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Brains of African mole-rats in numbers: Data for testing the social brain hypothesis

Buněčné složení mozku rypošů (Bathyergidae): data pro testování hypotézy sociálního mozku

Diplomová práce

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Kverková

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ABSTRACT

The social brain hypothesis (SBH) posits that complex social environments exert a major selection pressure driving the evolution of large brains and intelligence. The hypothesis was first proposed to explain the remarkable cognitive abilities of primates and has since been extended to other vertebrate groups and gained a substantial popularity. Nevertheless, the empirical support is equivocal in virtually every group where the hypothesis has been tested. In this thesis, the SBH is tested in the African mole-rats (Bathyergidae). Mole-rats share a subterranean mode of life and similar ecologies while covering the whole social spectrum, from solitary to "eusocial". The number of brain neurons is considered a better proxy for intelligence than relative or absolute brain size. Therefore, a novel approach, the isotropic fractionator, was used to estimate the total number of neurons and other cells in five brain parts (olfactory bulbs, cerebral cortex, cerebellum, diencephalon and basal ganglia, brain stem) of eleven bathyergid species. This simultaneously allows for examining if and how mole-rats differ from other rodents with respect to brain cellular scaling rules. We found that, contrary to expectations, mole-rats generally conform to these rules, with a few exceptions. They tend to have higher neuronal densities in the olfactory bulbs and deviate from the scaling rules in the cerebral cortex. Relative brain mass is not significantly associated with sociality in mole-rats. However, solitary species tend to have absolutely larger brains and more neurons in the cerebral cortex and diencephalon+striatum. This finding is contrary to the SBH, but consistent with the fact that, unlike in many vertebrate groups, solitary species in mole-rats are generally larger. The results further underscore that brain size is tightly coupled to body size and brain evolution should be interpreted in this light.

Key words: social brain hypothesis, isotropic fractionator, Bathyergidae, social complexity, neuronal numbers, glial cell numbers

ABSTRAKT

Hypotéza sociálního mozku (SBH) předpokládá, že složité sociální prostředí je zásadním selekčním tlakem na kognitivní schopnosti vedoucím v evoluci ke zvětšování mozku. Původně byla navržena jako vysvětlení mimořádných kognitivních schopností primátů, později však byla rozšířena i na další skupiny obratlovců a získala si značnou popularitu. Empirická data však přinášejí nesourodé výsledky prakticky v každé skupině živočichů, u níž byla hypotéza testována. V této diplomové práci je SBH testována u rypošů (Bathyergidae). Rypoši sdílejí podzemní způsob života a ekologii, ale pokrývají přitom celé sociální spektrum, od solitérně žijících po „eusociální". Počet neuronů je považován za lepší aproximaci míry inteligence než prostá relativní nebo absolutní velikost mozku. Proto jsme pomocí moderní metody, izotropní frakční homogenizace, stanovili počty neuronů a ostatních buněk v pěti částech mozku (čichové laloky, mozková kůra, mozeček, mezimozek a bazální ganglia, mozkový kmen) u jedenácti druhů rypošů. To současně umožnilo sledovat, zda a jak se mozky rypošů odlišují od pravidel buněčného škálování platných pro ostatní hlodavce. V rozporu s očekáváním jsme zjistili, že se rypoši obecně těmto pravidlům nevymykají, ale objevuje se u nich několik výjimek. Mají vyšší hustotu neuronů v čichových lalocích a odchylují se od pravidel buněčného škálování v mozkové kůře. Relativní hmotnost mozku není u rypošů spojena se sociálním uspořádáním. Solitérní druhy však mají absolutně větší mozek a více neuronů v mozkové kůře a mezimozku a striatu. Toto zjištění je v rozporu s hypotézou sociálního mozku, ale v souladu se skutečností, že na rozdíl od mnoha skupin obratlovců jsou solitérní druhy u rypošů v průměru větší. Výsledky tak podtrhují fakt, že rozdíly ve velikosti mozku jsou úzce spjaty s rozdíly ve velikosti těla, a evoluci mozku je třeba interpretovat v tomto kontextu.

Klíčová slova: hypotéza sociálního mozku, izotropní frakční homogenizace, Bathyergidae, sociální komplexita, počty neuronů, počty glií

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INTRODUCTION

The variation in brain sizes and cognitive abilities in vertebrates and their interrelationship and correlates have long been a central theme in comparative neuroscience, evolutionary biology and comparative psychology. Today we are in the midst of a boom of renewed interest in the topic. Just in the past few years there have been numerous studies dealing with correlates of brain size (e.g. Navarrete et al., 2011; Charvet & Finlay, 2012; Fonseca-Azevedo & Herculano-Houzel, 2012; Hager et al., 2012; van Schaik et al., 2012; Kotrschal et al., 2013; West, 2014; Chojnacka et al., 2015; Weisbecker et al., 2015) and comparative vertebrate cognition (reviewed in Beran et al., 2014). Modern methods and approaches have enabled unprecedented advances in the field and new unexpected facts are coming up both in neuroanatomy and cognitive science.

Interestingly, some of the most basic questions are still not settled: what measure of the brain is the best correlate of "cognitive abilities"? Is relative brain size important or does absolute brain size matter more? Should we focus on specific brain parts or consider the brain as a whole? The consensus now seems to be shifting in favour of absolute brain size (e.g. Deaner et al., 2007; MacLean et al., 2014; Dicke & Roth, 2016), but plenty of researchers are still opposed to this view. Moreover, new contradictory evidence based on behavioural studies has been brought forward recently (Benson-Amram et al., 2016; Jelbert et al., 2016, but cf. Vonk, 2016). Perhaps this tenacious resistance is based on an intuitive understanding that the relative size of an organ should give some inkling into its relative importance, coupled with the fact that humans have much larger brains than expected for body size.

Whichever stand we take in this dispute, there seems to be something missing from the story. After all, neurons are the computational units of the brain. Can we assume all brains are built the same, mass for mass? Hardly anyone would argue that, at least across broad taxonomic units. Yet this is exactly what comparative studies of brain size tacitly assume. A recent methodological advancement, the isotropic fractionator method (Herculano-Houzel & Lent, 2005) has made it possible to relatively quickly determine numbers of neurons and non-neuronal cells in whole brains and their parts. Results obtained using this method

highlighted the fact very clearly: not only are all brains *not* made the same way and distinct neuronal scaling rules apply in different mammalian lineages, but the differences can be striking (reviewed in Herculano-Houzel et al., 2015a). With that in mind, it might be best to limit comparative studies to closely related species, or to include data on neuronal numbers rather than just (relative or absolute) brain mass. Unfortunately, neither of these options might be feasible in every case.

In this thesis, I want to employ this modern technique to estimate cell numbers in whole brains and five brain regions in African mole-rats (Rodentia: Bathyergidae). There is very little published comparative data on mole-rat brains in general (Pirlot, 1989; Kruska & Steffen, 2009) and this is a first attempt at assessing the conservativeness of brain cellular scaling rules in a group of closely related mammals. Moreover, the naked mole-rat (*Heterocephalus glaber*) has been identified as an outlier among other rodents with respect to these rules (Herculano-Houzel et al., 2011), so comparative data on the whole family will shed some light on the reason of the deviation. This newly acquired information including data at the cellular level will then be used to test the social brain hypothesis (SBH), one of the most influential explanations for the evolution of large brains across vertebrates. This is also the first time direct data on neuronal numbers are used for evaluating an evolutionary hypothesis about the enhancement of cognitive abilities. Mole-rats serve as a suitable model in this case, because they are closely related, very uniform in their ecology, yet differ substantially in their social systems, ranging from solitary to the only vertebrates classified as "eusocial" (Jarvis & Bennett, 1993).

Before delving into the work itself, I want to give a brief overview of the social brain hypothesis, the cellular scaling rules described so far for vertebrate brains and a short introduction to the biology and social systems of the chosen model group, the African mole-rats (Bathyergidae), all of which should frame the findings presented later.

The social brain hypothesis

Brains vary tremendously in both absolute and relative size across the animal kingdom. Apart from the fact that the single best determinant of brain size is body size (Jerison, 1973), a number of other factors have been proposed to explain this variance, such as longevity, developmental mode, maternal investment, diet, mode of foraging, habitat complexity, home range, metabolic rate and trade-offs between metabolically expensive tissues (recently reviewed e.g. in Healy & Rowe, 2007; MacLean et al., 2012; Willemet, 2013; Isler & van Schaik, 2014).

Among these explanations, the social brain hypothesis is prominent. There are numerous versions of this hypothesis, but in the most general form, it contends that the demands imposed on individuals by a complex social environment exert a selection pressure favouring the evolution of large brains and complex cognitive abilities (Dunbar, 1998). It was originally proposed to explain the exceptional intelligence of primates (Humphrey, 1976; Byrne & Whiten, 1989; Dunbar, 1992), but it has since been extended to a wide range of taxa, including cetaceans (e.g. Marino, 1996), carnivores (e.g. Dunbar & Bever, 1998; Finarelli & Flynn, 2009; Arsznov & Sakai, 2013), bats (e.g. Barton & Dunbar, 1997), insectivores (Dunbar & Bever, 1998), ungulates (e.g. Pérez-Barbería & Gordon, 2005; Shultz & Dunbar, 2006), birds (e.g. Burish et al., 2004; Shultz & Dunbar,2010; West, 2014), cichlids (e.g. Pollen et al., 2007), and even insects (e.g. Ott & Rogers, 2010; Smith et al., 2010).

The SBH has a certain intuitive appeal and has gained great traction in evolutionary anthropology. Yet, what the underlying mechanisms are or how broadly it applies remains an area of active research. There is not a clear consensus about which proxy of cognitive abilities is the most suitable for testing the hypothesis. Direct behavioural tests are quite rare (e.g. MacLean et al., 2013) for the obvious reasons of practical difficulties with obtaining large enough sample, therefore some measure of brain size is commonly adopted. The most widely used is relative brain size (residuals from regression of brain mass on body mass) and neocortex ratio (the ratio of neocortex volume to the rest of brain volume) (Dunbar, 1992). The appropriateness of relating “brain size” with cognitive abilities will be discussed further in the next part of the introduction, but for now, I will just point out that neocortex ratio is more indicative of absolute brain size (see e.g. Finlay & Darlington, 1995) and neocortex size

is not necessarily reflective of intellectual abilities, since large areas are devoted to perceptual processing. For instance, the primary visual cortex in primates can account for over half of neocortex size [Van Essen et al., 1992]).

Another unsettled question is that of social complexity (for a review see Bergman & Beehner, 2015). For reasons of convenience, group size is most often chosen as a proxy, although it is not a very species-constant or reliable characteristic (Patterson et al., 2014) and does not necessarily correspond to complexity. Based on contrasting results in different animal groups, an important distinction has been made between primates and non-primates in this respect. In primates, neocortex ratio should correlate positively with group size; in other vertebrates, social monogamy should be associated with higher relative brain size (Shultz & Dunbar, 2007; Dunbar & Shultz, 2010).

This relates to the third unclear aspect: whether cooperation or competition is the main driving force of the cognitive enhancement mediated by sociality. Both factors have been implicated in primates – on the one hand, the original idea emphasized competition and tactical deception (as reflected in the name "Machiavellian intelligence"), but the mechanism was later reformulated by Dunbar as the need to maintain group cohesion through individual recognition and affiliative interactions to diffuse conflict. Such cognitively demanding behaviours are thought to take the form of behavioural coordination and pair bond formation in non-primates, but in primates have become generalized to all group members (reviewed in Dunbar & Shultz, 2007).

More recently, a related hypothesis, called the cooperative breeding hypothesis, has been put forward (Hrdy, 2005; Burkart & Schaik, 2010). Again, it was originally meant to explain the extraordinary human abilities and later applied to other lineages. The hypothesis posits that specific socio-cognitive domains can be enhanced by cooperative breeding, but also that increases in brain size might be in some cases facilitated by increased cognitive demands posed by allomaternal care in cooperative breeders. In this thesis, it is subsumed under the SBH umbrella (although the authors would probably protest), since it might be particularly relevant to African mole-rats, the ultimate cooperative breeders among mammals.

The empirical support of the SBH is not as sound as it may seem. For every group where more than one study was conducted there are opposing conclusions: for instance in primates (Kudo & Dunbar, 2001 vs. Schillaci, 2008), carnivores (Finarelli & Flynn, 2009 vs. Sakai et al., 2011), cetaceans (Marino, 1996 vs. Manger, 2006), bats (Barton & Dunbar, 1997 vs. Pitnick et al., 2006) birds (Burish et al., 2004 vs. Beauchamp & Fernández-Juricic, 2004) and cichlids (Pollen et al., 2007 vs. Reddon et al., 2016). And recent studies dealing with marsupials and wrasses failed to find a sound support for the claim that social complexity acts as a selection pressure for increasing brain size (Chojnacka et al., 2015; Weisbecker et al., 2015).

The hypothesis has not been tested in rodents until recently, which is surprising, given that they are the largest mammalian order and display a wide spectrum of social complexity. A possible reason is that they are not represented in the collections that served as the source of data on brain and brain compartment size for many such studies (such as Stephan et al., 1981; Jolicoeur et al., 1984). Matějů et al. (2016) were the first to fill this gap in knowledge and measured endocranial volumes in 63 species of the tribe Marmotini from skulls in museum collections. They showed that across ground squirrels, sociality is not associated with higher relative brain size (which, in some cases, is higher in solitary species), but that social species tend to have a larger body size and, correspondingly, absolutely larger brains. This does not support the SBH in the traditional sense, but authors invoke the possibility that a certain threshold brain size is needed to cope with the challenges of social lives in ground squirrels.

