyramidal neurons of the somatosensory (central column) and anterior cingulate cortices (right column). Triangles represent the means at each stage (all males) and bars are credible intervals.

In our results, as the brains varied in size seasonally, each region's size changed – or remained unchanged – independently of others. This variation might correspond to a mosaic adaptive development, which results in brain structure volumes that dynamically adjust to match the current cognitive demands and energetic constraints of the individuals. For example, summer juveniles would need to meet different cognitive requirements regarding territorial and reproductive behavior as they disperse and compete for territories (Moraleva and Telitzina 1994) than winter subadults, which minimize movement and social interactions to conserve energy. Cognitive demands would be different again in spring adults when shrews expand their home ranges for mate searching (Yaskin 2005; Gonda et al. 2013). This is consistent with our finding that in the winter, shrews underwent a decrease in hippocampal volume followed by regrowth in spring (Fig. 2.8). In other polygamous species where males show a greater expansion of home range than females, this has been linked to higher performance of males in spatial tests (Gaulin and Fitzgerald 1989; Galea et al. 1996). This is also congruent with the sexual dimorphism we found in the CA1 of adults (Fig. 2.10), as male common shrews enlarge their ranges more than females do (Stockley et al. 1994; Rychlik 1998; Stockley and Searle 1998). Furthermore, such a functional adaptive explanation is at least partially consistent with the observed decline in cortical regions in the winter. However, the lack of spring regrowth of the neocortex and the fairly extreme seasonal changes in other parts of the brain that are not associated with foraging and social functions, such as the thalamic regions remain difficult to account for.

Differences in the potential for plasticity between brain structures may also play an important role in constraining the pattern of seasonal variation in regional volumes that can occur. In a mammal with the usual curve of unidirectional brain growth (Dobbing and Sands 1973, 1979) the late developed regions – those where neurogenesis peaks occur later in ontogeny – tend to develop larger since they undergo more rounds of neurogenesis (Finlay and Darlington 1995; Clancy et al. 2001). This could translate into different capacity between regions to undergo plastic changes across seasons. Based on this, we expected the most plastic (latest developed) brain region to reveal the most drastic changes between seasons. However, ontogenetic timing does not seem to determine the intensity of change in our shrews' brain regions either, as both early (e.g.

thalamus) and late developing regions (e.g. neocortex) (Clancy et al. 2001) showed high seasonal variation. Attention should also be drawn to the changes in the cerebellum, which is one of the regions to develop latest in the mammalian brain: the sexual dimorphism in winter, which disappears later in adulthood, seems to be the result of differential timing in development between males and females. Consequently, male shrews reach adult cerebellar size earlier than females in ontogeny (Suárez et al. 1992; Fan et al. 2010; Tiemeier et al. 2011).

Regardless of the pattern of change, energetic limitations are likely to be a primary driver of variability in overall brain size across seasons. Energetic costs of brain computation function and tissue maintenance are extraordinarily high when compared to other physiological processes in different tissues (McNab and Eisenberg 1989; Aiello and Wheeler 1995; Laughlin et al. 1998). This energetic demand is considered an important constraint for development and evolution of brain size (Niven and Laughlin 2008; Bullmore and Sporns 2012). For this reason, the winter decrease in overall brain size of shrews has most commonly been proposed to be a strategy to reduce metabolic consumption during that period (Mezhzherin 1964; Pucek 1970), when food quality is lower (Churchfield et al. 2012) and therefore energy supply becomes a more limiting factor. Consequently, given that different brain structures have different metabolic demands due to their cellular architecture and activity level, we expected those brain regions with the highest metabolic costs to show the most pronounced winter shrinkage. However, such a scenario is unlikely to be the only explanation, based on our results: the magnitude of change of the different regions does not correlate to their metabolic scaling slope (Kaufman 2004; Karbowski 2007). For example, the thalamus which undergoes the strongest seasonal change shows one of the lowest mass-specific metabolic rates among brain regions (Kaufman 2004). Also, it does not explain why some regions do not regrow in the second spring/summer, when food availability is the same for adults and juveniles. Therefore, although energy limitation is probably an important factor to determine the changes in overall brain size it fails to completely explain the patterns in the different regions.

Although the overall seasonal variation in brain size may be caused by energetic limitation in winter, the variation in the different brain structures appears to be due to a combination of functional adaptations, as well as developmental constraints on plasticity. The size of each brain region is influenced by these factors to different degrees, and these influences may be different in the decrease and regrowth phases of the cycle.

Variation in neuron size and morphology. Our results on neuron morphology partially supported our expectations in the caudoputamen, but not in the cortical areas. The decline in caudoputamen volume from summer to winter (Fig. 2.9) was paralleled by a decrease in medium spiny neuron dendrite length and volume, spine number and soma size (Fig. 2.6, Table 2.2). These morphological changes resulted in neuronal retraction, which may have contributed to the observed decrease in the volume of the caudoputamen. We found a decrease in dendritic arbor length only in the anterior cingulate cortex. However, together with the decline in soma size in both the anterior cingulate and somatosensory cortices (Fig. 2.6, Table 2.2), this is unlikely to explain the -27.7% volume reduction in the neocortex, of which the somatosensory area makes up a large portion (Catania 2000). One possibility is that greater changes in dendritic trees might be located in other cortical areas, cell layers and/or neuron types. For pyramidal neurons where we assessed the morphology of basal dendrites, part of the seasonal variation in volume could be hidden in the apical dendrites. Also, we must consider alternative mechanisms that affect tissue volume. Because of methodological limitations, we did not quantify axon size and density in this study. In addition, seasonal variation in cell numbers throug cell death during autumn and cell recruitment in spring in the olfactory bulbs and dentate gyrus do not substantially contribute to the overall chance in hippocampus mass (Bartkowska et al. 2008). Adult neurogenesis in certain brain regions is a common process in mammals, but the rate of cell proliferation varies between species (Amrein 2015). Sorex shrews are an exceptional case with no adult neurogenesis in the dentate gyrus, in contrast with most of mammals (Bartkowska et al. 2008). Other brain regions have not yet been investigated for adult neurogenesis

Table 2.2. Mean values derived from neuron morphology during the three age stages, mean differences between stages and probabilities (P) of the difference from summer juveniles to winter subadults (s-w) and from winter subadults to spring-summer adults (w-a) as calculated from the posterior distributions.

	Summer juvenile		Winter subadult		Spring-summer adult					
	Mean ± SD	95% CrI	Mean ± SD	95% CrI	Mean ± SD	95% CrI	Difference summer-winter	P(s-w)	Difference winter-adult	P(w-a)
Somatosensory cortex										
dendrite length (µm)	2148 ± 418	1882/2409	2155 ± 660	1884/2425	2199 ± 595	1934/2463	7 (0.3%)	0.38	44 (2.0%)	0.46
dendr. volume (µm^3)	1498 ± 477	1214/1782	1433 ± 554	1145/1732	1410 ± 483	1127/1700	-65 (-4.3%)	0.62	-23 (-1.6%)	0.54
soma size (µm^2)	155 ± 36	137/172	127 ± 29	109/146	136 ± 32	119/153	-28 (-17.7%)	0.99	9 (7.0%)	0.75
spine number	2736 ± 1057	2332/3134	2703 ± 964	2283/3132	2324 ± 926	1918/2727	-33 (-1.2%)	0.46	-379 (-14.0%)	0.10
spine density (n/µm)	1.2 ± 0.3	1.1/1.4	1.2 ± 0.3	1.1/1.4	1.1 ± 0.3	0.9/1.2	<0.1 (-0.1%)	0.51	-0.2 (-15.2%)	0.97
Cingulate cortex										
dendrite length (µm)	1799 ± 521	1568/2035	1645 ± 720	1415/1874	1731 ± 470	1498/1959	-154 (-8.6%)	0.83	86 (5.2%)	0.70
dendr. volume (µm^3)	1120 ± 456	916/1320	841 ± 357	637/1041	965 ± 282	756/1174	-279 (-25.0%)	0.97	124 (14.8%)	0.80
soma size (µm^2)	171 ± 42	151/191	153 ± 32	133/173	137 ± 23	116/158	-18 (-10.4%)	0.89	-17 (-10.9%)	0.87
spine number	1912 ± 689	1519/2307	1965 ± 1365	1562/2366	2028 ± 821	1623/2435	53 (2.8%)	0.57	63 (3.2%)	0.58
spine density (n/µm)	1.1 ± 0.3	1.0/1.2	1.1 ± 0.4	1.0/1.3	1.2 ± 0.3	1.0/1.3	<0.1 (3.6%)	0.34	<0.1 (3.3%)	0.34
Caudoputamen										
dendrite length (µm)	2324 ± 672	2003/2645	2013 ± 453	1688/2342	1817 ± 549	1494/2137	-311 (-13.4%)	0.91	-196 (-9.7%)	0.80
dendr. volume (µm^3)	1725 ± 543	1472/1978	1348 ± 405	1093/1604	1157 ± 309	899/1412	-377 (-21.9%)	0.98	-191 (-14.2%)	0.98
soma size (µm^2)	114 ± 37	98/131	91 ± 21	74/108	82 ± 9	65/99	-23 (-20.2%)	0.97	-9 (-10.1%)	0.78
spine number	3243 ± 1105	2644/3842	2453 ± 930	1859/3060	2038 ± 503	1444/2637	-790 (-24.4%)	0.97	-415 (-16.9%)	0.83
spine density (n/µm)	1.4 ± 0.3	1.2/1.6	1.2 ± 0.3	1.0/1.4	1.2 ± 0.2	1.0/1.3	-0.2 (-15.0%)	0.94	-0.05 (-3.9%)	0.64

in shrews. But based on previous knowledge, the presence of adult neuron recruitment in other regions except potentially the olfactory bulb is unlikely (Amrein 2015). Volumetric changes are more likely to occur in the neuropil, which makes up the space between cells (Spocter et al. 2012). Finally, mammalian brains show high variation in white matter over the lifetime (Marner et al. 2003). Therefore future research should assess the seasonal variation in axonal innervation and the energetically costly myelin. If Dehnel's Phenomenon is an energy saving process, myelin would be expected to decrease in winter. Nonexclusively, in a less energetically demanding tissue, we would predict a decrease in the circulatory system to transports nutrients. Thus, we might observe a decrease in the density of microvessels, which may also impact tissue volume (Farkas and Luiten 2001).

Natural processes of neuron shrinkage are not uncommon over the course of ontogeny. During early development of most regions in the vertebrate nervous system there is a phase of initial overproduction of dendrites and synapses, followed by a period of elimination of surplus connections in an activity-dependent manner (Cowan et al. 1984; Clarke 1990). But this refinement phase often takes place at a perinatal stage in mammals, shortly after birth. In shrews, brain shrinkage is postnatal and lasts seven months, which constitutes half of their life span (Pucek 1970; Churchfield 1990). Dendritic and synaptic elimination do not seem to contribute significantly to the overt volumetric changes that are observed within brain regions in these shrews. There are also abundant instances of brain tissue deterioration in senescent mammals, including humans (Raz et al. 2005). This aging decrease in tissue volume correlates with neuron atrophy, which is caused by a decrease in soma size, decrease in dendritic arbors and spine numbers, both in the neocortex and hippocampus (Geinisman et al. 1978; Anderson and Rutledge 1996; Smith et al. 1999; Dickstein et al. 2007). Such changes in neuron morphology have been linked to a non-pathological decrease in cognitive performance during ageing in mammals (Duan et al. 2003; Burke and Barnes 2006). These cellular and cognitive changes might be comparable to the seasonal differences that we found between summer juveniles and winter subadults in brain morphology and cognition (Lázaro et al. 2018). Winter subadults performed more poorly than summer juveniles and adults in a learning test (Lázaro et al. 2018).

The seasonal brain shrinkage and regrowth that we describe result in a dramatic change in size and reorganization of neuroanatomy. Future research on these seasonal brain changes in shrews may leads towards biological and medical applications. The reversibility and rapid remodeling of brain tissue architecture makes the common shrew an optimal model for studying possible mechanisms to invert degenerative processes in the nervous system. Furthermore, the connection of brain structure with environmental fluctuations can provide with insights into the ecological pressures that shape the development and evolution of the mammalian brain.

Ethical approval. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All handling and sampling methods were approved by the Regierungspräsidium Freiburg, Baden-Württemberg (35-9185.81/G-11/21). This article does not contain any studies with human participants performed by any of the authors.

Acknowledgements

We thank Irmgard Amrein and Jan Taylor for their valuable input to the design of the study; and Alexander Bruttel, Sebastian Rikker, Lara Keicher, Cheryl Stimpson, Sebastian Stockmaier and Julian Wanner for their help in the field and laboratory.

Chapter 3

Cognitive skills of common shrews (*Sorex araneus*) vary with seasonal changes in skull size and brain mass

Abstract

In a rare phenomenon, shrews and few other species cope with seasonal environments by reducing and regrowing brain size, potentially at the cost of changes in cognitive abilities. Here, we confirm an extensive, seasonal shrinkage (21.4%) and regrowth (17.0%) of brain mass in winter and spring, respectively, in common shrew (*Sorex araneus* L.) in Southern Germany. In a spatial learning task experiment individuals with reduced winter brain size covered larger distances to find food, compared to the relatively large brained summer juveniles and regrown spring adults. By reducing their brain mass, these shrews may reduce their energetic demands, but at the cost of cognitive performance, implying a complex trade-off for coping with seasonally fluctuating resources. These results are relevant for our understanding of evolution and the dynamics of mammalian nervous systems in response to environmental changes.

Introduction

Organisms have developed a wide range of strategies to cope with seasonal fluctuations in resources and temperature, using a combination of changes in their morphology, physiology and behavior (Demas et al., 2010). During winter, vertebrates use two main strategies: migration or hibernation. These are usually combined with morphological adaptations such as improving insulating tissues, or changes in behavior including food storing (see below) or communal thermoregulation (Merritt and Zegers, 2014). This can then be associated with seasonal changes in certain cognitive capacities and the brain structures responsible for those skills (Sherry, 2006).