Given the above mentioned correlation of brain size with various life-history and ecology traits, testing of any specific effect in a group of animals homogenous in most other aspects is advisable. Mole-rats fit these requirements quite well and are thus the second rodent group to be examined with respect to the SBH. Can the pattern of contradictory results be broken?

Neuronal scaling rules and implications for cognitive abilities

Most comparative studies of brain evolution have used either brain mass or volume as the variable of interest. However, it is neurons and their connections that provide the substrate for cognition and simple volumes do not reflect either brain structure or complexity. With the establishment of the innovative isotropic fractionator method, it has become technically feasible to determine numbers of neurons and glia even in whole brains in a reasonable time-frame (Herculano-Houzel & Lent, 2005; Herculano-Houzel, 2012). The method has been independently shown to produce reliable results, comparable to the much more time-consuming and less effective stereological techniques (Bahney & von Bartheld, 2014; Miller et al., 2014).

Results obtained using this approach challenged a number of fallacies and misconceptions about brain composition. For instance the human brain does not contain 100 billion neurons and ten times more glial cells, as widely cited (e.g. Kandel et al., 2000), instead, the number is around 85 billion and the ratio of glia to neurons is close to 1:1 and most neurons are not located in the largest compartment (cerebral cortex) but in the cerebellum (80 %) (Azevedo et al., 2009).

It also transpired that brain cellular scaling rules differ in phylogenetic lineages, both between and within high level taxa, and even individual species can deviate from these rules in some way. Within mammals, primates are exceptional in that their numbers of cortical neurons scale almost linearly with cortical volume (Herculano-Houzel et al., 2007; Gabi et al., 2010), whereas in all other mammalian groups examined so far (rodents, insectivores, artiodactyls, afrotheres) they scale with negative allometry, meaning that larger brains show lower neuronal densities, and with different exponents in different groups (Herculano-Houzel et al., 2014b; Herculano-Houzel et al., 2015a). Conversely, it seems that scaling rules in the “rest of brain” (comprising the brain stem, diencephalon and basal ganglia) are shared by most mammals (Herculano-Houzel et al., 2015a). Neuronal scaling rules are not shared between Afrotheria and Eulipotyphla, some members of which were in the past classified together as “basal insectivores” based on their brain morphology (Stephan, 1972). For instance, eulipotyphlan insectivores have higher neuronal densities in the cerebellum (Sarko et al., 2009; Neves et al., 2014). The African elephant (*Loxodonta africana*) is a special case:

its cerebellum defies the patterns common to Afrotheria and all other mammals. It contains twice as many neurons than would be expected for the mass and more than ten times the neurons expected from the concerted relationship between cortex and cerebellum (Herculano-Houzel et al., 2014a). Artiodactyls are characterized by very low neuronal densities in the cortex (the neuronal fraction can be as low as 6 % in the giraffe), and surprisingly, deviate from the scaling rules in the "rest of brain" (Kazu et al., 2014).

Intriguingly, brains of songbirds and parrots feature exceptionally high neuronal densities, surpassing even those of primates (Olkowicz et al., under review). These avian brains thus contain up to 5 times the number of neurons compared to primate brains of the same size and, moreover, disproportionately more of all brain neurons are allocated in the telencephalon (up to 85 %, compared to around 20 % in mammals) and their numbers scale linearly with telencephalon size, providing a possible explanation for the remarkable cognitive abilities of some large corvids and parrots. Other bird groups examined do not reach these extreme levels, but still feature neuronal densities comparable to or higher than those of primates. These findings might be important not only in terms of absolute numbers of neurons, as the units of processing capacity, but also because of higher packing densities potentially increasing the processing speed and thus streamlining cognitive processes (Dicke & Roth, 2016). In gallinaceous birds (basal Neognathae), absolute numbers of brain neurons are 4-5 times lower, telencephalic neurons scale with negative allometry, and with growing brain size, disproportionately more brain neurons are allocated in the cerebellum (Kocourek et al., 2016). From other bird orders examined, owls reach similar neuronal densities to songbirds and parrots, while ratites and pigeons share lower encephalization, lower fraction of neurons allocated to the telencephalon and overall lower neuronal densities with gallinaceous birds (Olkowicz et al., in prep.).

To adopt a critical stance, many of these studies have used an objectionably low number of species (and individuals). Namely, the neuronal scaling rules in Eulipotyphla (Sarko et al., 2009), Afrotheria (Neves et al., 2014) and Artiodactyla (Kazu et al., 2014) have been established based on just five species and a significant proportion of species across the studies was represented by a single individual, which is perhaps understandable in the case of an elephant, but less so in a rabbit. Nevertheless, the rules seem to hold even after

including other species not present in the original dataset (Gabi et al., 2010; Herculano-Houzel et al., 2011).

Altogether these data make a strong case in favour of the absolute number of cortical neurons and neuronal densities being a highly important factor for animal "intelligence". These parameters are maximised in primates and large corvids and parrots, all of which are capable of advanced cognition (e.g. Emery, 2006; Seed et al., 2009). On the other hand, vertebrate brains seem to be extraordinarily inefficient compared to invertebrates. Many potentially demanding cognitive feats are apparently easily accomplished with miniature insect brains (e.g. Chittka & Skorupski, 2011; Avarguès-Weber & Giurfa, 2013; Giurfa, 2013). Darwin himself was struck by this, as he noted in *The Descent of Man*: "The brain of an ant is one of the most marvellous atoms of matter in the world, perhaps more so than the brain of a man." (Darwin, 1888). Even if we limit ourselves to vertebrates, neuronal numbers and densities are certainly not the best imaginable proxy for cognitive abilities, but they seem to provide the best feasible approximation.

African mole-rats

African mole-rats are a monophyletic family of hystricomorph rodents endemic to sub-Saharan Africa, comprising at least 22, but probably over 30, species in 6 genera (*Heterocephalus*, *Heliophobius*, *Bathyergus, Georychus*, *Cryptomys* and *Fukomys*) (Van Daele et al., 2007; Faulkes et al., 2010; Faulkes et al., 2011). Recently, it was suggested that *Heterocephalus glaber* be moved into its own family Heterocephalidae based on the time of divergence (Patterson & Upham, 2014). Since this is purely a matter of taxonomy and does not change the phylogenetic relationships in any way, all the species are treated here as belonging to the family Bathyergidae.

All mole-rats are strictly subterranean, living in burrows consisting of complicated tunnel systems, which they dig in the soil using their chisel-like incisors or, in the case of *Bathyergus*, forefeet (Bennett & Faulkes, 2000). Such environment provides relative safety from predators and protection from climatic extremes, but presents also challenges in the form of high relative humidity and low oxygen and high carbon dioxide concentrations (Nevo, 1979 but cf. Roper et al., 2001; Šumbera et al., 2004). In line with this underground

existence in virtual absence of light, all species are microphthalmic and have greatly reduced optic nerves and visual systems (reviewed in Němec et al., 2008). Consequently, other senses might be more developed, and magnetic orientation plays probably an important role in their navigation (reviewed in Moritz et al., 2007). Communication takes form of various vocalizations and drumming (e.g. Narins et al., 1992; Credner et al., 1997; Bednářová et al., 2013). They feed on underground parts of plants, such as bulbs, roots and tubers, that are available year-round but can be patchily distributed (Bennett & Faulkes, 2000). Despite the uniform fossorial lifestyle, African mole-rats cover the whole social spectrum: most species are cooperative breeders, some of which have been described as "eusocial" (Jarvis, 1981; Burda et al., 2000), while three genera are solitary dwelling and territorial with aggressive behaviour towards conspecifics (Bennett & Faulkes, 2000).

Bathyergids are among the most studied subterranean rodents and their biology has many intriguing aspects. Compared with other similar sized rodents, mole-rats are relatively long-lived (Sherman & Jarvis, 2002; Dammann et al., 2011). The naked mole-rat also shows increased tolerance to oxidative stress (Peréz et al., 2009) and certain types of painful stimuli (Park et al., 2008; Lavinka et al., 2009) and decreased susceptibility to cancer (Buffenstein, 2008). For these reasons they received a lot of attention and, lately, the naked mole-rat has become an important model species in research of aging, cancer, hypoxia and nociception (reviewed in Schuhmacher, 2015). Moreover, the naked mole-rat is, apparently, a poikilothermic mammal and has a very low resting metabolic rate (Buffenstein & Yahav, 1991; McNabb, 1996). Owing to these traits and the importance of mole-rats as a model for the study of mammalian sociality, a relative abundance of information is available about their physiology, ecology and social systems. In the context of this thesis, the differences between social and solitary species and the social organization are the most relevant and will be further described.

Solitary mole-rats

Mole-rats in the three solitary genera *Heliophobius*, *Bathyergus* and *Georychus* are less well studied than the social ones. They are generally larger in body size, are restricted to more mesic regions of the range with softer soils (Bennett & Faulkes, 2000) and breed seasonally after winter rainfall, with presumably promiscuous mating (Patzenhauerová et al., 2010;

Bray et al., 2012) and only a brief period of contact between the mating partners. The female cares for the pups alone and they start displaying aggressive behaviour shortly after weaning and subsequently disperse (Bennett & Jarvis, 1988; Šumbera et al., 2003; Herbst et al., 2004).

Social mole-rats

The situation in social bathyergids (*Heterocephalus, Cryptomys* and *Fukomys*) is almost completely opposite. They tend to attain smaller sizes (although *F. mechowii* is the second largest in the family) and occur over a wider geographic range, both in relatively mesic and in arid regions, where the soils can get extremely hard during droughts, preventing efficient burrowing (Bennett & Faulkes, 2000). They are continuous breeders and most are probably socially monogamous (e.g. Bappert et al., 2012; Patzenhauerová et al., 2013) but several males have been reported to mate with the reproductive female (e.g. Jarvis 1981; Faulkes et al. 1997). Most importantly, cooperative breeding is an integral part of their reproduction, with one female ("the queen") monopolizing reproduction and the offspring of the reproductive pair staying for prolonged periods in the natal colony as non-breeding helpers. The non-reproductive animals are mostly responsible for digging and maintaining burrows, foraging for food and bringing it to communal storage, colony defence against predators and foreign conspecifics, but also taking care of the pups – grooming, huddling, returning them to the nest chamber when they wander off and providing them with cecotrophs (Jarvis, 1981; Bennett, 1990; Burda & Kawalika, 1993; Jarvis & Bennett, 1993; Burda, 1995). In some species there is evidence of morphologically distinct "infrequent workers", characterized by larger body size and more fat reserves, that may function as a dispersive caste (e.g. O'Riain et al., 1996; Scantlebury et al., 2006). Pregnancy and lactation in social mole-rats is longer and the young grow more rapidly compared with solitary ones (Burda, 1990; Bennett et al., 1991).

There are also some differences between the social genera. *Heterocephalus* tends to have the largest colonies, suppression of reproduction in subordinates of both sexes is physiological and hormonally mediated (Faulkes et al., 1990; 1991), probably induced by aggression from the breeding female. When a reproductive animal is removed, it is replaced from within the colony after aggressive struggles for the queen status among the dominant non-reproductive females (Clarke & Faulkes, 1997). This results in high levels of inbreeding

(Reeve et al., 1990), but newer data indicate that outbreeding is still preferred even in this species (Braude, 2000; Ciszek, 2000). In contrast, in *Fukomys* and *Cryptomys*, incest avoidance seems to be the main mechanism of reproductive suppression and subordinate animals are fertile and able to mate with unfamiliar conspecifics (Burda, 1995). When reproductive animals die, the colony either halts reproduction until it is joined by a foreign conspecific or it dissolves (Jarvis & Bennett, 1993). However, the above described situation is not so clear-cut and, occasionally, adult and subadult foreign conspecifics can be found in the colonies and subordinate or even extra-colony males can father offspring (Bishop et al., 2004; Burland et al., 2004). In *Cryptomys*, reproduction is thought to be seasonal (Spinks et al., 1997; Rensburg et al., 2002), but this may not be universal (Oosthuizen et al., 2008), and groups tend to be smaller and much less stable, especially in the mesic parts of the range (Spinks et al., 2000).

Evolution of sociality

There is some debate about whether social or solitary organization is derived in mole-rats. The assumption of sociality being ancestral is sound on the grounds that the closest recent relatives to bathyergids are also social. The cane rats (genus *Thryonomys*) live in small family groups (Fitzinger, 1995) and the dassie rat (*Petromus typicus*) displays social monogamy with strong pair bonds (Rathbun & Rathbun, 2006). On the other hand, sociality is rather uncommon among subterranean mammals, and bathyergids from the fossil record are large just like recent solitary species, which has led some authors to look for an adaptive explanation for the emergence of sociality (e.g. Jarvis & Bennett, 1991).
The aridity food-distribution hypothesis (AFDH) (reviewed in Jarvis et al., 1994) posits that (eu)sociality in mole-rats evolved in response to unpredictable rainfalls in their geographic range and the associated scarcity and patchy distribution of food resources. Under such conditions, the risks of food shortage and the costs of dispersal might be too high and favour philopatry. This has been criticised on the grounds that the distribution of social species overlaps in some regions with that of solitary ones (Burda, 1990), but it is important to note that according to this hypothesis, the constraint only works in one direction and nothing prevents the social mole-rats from moving into mesic areas, but rather the solitary species are restricted to more favourable environments. Regardless of whether sociality in the family was ancestral or not, based on the current consensus on mole-rat phylogeny and the

principle of parsimony, it must have been either lost twice independently, or lost once and regained once. (Phylogenetic tree of Bathyergidae with social system mapped is provided bellow in Figure 5).

Eusociality

The most remarkable aspect of mole-rat sociality is that it has in some cases warranted the label of "eusociality" (reviewed in Burda et al., 2000). Eusociality in mole-rats was first identified in *Heterocephalus glaber* (Jarvis, 1981) based on the criteria of the original definition applying to social insects: reproductive division of labour (i.e. existence of a reproductive caste), cooperative care of the young and overlapping adult generations (Wilson, 1971). Questions concerning the factors leading to the evolution of eusociality, or indeed the criteria that should apply in classifying mole-rat species as eusocial, are still not settled. According to Burda (1999), life-long philopatry in most individuals (assessed indirectly by the number of overlapping generations) should be the decisive factor. Alternatively, colony size and reproductive skew can be used as indicators of eusociality (e.g. Sherman et al., 1995; Faulkes et al., 1997), but all these criteria are actually related and give similar results. For the purposes of this thesis, species are assigned to the "eusocial" and "social" categories based on Burda (1999).

African mole-rats are an ideal group to provide answers to some of the unanswered questions concerning the SBH without introducing confounding factors associated with substantial differences in general biology and ecology. Moreover, due to the special nature of their social systems, they might give more insight into the underlying mechanisms of the association between sociality and brain size.

AIMS

To estimate neuronal and non-neuronal cell counts in the brains and five brain regions (cerebral cortex, cerebellum, olfactory bulbs, diencephalon and basal ganglia, brain stem) of 11 species of African mole-rats.

To see how African mole-rats compare to other rodents examined with respect to numbers of neurons and associated brain scaling rules.

To test whether sociality is associated with absolute or relative brain size and numbers of neurons in African mole-rats.

MATERIALS AND METHODS

Animals

Eleven species of African mole-rats (Bathyergidae) were used for the analysis. Three solitary: the Cape dune mole-rat *Bathyergus suillus* (BS), the Cape mole-rat *Georychus capensis* (GC), the silvery mole-rat *Heliophobius argenteocinereus* (HA); four classified as social: the common mole-rat *Cryptomys hottentotus* (CH), the highveld mole-rat *Cryptomys pretoriae* (CP), the Natal mole-rat *Cryptomys natalensis*, the Mashona mole-rat *Fukomys darlingi* (FI), and four classified as eusocial: the Ansell's mole-rat *Fukomys anselli* (FA), the Damaraland mole-rat *Fukomys damarensis* (FD), the giant mole-rat *Fukomys mechowii* (FM), the naked mole-rat *Heterocephalus glaber* (HG). Three adult individuals of each species were processed, including at least one of each sex (in social species, only non-reproductive individuals were used). Some animals were obtained from colonies in the University of South Bohemia (HG, FI, FM) and from colonies in the University of Duisburg-Essen (FA). All the other species were wild-caught (BS, CH, CP, CN, FD, GC, HA).

Tissue preparation and brain dissection

Animals were killed by halothane overdose, weighed and measured, then perfused transcardially with heparinized phosphate-buffered saline (PBS), followed by 4% phosphate-buffered paraformaldehyde (PFA). Afterwards, the animals were dissected for sex determination. Brains were harvested immediately after perfusion, the spinal cord was transected at the level of the first spinal nerve and the optic nerve cut rostrally to the optic chiasm, meninges were removed, and the brains were blotted with a paper towel and weighed. Brains were then post-fixed in the same fixative for at least 2 weeks.

After fixation, brains were dissected under an Olympus SZX 16 stereomicroscope into the following five parts: olfactory bulbs, cerebral cortex (including the underlying white matter), cerebellum, diencephalon and basal ganglia, brain stem. When dissecting the cerebellum, cerebellar peduncles were cut at the surface of the brain stem. Olfactory bulbs were separated by transecting the olfactory tract. Cerebral cortex includes the hippocampus and piriform cortex and the underlying white matter and was dissected by peeling it away from the subcortical structures (in a manner consistent with Herculano-Houzel et al., 2006). Additionally, the brain stem (comprising the mesencephalon and medulla oblongata) was

separated from the ensemble of basal ganglia and diencephalon (hereafter referred to as diencephalon) by cutting along the plane connecting the posterior commissure dorsally and hypothalamus-mesencephalon boundary ventrally. Each part was then blotted and weighed using analytical scales to the tenth of a milligram. Mass was corrected for shrinkage during fixation by multiplying with the ratio of original brain mass to postfixed brain mass. When comparing mole-rats to other rodents, the diencephalon and brain stem are pooled together as the "rest of brain".

Figure 1. Dissected brain of the giant mole-rat *Fukomys mechowii*, showing the olfactory bulbs, cerebral hemispheres, diencephalon, cerebellum and brain stem.

Sample preparation

To obtain a suspension of free nuclei, each brain part was manually homogenized using an appropriately sized Tenbroeck tissue grinder (2ml, 7ml, or 15ml) in a dissociation solution (40 mM natrium citrate with 1% Triton X-100 (Sigma-Aldrich)), until no visible pieces of tissue were present. The quality of homogenate was checked using the microscope, to verify that no large clumps of cells were present. In case the homogenate was not ground adequately, sample was reground to ensure reliable results. The homogenate was then quantitatively transferred to a centrifuge tube and the volume was adjusted with PBS to a precise value. To be able to visualize the nuclei under a fluorescent microscope, 5 vol% of 1% solution of DAPI (2-(4-amidinophenyl)-1H-indole-6-carboxamidine, Sigma-Aldrich) in PBS was added.

Cell counting

Samples were well mixed, taking care not to create bubbles, so as to obtain a homogenous suspension. Four samples of 10 µl were then taken, applied to an improved Neubauer counting chamber (Karl Hecht GmbH & Co KG; Figure 2) and examined using a fluorescent microscope (Olympus BX 51) at 400x magnification. All the nuclei in the area of one big square (25 inners squares) were counted manually using a clicker and coefficient of variation (CV - the ratio of standard deviation to mean) for the 4 samples was calculated. To ensure reliable counts, if the CV was over 10 % more samples were processed until the criterion was reached.

The mean of all the samples for the given structure (number of nuclei per 0.1 µl) was then multiplied by 10,000 to obtain the number in 1 ml and by the total volume of the homogenate in ml to derive the actual number of cells in the structure.

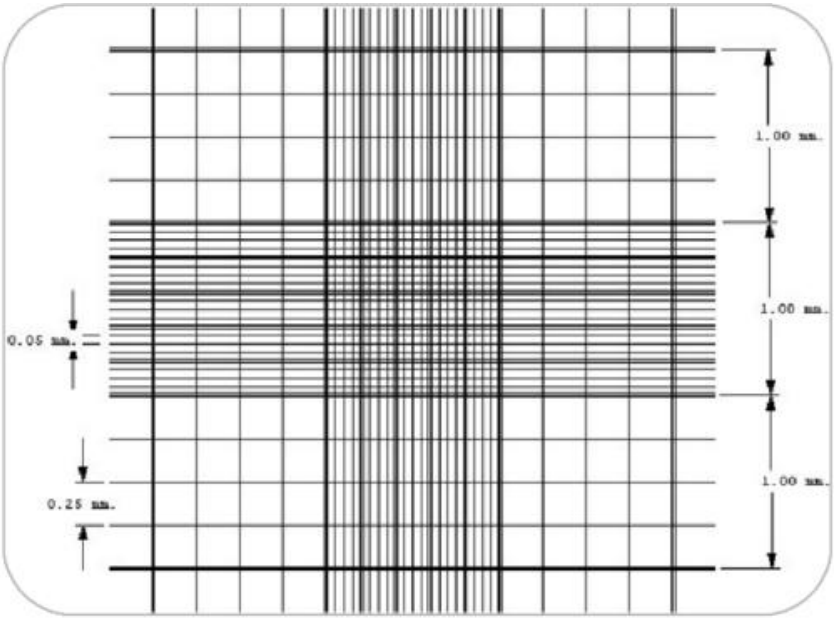

Figure 2. The counting grid of the Neubauer chamber. Nuclei were counted in the central big square, divided into 25 inner squares. This corresponds to a volume of 0.0001 ml. Figure comes from a technical manual (Bastidas, 2013).

Immunostaining

After counting the absolute number of cells, 1ml samples were centrifuged, washed in PBS three times and stained immunocytochemically for a nuclear neuronal marker NeuN. This marker is highly conserved across mammals and even vertebrates and stains all the major neuronal types in the brain, with the exception of cerebellar Purkinje cells and mitral cells (Mullen et al., 1992). This is not a concern in this type of study, since these likely account for a negligible proportion of all brain neurons (Andersen et al., 1992).

The samples were incubated overnight at 4°C with anti-NeuN rabbit polyclonal antibody (Merck Millipore, dilution 1:800) with the addition of 5 % normal goat serum and subsequently washed in PBS with 1% Triton-X (PBS-T) three times and incubated for 1 hour at room temperature with secondary anti-rabbit antibody conjugated with Alexa Fluor 594 (Invitrogen, dilution 1:400). 5 % DAPI was added again to counterstain the nuclei. The proportion of NeuN-positive nuclei was then determined for each sample by applying a 10 µl sample in the hemocytometer and examining at least 500 individual nuclei.

The percentage of immunopositive nuclei was then applied to the previously determined cell count to derive the absolute number of neurons and non-neuronal cells.

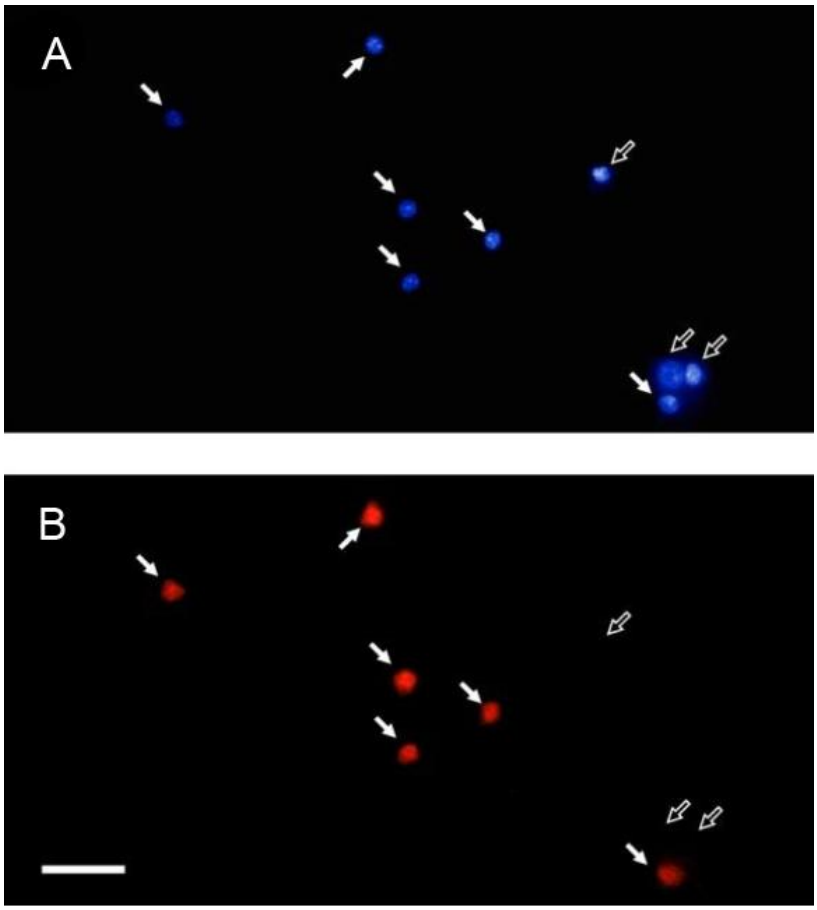

Figure 3. High-power micrographs showing a sample of homogenate from the cerebral hemispheres (A) dissociated nuclei stained with DAPI (B) immunolabeled with anti-NeuN antibody and secondary antibody conjugated with Alexa Fluor 594. Scale bar at 50 µm.

Coding of sociality

Given the lack of a generally accepted measure of social complexity and problems associated with even simple measures such as group size (Patterson et al. 2014), we decided to adopt a clean categorical approach. While crude, it is not subject to much variation and research effort bias, and association with brain measures should be apparent. Sociality was coded as either two-level (solitary, social) or three-level (solitary, social, eusocial) categorical variable. For the purposes of this thesis, species are assigned to the "eusocial" category according to Burda (1999). All the other cooperatively breeding species are considered "social". Although there is some controversy over these labels, this is not a central question of this thesis. It merely serves as a potentially interesting distinction, allowing for a more detailed study of factors affecting brain size and composition. In any case, the main distinction between solitary and social, important for testing of the SBH, is very clear.

These categories also roughly correspond to group size (Faulkes et al., 1997). For the sake of completeness, sociality was also treated as a numerical variable in the form of maximum group size. Group sizes were collated from the literature (Faulkes et al., 1997; Sichilima et al., 2008; Sichilima et al. 2012).

Statistical analysis

Data analyses were performed in R Studio with R 3.1.2 (R Core Team, 2015). Data were log-transformed when warranted to get normal distribution. Regressions were done using phylogenetic least squares (PGLS), as implemented in the R package *nlme*, with simultaneous estimation of the phylogenetic signal with the regression parameters (Revell, 2010). For the sake of ensuring comparability with previously published data on rodents and other mammals, in the descriptive part, linear regression coefficients were also determined by the ordinary least squares (OLS) method from species averages. To avoid cluttering the text, the phylogenetically informed statistics are reported only where the two methods are not in agreement. Statistical significance was evaluated at α level of 0.05. For analyses involving whole brain mass without neuronal counts, an extended data set with 106 individuals was used.