Seasonally food-storing birds and mammals such as black-capped chickadees (*Poecile atricapillus* L.; Smulders et al., 1995), Richardson's ground squirrels (*Urocitellus richardsonii* Sabine; Burger et al., 2013) and grey squirrels (*Sciurus carolinensis* Gmelin; Jacobs and Liman, 1991; MacDonald, 1997) exhibit elevated spatial cognitive capacities during the caching season along with a reversible increase of hippocampus size, a brain region responsible for spatial memory and learning (Burger et al., 2013; Lavenex et al., 2000b; Sherry et al., 1992). Similarly, voles (*Myodes glareolus* Schreber, *Microtus* spp.) and mice (*Peromyscus* spp.) exhibit improved spatial learning capacities along with an increase in hippocampal size during the mating season, when their home ranges increase as they search for mates (Galea et al., 1994; Jacobs et al., 1990; Pyter, 2005; Pyter et al., 2006; Workman et al., 2009; Yaskin, 2013). In birds, this increased ability to cache and find food coincides with higher rates of adult neurogenesis and increased hippocampal cell numbers (Smulders et al., 2000; Tramontin et al., 1998). In contrast, in the mammals studied to date, hippocampus size changes are not reflected in cell numbers (Barker et al., 2003; Lavenex et al., 2000a).

In rare cases plastic seasonal adaptations at the morphological and behavioral level can be even more pronounced, i.e. in species that are unable to migrate or hibernate, such as some red-toothed shrews (*Sorex* spp.) and small mustelids (*Mustela* spp.; Dechmann et al., 2017; LaPoint et al., 2017). The red-toothed shrews, in which this has been most intensively studied, occur in some of the most variable environments, including the arctic (Mackiewicz et al., 2017), despite their extremely small body size and high metabolic rate (Ochocińska and Taylor, 2005). They have developed a remarkable strategy to cope with harsh winter conditions: they reduce their overall body size, including the brain, several other organs, as well as skeletal elements and spine length, presumably to minimize absolute metabolic demands when conditions are most harsh and thus increasing their chance of survival (Pucek, 1965a; Pucek, 1970; Saure and Hyvärinen, 1965; Taylor et al., 2013). They then regrow in spring as they become sexually mature just before their single lifetime reproductive period. In our study species, the common shrew (*Sorex araneus* L.), the winter decrease in skull size and correlated decrease in brain mass is up to 20%, followed by partial regrowth of 15%, a process known as Dehnel's phenomenon (Bielak and Pucek, 1960; Dehnel, 1949a). To our knowledge, this is the most extreme individual reversible change of total brain size reported in a vertebrate, but whether it is linked to changes in cognitive abilities is unknown.

Shrews are efficient hunters and seem to incorporate memory of their previous foraging paths (Barnard and Brown, 1985; Pierce, 1987; Saarikko, 1989). The seasonal change in brain size has never been considered in these experiments however, and in fact summer shrews performed poorly in an associative learning task (Page et al., 2012). Their smaller winter territories coincide with the reduced brain size, and in spring both their territories and their brains expand in size (Stockley and Searle, 1998). Reduced home ranges have been associated with lower spatial capacities during winter in other species (see above) which would predict lower spatial skills in shrews during winter (Yaskin, 2011). Winters' reduced spatial cognition demands may allow individuals to reduce the size of their energetically demanding brain at this time. Several brain regions may be linked to integrative information, especially the neocortex and the thalamus show the most pronounced winter decrease (27% in volume each; Yaskin, 1994). There is also a winter decrease followed by a spring regrowth in the hippocampus (Yaskin, 1994). This has been hypothesized as a link between spatial cognition and home range size (Yaskin, 2005; Yaskin, 2011).

We designed a spatial learning test to quantify seasonal changes in shrew cognitive abilities as determined from their ability to use a reference cue to find food. We aimed to 1) describe the seasonal pattern reported in previous studies in skull size and brain mass from year-round sampling in our population of common shrews from Southern Germany; and 2) compare the spatial cognitive skills between summer juveniles, wintering individuals and spring adults in a reference cue test. We predicted that brain size in shrews is linked to cognitive ability and thus the seasonal changes in brain size would lead to decreased cognitive abilities in the winter. This study is a first step towards understanding the cognitive consequences of Dehnel's phenomenon (Yaskin, 2011).

Materials and Methods

Trapping and processing of specimens. All handling and sampling methods were approved by the Regierungspräsidium Freiburg, Baden-Württemberg (35-9185.81/G-11/21, 35-9185.81/G-14/28). We trapped shrews in Möggingen Germany (47°46'04.70"N, 8°59'47.11"E) between August 2013 and October 2015 with wooden live-traps (PPUH A. Marcinkiewicz, Rajgród, Poland) baited with mealworms (*Tenebrio molitor* L.) and checked at $\leq$ 2 hr intervals. To extract the skulls and brains we anesthetized shrews with isoflurane and then increased the flow rate to lethal levels (*N* =58 males, 35 females, 13 undetermined sex). We placed sacrificed shrews in a laboratory colony of *Dermestes maculatus* (De Geer) to obtain clean skeletons. We captured shrews for the behavioral test from the same study area using the same capture methods.

For age determination, we classified the individuals as immature juveniles or adults based on the development of testes or mammary glands, tooth wear, and fur appearance (Churchfield, 1990; Pankakoski, 1989). In immature shrews we determined the sex using a PCR-based gonosomal sexing method (C. Roos, DPZ Göttingen, unpublished data). DNA was extracted from tail tip samples using Qiagen’s DNAeasy kit (Qiagen, GmbH, Hilden). We lacked sufficient material to sex all immature individuals.

Skull and brain measurements. We recorded the following measurements from cleaned skulls: skull length (SKL), from the anterior most projection of incisor 1 to the occipital condyle; braincase width (BCW), the greatest lateral diameter of the braincase; and braincase height (BCH), from the tympanic rings to the dorsal surface of the braincase. All linear measurements were taken with digital calipers to the nearest 0.01 mm, except tooth row length (see below). To obtain brain mass we weighed the fresh extracted brains with an electronic scale (Kern & Son, AES 200-4cm, Balingen, Germany) to the nearest 0.001 g. We size-corrected all skull measurements and brain mass by the non-changing maxillary tooth row length (i.e., $\mathrm{BCH_{cor}}$, $\mathrm{BCW_{cor}}$, $\mathrm{SKL_{cor}}$ and brain $\mathrm{mass_{cor}}$) (Lázaro et al., in press). The tooth row length is defined as the distance from the uppermost edge between the premaxilla and incisor 1 to the basal junction of molars 2 and 3 and was measured via digital X-ray images of the skulls in lateral view using Image-J (Schneider et al., 2012). To obtain the X-ray images, the skulls were placed on a wooden base which allowed standardized position for all individuals in lateral view. We used a Faxitron MX 20 cabinet (26 kV, 6 sec), an OPG Imaging Plate (Gendex) and a scanner (DenOptix /Gendex). All measurements were taken by a single observer (JL).

Skull measurement error. We estimated the technical error of measurement (Harris and Smith, 2009) based on repeated measurements of ten extracted skulls. The same observer re-measured each individual in five independent sessions. We quantified the technical error of measurement as the within-subject standard deviation based on these repeated measurements. This gives 95% confidence intervals for repeatability error in normally distributed data (Harris and Smith, 2009) (Table C.1).

Behavior test. For the behavior tests, we trapped different individuals during the seasons of peak sizes as follows: large summer juveniles in August 2015 (N = 8, sex undetermined); size-decreased winter subadults in January-February 2015(N = 2 males, 2 females, 4 undetermined); regrown spring adults in April-May 2015 (N = 1 female, 6 males). Shrews used in behavior tests were allowed to recover from capture and habituate to captivity in a double-cage system for one day. One cage (38x25x30 cm) contained natural soil, a running wheel, hay for bedding, an inverted terracotta flowerpot, and *ad libitum* water. This cage was connected to the second, equally sized, cage via a plastic tube and contained a food dish and a thin layer of sand as substrate to familiarize the shrews with the substrate of the experimental arena (see “Behavior test” section). The experimental room was illuminated with the natural light/dark regime; the temperature was 18°C. Although temperature might have an effect on the test performance, carrying out all tests at the same temperature allowed for standardized conditions in all animals. The food was a mixture of meat, earthworms and mealworms (Searle, 1984). Pregnant females were not included. Immediately after completion of ten trials, we marked individuals with subcutaneous passive integrated transponders (7x1.5mm , UNO PICO) and we released them at the site of capture. Note that we caught new individuals during every season.

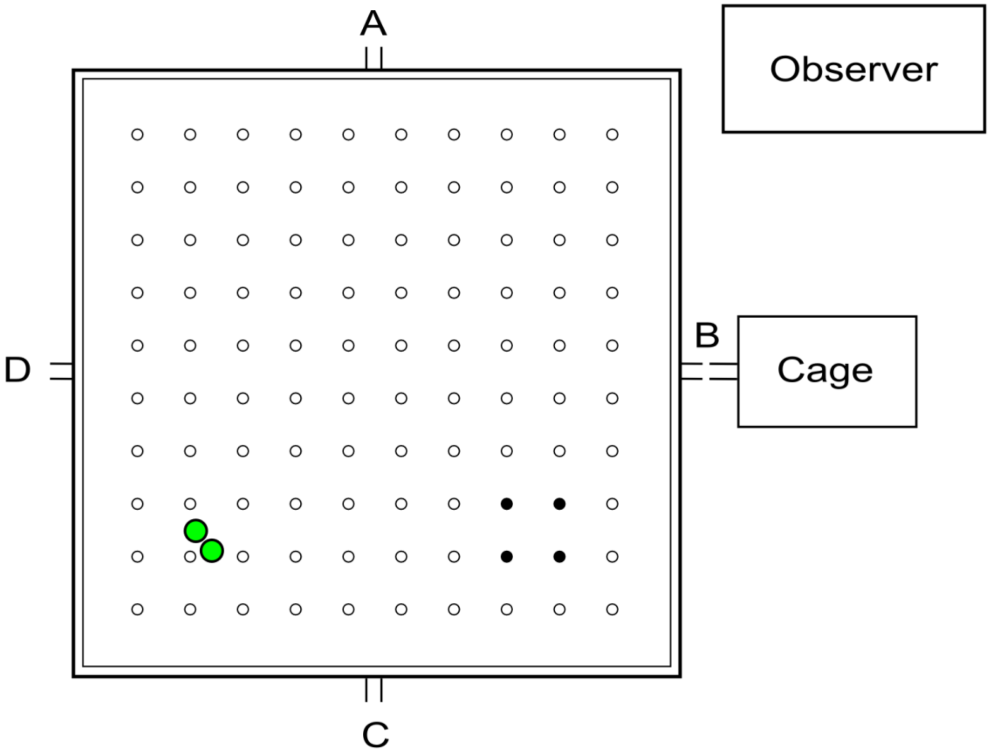

Figure 3.1. Experimental setting for behavioral tests. Open circles represent empty wells; green circles indicate the location of the light cue and black circles indicate the wells with food items; the four entrances are located at the cardinal points (A, B, C, D). The cage was connected to a randomly chosen one during each trial. See text for details.

We deprived shrews of food for two hours prior to testing. The tests started at 5 pm for all individuals and then lasted varying amounts of time depending on each individual's performance. The experimental setup was a square arena (110 by 110 cm) (Fig. 3.1). The floor of the arena was a flat surface covered with compressed sand into which 100 wells (ø 18 mm, depth 15 mm) were pressed with a convex template. The arena was symmetrical except for the placement of the food and cues. Four adjacent wells, located at the southeast corner, contained one immobile mealworm pupa each. The arena sand was sprinkled with mealworm bedding material to ensure mealworm odor was distributed evenly. We used a pair of small, green glow sticks (35 mm long; Suxxes-Fisherman’s Partner, Germany) near the southwest corner as reference cues. This spatial arrangement remained unaltered throughout all trials. The arena was covered by a transparent acrylic glass plate ceiling, creating a 2.4 cm high space between the sand surface and the transparent ceiling. It was surrounded by 65 cm-high uniformly painted oriented strand board walls on each side, as sight barriers. Thus, shrews could move around the arena freely, but remained in constant contact with the ceiling or walls as they avoid open spaces in natural habitat. The arena had four entrances, one at the center of each of the four sides: for reference henceforth called A, B, C and D (Fig. 3.1). At the beginning of each trial we attached the habituation cage to an entrance, allowing the shrew to freely enter the arena through a connecting tube. The remaining entrances were blocked. We generated a random sequence of entrances for the 10 trials and then always used this sequence. The sequence was: D, B, A, B, D, C, B, A, C, A. The trial ended when the shrew found the first food item. Each individual was subjected to 10 consecutive trials at 20 minute intervals. If the shrew had not found the food within three hours after a trial had started we would have cancelled the trial to avoid risk of starvation, but that limit was never reached. Before and between trials we rinsed all parts of the arena with an alcohol-based window cleaner and mixed the sand to break up olfactory trails. We used fresh cues and food items in each trial. We replaced the sand between individuals.

The trials were carried out in a room illuminated by a faint red led light bulb and the arena was directly illuminated with an infrared light. We recorded the complete trials with a consumer video camera sensitive to near infrared light (Somikon PX8262675, Buggingen, Germany) and one observer was always present, sitting in the same position (Fig. 3.1). The movement paths were extracted blind to the trial and shrew using the tracking software "Tracker V 4.87" (Brown, 2009) by a single observer (MS). Path lenghts were calculated with the move package (Kranstauber and Smolla, 2016) in R version 3.2.3 (R Core Team, 2015). We also measured the search time as the time from the shrew's entrance to the arena until it finds the first food item.