Due to the small number of species in each sociality group, inadequate for the use of ANOVA or ANCOVA, tests of the effect of sociality were performed on individual data using Bayesian generalised linear mixed models with Markov chain Monte Carlo (MCMC) estimation in the R package *MCMCglmm* (Hadfield, 2010). These models allow for including phylogenetic data

and multiple measurements per species as random effects. Non-informative priors were defined for both random effects and the residual variance as *V*=1 and *nu*=0.002, which corresponds to an inverse gamma distribution. Each model was run for 5 million iterations, with a burnin of 5000, and a thinning interval of 1000, that means approx. 5000 estimations were sampled. This resulted in complete convergence, with no autocorrelation between the successive iterations in the Markov chain. Estimates of the differences between the levels of sociality were calculated from a posterior distribution created by subtracting the estimates for each level obtained during each MCMC iteration. Parameter estimates were considered statistically significant when 95% high posterior density intervals (HPD) did not include 0. Mole-rat phylogeny was constructed from Faulkes et al. (2004), Glires phylogeny was constructed from Blanga-Kanfi et al. (2009) in R package *ape* (Paradis et al., 2004) and proportional branch lengths were assumed (Figure 4).

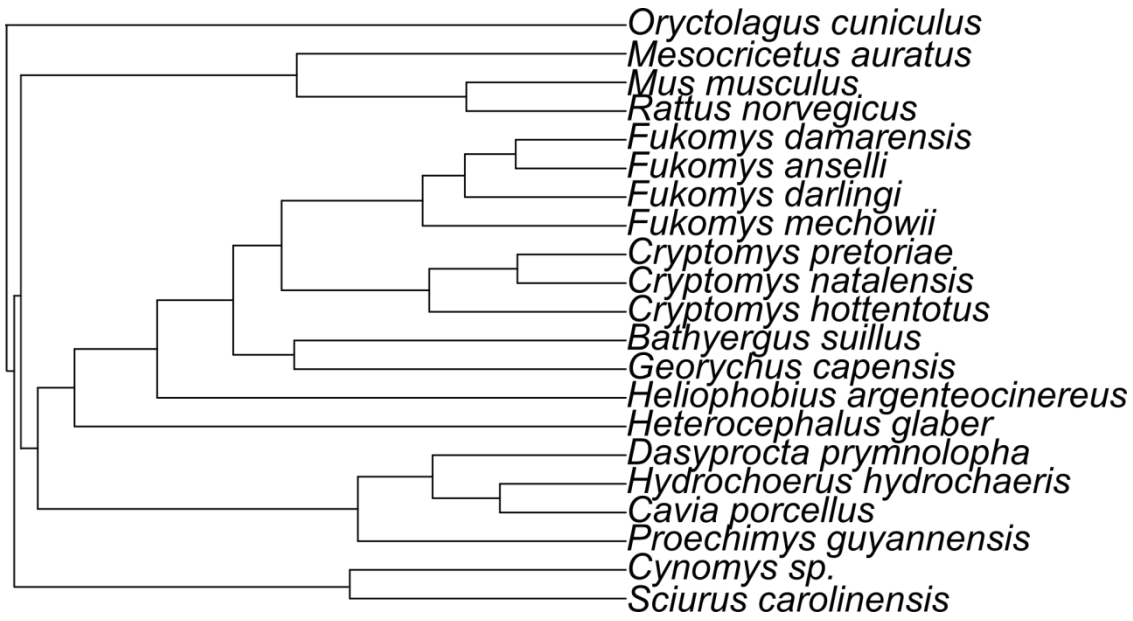

Figure 4. Combined phylogenetic tree of the Glires species included in Herculano-Houzel et al. (2011) and the mole-rat species included in this thesis. Constructed from Blanga-Kanfi et al. (2009) and Faulkes et al. (2004) and made ultrametric.

Graphing

All graphs were plotted in R package *ggplot2* (Ginestet, 2011) and additional labels and aesthetics were added in the vector graphics editor Inkscape 0.91.

RESULTS

Results are presented in two parts corresponding to the two aims of this thesis: first, the descriptive data on mole-rat brains are presented in context of other rodent species, and then these data are used to look for a relationship with sociality. Each part is concluded with a short summary of main findings and a general discussion follows.

Mole-rat brains in numbers

All quantitative data collected for this thesis are presented in Table 1.

Table 1. Summary of all the data averaged by species.

Species	*B. suillus*	*G. capensis*	*H. argenteocinereus*	*C. hottentotus*	*C. natalensis*	*C. pretoriae*
Body mass (g)	1120.50 ± 484.40	151.50 ± 40.93	170.60 ± 17.15	68.70 ± 7.03	88.77 ± 40.45	109.33 ± 7.77
Whole brain mass (mg)	3818.90 ± 107.99	1671.40 ± 184.82	1603.33 ± 99.38	845.67 ± 22.05	1261.50 ± 108.77	1461.33 ± 53.26
neurons (mil.)	361.29 ± 24.4	169.67 ± 11.42	147.94 ± 6.80	96.24 ± 9.65	149.98 ± 8.50	169.16 ± 16.23
other cells (mil.)	236.03 ± 19.62	120.50 ± 16.11	110.01 ± 4.34	66.07 ± 5.57	83.44 ± 0.79	104.09 ± 5.73
Cerebral cortex mass (mg)	1566.87 ± 94.31	738.97 ± 82.73	695.63 ± 45.95	342.47 ± 35.27	533.93 ± 53.97	649.87 ± 2.42
neurons (mil.)	43.48 ± 5.11	25.60 ± 2.09	25.44 ± 2.23	11.73 ± 0.22	17.46 ± 1.39	20.56 ± 0.90
other cells (mil.)	68.64 ± 3.24	34.93 ± 3.12	30.10 ± 3.41	15.85 ± 0.81	21.22 ± 1.27	28.73 ± 1.29
Cerebellum mass (mg)	711.77 ± 47.87	244.67 ± 37.60	232.90 ± 11.27	139.90 ± 11.86	229.53 ± 26.93	243.37 ± 29.86
neurons (mil.)	268.89 ± 17.47	116.74 ± 10.31	94.82 ± 1.97	68.81 ± 9.02	109.75 ± 8.75	120.58 ± 16.69
other cells (mil.)	69.5 ± 16.24	28.63 ± 6.77	25.75 ± 2.80	17.64 ± 2.31	21.52 ± 4.62	26.51 ± 3.35
Olfactory bulbs mass (mg)	188.50 ± 25.38	73.83 ± 5.98	59.60 ± 10.71	48.43 ± 3.42	70.77 ± 11.36	62.97 ± 6.98
neurons (mil.)	23.81 ± 2.96	12.46 ± 1.42	10.30 ± 2.15	7.99 ± 0.46	11.85 ± 0.29	12.07 ± 1.41
other cells (mil.)	29.09 ± 5.57	11.85 ± 2.02	9.32 ± 1.04	7.78 ± 0.37	10.77 ± 2.04	11.82 ± 0.67
Dienc. +striatum mass (mg)	676.73 ± 22.16	337.43 ± 43.97	356.23 ± 37.59	177.97 ± 7.98	250.23 ± 35.15	295.17 ± 25.94
neurons (mil.)	16.42 ± 0.75	9.06 ± 1.13	11.92 ± 1.13	4.55 ± 0.69	6.82 ± 0.81	9.92 ± 0.62
other cells (mil.)	37.78 ± 5.020	23.95 ± 2.31	23.43 ± 4.00	13.10 ± 1.61	15.26 ± 1.45	19.71 ± 2.03
Brain stem mass (mg)	674.97 ± 62.73	276.50 ± 16.39	259.13 ± 9.66	159.23 ± 8.20	177.23 ± 17.03	209.83 ± 5.33
neurons (mil.)	8.67 ± 0.60	5.81 ± 0.34	5.46 ± 0.84	3.17 ± 0.36	4.11 ± 0.14	6.03 ± 0.35
other cells (mil.)	31.01 ± 0.47	21.14 ± 2.16	21.42 ± 1.10	11.69 ± 0.79	14.68 ± 0.89	17.33 ± 1.28

Table 1. Continued

Species	*F. darlingi*	*F. anselli*	*F. damarensis*	*F. mechowii*	*H. glaber*
Body mass (g)	154.37 ± 41.64	82.67 ± 10.26	96.30 ± 33.34	308.63 ± 44.69	24.00 ± 2.33
Whole brain					
mass (mg)	1344.67 ± 42.16	1148.67 ± 16.29	1357.20 ± 52.86	1820.33 ± 93.00	399.67 ± 30.07
neurons (mil.)	113.35 ± 3.79	102.82 ± 12.28	178.3 ± 19.08	174.24 ± 14.60	33.62 ± 3.66
other cells (mil.)	82.76 ± 6.94	72.84 ± 3.41	100.21 ± 6.64	131.89 ± 2.13	29.83 ± 1.81
Cerebral cortex					
mass (mg)	624.60 ± 16.53	480.27 ± 14.99	558.47 ± 16.48	790.20 ± 29.43	170.37 ± 24.69
neurons (mil.)	12.49 ± 0.53	9.73 ± 2.23	21.27 ± 0.59	23.32 ± 0.94	5.31 ± 0.31
other cells (mil.)	20.10 ± 1.68	17.12 ± 0.81	25.09 ± 1.52	38.38 ± 1.81	7.34 ± 0.53
Cerebellum					
mass (mg)	202.13 ± 10.76	160.20 ± 11.23	247.77 ± 19.90	284.90 ± 20.62	51.57 ± 7.89
neurons (mil.)	84.91± 3.95	75.44 ± 8.90	129.96 ± 18.15	122.64 ± 14.34	20.52 ± 3.01
other cells (mil.)	25.79 ± 4.71	18.83 ± 1.87	25.02 ± 1.73	33.61 ± 3.14	9.19 ± 1.46
Olfactory bulbs					
mass (mg)	55.167 ± 1.53	49.90 ± 5.23	66.87 ± 6.73	78.50 ± 6.91	19.37 ± 2.36
neurons (mil.)	7.60 ± 1.25	8.88 ± 0.64	12.55 ± 1.82	14.11 ± 2.13	3.01 ± 0.33
other cells (mil.)	8.68 ± 1.09	8.38 ± 0.12	10.86 ± 1.71	14.71 ± 1.94	3.24 ± 0.33
Dienc. + striatum					
mass (mg)	260.20 ± 11.19	271.90 ± 14.90	265.20 ± 15.54	382.80 ± 28.97	89.73 ± 7.20
neurons (mil.)	5.19 ± 0.81	4.86 ± 0.90	9.58 ± 1.69	9.42 ± 0.88	2.86 ± 0.50
other cells (mil.)	14.62 ± 1.75	13.47 ± 0.42	19.97 ± 1.83	25.42 ± 1.42	4.96 ± 0.56
Brain stem					
mass (mg)	202.50 ± 18.95	186.37 ± 15.05	219.83 ± 18.66	283.93 ± 27.04	68.37 ± 5.78
neurons (mil.)	3.14 ± 0.15	3.90 ± 1.16	4.94 ± 1.10	4.75 ± 0.98	1.91 ± 0.28
other cells (mil.)	13.57 ± 0.70	15.04 ± 1.13	19.28 ± 0.83	19.78 ± 1.47	5.10 ± 0.67

Absolute and relative brain size

The studied species range in average body mass from 37 g to 908 g and in average brain mass from 400 mg to 3.82 g (Figure 5). The relationship between brain mass and body mass can be described by the equation:

$$\log(\text{brain mass}) = 4.166 + 0.624 \times \log(\text{body mass})$$

($R^2 = 0.92$, $p < 0.0001$, intercept 95% CI = 3.483-4.848, slope 95% CI = 0.486-0.762). Species averages for establishing this power law were taken from a larger number of individuals (n=106) used in Kverková et al. (in prep.). Nevertheless, the results are not significantly different when using just the 33 individuals included in the present work.

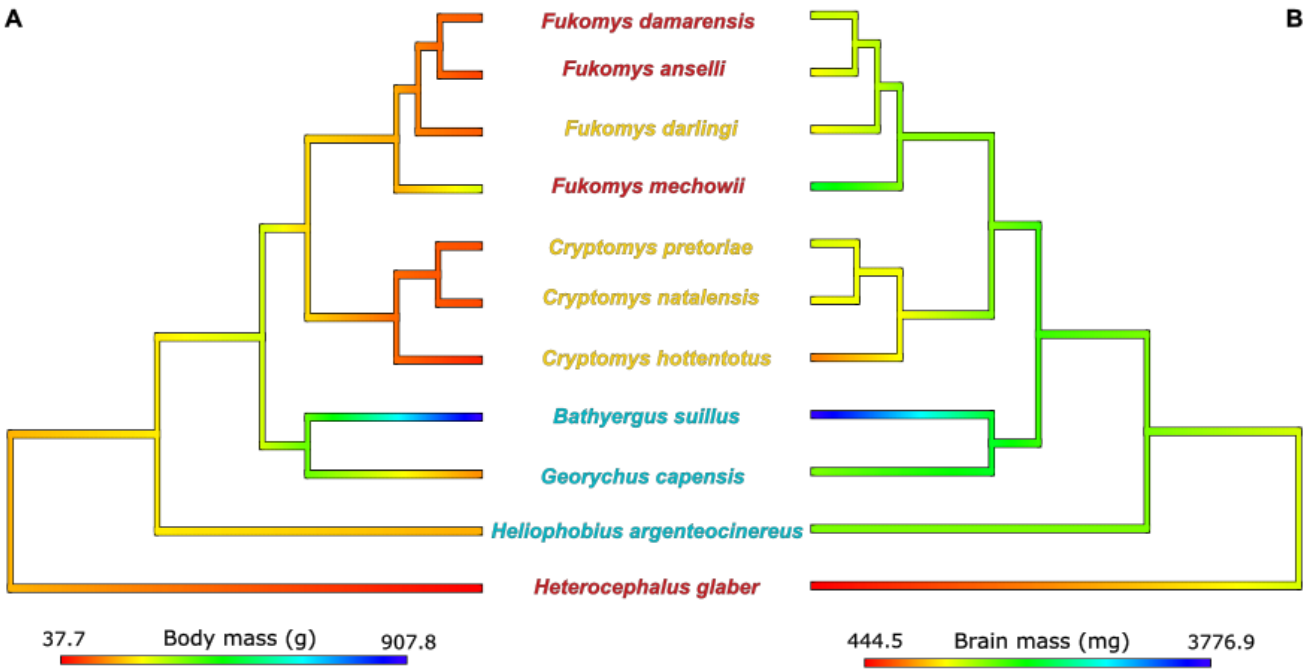

Figure 5. Mapping of body mass and brain mass on a phylogenetic tree of Bathyergidae. The phylogeny of the 11 species of Bathyergidae included in the analyses with (A) body mass and (B) brain mass mapped as a continuous trait. Species names are colour-coded by sociality: red – eusocial, yellow – social, blue – solitary.

When compared with the brain-body relationship in other rodents, mole-rats show lower than average encephalization. They fall below the regression line *log (brain mass) = 4.482 + 0.642 × log (body mass)* (R^2 = 0.92, p < 0.0001, intercept 95% CI = 4.388-4.575, slope 95% CI = 0.624-0.660), but within the 95% prediction interval of the expected brain-body relationship for Rodentia, with the exception of the naked mole-rat *Heterocephalus glaber* and the Cape dune mole-rat *Bathyergus suillus*, the smallest and largest mole-rat species, respectively (Figure 6).

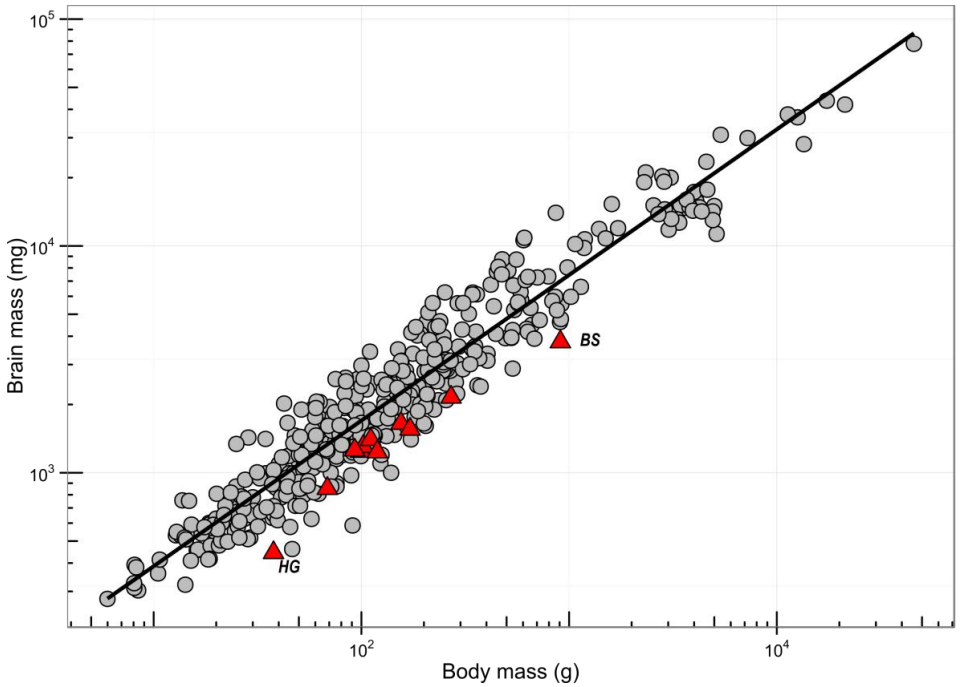

Figure 6. Brain-body scaling in a large dataset of rodent species (n=414) collated from the literature and kindly provided by Zuzana Pavelková. Data points represent species averages. Mole-rat species included in this study are represented by red triangles. Regression line shown is for OLS regression calculated for rodents excluding mole-rats. *Bathyergus suillus* is labelled BS and *Heterocephalus glaber* is labelled HG.

Relative size of individual brain parts

Bathyergids are relatively small-brained rodents, but is the brain reduced in size uniformly, or is there some selective reduction? Just as brain mass scales tightly with body mass, different brain structures change in size predictably as brain mass increases. Mass of all the brain structures correlates highly with overall brain mass (R^2=0.934–0.998, p <0.0001 in all cases) and mole-rats share these scaling rules with other rodents (Figure 7).

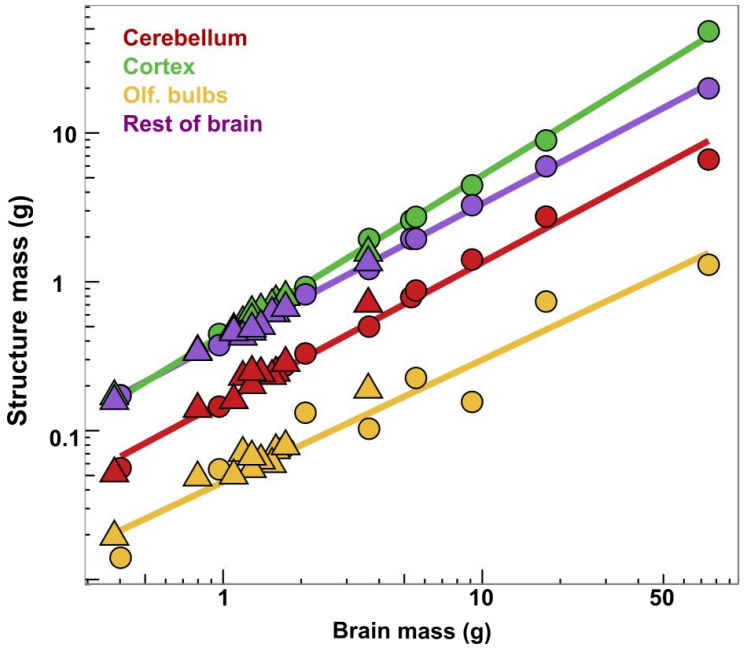

Figure 7. Scaling of structure mass with whole brain mass. Data points are species averages. Mole-rats are represented by triangles, other rodents are represented by circles. Regression lines shown are from OLS regression of structure mass on brain mass in the Glires dataset (Herculano-Houzel et al., 2011). Whole brain does not include olfactory bulbs for the sake of comparability with the published data. Axes are log-transformed.

While, as shown above, individual structures scale predictably with overall brain size, due to the different slopes their proportions may change as brains get larger. In rodents, this happens in the cortex and "rest of brain". As the cortex gets disproportionately larger with increasing brain mass (OLS: slope=0.0374, p=0.0014), the "rest of brain" gets correspondingly smaller (OLS: slope= -0.0251, p=0.0003), and the cerebellum and olfactory bulbs maintain about the same proportion. Within mole-rats, none of these trends reaches statistical significance, but most strikingly, the proportion of cortex does not seem to depend on brain size (Figure 8A). This might be due to the fact that the range of mole-rat brain sizes is rather limited compared to the range of the other rodent species included. In any case, the absence of this relationship may be important for interpreting the differences between social and solitary mole-rats.

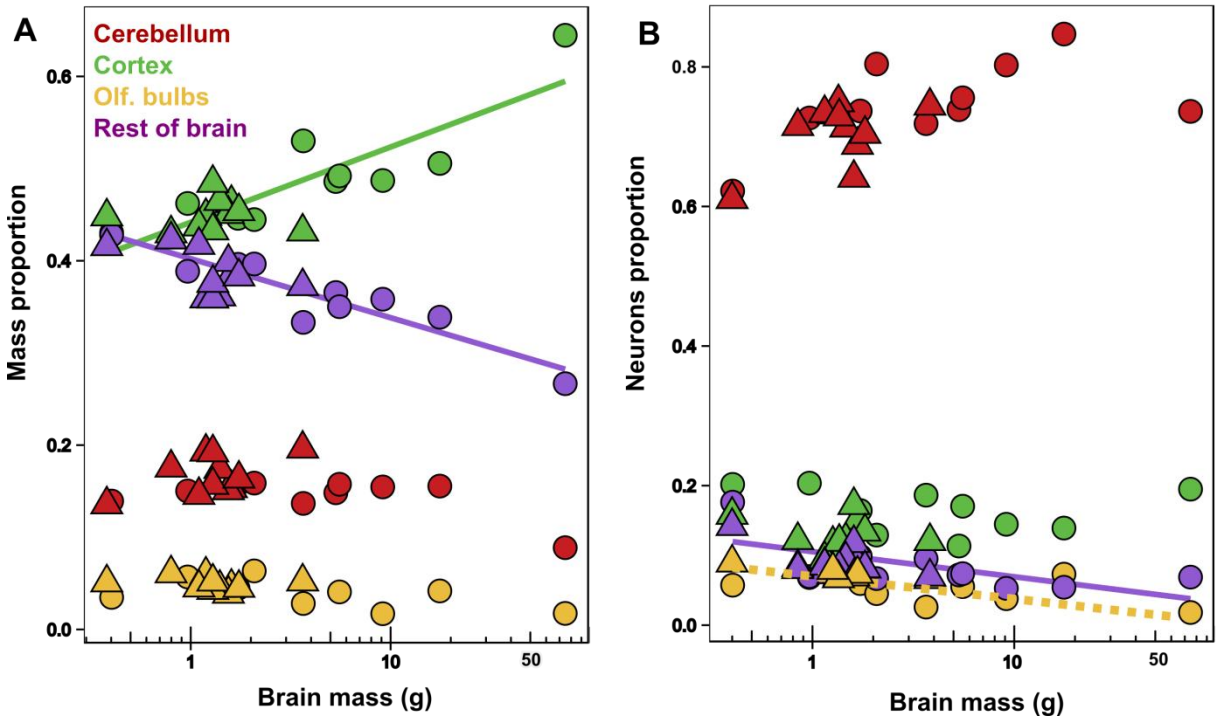

Figure 8. Scaling of brain structure proportions with brain mass. (A) Proportion of brain mass. (B) Proportion of brain neurons. Data points are species averages. Mole-rats are represented by triangles, other rodents are represented by circles (data from Herculano-Houzel et al., 2011). Regression lines shown for the significant relationships are from OLS regression of structure mass/neurons proportion on brain mass (dotted line – mole-rats, full line – other rodents). Whole brain does not include olfactory bulbs for the sake of comparability with the Glires dataset. Axes are log-transformed.

Proportions of different brain parts in mass and neurons

While whole brain and brain region masses are somewhat informative, the focus of this thesis is on the scaling and allocation of neurons. Like in all mammalian groups studied so far, mole-rats show stark differences in brain composition in terms of mass and neuronal numbers (Figures 9, 10). Whereas the cortex accounts for almost half of the brain mass (39-46 %), it contains only 10-17 % of all brain neurons. Conversely, the cerebellum takes up just 13-18 % of brain mass, but houses an overwhelming majority of neurons (61-75 %). This is comparable to other rodents examined, where the cortex accounts for 12-19% and the cerebellum for 56-75% of brain neurons (Herculano-Houzel et al., 2011).

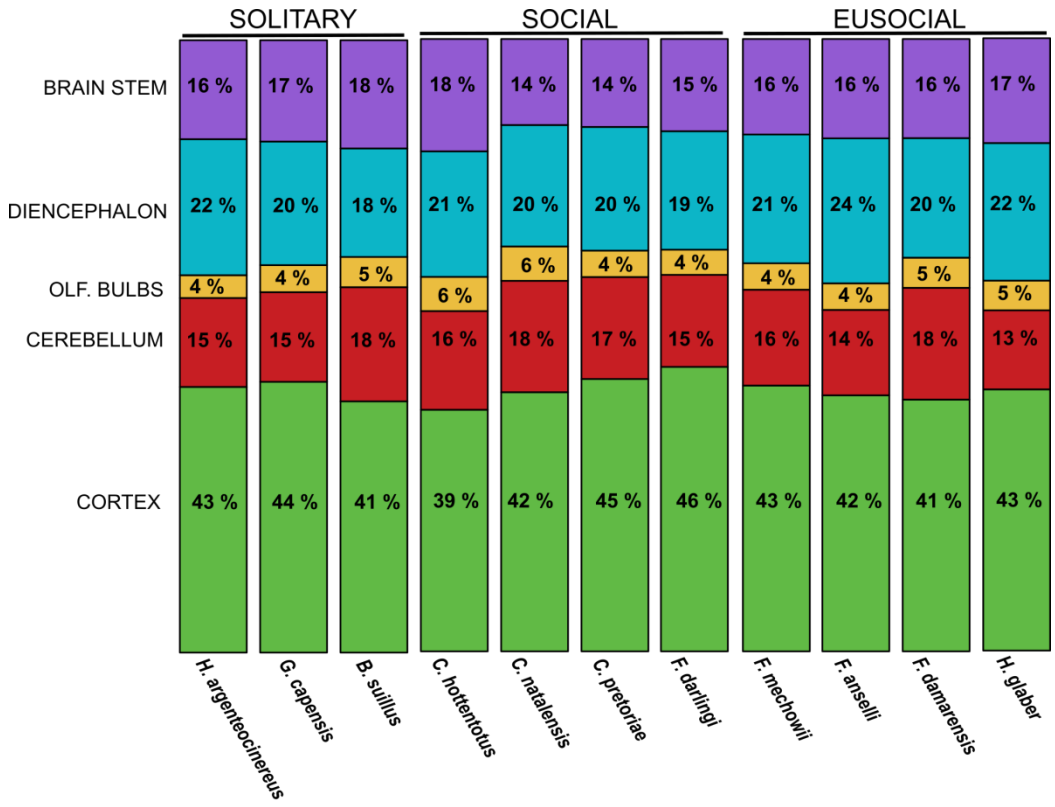

Figure 9. Average mass percentages of different brain regions in the 11 species of mole-rats.