Statistical analyses. All analyses were performed in R (R Core Team, 2015). To quantify the seasonal patterns in skull dimensions and brain mass we used the seasonal non-linear predictions of the change for our variables of interest (i.e., BCH_{cor},, BCW_{cor}, SKL_{cor} and brain $mass_{cor}$) to formulate our *a priori* expectations. We used generalized additive models (GAM) (Wood, 2006) with sex as a parametric term and an age-corrected Julian capture day of the year (*jday*) as a non-parametric term to quantify potential nonlinear patterns. For calculating *jday* for the juveniles, we shifted June 1st of year one (the year when the shrew is born) to *jday* = 1 until May 31st of the following year (*jday* = 365). For adults, *jday is* 365 + *jday* of year two (adults are trapped in their year two). For example, a juvenile captured on October 12th, *jday* = 134. Whereas, an adult captured on July 19th, *jday* = 413.We used a Gaussian distribution and added a smoothing function to *jday* that included 5 knots in a thin plate regression spline. We restricted the number of knots to identify the *a priori* pattern while reducing the risk of model overfitting. We ran this initial GAM on both skull measurements and brain mass data for individuals with known sex to verify there was no sexual dimorphism in the patterns. Then, we ran the models again with combined sexes. GAMs were performed using the R package *mgcv* (Wood, 2015).

To assess seasonal differences in food searching behavior during the tests we calculated search path length as path efficiency ratio, i.e. path length divided by the straight-line distance between the entrance and the central point between the four food wells in each trial. Henceforth we will refer to it as search path length. We used linear mixed-effects models to analyze the search path length (log transformed to obtain a Gaussian distribution) and compared these models via analysis of variance. In the first model (M1) we treated *trial* and *season* as factors and included *individual* as a random effect. We removed *season* from the second model (M2) to assess its influence and compared both models by analysis of variance. We then ran a third model (M3) with a *trial* and *season* interaction term. We analyzed the variation in log transformed search time using the same method as with search path length. We used the three models and ANOVA comparisons as described above, replacing the response variable for search time, i.e. M4, M5 and M6, respectively. Linear mixed-effects models were performed with the R package *lme4* (Bates et al., 2014).

Results and discussion

Seasonal variation in skull size and brain mass. Our results confirm decreases in both skull size and brain mass from summer juveniles to winter subadults and regrowth in spring adults. We found this seasonal pattern in each of the four analyzed metrics (BCHcor, BCWcor, SKLcor and brain masscor), although the seasonal pattern in BCWcor and SKLcor was less pronounced than in BCHcor (Fig. C.1, C.2). The focal region of change is therefore the post-rostral cranium, of which the braincase forms the largest portion. Thus we will focus the presentation of our results and discussion on fluctuations in brain masscor and BCHcor which shows the strongest change among skull size parameters (Fig. 3.2A).

In accordance with previous studies (Bielak and Pucek, 1960; Pucek, 1970) we found no significant difference in BCHcor or brain masscor between sexes (BCHcor: GAM, $n = 85$, males estimate = 0.838929, females estimate = 0.836018, $P > 0.1$; brain masscor: GAM, $n = 71$, males estimate = 0.0332797, females estimate = 0.0329003, $P > 0.1$). The sexes also did not differ when we included an interaction between sex and jday (BCHcor: GAM, $n = 85$, e.d.f.(females) = 2.354, e.d.f.(males) = 3.865, P(smooth t.) < 0.001, deviance explained = 67.4%; brain mass: GAM, $n = 71$, e.d.f.(females) = 2.120, e.d.f.(males) = 3.384, P(smooth t.) < 0.01, deviance explained = 44.5%).

The final model for BCHcor (Fig. 3.2B) indicated a significant non-linear pattern matching our a priori seasonal predictions (GAM, $n = 98$, e.d.f. (smooth term) = 3.847, $P < 0.\ 001$, deviance explained = 68.4%). The average decrease in BCHcor from July

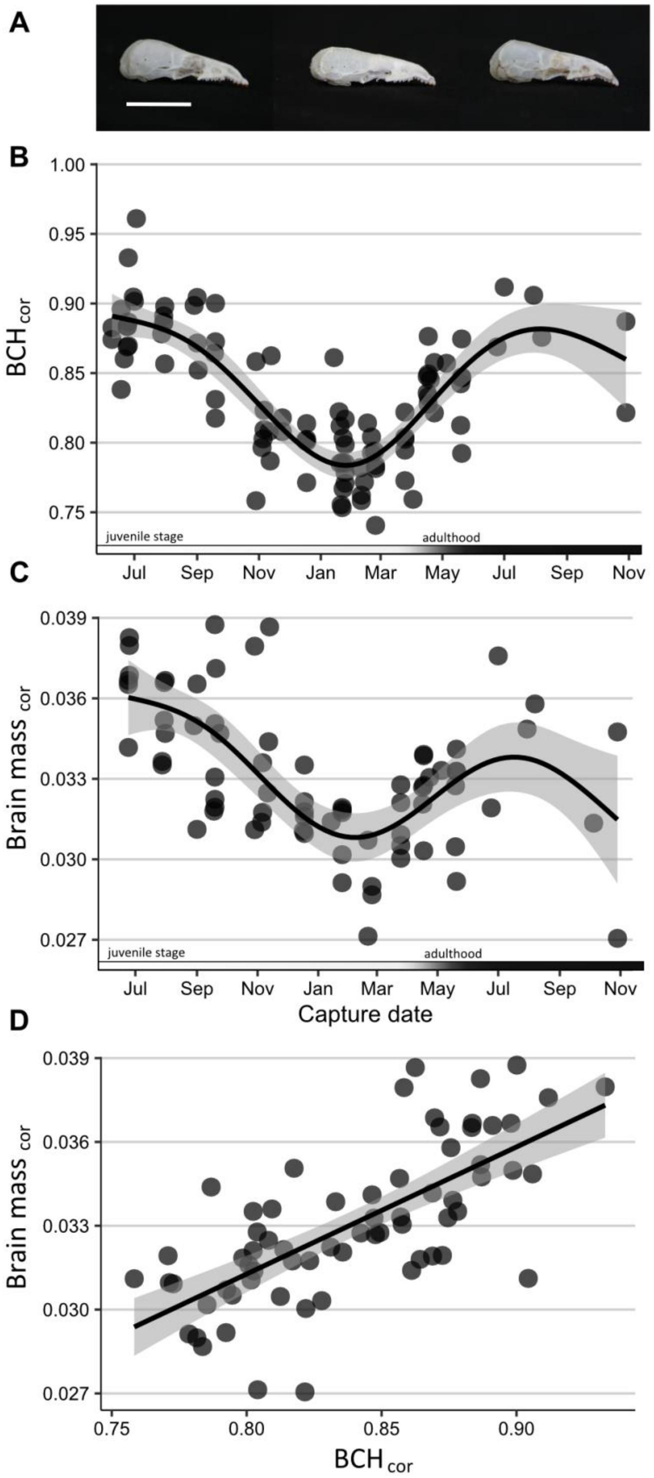

Figure 3.2. Seasonal pattern in relative skull height (BCHcorr) and brain mass from post-mortem specimens. A, Three representative skulls from the main stages of the cycle (from left to right): July-juvenile, size-decreased February-subadult, and regrown May-adult. White scale bar is 10 mm. **B**, BCHcor over time with fitted GAM. Solid line and shaded area represent fitted values and standard error of the model. The shaded x-axis indicates the two main age life stages. **C**, Brain mass over time with fitted model as depicted in (B). (D) Correlation between corrected brain mass and BCHcor with fitted regression line and 95% confidence intervals ($P < 0.001$, adj. $r2 = 0.48$).

juveniles to February subadults was -11.9% and the regrowth to adults (June -August) was +12.9%. Previous studies used BCH as a proxy metric for overall braincase capacity and brain mass (Bielak and Pucek, 1960). Our results corroborate this correlation between BCHcor and brain masscor (Fig. 3.2D, Pearson's product-moment correlation, $t = 8.0959$, $P < 0.001$, $r = 0.70$). Brain masscor also exhibited significant non-linearity across seasons (Fig. 3.2C, GAM, $n = 72$, e.d.f. (smooth term) = 3.862, $P < 0.001$, deviance explained = 44.4%) which included a mean decrease of -21.4% from July to February and a regrowth of 17.0% to summer (June-August). We thus confirm that our study population exhibits this large postnatal reversible change in brain mass. Both BCHcor and brain masscor showed a second decline in old adults during their second autumn, but sample sizes were too low for rigorous analyses.

Differences in behavioral performance. The behavior experiment revealed that most individuals attained and maintained short search paths within the first four trials, but this varied between seasons (Fig. 3.3). The comparison between the first model (M1, N (individuals) = 23, n (observations) = 225, s.d. (individual) = 0.277, F (trial) = 9.831, F (season) = 4.856, Second-order Akaike's Information Criterion (AICc) = 659.04) and the second model (M2, s.d. (individual) = 0.368, F (trial) = 9.839, AICc = 660.27) revealed a significant effect of season at the factor level (ANOVA, $P < 0.05$). The comparison of M2 with the third model (M3, s.d. (individual) = 0.275, F (trial) = 9.873, F (season) = 4.897, F (interaction) = 1.056, AICc = 675.88) revealed no significant effect of season at the interaction level (ANOVA, $P = 0.07$). Large summer juveniles and regrown spring adults showed similar learning curves, while small winter animals generally exhibited longer search paths especially during the second trial. This result matches the seasonal variation in overall brain mass (Fig. 3.2C).

The results on search time revealed a learning curve for the three seasons, but the large amount of variation between and within trials made the between-season differences less clear (Fig. C.3). Similar to path length, the comparison between the first model for search time (M4, N (individuals) = 23, n (observations) = 225, s.d. (individual) = 0.076, F (trial) = 8.362, F (season) = 3.409, AICc = 344,97) and the second model (M5, s.d. (individual) = 0.109, F (trial) = 8.364, AICc = 340.30) revealed a significant difference between models (ANOVA, $P < 0.05$), but the AICc values indicate M5 (without season) as preferred model. The comparison of M5 with the third model (M6, s.d. (individual) = 0.080, F (trial) = 8.488, F (season) = 3.414, F (interaction) = 1.164, AICc = 386.36) revealed no significant effect of season at the interaction level (ANOVA, P = 0.08).

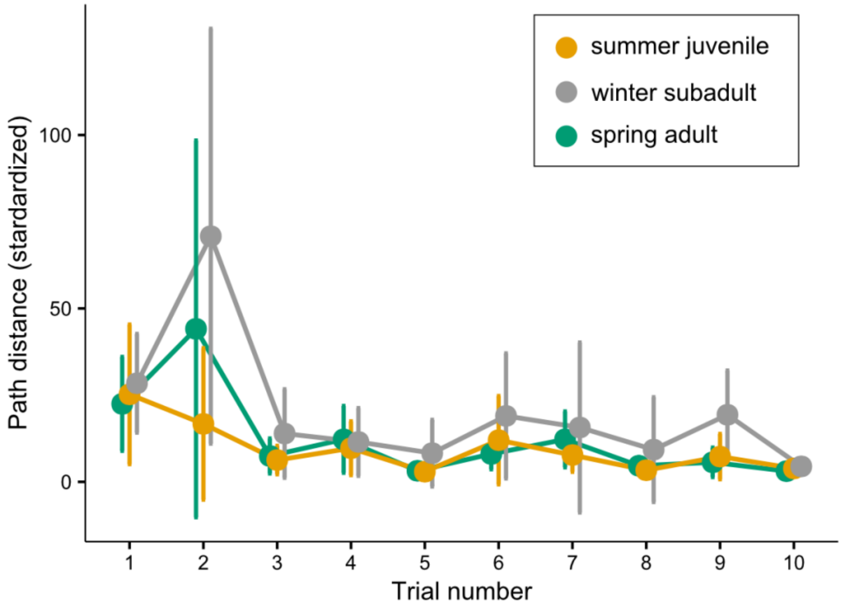

Figure 3.3. Learning performance of individuals across three seasons. Small winter subadults (N = 8 individuals) used longer search paths than large summer juveniles (N = 8) and regrown spring adults (N = 7) to find food. Circles and bars represent average distance (standardized by distance from the entrance to the food) ± s.d.

We postulate that the spatial learning impairment in winter is a direct consequence of the decrease in brain size and changes in brain internal structure. In particular, it might be linked to the winter shrinkage observed in specific brain regions as the neocortex and hippocampus (Yaskin, 1994) that process cue integration and spatial cognition, as it has been observed in other mammals (Martin et al., 2017; Sherry et al., 1992). A size decrease of the hippocampus may affect not only spatial cognition but also the capability of object recognition which is crucial in a cue-based test (Martin et al., 2017). As we performed our trials with different animals during each season this can clearly be distinguished from a memory loss. In contrast, the least shrew Cryptotis parva, which does not exhibit the seasonal change in brain mass, does not show a seasonal pattern in spatial learning abilities, but a steady decline associated with senescence (Punzo and Chavez, 2003). However, the neurological mechanisms underlying the observed cognitive differences remain unclear (Bartkowska et al., 2008).

Reducing energetically expensive tissue such as the brain (Niven and Laughlin, 2008) might provide a survival advantage during the period of resource scarcity (Kotrschal et al., 2013; Pucek, 1970). In wintering shrews this need for energy saving may outweigh the potential cognitive advantages of a large brain. The result of this trade-off translates into an acceptable cognitive impairment in the small-brained wintering shrews which might, however, cause an adaptive constraint for demanding spatial tasks such as foraging (but see below). In summer and spring this balance switches, leading to larger body and brain size as food resources increase and shrews meet more diverse spatial tasks. During the first summer, juveniles disperse and establish their own territories which are critical for wintering. This requires intensive spatial exploration and direct intraspecific competition, even displacing the old adults (Churchfield, 1990). In spring, after reaching sexual maturity they dramatically enlarge their home ranges again, meeting increased competitive and spatial demands. In this polygamous species the expansion of home ranges is especially strong in males, which increase their mobility as they search for mates (Stockley and Searle, 1998). This sexual difference in adult spatial behavior would predict higher spatial capacity in adult males than in females (Yaskin, 2005; Yaskin, 2011) as has been observed in polygamous rodents (Galea et al., 1996; Gaulin and Fitzgerald, 1989), but the exclusion of pregnant females (most females are pregnant in spring) for our tests impeded such a comparison in our study. Moreover, the general activity of all organisms is higher in spring and summer, which leads to a more dynamic environment. Productivity of organisms such as the growth and replacement of plants, or products from other animals' activity such as galleries from burrowers produce a more dynamic micro-topography. These dynamic habitat structures probably require higher explorative effort and spatial memory than in the more steady winter landscape, similar to the case of flying vertebrates in dense versus open habitats (Safi et al., 2005).

Alternatively we must also consider non-cognitive explanations, especially in these freshly trapped wild animals which were confronted with a new artificial environment during the experiments. The decrease in path length variation along the trials could be partly caused by an effect of habituation to the experimental setting and a gradual decrease in stress. Also, right after shrews enter the arena for first time, explorative movements could partially explain the longer paths in the first trials. However, it is important to notice that several individuals from the three groups increased their path length from trial 1 to 2 (Fig. C.4). This cannot be explained by explorative behavior. In winter, when productivity is lower, prey replenishment would slow down in their natural habitat. This would lead us to predict a more pronounced increase in trial 2 in the winter shrews than in other seasons in our test. However, we found no significant differences in search path between seasons in trial 2 (results Kruskal-Wallis). In fact, there is no evidence for a decline in the populations of active prey in winter according to the literature. Soil cores reveal no reduction of prey abundance in winter (Churchfield et al., 2012), but a possible reduction in prey quality. Earthworms, the main prey for shrews in summer (Churchfield et al., 2012; Pernetta, 1976), migrate into deeper soil layers and remain mostly inactive during winter (Nordström, 1975; Rundgren, 1975; but see Churchfield et al., 2012). Thus, shrews switch to other less nutritive but still active prey (e.g., beetles, snails, spiders and opilionids), some of which have autumn and winter activity peaks (Gongal'skii et al., 2003; Hågvar and Hågvar, 2011; Jaskuła and Soszyńska-Maj, 2011; Merriam et al., 1983; Pernetta, 1976).

The seasonal variations we describe illustrate a rarely investigated but highly interesting coping strategy for resource seasonality. This involves processes at the morphological, neuronal and behavioral level. The common shrew offers a unique opportunity to understand the bidirectional selective forces that shape the size and structure of the mammalian brain, not only in the same population, but within individuals. Apart from its implications in evolutionary biology, such reversible changes in brain mass will be of interest for medical research on neurodegenerative diseases and cognitive disorders related to ageing.

Acknowledgments

We thank Teague O’Mara, Alexander Steinbrecht, Anneke van Heteren and Sharon Swartz for comments on early versions of this manuscript and Marion Muturi for assistance in the field and laboratory. This project was funded by the Max Planck Society through the Max Planck - Poland Biodiversity Initiative.

General discussion

In my dissertation I aimed at elucidating a series of open questions on the seasonal changes in anatomy and behavior of the common shrew. In the first chapter I provided the confirmation that seasonal reversible changes in braincase size occur within individuals (Fig. 1.1). For my descriptions, instead of using complex techniques based on geometric morphometrics, I chose a rather straightforward approach based on linear measurements. These simple measurements were thus easily comparable with the observations reported in previous studies which gauged skull dimensions with calipers (Crowcroft and Ingles, 1959; Dehnel, 1949a; Pucek, 1963; Yaskin, 1984). Thus, my results also verify that all those excellent descriptions of seasonal anatomical changes at the population level reflect indeed changes at the individual level as they predicted.

The skull metric that revealed the most intense individual seasonal changes was braincase height. In Chapter 3, I report a high correlation between braincase height and brain mass (Fig.2.2C); in other words, braincase height is a good predictor of brain mass in shrews. This evidence is sufficient to conclude that the changes in brain mass reported here (Fig.2.2B) and in previous studies (Bielak and Pucek, 1960; Yaskin, 1994) also reflect changes within individuals.

My results are also the first evidence of Dehnel phenomenon in Southern Germany. The presence of seasonal morphological effects at this location is interesting because seasonality is less severe here than in the study locations of previous works, where they measured the phenomenon at higher latitudes. In fact, the shrink/regrow phases in braincase height that I report here (Fig. 1.1, 3.2) are less pronounced than in northeastern Poland (-17.7, +12.8%) (Dehnel, 1949a) and the Moscow region (-17.8,

+14.6%) (Yaskin, 1994).Some authors have described a pattern in the geographical variability of Dehnel phenomenon, at least in Europe, being more intense in northern and eastern populations than in southern and western ones (Churchfield, 1990; Pucek, 1963; Pucek, 1970; Pucek and Markov, 1964). These differences have been attributed to climate and ecological factors, since those populations inhabiting areas with more severe winters would suffer higher limitations in prey availability during this period, so a more pronounced decrease in body size would mean an advantage. On the other hand, this prediction contradicts the classic rules of thermoregulation, which maintain that in colder environments mammals increase in size, reducing the proportional body surface and hence the heat loss – this is the most common explanation of the Bergmann's rule. However, it must be pointed out that *Sorex* shrews in fact follow the inverse to the Bergmann's rule (McNab, 2010; Ochocińska and Taylor, 2003), which is in consonance with the body size shrinkage in winter.

In Chapter 2, I quantified the seasonal changes in different brain regions separately. I concluded that different regions contribute to the change to different extents, and some regions showed different patterns for males and females (Fig. 2.2-2.5). Also I discussed few hypotheses to explain the different patterns between regions and sexes. Although a similar study was attempted in a Russian population (Yaskin, 1994), several differences arouse in the intensity of change in different brain regions between our study and Yaskin's observations. Thus, there is not only geographical variation in the change of overall brain size as discussed above, but also in the seasonal structural changes. For example, hippocampal changes seem more intense in Yaskin's report. This brings up the question whether these geographical differences respond to a general latitudinal gradient or to the adaptive demands from specific characteristics of the local habitats.

Also in Chapter 2 I attempted to quantify the seasonal differences in neuron size and morphology. The aim of this analysis was to establish some of underlying cellular changes that could explain the overall variations in tissue volume. I found a decrease in neuron soma size, dendritic length and volume, and spine number and density in the caudoputamen, which could partially contribute to the winter shrinkage in this particular brain region (Fig.2.6). In the neocortex, a region which was expected to show more drastic changes, only the anterior cingulate cortex showed a subtle shrinkage in dendrites, plus a decrease in soma size in both cingulate and somatosensory cortices (Fig. 2.6). These results do not however discard that changes in neuron size do not contribute to the overall seasonal change. The cellular mechanisms can vary between brain regions. For example, in the canaries' song nuclei some regions change in size seasonally in correlation to cell number, while in other regions the change is associated with neuron size (Smith et al., 1999; Smulders et al., 2000; Tramontin and Brenowitz, 2000; Tramontin et al., 1998). Nevertheless, I hope this study will constitute a start point for a series of studies aiming to explore other potential causes for the seasonal tissue changes.

In Chapter 3 I also reported behavioral differences between shrews of the three cycle stages. In a spatial learning test winter shrews performed worse than summer juveniles and spring adults (Fig.2.3). Thus, the cognitive performance not only parallels overall brain mass along seasons, but also the variation in hippocampal volume reported in Chapter 2 (Fig. 2.2). As the hippocampus is responsible for navigation and spatial memory (Sherry et al., 1992), I hypothesize that the winter hippocampal shrinkage contributes to the impairment of spatial skills described in the test. Moreover, in Chapter 2 (Fig. 2.3) I reported that the hippocampal sub-region CA1 is smaller in adult females than in males. In the behavioral experiment I was not able to test females, but based on my hypothesis I predict that adult females would perform worse than adult males. As discussed in Chapter 3, this sexual dimorphism matches animals' behavior in the wild. In spring, males expand their territories and increase exploring behavior for mate searching, which probably increases the demand for spatial cognitive skills (Stockley and Searle, 1998).

My behavioral test is the first attempt to study the cognitive aspect the Dehnel phenomenon. In addition, as shrews are not usual laboratory model species and there are no standard procedures for them, the experimental setting that I and my co-authors designed can be used by other scientists interested in shrew cognition.

Overall, in my dissertation I was able to address different aspects of the seasonal changes in the skull and brain size of the common shrew. I emphasized the interest that this study case has as a unique biological system. But I also used shrews as model to tackle questions of general interest for science. I expect that this study system will become a common target for evolutionary biologists who aim to analyze the environmental forces that shape mammalian anatomy, particularly braincase and brain size and its structure. One of the several directions for future research should address the environmental factors that might trigger the seasonal changes, namely temperature, light regime and diet. Experiments in captivity under controlled conditions could clarify which factors cause or modulate the seasonal changes. Also, to analyze the proximate causes, we should attempt to identify the genetic pathways and molecule cascades that initiate and control the underlying physiological process. Additional histological studies will be also required to characterize the tissue micro-structural changes in both brain and bone. Moreover, although my behavioral experiment provides a first hint on the seasonal effects on spatial learning, more tests could target different cognitive aspects of the seasonal brain changes. This would broaden the knowledge on the consequences of Dehnel phenomenon on cognition. Additionally, this laboratory experiments should be complemented by field studies to analyze the movement patterns of shrews in the wild. Quantifying the extent and complexity of movement in natural conditions would help understating how the seasonal cognitive changes would mean an adaptation – or a constraint – to the environment.

Acknowledgements

This project would have been impossible without Dina. Thank you for enable me to be part of your team and for your guidance and support along all these years. Also to Moritz, thank you for your wise advice in every moment. Thanks to your patient supervision I have learnt a lot from both of you, and not only science.

To Martin, thank you also for giving me this opportunity and for your support all these years.

A big thank goes also to Chet. Thank you and your team for welcoming me in your lab at the George Washington University. That was a great experience.

I am also grateful to Jan for sharing his knowledge on shrew biology, as well as the wonderful field station of the Białystok University. I will keep great memories of our trapping days in the Biebrza marshes. Also thank you Jan, Dina, Moritz and Chet for being part of my PAC.

I could not have accomplished all the work without Marion. Thank you for all your help in the field and in the laboratory. Also thank to all students and assistants who helped me in the field and in the lab: Alex, Sebas, Bethany, Matthias, Julian, Lara, Karla, Toni, Basti, Hans, Jake, Jacob and Jasmine. And thanks to the animal caretakers and technicians for all their hard work.

I have received so much help from colleagues and collaborators during the project. Scott, I have learnt a lot from your mathematical and critic view. Sophie, thank you for all the discussions about behavior and ecology, it was so nice to have you around from time to time. Yann, thank you for making the long office days more pleasant. Thanks to all Kami's and Dina's labs members for all the great meetings.

I also want to thank the IMPRS, it was great to be part of it and to meet all those wonderful colleagues. Mäggi, thank you for all your help.

To all the staff at the Max Planck Institute for Ornithology in Radolfzell. I feel truly lucky for working in such a nice atmosphere. And to MaxCine. Babette, thank you for making me part of it.

To my parents and my sister. You have shown me the way to grow. You have supported my whole career and encouraged me in all my decisions, even if that means living thousands of kilometers away from you.

And Monia. You have shown me the way to go. Thank you for being there in the happy moments as well as the hard times. Thank you for all your support and understanding in all situations. Thank you.

Author contributions

Chapter 1. Profound reversible seasonal changes of individual skull size in a mammal

Javier Lázaro: performed all the data collection and most of the analyses, designed the study and wrote the paper.

Dina K. N. Dechmann: conceived the project, designed the study and wrote the paper.

Scott LaPoint: supervised and performed statistical analyses.

Martin Wikelski: conceived the project and contributed to discussion.

Moritz Hertel: conceived the project, designed the study and wrote the paper.

Chapter 2. Profound seasonal changes in brain size and architecture in the common shrew.

Javier Lázaro: designed the study, performed the neuron tracings, performed the data analyses and wrote the paper.

Moritz Hertel: conceived the project and designed the study.

Chet C. Sherwood: designed the study and wrote the paper.

Marion Muturi: performed the 3D brain reconstructions.

Dina K. N. Dechmann: conceived the project, designed the study and wrote the paper.

Chapter 3. Cognitive skills of common shrews (*Sorex araneus)* vary with seasonal changes in skull size and brain mass

Javier Lázaro: performed most of the data collection and analyses, designed the study and wrote the paper.

Moritz Hertel: conceived the project, designed the study and wrote the paper.

Scott LaPoint: conceived the project, supervised and helped perform statistical analyses.

Martin Wikelski: conceived the project and contributed to discussions.

Matthias Stiehler: processed the video data and ran tests during two of the seasons.

Dina K. N. Dechmann: conceived the project, designed the study and wrote the paper.

References

Aiello, L. C. and Wheeler, P. (1995). The Expensive-Tissue Hypothesis. *Curr. Anthropol.* **36**, 199–221.

Amrein, I. (2015). Adult hippocampal neurogenesis in natural populations of mammals. *Cold Srping Harb Perspect Biol* **7**, a021295.

Anderson, B. and Rutledge, V. (1996). Age and hemisphere effects on dendritic structure. *Brain* **119**, 1983–1990.

Barker, J. M., Boonstra, R. and Schulte-Hostedde, A. I. (2003). Age determination in yellow-pine chipmunks (*Tamias amoenus*): a comparison of eye lens masses and bone sections. *Can. J. Zool.* **81**, 1774–1779.

Barker, J. M., Wojtowicz, J. M. and Boonstra, R. (2005). Where's my dinner? Adult neurogenesis in free-living food-storing rodents. *Genes, Brain Behav.* **4**, 89–98.

Barnard, C. J. and Brown, C. A. J. (1985). Risk-sensitive foraging in common shrews (*Sorex araneus* L.). *Behav. Ecol. Sociobiol.* **16**, 161–164.

Barnea, A. and Nottebohm, F. (1994). Seasonal recruitment of hippocampal neurons in adult free-ranging black-capped chickadees. *Proc. Natl. Acad. Sci. U. S. A.* **91**, 11217–11221.

Barnea, A. and Nottebohm, F. (1996). Recruitment and replacement of hippocampal neurons in young and adult chickadees: an addition to the theory of hippocampal learning. *Proc. Natl. Acad. Sci. U. S. A.* **93**, 714–718.