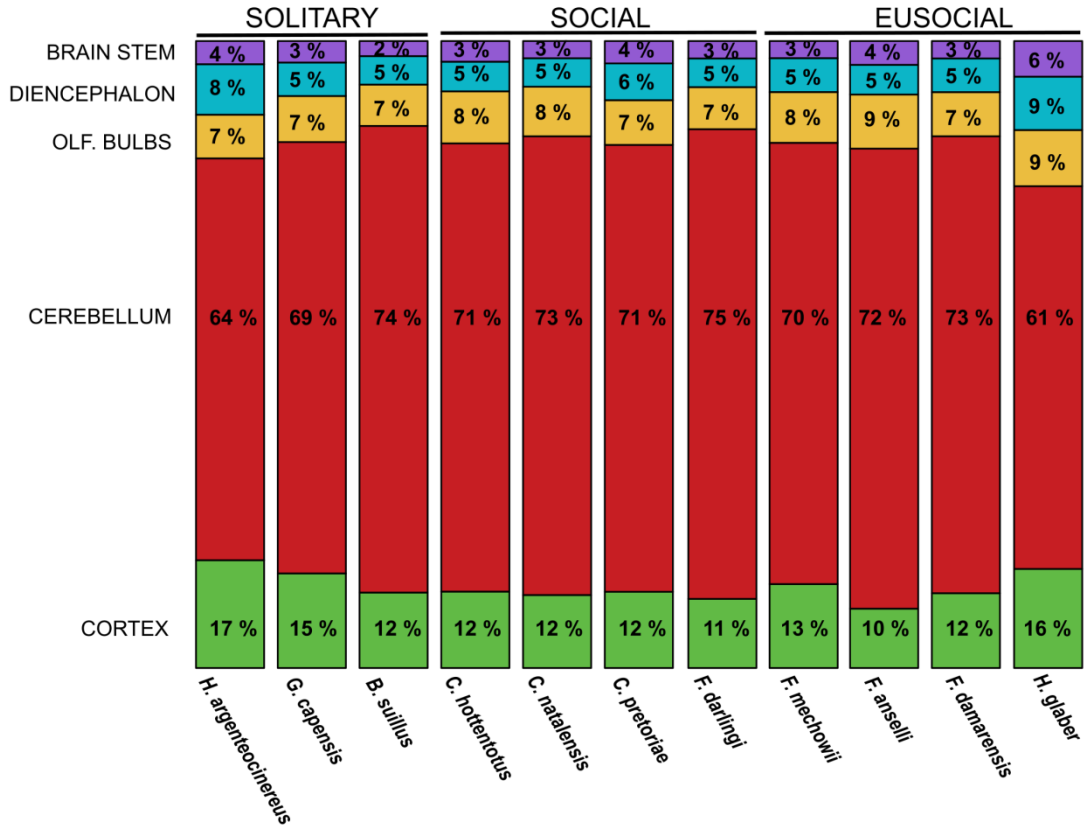

Figure 10. Average percentages of all brain neurons allocated in different brain regions in the 11 species of mole-rats.

In contrast to structure mass, the fraction of neurons contained in different structures does not seem to change much with increasing brain mass in rodents (Figure 8B). The only significant decrease in neuronal proportion is in the "rest of brain", but the slope is quite low (OLS: slope=-0.0145, p=0.0472). Additionally, in mole-rats only, there seems to be a similarly slow decline in the fraction of neurons contained in the olfactory bulbs (OLS: slope =-0.0084, p=0.0169).

Cellular scaling rules in mole-rat brains

Total brain neurons in the studied species of mole-rats range from 34 million in the naked mole-rat to 361 million in the Cape dune mole-rat. When it comes to scaling of brain neurons with body mass, mole-rats conform to the previously described relationship in Glires (Herculano-Houzel et al., 2011):

$$log(brain\ neurons) = 16.534 + 0.451 \times log(body\ mass)$$

Not even the naked-mole rat falls outside the 95% prediction interval, neither the slope (ANCOVA: p=0.504) nor intercept (ANCOVA: p=0.464) for mole-rats is significantly different from that of other rodents. (Figure 11A).

However, when we consider the scaling of brain neurons with brain mass (Figure 11B), bathyergids depart significantly from the Glires dataset

$$log(brain\ neurons) = 18.609 + 0.641 \times log(body\ mass)$$

by having both lower intercept (ANCOVA: p=0.0470) and higher slope (ANCOVA: p=0.0028). Nevertheless, this difference disappears after excluding the two species with the smallest and largest brain – the naked mole-rat and the capybara. These two species are influential points crossing the Cook's distance (Cook, 1977) and are largely responsible for the difference in slopes. Pooling all the mole-rat species together with the original rodent data set (note that this doubles the number of observations) does not result in changing the previously published relationship substantially (dashed line in Figure 11B). Thus, it seems that these scaling rules are rather robust. Even though the naked mole-rat was excluded from the published data as an outlier, the other species fit in quite well. Having low neuronal numbers is therefore probably not a peculiarity of microphthalmic subterranean rodents, but the naked mole-rat seems to be a special case.

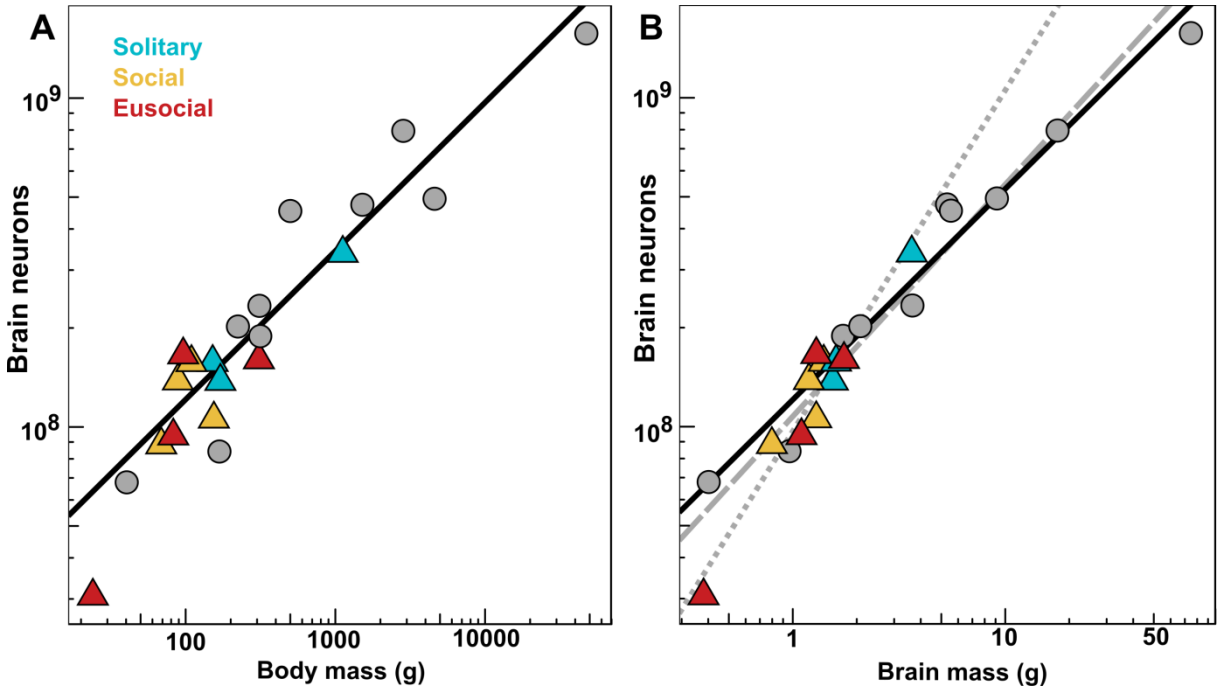

Figure 11. Scaling of brain neurons with (A) body mass, (B) brain mass. Data points are species averages. Mole-rats are represented by triangles, other rodents are represented by circles (data from Herculano-Houzel et al., 2011). Lines shown are from OLS regression of total brain neurons on body/brain mass (solid – original scaling relationship for rodents, dashed – scaling relationship after including mole-rats, dotted – scaling rules for mole-rats including HG). PGLS regression for the pooled gives virtually the same results and is not shown. Axes are log-transformed.

The number of structure neurons scales somewhat less predictably than structure mass with brain mass (R^2=0.755-0.963, $p < 0.0001$ in all cases), and mole-rats do differ from the other rodents in some respects (Figure 12). The slopes are significantly steeper in the cerebellum (OLS: p=0.0044) and in the cerebral cortex (OLS: p=0.0067), where the intercept is significantly lower as well (OLS: p=0.0006). Although the naked mole-rat is an influential point, the difference is still significant even after excluding it.

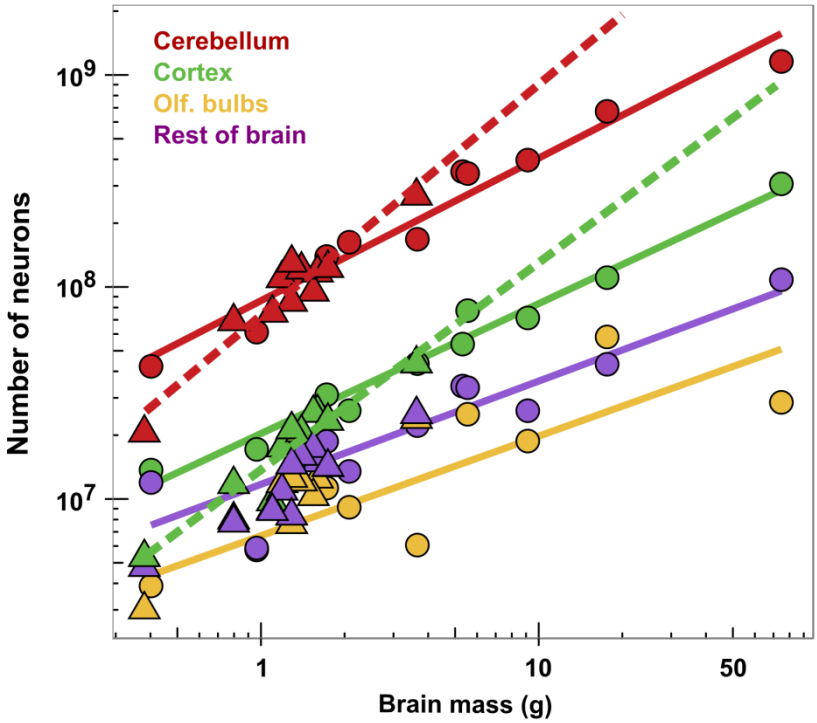

Figure 12. Scaling of neurons in different brain structures with whole brain mass. Data points are species averages. Mole-rats are represented by triangles, other rodents are represented by circles (data from Herculano-Houzel et al., 2011). Regression lines are from OLS regression of structure neurons on brain mass (dotted line – mole-rats, full line – other rodents). Whole brain does not include olfactory bulbs for the sake of comparability with the Glires dataset. Axes are log-transformed.

It is of note that the slopes for cerebellum and cortex in mole-rats remain roughly parallel to each other. Indeed, numbers of cerebellar and cortical neurons are highly correlated (OLS: $r=0.972$, $p<0.001$) across all the rodents (Figure 13). This is in line with the finding that numbers of cortical and cerebellar neurons scale closely together across four mammalian orders, although the size of these structures does not (Herculano-Houzel, 2010).

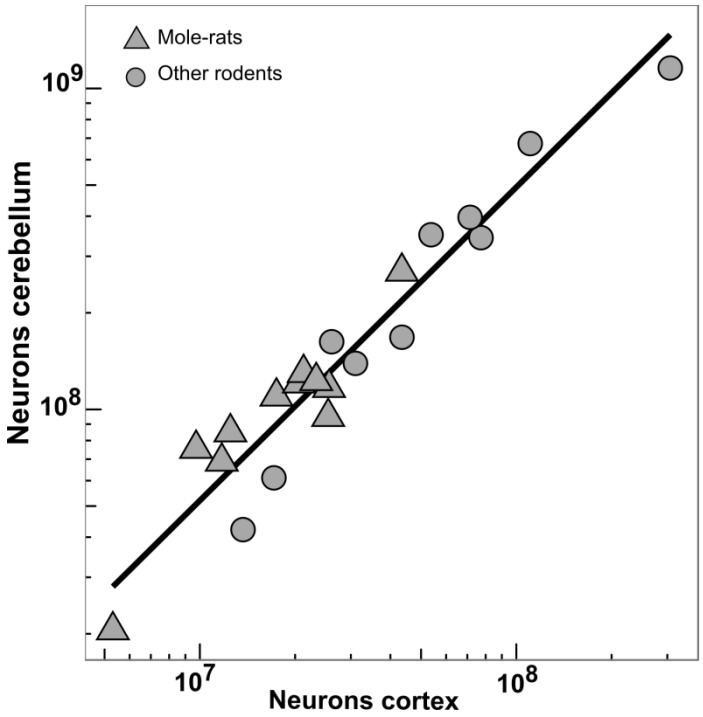

Figure 13. Scaling of cerebellar neurons with cortical neurons. Data points are species averages. Mole-rats are represented by triangles, other rodents are represented by circles (data from Herculano-Houzel et al., 2011). Regression line shown is from OLS regression of cerebellar neurons on cortical neurons [log(cerebellar neurons) = 2.032 + 0.976 × log(cortical neurons)]. Axes are log-transformed.