Bartkowska, K., Djavadian, R. L., Taylor, J. R. E. and Turlejski, K. (2008). Generation recruitment and death of brain cells throughout the life cycle of Sorex shrews (*Lipotyphla*). *Eur. J. Neurosci.* **27**, 1710–1721.

Bates, D., Mächler, M., Bolker, B. M. and Walker, S. C. (2014). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48.

Bielak, T. and Pucek, Z. (1960). Seasonal changes in the brain weight of the common shrew (*Sorex araneus* Linnaeus: 1758). *Acta Theriol.* **13**, 297–300.

Bolhuis, J. J. and Macphail, E. M. (2001). A critique of the neuroecology of learning and memory. *Trends Cogn. Sci.* **5**, 426–433.

Brenowitz, E. a, Nalls, B., Wingfield, J. C. and Kroodsma, D. E. (1991). Seasonal changes in avian song nuclei without seasonal changes in song repertoire. *J. Neurosci.* **11**, 1367–1374.

Brown, D. (2009). Tracker video analysis and modeling tool. *Open source, Phys.* **4**,.

Buchalczyk, T. and Ruprecht, A. L. (1977). Skull Variability of *Mustela putorius* Linnaeus, 1758. *Acta Theriol.* **22**, 87–120.

Bullmore, E. and Sporns, O. (2012). The economy of brain network organization. *Nat. Rev. Neurosci.* **13**, 336–349.

Burger, D. K., Saucier, J. M., Iwaniuk, A. N. and Saucier, D. M. (2013). Seasonal and sex differences in the hippocampus of a wild rodent. *Behav Brain Res* **236**, 131–138.

Burke, S. N. and Barnes, C. A. (2006). Neural plasticity in the ageing brain. *Nat. Rev. Neurosci.* **7**, 30–40.

Caboń, K. (1956). Untersuchungen über die saisonale Veränderlichkeit der Gehirnes bei der kleinen Spitsmaus (*Sorex minutus minutus* L.). *Ann. Univ. Marie Curie-Sklodowska, Sect. C* **10**, 93–105.

Catania, K. C. (2000). Cortical organization in insectivora: the parallel evolution of the sensory periphery and the brain. *Brain. Behav. Evol.* **55**, 311–21.

Catania, K. C., Lyon, D. C., Mock, O. B. and Kaas, J. H. (1999). Cortical organization in shrews: evidence from five species. *J. Comp. Neurol.* **410**, 55–72.

Charvet, C. J., Striedter, G. F. and Finlay, B. L. (2011). Evo-devo and brain scaling: candidate developmental mechanisms for variation and constancy in vertebrate brain evolution. *Brain. Behav. Evol.* **78**, 248–57.

Churchfield, S. (1981). Water and fat contents of British shrews and their role in the seasonal changes in body weight. *J. Zool.* **194**, 165–173.

Churchfield, S. (1990). *The Natural History of Shrews*. Bromley: Christopher Helm.

Churchfield, S., Rychlik, L. and Taylor, J. R. E. (2012). Food resources and foraging habits of the common shrew, *Sorex araneus*: does winter food shortage explain Dehnel’s phenomenon? *Oikos* **121**, 1593–1602.

Clancy, B., Darlington, R. B. and Finlay, B. L. (2001). Translating developmental time across species. *Neuroscience* **105**, 7–17.

Clarke, P. G. (1990). Developmental cell death: morphological diversity and multiple mechanisms. *Anat. Embryol. (Berl).* **181**, 195–213.

Cowan, W. M., Fawcett, J. W., O’Leary, D. D. and Stanfield, B. B. (1984). Regressive events in neurogenesis. *Science* **225**, 1258–65.

Crowcroft, P. and Ingles, J. M. (1959). Seasonal changes in the brain-case of the common shrew (*Sorex araneus* L.). *Nature* **193**, 907–908.

Dapson, R. W. (1968). Growth patterns in a post-juvenile population of short-tailed shrews (*Blarina brevicauda*). *Am. Midl. Nat.* **79**, 118–129.

de Sousa, A. A., Sherwood, C. C., Mohlberg, H., Amunts, K., Schleicher, A., MacLeod, C. E., Hof, P. R., Frahm, H. and Zilles, K. (2010). Hominoid visual brain structure volumes and the position of the lunate sulcus. *J. Hum. Evol.* **58**, 281–292.

Dechmann, D. K. N., LaPoint, S., Dullin, C., Hertel, M., Taylor, J. R. E., Zub, K. and Wikelski, M. (2017). Profound seasonal shrinking and regrowth of the ossified braincase in phylogenetically distant mammals with similar life histories. *Sci. Rep.* **7**, 42443.

Dehnel, A. (1949a). Studies on the genus Sorex L. *Ann. Univ. Marie Curie-Sklodowska, Sect. C* **4**, 17–102.

Dehnel, A. (1949b). Studies on the genus Sorex L. *Ann. Univ. Marie Curie-Sklodowska, Sect. C* **4**, 17–102.

Dehnel, A. (1950). Studies on the genus Neomys Kaup. *Ann. Univ. Marie Curie-Sklodowska, Sect. C* **4**, 1–63.

Demas, G. E., Weil, Z. M. and Nelson, R. J. (2010). Photoperiodism in mammals: regulation of nonreproductive traits. In *Photoperiodism: The Biological Calendar* (ed. Nelson, R. J., Delinger, D. L., and Somers, D. E.), pp. 461–502. New York: Oxford University Press.

Dickstein, D. L., Kabaso, D., Rocher, A. B., Luebke, J. I., Wearne, S. L. and Hof, P. R. (2007). Changes in the structural complexity of the aged brain. *Aging Cell* **6**, 275–284.

Dobbing, J. and Sands, J. (1973). Quantitative growth and development of human brain. *Arch. Dis. Child.* **48**, 757–767.

Dobbing, J. and Sands, J. (1979). Comparative aspects of the brain growth spurt. *Early Hum. Dev.* **311**, 79–83.

Duan, H., Wearne, S. L., Rocher, A. B., Macedo, A., Morrison, J. H. and Hof, P. R. (2003). Age-related dendritic and spine changes in corticocortically projecting neurons in macaque monkeys. *Cereb. Cortex* **13**, 950–961.

Fan, L., Tang, Y., Sun, B., Gong, G., Chen, Z. J., Lin, X., Yu, T., Li, Z., Evans, A. C. and Liu, S. (2010). Sexual dimorphism and asymmetry in human cerebellum: An MRI-based morphometric study. *Brain Res.* **1353**, 60–73.

Farkas, E. and Luiten, P. G. M. (2001). Cerebral microvascular pathology in aging and Alzheimer's disease. *Prog. Neurobiol.* **64**, 575–611.

Finlay, B. L. and Darlington, R. B. (1995). Linked regularities in the development and evolution of mammalian brains. *Science* **284**, 1578–1584.

Galea, L. A. M., Kavaliers, M., Ossenkopp, K. P., Innes, D. L. and Hargreaves, E. L. (1994). Sexually dimmorphic spatial learning varies seasonally in two populations of deer mice. *Brain Res.* **635**, 18–26.

Galea, L. A. M., Kavaliers, M. and Ossenkopp, K. (1996). Sexually dimorphic spatial learning in meadow voles *Microtus pennsylvanicus* and deer mice *Peromyscus maniculatus*. *J. Exp. Biol.* **199**, 195–200.

Gaulin, S. J. C. and Fitzgerald, R. W. (1989). Sexual selection for spatial-learning ability. *Anim. Behav.* **37**, 322–331.

Geinisman, Y., Bondareff, W. and Dodge, J. T. (1978). Dendritic atrophy in the dentate gyrus of the senescent rat. *Am. J. Anat.* **152**, 321–329.

Gelman, A. and Su, Y.-S. (2015). arm: Data analysis using regression and multilevel/hierarchical models. R package version 1.8-6.

Gliwicz, J. and Taylor, J. R. E. (2002). Comparing life histories of shrews and rodents. *Acta Theriol.* **47**, 185–208.

Gonda, A., Herczeg, G. and Merilä, J. (2013). Evolutionary ecology of intraspecific brain size variation: a review. *Ecol. Evol.* **3**, 2751–2764.

Gongal’skii, K. B., Savin, F. A., Panchenko, I. A. and Pokarzhevskii, A. D. (2003). Evaluation of winter activity of soil invertebrates. *Dokl. Biol. Sci.* **392**, 416–418.

Hågvar, S. and Hågvar, B. (2011). Invertebrate activity under snow in a South-Norwegian spruce forest. *Soil Org.* **83**, 287–306.

Harris, E. F. and Smith, R. N. (2009). Accounting for measurement error: A critical but often overlooked process. *Arch. Oral Biol.* **54**, 107–117.

Hofman, M. a and Swaab, D. F. (1992). Seasonal changes in the suprachiasmatic nucleus of man. *Neurosci. Lett.* **139**, 257–60.

Hofman, M. A. and Swaab, D. F. (2002). A brain for all season: cellular, and molecular mechanism of photoperiodic plasticity. In *Plasticity in the adult brain: from genes to neurotherapy, progress in brain research* (ed. Hofman, M. A., Boer, G. J., Holtmaat, A. J. G. D., van Someren, E. J. W., Verhaagen, J., and Swaab, D. F.), pp. 255–280. Amsterdam: Elsevier.

Hyvarinen, H. (1969). On the seasonal changes in the skeleton of the common shrew (*Sorex araneus* L.) and their physiological background. *Aquil. Series Zool.* **7**, 2–32.

Jacobs, L. F. and Liman, E. R. (1991). Grey squirrels remember the locations of buried nuts. *Anim. Behav.* **41**, 103–110.

Jacobs, L. F., Gaulin, S. J., Sherry, D. F. and Hoffman, G. E. (1990). Evolution of spatial cognition: sex-specific patterns of spatial behavior predict hippocampal size. *Proc. Natl. Acad. Sci. U. S. A.* **87**, 6349–52.

Jaskuła, R. and Soszyńska-Maj, A. (2011). What do we know about winter active ground beetles (Coleoptera, Carabidae) in Central and Northern Europe? *Zookeys* **100**, 517–532.

Karbowski, J. (2007). Global and regional brain metabolic scaling and its functional consequences. *BMC Biol.* **5**, 18.

Kaufman, J. A. (2004). Pattern and scaling of regional cerebral glucose metabolism in mammals. PhD dissertation, Washington.

Kotrschal, A., Rogell, B., Bundsen, A., Svensson, B., Zajitschek, S., Brannstrom, I., Immler, S., Maklakov, A. A. and Kolm, N. (2013). Artificial selection on relative brain size in the guppy releals costs and benefits of evolving a larger brain. *Curr. Biol.* **23**, 168–171.

Kranstauber, B. and Smolla, M. (2016). move: Visualizing and Analyzing Animal Track Data. R package version 2.0.0.

Krebs, J. R., Sherryt, D. F., Healy, S. D., Perryt, V. H. and Vaccarino, A. L. (1989). Hippocampal specialization of food-storing birds. *Neurobiology* **86**, 1388–1392.

Kruska, D. (1993). Evidence of decrease in brain size in Ranch Mink, Mustela vison f. dom., during subadult postnatal ontogenesis. *Brain. Behav. Evol.* **41**, 303–315.

LaPoint, S., Keicher, L., Wikelski, M., Zub, K. and Dechmann, D. K. N. (2017). Growth overshoot and seasonal size changes in the skulls of two weasel species. *Open Sci.*

Laughlin, S. B., de Ruyter Van Steveninck, R. R. and Anderson, J. C. (1998). The metabolic cost of neural information. *Nat. Neurosci.* **1**, 36–41.

Lavenex, P., Steele, M. A. and Jacobs, L. F. (2000a). The seasonal pattern of cell proliferation and neuron number in the dentate gyrus of wild adult eastern grey squirrels. *Eur. J. Neurosci.* **12**, 643–648.

Lavenex, P., Steele, M. A. and Jacobs, L. F. (2000b). Sex differences , but no seasonal variations in the hippocampus of food-caching squirrels: a stereological study. *J. Comp. Neurol.* **425**, 152–166.

Lázaro, J., Dechmann, D. K. N., LaPoint, S., Wikelski, M. and Hertel, M. (2017a). Profound reversible seasonal changes of individual skull size in a mammal. *Curr. Biol.* **27**, R1106–R1107.

Lázaro, J., Dechmann, D., LaPoint, S., Wikelski, M. and Hertel, M. (2017b). Data from: Profound reversible seasonal changes of individual skull size in a mammal. *Dryad Digit. Repos.*

Lázaro, J., Hertel, M., LaPoint, S., Wikelski, M., Stiehler, M. and Dechmann, D. K. N. (2018). Cognitive skills of common shrews (Sorex araneus) vary with seasonal changes in skull size and brain mass. *J. Exp. Biol.* **221,** jeb-166595.

MacDonald, I. M. V. (1997). Field experiments on duration and precision of grey and red squirrel spatial memory. *Anim. Behav.* **54**, 879–891.

Mackiewicz, P., Moska, M., Wierzbicki, H., Gagat, P., Mackiewicz, D. and Mazzotti, S. (2017). Evolutionary history and phylogeographic relationships of shrews from Sorex araneus group. *PLoS One* **12**, e0179760.

Magariños, A. M., McEwen, B. S., Saboureau, M. and Pevet, P. (2006). Rapid and reversible changes in intrahippocampal connectivity during the course of hibernation in European hamsters. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 18775–18780.

Marner, L., Nyengaard, J. R., Tang, Y. and Pakkenberg, B. (2003). Marked loss of myelinated nerve fibers in the human brain with age. *J. Comp. Neurol.* **462**, 144–152.

Martin, S., Lazzarini, M., Dullin, C., Balakrishnan, S., Gomes, F. V., Ninkovic, M., El Hady, A., Pardo, L. A., Sttühmer, W. and Del-Bel, E. (2017). SK3 Channel overexpression in mice causes hippocampal shrinkage associated with cognitive impairments. *Mol. Neurobiol.* **54**, 1078–1091.

Mcnab, B. K. and Eisenberg, J. F. (1989). Brain size and its relation to the rate of metabolism in mammals. *Am. Nat.* **133**, 157–167.

McNab, B. K. (2010). Geographic and temporal correlations of mammalian size reconsidered: a resource rule. *Oecologia* **164**, 13–23.

Merriam, G., Wegner, J. and Caldwell, D. (1983). Invertebrate activity under snow in deciduous woods. *Holarct. Ecol.* **6**, 89–94.

Merritt, J. F. and Zegers, D. A. (2014). Social thermoregulation in least shrews, *Cryptotis parva*. *Mammalia* **78**, 11–22.

Mezhzherin, V. A. (1964). Dehnel's phenomenon and its possible explanation. *Acta Theriol.* **8**, 95–114.

Moraleva, N. and Telitzina, A. (1994). Territoriality in juveniles of the common shrew (*Sorex araneus*) in prepeak and peak years of population density. *Spec. Publ. Carnegie Museum Nat. Hist.* **18**, 67–76.

Naumann, R. K., Anjum, F., Roth-Alpermann, C. and Brecht, M. (2012). Cytoarchitecture, areas, and neuron numbers of the Etruscan shrew cortex. *J. Comp. Neurol.* **520**, 2512–30.

Niven, J. E. and Laughlin, S. B. (2008). Energy limitation as a selective pressure on the evolution of sensory systems. *J. Exp. Biol.* **211**, 1792–1804.

Nordström, S. (1975). Seasonal activity of lumbricids in Southern Sweden. *Oikos* **26**, 307–315.

Nottebohm, F. (1981). A brain for all seasons: cyclical anatomical changes in song control nuclei of the canary brain. *Science* **214**, 1368–1370.

Ochocińska, D. and Taylor, J. R. E. (2003). Bergmann's rule in shrews: geographical variation of body size in Palearctic *Sorex species*. *Biol. J. Linn. Soc.* **78**, 365–381.

Ochocińska, D. and Taylor, J. R. E. (2005). Living at the physiological limits: field and maximum metabolic rates of the common shrew (*Sorex araneus*). *Physiol. Biochem. Zool.* **78**, 808–18.

Page, R. A., von Merten, S. and Siemers, B. M. (2012). Associative memory or algorithmic search: A comparative study on learning strategies of bats and shrews. *Anim. Cogn.* **15**, 495–504.

Pankakoski, E. (1989). Variation in the tooth wear of the shrews *Sorex araneus* and *S. minitus*. *Ann. Zool. Fennici* **26**, 445–457.

Paxinos, G. and Franklin, K. B. J. (2013). *The Mouse Brain in Stereotaxic Coordinates*. 4th ed. San Diego: Acadmic Press.

Pernetta, J. C. (1976). Diets of the shrews *Sorex araneus* L . and *Sorex minutus* L . in Wytham grassland. *J. Anim. Ecol.* **45**, 899–912.

Pierce, G. J. (1987). Search paths of foraging common shrews Sorex araneus. *Anim. Behav.* **35**, 1215–1224.

Pinheiro, J., DebRoy, S., Sarkar, D. and Team, R. C. (2016). nlme: Linear and Nonlinear Mixed Effects Models.

Popov, V. I. and Bocharova, L. S. (1992). Hibernation-induced structural changes in synaptic contacts between mossy fibers and hippocampal pyramidal neurons. *Neuroscience* **48**, 53–62.

Popov, V. I., Bocharova, L. S. and Bragin, a. G. (1992). Repeated changes of dendritic morphology in the hippocampus of ground squirrels in the course of hibernation. *Neuroscience* **48**, 45–51.

Pucek, Z. (1963). Seasonal changes in the braincase of some representatives of the genus *Sorex* from the Paleartic. *J. Mammal.* **44**, 523–536.

Pucek, Z. (1965a). Seasonal and age changes in the weight of internal organs of shrews. *Acta Theriol.* **10**, 369–438.

Pucek, M. (1965b). Water contents and seasonal changes of the brain-weight in shrews. *Acta Theriol.* **10**, 353–367.

Pucek, Z. (1970). Seasonal and age change in shrews as an adaptive process. *Symp. Zool. Soc. Lond.* **26**, 189–207.

Pucek, Z. and Markov, G. (1964). Changes in the skull of the common shrew from Bulgaria. *Acta Theriol.* **9**, 363–366.

Punzo, F. and Chavez, S. (2003). Effect of aging on spatial learning and running speed in the shrew (*Cryptotis parva*). *J. Mammal.* **84**, 1112–1120.

Pyter, L. M. (2005). Short photoperiods impair spatial learning and alter hippocampal dendritic morphology in adult male white-footed mice (*Peromyscus leucopus*). *J. Neurosci.* **25**, 4521–4526.

Pyter, L. M., Trainor, B. C. and Nelson, R. J. (2006). Testosterone and photoperiod interact to affect spatial learning and memory in adult male white-footed mice (*Peromyscus leucopus*). *Eur. J. Neurosci.* **23**, 3056–3062.

R Core Team (2015). R: A language and environment for statistical computing.

Raz, N., Lindenberger, U., Rodrigue, K. M., Kennedy, K. M., Head, D., Williamson, A., Dahle, C., Gerstorf, D. and Acker, J. D. (2005). Regional brain changes in aging healthy adults: General trends, individual differences and modifiers. *Cereb. Cortex* **15**, 1676–1689.

Rundgren, S. (1975). Vertical distribution of lumbricids in Southern Sweden. *Oikos* **26**, 299–306.

Rychlik, L. (1998). Evolution of social systems in shrews. In *Evolution of Shrews* (ed. Wojcik, J. M. and Woslan, M.), pp. 347–405. Bialowieza: Mammal Research Institute, Polish Academy of Sciences.

Saarikko, J. (1989). Foraging behaviour of shrews. *Ann. Zool. Fennici* **26**, 411–423.

Safi, K., Seid, M. A. and Dechmann, D. K. N. (2005). Bigger is not always better: when brains get smaller. *Biol. Lett.* **1**, 283–6.

Saure, L. and Hyvärinen, H. (1965). Seasonal changes in the histological structure of the spinal column of *Sorex araneus* (L.). *Nature* **208**, 705–706.

Scheibel, M. E. and Scheibel, A. B. (1978). The methods of Golgi. In *Neuroanatomical research techniques* (ed. R. T. Robertson), pp. 98–144. New York, NY: New York Academic Press.

Schmidt, K. (1992). Skull variability of *Mustela nivalis* Linnaeus , 1766 in Poland. *Acta Theriol.* **37**, 141–162.

Schneider, C. A., Rasband, W. S. and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **9**, 671–675.

Searle, J. B. (1984). Breeding the common shrew (*Sorex araneus*) in captivity. *Lab. Anim.* **18**, 359–363.

Serafinski, W. (1955). Morphological and ecological investigations on Polish species of the genus *Sorex* L. (Insectivora, Soricidae). *Acta Oecologica* **1**, 27–86.

Sherry, D. F. (2006). Neuroecology. *Annu. Rev. Psychol.* **57**, 167–197.

Sherry, D. F., Jacobs, L. F. and Gaulin, S. J. (1992). Spatial memory and adaptive specialization of the hippocampus. *Trends Neurosci.* **15**, 298–303.

Smith, G. T., Brenowitz, E. A., Beecher, M. D. and Wingfield, J. C. (1997). Seasonal changes in testosterone, neural attributes of song control nuclei, and song structure in wild songbirds. *J. Neurosci.* **17**, 6001–6010.

Smith, D. E., Roberts, J., Gage, F. H. and Tuszynski, M. H. (1999). Age-associated neuronal atrophy occurs in the primate brain and is reversible by growth factor gene therapy. *Proc. Natl. Acad. Sci.* **96**, 10893–10898.

Smulders, T. V., Sasson, A. D. and Devoogd, T. J. (1995). Seasonal variation in hippocampal volume in a food-storing bird, the black-capped chickadee. *J. Neurobiol.* **27**, 15–25.

Smulders, T. V., Shiflett, M. W., Sperling, A. J. and Devoogd, T. J. (2000). Seasonal changes in neuron numbers in the hippocampal formation of a food-hoarding bird: The black-capped chickadee. *J. Neurobiol.* **44**, 414–422.

Spocter, M. a, Hopkins, W. D., Barks, S. K., Bianchi, S., Hehmeyer, A. E., Anderson, S. M., Stimpson, C. D., Fobbs, A. J., Hof, P. R. and Sherwood, C. C. (2012). Neuropil distribution in the cerebral cortex differs between humans and chimpanzees. *J. Comp. Neurol.* **520**, 2917–29.

Stephan, H. (1960). Methodische Studien über den quantitativen Vergleich architektonischer Struktureinheiten des Gehirns. *Z. Wiss. Zool.* **164**, 143–172.

Stockley, P. and Searle, J. B. (1998). Shrew mating systems. In *Evolution of Shrews* (ed. Wójcik, J. M. and Wolsan, M.), pp. 407–424. Białowieża: Mammal Research Institute, Polish Academy of Sciences.

Stockley, P., Searle, J. B., Macdonald, D. W. and Jones, C. S. (1994). Alternative reproductive tactics in male common shrews: relationships between mate-searching behaviour, sperm production, and reproductive success as revealed by DNA fingerprinting. *Behav. Ecol. Sociobiol.* **34**, 71–78.

Striedter, G. F. (2005). *Principles of Brain Evolution*. Sunderland, MA: Sinauer.

Suárez, I., Bodega, G., Rubio, M. and Fern??ndez, B. (1992). Sexual dimorphism in the hamster cerebellum demonstrated by glial fibrillary acidic protein (GFAP) and vimentin immunoreactivity. *Glia* **5**, 10–16.

Szafrańska, P. a., Zub, K. and Konarzewski, M. (2013). Seasonal variation of resting metabolic rate and body mass in free-living weasels Mustela nivalis. *Physiol. Biochem. Zool.* **86**, 791–798.

Taylor, J. R. E. (1998). Evolution of energetic strategies in shrews. In *Evolution of Shrews* (ed. Wojcik, J. M. and Woslan, M.), pp. 309–346. Bialowieza: Mammal Research Institute, Polish Academy of Sciences.

Taylor, J. R. E., Rychlik, L. and Churchfield, S. (2013). Winter reduction in body mass in a very small, nonhibernating mammal: consequences for heat loss and metabolic rates. *Physiol. Biochem. Zool.* **86**, 9–18.

Tiemeier, H., Lenroot, R. K., Greenstein, D. K., Tran, L. and Giedd, J. N. (2011). Cerebellum development during childhood and adolescence: a longitudinal morphometric MRI study. *Neuroimage* **49**, 63–70.

Tramontin, A. D. and Brenowitz, E. A. (2000). Seasonal plasticity in the adult brain. *Trends Neurosci.* **23**, 251–258.

Tramontin, A. D., Smith, G. T., Breuner, C. W. and Brenowitz, E. A. (1998). Seasonal plasticity and sexual dimorphism in the avian song control system: Stereological measurement of neuron density and number. *J. Comp. Neurol.* **396**, 186–192.

Weiler, E. (1992). Seasonal changes in adult mammalian brain weight. *Naturwissenschaften* **79**, 474–476.

West, G. B., Brown, J. H. and Enquist, B. J. (2001). A general model for ontogenetic growth. *Nature* **413**, 628–631.

Wood, S. N. (2006). *Generalized Additive Models: an Introduction with R*. Boca Raton: Chapman & Hall.

Wood, S. N. (2015). Stable and efficient multiple smoothing parameter estimation for generalized additive models. *J. Am. Stat. Assoc.* **99**, 673–686.

Workman, J. L., Bowers, S. L. and Nelson, R. J. (2009). Enrichment and photoperiod interact to affect spatial learning and hippocampal dendritic morphology in white-footed mice (*Peromyscus leucopus*). *Eur. J. Neurosci.* **29**, 161–170.

Yaskin, V. a (1984). Seasonal changes in brain morphology in small mammals. *Spec. Publ. Carnegie Museum Nat. Hist.* **10**, 183–191.

Yaskin, V. A. (1994). Variation in brain morphology of the common shrew. In *Advances in the Biology of Shrews* (ed. Merrit, J. F., Kirkland, G. L. and Rose, R. K.), pp. 155–161. Pittsburgh: Carnegie Museum of Natural History, special publication.

Yaskin, V. A. (2005). The annual cycle of spatial behavior and hippocampal volume in Sorex. In *Advances in the Biology of Shrews II* (ed. Merrit, J. F., Churchfield, S., Hutterer, R. and Sheftel, B. I.), pp. 373–385. New York, NY: International Society of Shrew Biologists.

Yaskin, V. A. (2011). Seasonal changes in hippocampus size and spatial behaviour in mammals and birds. *Biol. Bull. Rev.* **1**, 279–288.

Yaskin, V. A. (2013). Seasonal modulation of sex-related differences in hippocampus size and spatial behavior in bank voles, *Clethrionomys glareolus* (Rodentia, Cricetidae). *Russ. J. Ecol.* **44**, 221–226.

Yopak, K. E., Lisney, T. J., Darlington, R. B., Collin, S. P., Montgomery, J. C. and Finlay, B. L. (2010). A conserved pattern of brain scaling from sharks to primates. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 12946–51.

Zub, K., Borowski, Z., Szafrańska, P. a., Wieczorek, M. and Konarzewski, M. (2014). Lower body mass and higher metabolic rate enhance winter survival in root voles, *Microtus oeconomus*. *Biol. J. Linn. Soc.* 297–309.

Appendix A.

Supplementary material of Chapter 1

Skull measurements and technical measurement error

We recorded the following measurements: skull length (SKL), from the anterior most projection of I1 to the occipital condyle; braincase width (BCW), greatest lateral diameter of the braincase; and braincase height (BCH), from the dorsal outline of the braincase to the orthogonal line defined by the ventral outlines of the pterygoid process and occipital condyle, passing over a constant proportional distance (k) on the line defined by SKL (Fig. A.1), gauged on the digital X-ray files using Image-J (Schneider et al., 2012). All measurements on the X-ray images were taken blind by a single observer (JL) regarding capture date and sex to avoid biases. We size corrected all skull measurements by dividing by the non-changing maxillary tooth row length (Fig. A.1A, A.2A).