Scaling of structure mass with the number of neurons and non-neuronal cells

The brain is not uniform in its composition and so different structures can be expected to scale differently with the number of neurons and other cells. For the sake of comparability with the data published on other rodents (and mammals in general), the results are presented in the form where structure mass is dependent on the number of cells. While a bit harder to interpret initially, there is a logical justification to it – brain mass does in fact depend on the number of cells that make it up and not the other way round. Figures 14 and 15 present the scaling rules for neurons and non-neuronal cells in a separate dataset of 10 Glires species (Herculano-Houzel et al., 2011) with the addition of the newly acquired data for mole-rats. Note that non-neuronal cells scale very similarly across brain structures and species, while neurons follow distinct scaling rules with varying intercepts and slopes in different brain parts. Generally, in rodents the mass of a brain structure increases more steeply than the number of neurons. This might be interpreted as the neurons getting larger on average, while the glia remaining the same size (Herculano-Houzel et al., 2006; Mota &

Herculano-Houzel, 2014). Although this is the canonic explanation, it has not been tested directly and other factors might contribute to the same result, such as increasing thickness of the myelin sheath (which has been linked with postadolescent brain growth, e.g. Sowell et al., 2010) or simply expansion of the interstitial space.

In general, mole-rats conform to the scaling rules for rodent brains, with the exception of cortex, where they have higher intercepts for both neurons (ANCOVA: $p < 0.0001$) and other brain cells (ANCOVA: $p < 0.0001$) and a lower slope for neurons (ANCOVA: $p < 0.0001$). This means mole-rats have generally slightly lower densities of other cells and mostly constant densities of neurons, i.e. lower than expected in smaller brains but they do not decrease much in larger brains. Additionally, in the olfactory bulbs, mole-rats have a lower intercept for neurons, but only when OLS is used to assess the relationship (OLS: $p=0.018$, PGLS: $p=0.190$). This would mean that in mole-rats and other rodents the mass of the olfactory bulbs changes at the same rate with the number of neurons, but mole-rats tend to have more neurons for a given mass.

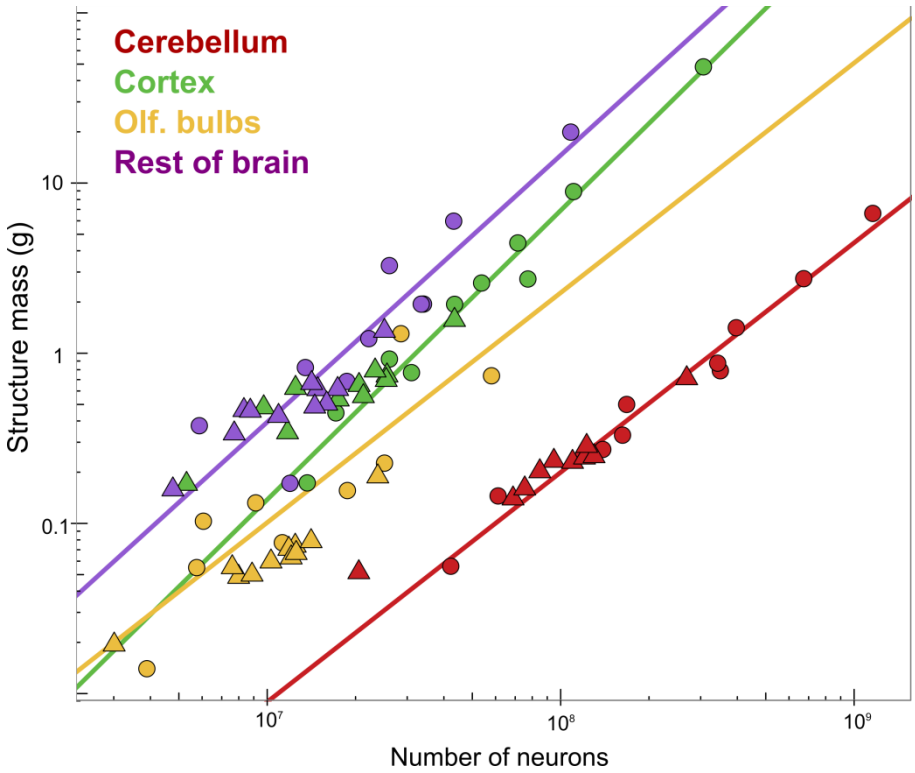

Figure 14. Scaling of structure mass with the number of neurons in mole-rats and other rodents. Data points are species averages. Regression lines shown are from OLS regression in the Glires data set excluding mole-rats (Herculano-Houzel et al., 2011). Axes are log-transformed.

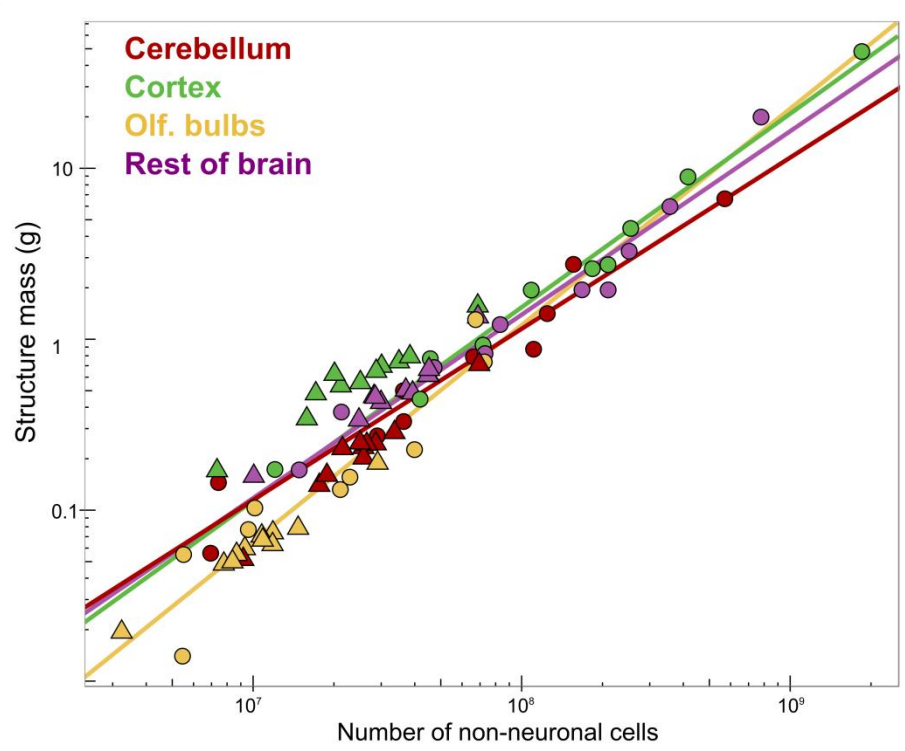

Figure 15. Scaling of structure mass with the number of non-neuronal cells in mole-rats and other rodents. Data points are species averages. Regression lines shown are from OLS regression in the Glires data set excluding mole-rats (Herculano-Houzel et al., 2011). Axes are log-transformed.

Neuronal and other cell densities

Across rodents, neuronal densities decrease with increasing brain structure mass (Figure 16A). When mole-rats are evaluated separately, the density of neurons in the olfactory bulbs and "rest of brain" decreases in a similar way, but there is no significant relationship between neuronal density and structure mass in the cortex (OLS: p=0.817) and the cerebellum (OLS: p=0.802). Since the cerebellum and cortex combined account for over 80% of neurons in the brain, it is not surprising that the same divergence from rodent rules can be seen in the whole brain: mole-rats have a lower intercept (OLS: p=0.047; PGLS: p=0.0713) and a higher slope (OLS: p=0.0028). While this might look a bit surprising at first, a simple explanation is possible. These rules hold over much larger scales, whereas the mole-rat brains range only over about 3 grams. Generally, neuronal densities in mole-rats are comparable with those of other rodents, but several species *(H. glaber*, *F. anselli*, *F. darlingi*) have notably low densities in the cortex.

The number of non-neuronal cells remains relatively constant compared with neurons, but it is not completely fixed. There is no relationship between non-neuronal density and structure

mass in the cerebellum and rest of brain in either mole-rats or other rodents, but the density is falling, albeit less steeply than in the case of neurons, in the olfactory bulbs (OLS: p=0.0169) and in the cortex (OLS: p=0.0022). Just like with neurons, mole-rats again differ from the Glires data set (OLS: $p < 0.0001$) in that the non-neuronal density in the cortex does not decrease with increasing mass. Thus the densities of both cortical neurons and non-neuronal cells show no association with brain mass in mole-rats, in contrast to all the other species examined.

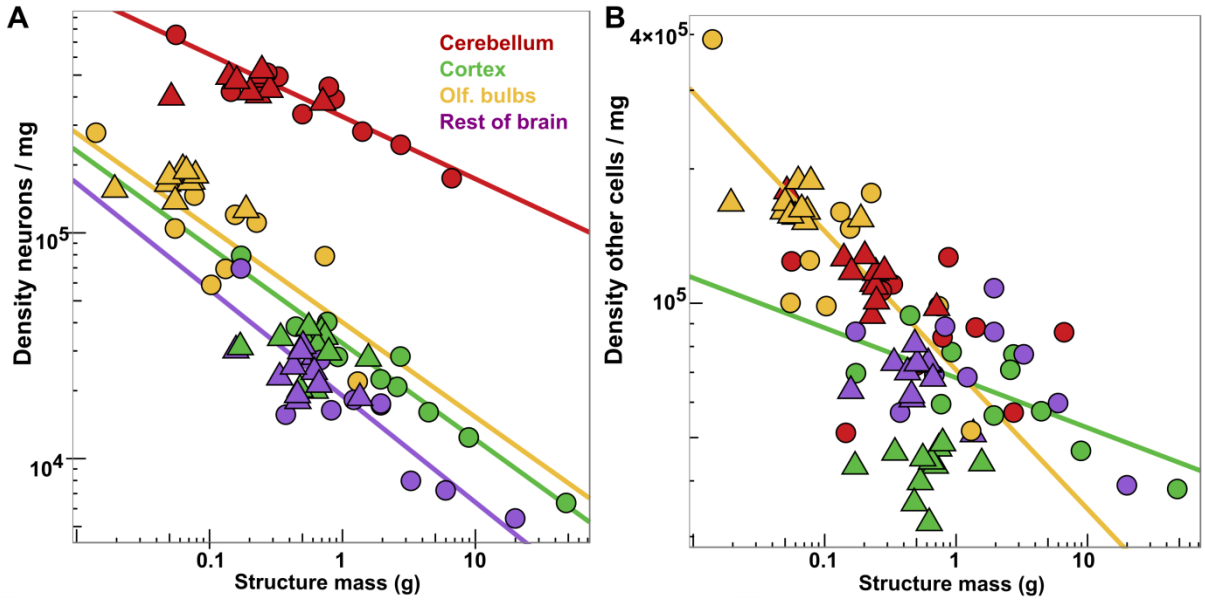

Figure 16. Scaling of cellular densities with brain structure mass. (A) Neuronal densities (B) Non-neuronal densities. Data points are species averages. Mole-rats are represented by triangles, other rodents are represented by circles (data from Herculano-Houzel et al., 2011). Regression lines shown for the significant relationships are from OLS regression of cellular density per mg of tissue on the respective structure mass in the Glires data set excluding mole-rats. Axes are log-transformed.

Glia/neurons ratio

Another interesting rule established using the isotropic fractionator is the scaling of the ratio of glial cells to neurons. Across all brain structures and taxonomic units examined, this ratio goes down with increasing neuronal density (Mota & Herculano-Houzel, 2014). Given that, as was just shown, neuronal density in rodent brains gets lower as brain mass gets higher, the ratio of glia to neurons is highest in the largest rodents. Mole-rats are not an exception to this general rule that seems to be shared across all mammals (Figure 17).

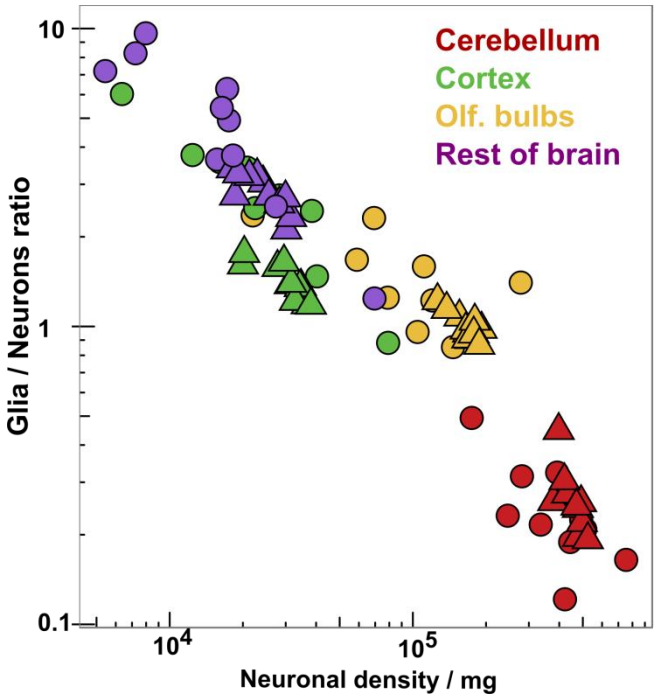

Figure 17. Ratio of non-neuronal to neuronal cells as a function of neuronal density. Data points are species averages. Mole-rats are represented by triangles, other rodents are represented by circles (data from Herculano-Houzel et al., 2011). Axes are log-transformed.