We estimated the technical error of measurement (Harris and Smith, 2009) based on repeated measurements of ten individuals. The same observer re-measured each individual in five independent sessions. Each session included the entire X-raying process, from the placement of the shrew under the X-ray source to the picture measurement using Image-J (Schneider et al., 2012). We quantified the technical error of measurement as the within-subject standard deviation based on these repeated measurements. This gives 95% confidence intervals for repeatability error in normally distributed data (Harris and Smith, 2009). The technical measurement error as % of the mean common SD is 2.04% for BCHcor, 1.35% for SKLcor, 1.89% for BCWcor and 1.64% for tooth row.

Age and sex determination

We classified individuals as summer juvenile, winter subadult or adult based on the annual life cycle of the shrews and external morphological characteristics. In the wild *S. araneus* has a maximum life span of ~18 months (Churchfield, 1990). Young shrews reach their first summer peak in body size after three weeks, pass the winter in an immature, subadult stage and reach sexual maturity in spring (Churchfield, 1990). Shrews mate only as adults during their second summer (June – September) and die shortly thereafter. There is only a brief period, June – September, when two generations overlap, only one of them sexually mature. During this period of overlap, we classified "summer juveniles" as follows: no developed testes or mammary glands present, little tooth wear usually with pigmented tooth tips (Pankakoski, 1989), furry tails, and body mass typically < 10 g. We classified individuals as "adults" if their teeth were blunt, tooth pigmentation was reduced or absent, tails were hairless and often scarred, and body mass was > 10 g.

To determine the sex of immature individuals (all individuals until the second summer) we used a PCR-based gonosomal sexing method (C. Roos; DPZ Göttingen unpublished). DNA was extracted from tail tip samples using Qiagen's DNAeasy kit /(Qiagen, GmbH, Hilden). Adults were sexed by the presence of testes or mammary glands.

Quantifying seasonal patterns in skull dimensions

We used the seasonal nonlinear predictions of the change for our variables of interest (i.e., BCH_{cor}, SKL_{cor}, and BCW_{cor}) to formulate our a priori expectations. We used generalized additive mixed models (GAMM) (Wood, 2006) with sex as a parametric term, 'individual' as a random effect and age-corrected capture Julian day of the year (jday) as a non-parametric term to quantify potential nonlinear patterns. We used a Gaussian distribution and added a smoothing function to jday that included 5 knots in a thin plate regression spline. We restricted the number of knots to identify the a priori pattern while reducing the risk of model overfitting. To calculate jday, we first shifted June 1st of year one to jday = 1 until May 31st of the following year = jday 365, for the juveniles. For adults, the jday is calculated as 365 + jday of year one. For example, a juvenile captured on October 10th, jday = 132 and for an adult captured on July 18th, jday = 412. As shrews do not live beyond 18 months, there is no possibility of further jday cycles (i.e., our maximum jday = 515).This first model suggested no differences between the sexes at the factor level (BCH_{cor}: GAMM, n = 89, males estimate = 0.0007581, $P > 0.1$), nor did the model with an added interaction between sex and jday (BCH_{cor}: GAMM, n = 89, e.d.f.(females) = 3.812, e.d.f.(males) = 3.689, P(smooth t.) < 0.001 for both sexes). We then combined sexes and re-ran the model. Final models for SKL_{cor} and BCW_{cor} are depicted in Fig. A.2. All analyses were performed in R (ver. 3.2.3) (R Core Team, 2015), using the R package nlme (Pinheiro et al., 2016).

In addition, we tested for significance of within-subject change of skull measures with paired t-tests. For these tests, the closest value to each cycle peak (summer juvenile/winter subadult/spring-summer adult) was chosen for each recaptured individual. One test was performed for the shrinking phase (summer to winter) and a second one for the regrowth (winter to spring-summer).

Data availability

Data used for this paper can be found in Dryad: http://dx.doi.org/10.5061/dryad.15r51 (Lázaro et al., 2017b).

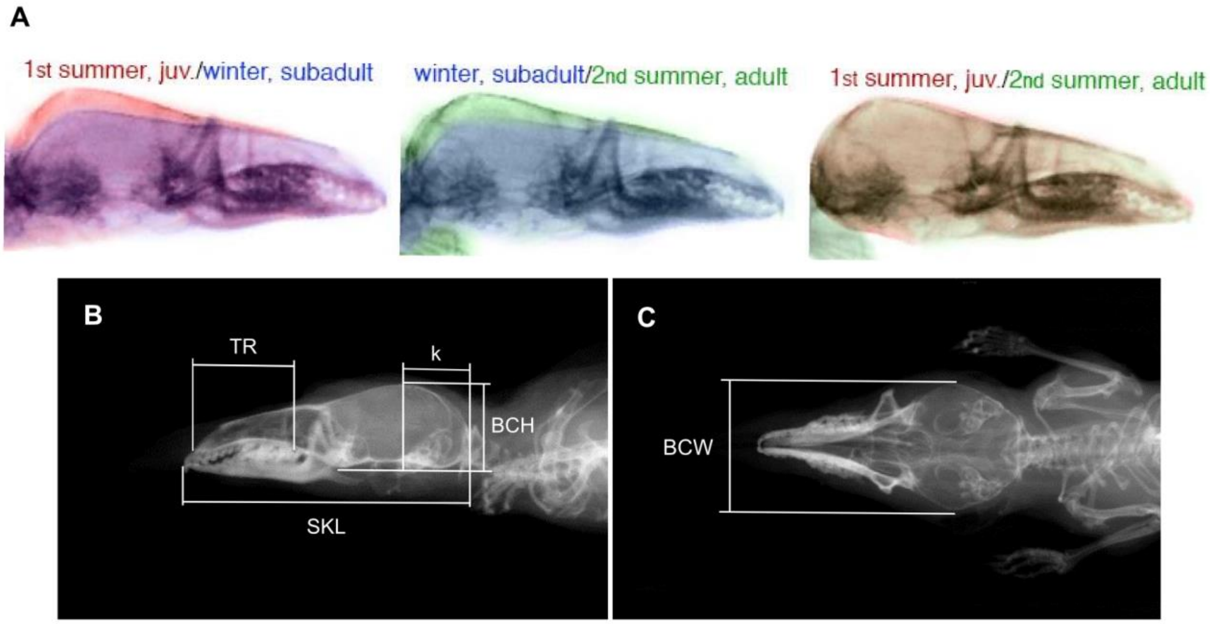

Figure A.1. Overlapping X-ray images and linear measurements taken from X-ray images. A, Overlapping X-ray images from the skull of the same individual as summer juvenile in July and winter subadult in February (left), winter subadult and adult in May (center) and summer juvenile and adult (right).**B**, linear measurements we recorded from x-rays: skull length (SKL),,braincase height (BCH), tooth row length (TR) and **C**, braincase width (BCW). See text for details.

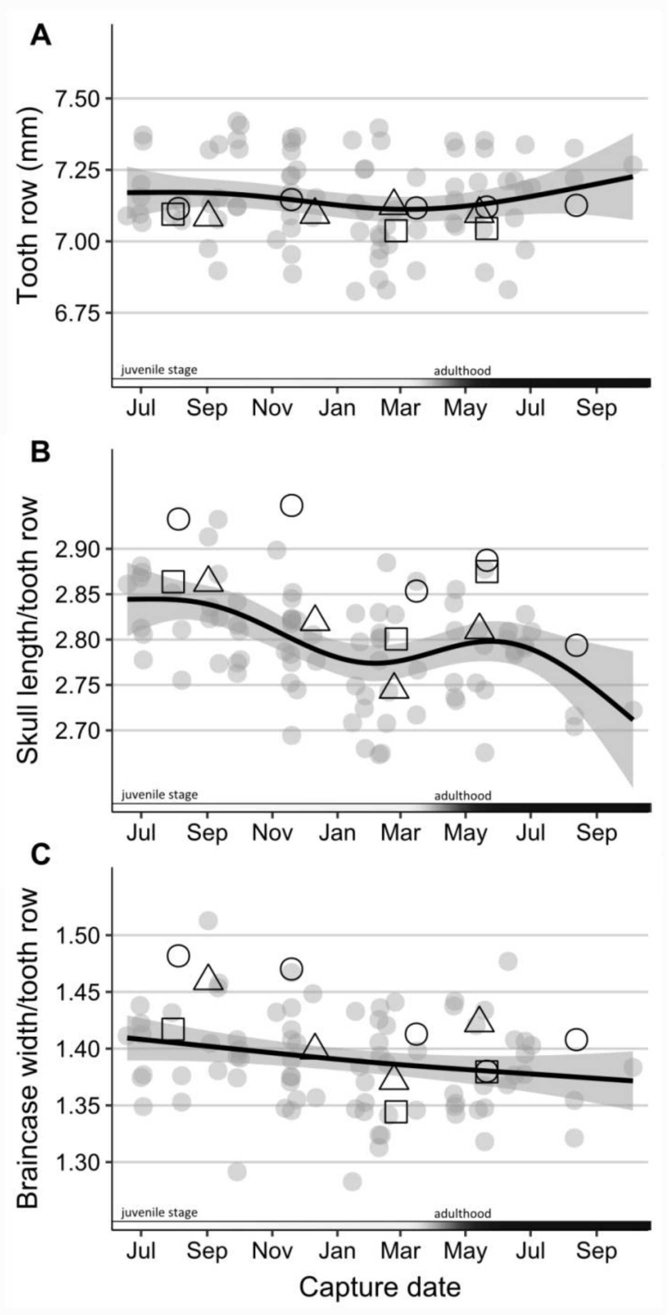

Figure A.2. Seasonal changes in tooth row length, $\mathrm{SKL_{cor}}$ and $\mathrm{BCW_{cor}}$. A, Measurements of tooth row length from recaptured individuals show no seasonal (GAMM, e.d.f = 1, n = 95, P > 0.1) or sex-specific (GAMM, e.d.f = 1, male estimate = +0.0776 mm, P > 0.05) patterns. **B**, Seasonal pattern in $\mathrm{SKL_{cor}}$ and C) $\mathrm{BCW_{cor}}$; see model details in text. Solid line and shaded area represent fitted values and standard error of the model. Symbols highlight the same individuals as in Fig 1. The shaded bar along the x-axis indicates major age stages of individuals: juvenile and subadult (white portion of the bar) and adult (black).

Appendix B.

Supplementary material of Chapter 2

Table B.1. Absolute values for entire hemisphere volumes in the three stages and in both sexes. Values are in mm^3.

	Summer juvenile		Winter subadult		Spring-summer adult					
	Mean ± SD	95% CrI	Mean ± SD	95% CrI	Mean ± SD	95% CrI	Difference summer-winter	P(s-w)	Difference winter-adult	P(w-a)
both sexes	122.9 ± 4.7	119.0/127.0	102.9 ± 6.4	99.1/106.7	112.5 ± 7.0	108.9/116.0	-20.0 (-16.3%)	>0.99	9.5 (9.3%)	>0.99
males	122.1 ± 4.6	117.0/127.0	106.6 ± 2.7	101.4/111.7	115.6 ± 6.7	111.3/120.0	-15.6 (-12.7%)	>0.99	9.1 (8.5%)	>0.99
females	123.8 ± 5.1	118.7/129.0	99.9 ± 7.3	95.2/104.6	108.1 ± 4.9	102.9/113.3	-23.9 (-19.3%)	>0.99	8.1 (8.2%)	0.99

Table B.2. Corrected volumes for striatal subregions in the three age stages, mean differences between stages and probabilities (P) of the difference from summer juveniles to winter subadults (s-w) and from winter subadults to spring-summer adults (w-a) as calculated from the posterior distributions. The values of each sex are depicted only for the region that revealed sex-specific differences. Volumes are given in μm^3 and are measured in one hemisphere.

	Summer juvenile		Winter subadult		Spring-summer adult					
	Mean ± SD	95% CrI	Mean ± SD	95% CrI	Mean ± SD	95% CrI	Difference summer-winter	P(s-w)	Difference winter-adult	P(w-a)
Nucleus accumbens	2.7 ± 0.6	2.1/3.2	2.6 ± 0.6	2.1/3.1	2.5 ± 0.4	2.1/3.0	-0.1 (-2.6%)	0.62	-0.1 (-2.7%)	0.38
Amygdala	1.5 ± 0.3	1.0/2.0	1.3 ± 0.2	0.8/1.8	1.5 ± 0.3	1.0/1.9	-0.3 (-17.0%)	0.98	0.2 (16.9%)	0.96
Caudoputamen	10.7 ± 1.7	10.2/11.2	8.5 ± 1.1	8.0/9.0	8.5 ± 0.1	8.0/9.0	-2.2 (-20.7%)	>0.99	0.01 (0.1%)	0.51
males	9.8 ± 0.9	9.1/10.5	8.2 ± 1.5	7.5/8.9	8.5 ± 1.0	7.9/9.0	-1.6 (-16.0%)	0.97	0.2 (2.7%)	0.62
females	11.6 ± 1.9	10.9/12.3	8.7 ± 0.8	8.1/9.3	8.6 ± 1.0	7.9/9.2	-2.9 (-25.0%)	>0.99	-0.2 (-1.7%)	0.43

Table B.3. Corrected volumes for hippocampal subregions in the three age stages, mean differences between stages and probabilities (P) of the difference from summer juveniles to winter subadults (s-w) and from winter subadults to spring-summer adults (w-a) as calculated from the posterior distributions. The values of each sex are depicted only for the region that revealed sex-specific differences. Volumes are given in µm^3 and are measured in one hemisphere.

	Summer juvenile		Winter subadult		Spring-summer adult					
	Mean ± SD	95% CrI	Mean ± SD	95% CrI	Mean ± SD	95% CrI	Difference summer-winter	P(s-w)	Difference winter-adult	P(w-a)
CA1	6.1 ± 0.9	5.7/6.6	5.4 ± 0.7	5.0/5.8	6.1 ± 0.9	5.7/6.5	-0.8 (-12.6%)	0.97	0.7 (13.5%)	0.97
males	6.5 ± 0.5	5.9/7.0	5.6 ± 0.5	5.0/6.2	6.7 ± 0.6	6.2/7.2	-0.8 (-13.0%)	0.96	1.0 (18.4%)	0.99
females	5.8 ± 1.2	5.2/6.4	5.1 ± 0.9	4.6/5.7	5.3 ± 0.7	4.7/5.9	-0.7 (-11.4%)	0.92	0.1 (2.7%)	0.62
CA2	2.3 ± 0.3	1.9/2.8	2.0 ± 0.8	1.6/2.4	2.4 ± 0.4	2.0/2.8	-0.4 (-15.8%)	0.94	0.5 (24.2%)	0.98
CA3	2.5 ± 0.4	2.1/2.9	2.7 ± 0.4	2.3/3.1	2.6 ± 0.4	2.2/3.0	0.2 (8.3%)	0.13	-0.1 (-2.6%)	0.34
Dentate gyrus	6.3 ± 0.9	5.8/6.7	5.3 ± 1.0	4.9/5.7	6.1 ± 0.9	5.7/6.5	-1.0 (-15.2%)	0.99	0.8 (15.6%)	0.98
Subbiculum	2.2 ± 0.6	1.8/2.6	2.0 ± 0.4	1.6/2.4	2.0 ± 0.5	1.6/2.4	-0.2 (-8.5%)	0.81	-0.02 (-1.2%)	0.46

Appendix C.

Supplementary material of Chapter 3

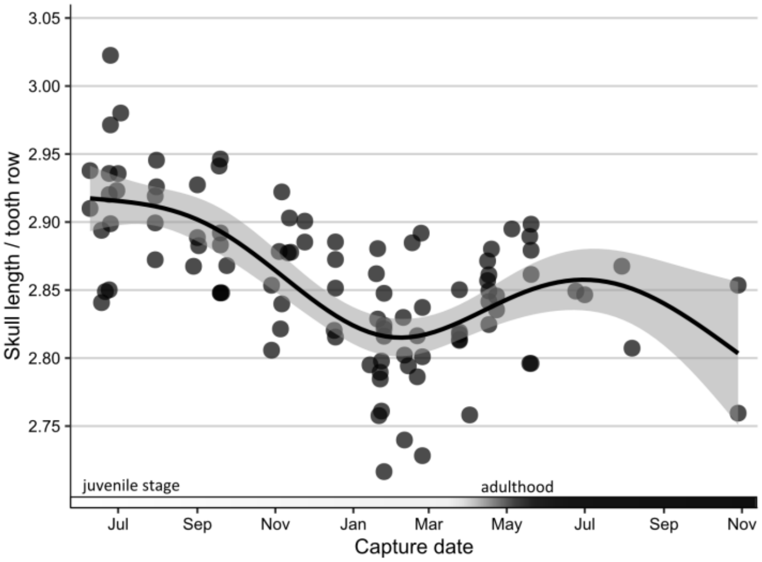

Figure C.1. **SKLcor over time with fitted GAM.** Solid line and shaded area represent fitted values and standard error of the model. The shaded x-axis indicates the two main age life stages. The first two models which included the individuals with known sex suggested neither differences between males and females at the factor level (GAM, n = 87, males estimate = 2.859958, females estimate = 2.859958, P > 0.5) nor with added interaction between sex and jday (GAM, n = 87, e.d.f.(females) = 3.446, e.d.f.(males) = 3.127, P(smooth t.) < 0.001, deviance explained = 46.5%). The final model for SKLcor in which sex was excluded (represented in the figure) revealed a significant non-linear pattern (GAM, n = 100, e.d.f.(smooth term) = 3.761, P < 0. 001, deviance explained = 47.9%), although the difference between seasons is not as pronounced as in BCHcor.

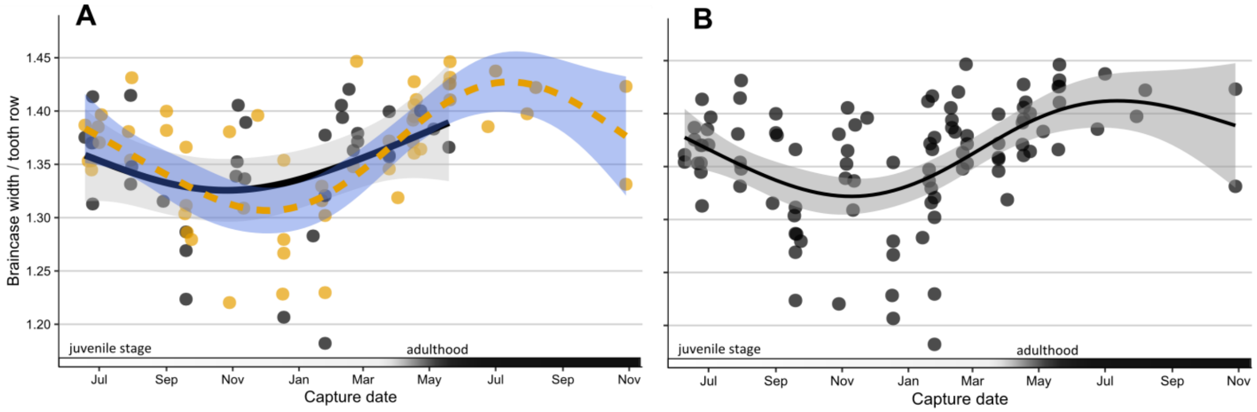

Figure C.2. BCWcor over time. A, Model including sex: orange = males; black = females. **B**, Model without sex. Lines and shaded areas represent fitted values and standard error of the model respectively. The shaded x-axis indicates the two main age life stages. The initial model with sex added as a factor revealed no sex differences (GAM, n = 87, males estimate = 1.356564, females estimate = 1.3562332, P > 0.5). The second model with an interaction between sex and jday indicated a slight difference between sexes, with males showing a stronger non-linear pattern (GAM, n = 87, e.d.f.(females) = 1.964, P(smooth t. females) <0.05, e.d.f.(males) = 3.597, P(smooth t. males) < 0.001, deviance explained = 35.2%) although the high overlap between the confidence intervals of the fitted values suggests no significance. The third model which excluded sex also showed a significant non-linear pattern (GAM, n = 100, e.d.f. (smooth term) = 3.475, P < 0. 001, deviance explained = 27.5%).

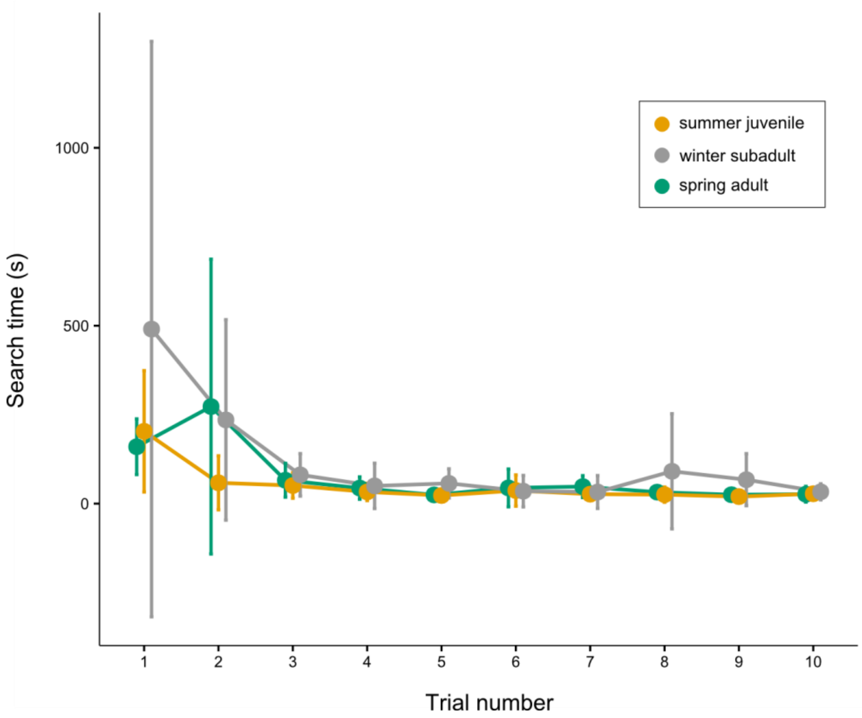

Figure C.3. Variation in search time in the three seasons. Circles and bars represent average latency ± s.d, in each trial.

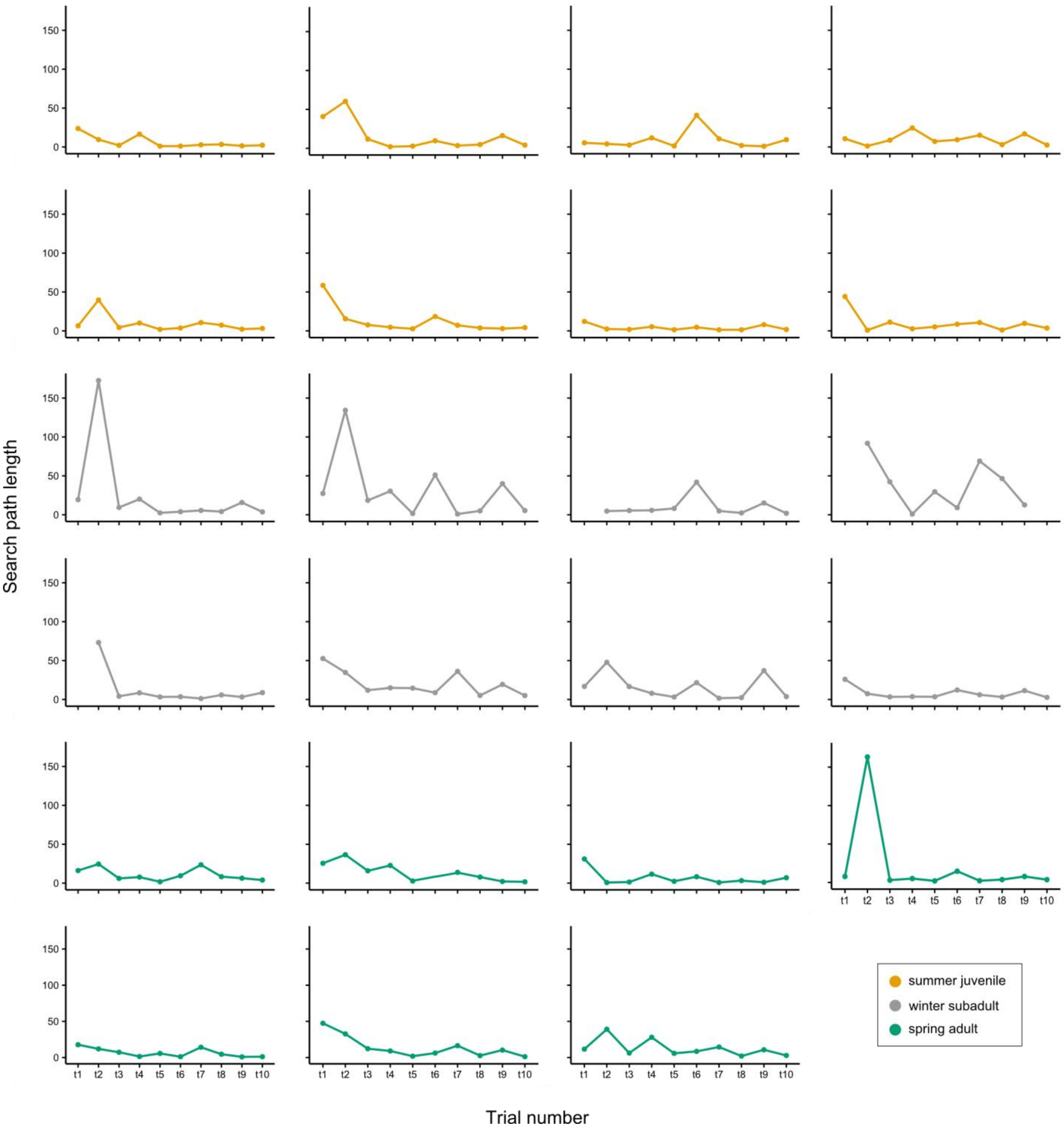

Figure C.4. Search path length along trials in each individual. Each graph illustrates the results of one individual along the ten trials. Units of path length are expressed as a path efficiency ratio, see text for details.

Table C.1. Technical measurement error based on repeated caliper measurements (mm).

	Mean	Residuals Mean sq.	Absolute error (common s.d.)	Error in % of the mean
BCH	6.61	0.0008	0.02828	0.46
SKL	20.17	0.0010	0.03162	0.16
BCW	9.64	0.0009	0.03033	0.31

List of publications

Wieczorek, M., Szafrańska, P.A., Labecka, A.M., **Lázaro, J.**, Konarzewski, M. (2015) Effect of the abrasive properties of sedges on the intestinal absorptive surface and resting metabolic rate of root voles. *J. Exp. Biol*.; **218**, 309-315. doi:10.1242/jeb.117168

Lázaro, J., Dechmann, D.K.N., LaPoint, S., Wikelski, M., Hertel, M. (2017). Seasonal, reversible changes in individual skull and brain size alter cognitive abilities of a mammal during winter. *Curr. Biol.* 27:R1106–R1107

Lázaro, J., Hertel, M., LaPoint, S., Wikelski, M., Stiehler, M., Dechmann, D.K.N. (2018). Cognitive skills of common shrews (*Sorex araneus*) vary with seasonal changes in skull size and brain mass. J Exp Biol. 221. doi: 10.1242/jeb.166595

Lázaro, J., Hertel, M., Sherwood, C.C., Muturi, M.,Dechmann, D.K.N. (2018). Profound seasonal changes in brain size and architecture in the common shrew. *Brain Struct. Func*. doi.org/10.1007/s00429-018-1666-5