Summary of the main findings:

- Mole-rats **share the brain-body scaling** and **the structure mass-brain mass scaling** rules in the 4 brain compartments with other rodents, but have **relatively small brains** for their body size.
- Mole-rats **share** the brain **scaling rules for non-neuronal cells** with other rodents.
- Mole-rats **share** the coordinated **scaling of cortical and cerebellar neurons** with other rodents.
- Mole-rats **share** the **scaling of the glia/neurons ratio** with other rodents.
- Mole-rats tend to have **higher neuronal densities** in the **olfactory bulbs** than other rodents.
- Differences between mole-rats and other rodents are the most pronounced in the cerebral cortex. The **proportion of cortex does not increase** with brain size and **cellular scaling rules** in the cortex **differ**, both for neurons and non-neuronal cells.

Testing the social brain hypothesis: applying the data

Equipped with these quantitative data, we can turn to the central question: is sociality in mole-rats associated with brain size or composition and, if so, how?

Starting at the highest level and simply looking at relative and absolute brain size (Figure 18), it is apparent that relative brain mass is the same across all the sociality grades (solitary vs. social: pMCMC = 0.637, social vs. eusocial: pMCMC = 0.684), whereas absolute brain size tends to be higher in solitary species (posterior mean = 0.6486, HPD = [-0.0018, 1.4556], pMCMC = 0.0741). This is not surprising given the larger body sizes of solitary mole rats. In fact, they are significantly larger than social ones (posterior mean = 1.1089, HPD = [0.1481, 2.2049], pMCMC = 0.0321) (Figure 19). Sociality has no significant effect on the relationship between brain and body mass, although solitary mole-rats tend to have higher intercept (posterior mean = 1.4472, HPD = [-0.8560, 3.5967], pMCMC = 0.1638) and lower slope (posterior mean = - 0.2969, HPD = [-0.7069, 0.1536], pMCMC = 0.1345).

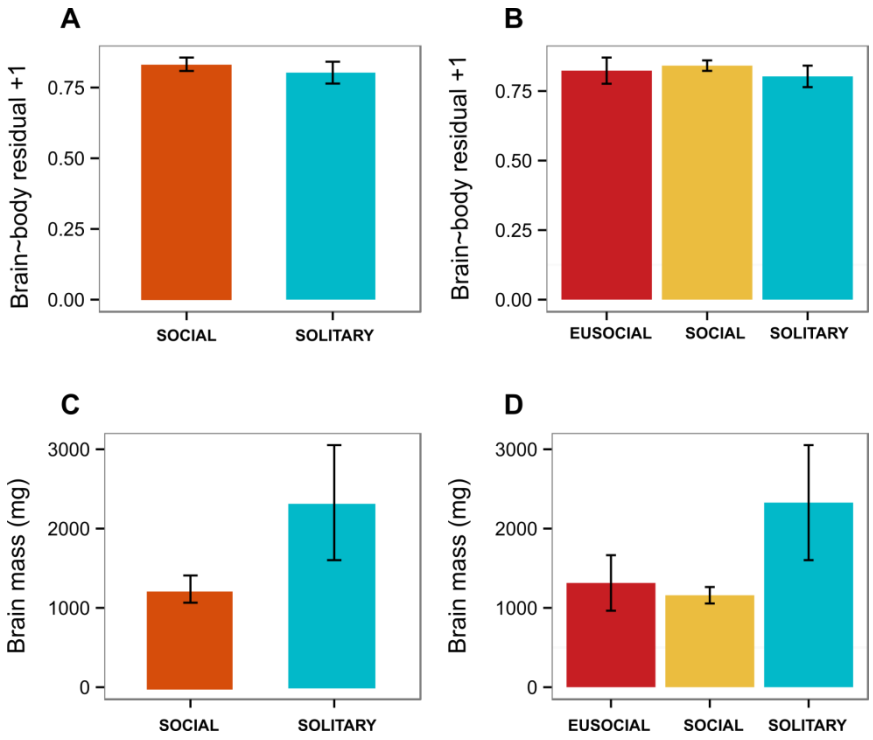

Figure 18 Absolute and relative brain size by sociality.(A and B): Bar plots illustrating the differences in relative brain size (expressed as a residual from the brain~body regression line for rodents, with 1 added to get positive numbers) between (A) social and solitary, and (B) eusocial, social and solitary species. No groups are significantly different. (C and D): Bar plots illustrating the differences in absolute brain size between (C) social and solitary, and (D) eusocial, social and solitary species of mole-rats. No groups are significantly different. Data are represented as mean ± SEM.

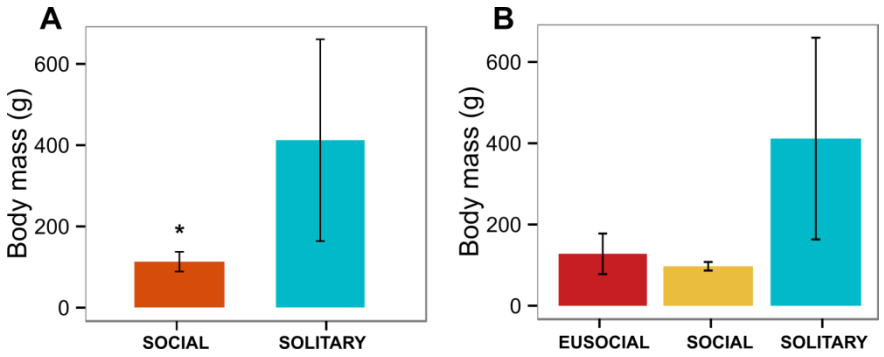

Figure 19. Body mass by sociality. Bar plots illustrating the differences in body mass between (A) social and solitary, and (B) eusocial, social and solitary species of mole-rats. Social species are significantly smaller than solitary ones (posterior mean = 1.1089, pMCMC=0.0321). Data are represented as mean ± SEM.

As for the absolute numbers of neurons in the brain, there is no significant association with sociality, although solitary species again tend to have more neurons (pMCMC=0.151), while eusocial and social species do not seem to differ (pMCMC=0.588) (Figure 20).

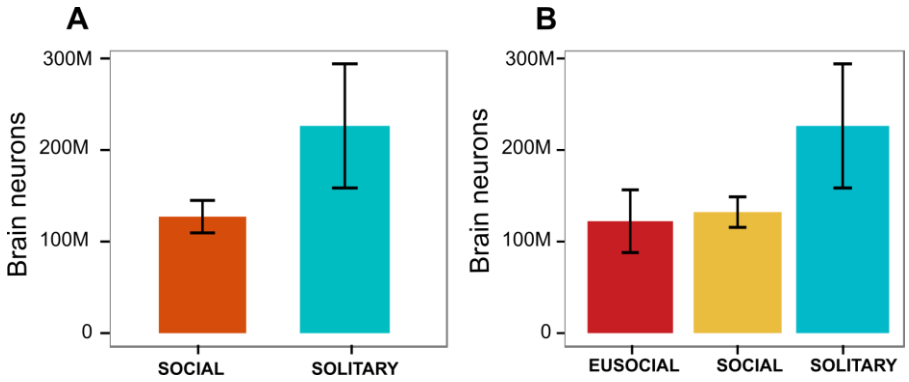

Figure 20. Neurons in the whole brain by sociality. Bar plots illustrating the differences in total brain neurons between (A) social and solitary, and (B) eusocial, social and solitary species of mole-rats. No groups are significantly different, although solitary species tend to have more neurons than social ones (pMCMC=0.163). Data are represented as mean ± SEM.

The neuronal index has been proposed by Herculano-Houzel (2007) as an adequate proxy for cognitive abilities. It refers to the “excess neurons”, that is neurons above (or bellow) what is predicted from the neurons-body relationship for the given taxonomic group. No association between the neuronal index and sociality is detectable (solitary vs. social pMCMC=0.7572, social vs. eusocial pMCMC=0.500) (Figure 21).

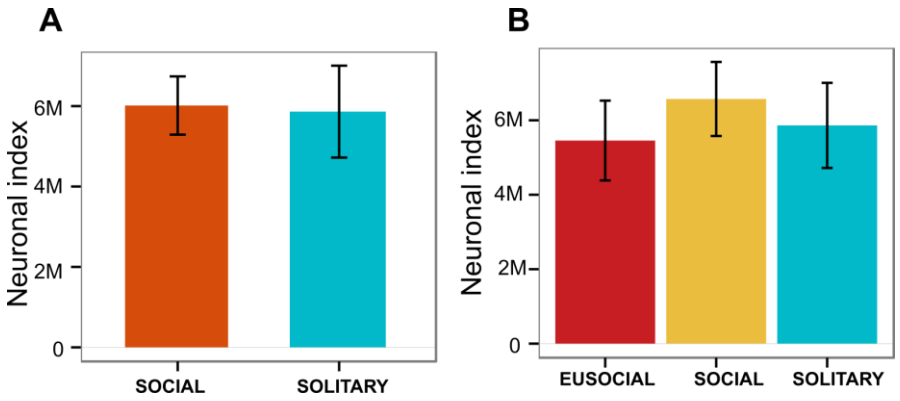

Figure 21. Neuronal index by sociality. Bar plots illustrating the differences in neuronal index (the residual from the body mass~neurons regression line for Rodentia, adjusted by adding the largest negative value to get positive numbers) between (A) social and solitary, and (B) eusocial, social and solitary species of mole-rats. No groups are significantly different. Data are represented as mean ± SEM.

Now differences in the absolute and relative numbers of neurons in the individual brain structures are left to be examined. Solitary mole-rats have significantly more neurons in the cerebral cortex and the diencephalon and marginally significantly more neurons in the brain stem, but not in the olfactory bulbs or the cerebellum (which is consistent with the results for the whole brain). There is no discernible trend concerning the social and eusocial groups (Table 2).

Table 2. Absolute numbers of neurons by sociality.

Brain part	Differences in absolute numbers of neurons (solitary vs. social, 2-level sociality)			Differences in absolute numbers of neurons (social vs. eusocial, 3-level sociality)		
	Posterior mean	HPD	pMCMC	Posterior mean	HPD	pMCMC
Whole brain	0.5982	[-0.3312, 1.4613]	0.1630	0.2357	[-0.7411, 1.1657]	0.5880
Cortex	**0.79276**	**[0.0694, 1.5191]**	**0.0396**	0.18852	[-0.6152, 1.0791]	0.6214
Cerebellum	0.5692	[-0.4279, 1.5111]	0.2130	0.2937	[-0.7110, 1.3721]	0.5360
Diencephalon	**0.68836**	**[0.0306, 1.3882]**	**0.0480**	0.05740	[-0.7410, 0.7963]	0.8456
Olfactory bulbs	0.4878	[-0.3242, 1.2337]	0.1940	0.1623	[-0.7803, 1.0480]	0.6680
Brain stem	0.54626	[-0.0172, 1.0851]	0.0484	0.0998	[-0.4819, 0.7439]	0.7175

The relative numbers of neurons were determined in two ways. The first one is analogous to the “neocortex ratio”, introduced by Dunbar for testing the SBH (Dunbar, 1992). He reasoned that the fraction of neocortex in the brain should reflect its “importance” but because it also accounts for most of the brain volume, it makes sense to use instead its ratio to the rest of the brain. Since, similarly, the cerebellum accounts for most of the brain neurons, it would

be more appropriate to use the ratio of neurons in the structure of interest to the brain stem, presumably the most conserved brain part, not much involved in cognition. The ratio of cortical volume to medulla volume has also been suggested as an appropriate proxy for cognitive abilities (Passingham, 1975).

There are no significant differences between the groups in this parameter, although the solitary mole-rats tend to have a higher ratio of cortical/brain stem neurons (Table 3).

Table 3. Ratios of structure neurons to the brain stem by sociality.

Brain part	Differences in the ratio of structure : brain stem neurons (solitary vs. social, 2-level sociality)			Differences in the ratio of structure : brain stem neurons (social vs. eusocial, 3-level sociality)		
	Posterior mean	HPD	pMCMC	Posterior mean	HPD	pMCMC
Cortex	0.9234	[-0.2615, 2.0751]	0.113	0.1201	[-1.2499, 1.3797]	0.836
Cerebellum	0.3054	[-8.3405, 9.7931]	0.948	2.7180	[-7.2030, 12.816]	0.550
Diencephalon	0.2284	[-0.2396, 0.7151]	0.306	-0.1041	[-0.6129, 0.4542]	0.667
Olfactory bulbs	-0.1922	[-0.9735, 0.5382]	0.575	0.0344	[-0.7897, 0.9039]	0.931

Another way to derive a relative number is to use the difference between the observed and expected number of neurons in a given structure (similarly to the neuronal index). The scaling rules for rodent brains, excluding mole-rats, were used to provide an unbiased reference. Data for the diencephalon and brain stem were pooled to correspond to the "rest of brain". Again, there are no significant differences, but solitary mole-rats tend to have higher numbers for the cerebral cortex.

Table 4. Relative numbers of structure neurons by sociality.

Brain part	Differences in the relative neuronal numbers (solitary vs. social, 2-level sociality)			Differences in the relative neuronal numbers (social vs. eusocial, 3-level sociality)		
	Posterior mean	HPD	pMCMC	Posterior mean	HPD	pMCMC
Cortex	0.4113	[-0.0363, 0.8235]	0.0585	0.08425	[-0.3738, 0.5776]	0.7027
Cerebellum	0.0842	[-0.2568, 0.4519]	0.6020	0.1093	[-0.2764, 0.5050]	0.5260
Olfactory bulbs	0.1466	[-0.2284, 0.5500]	0.4000	0.0291	[-0.3903, 0.4685]	0.8780
Rest of brain	0.2772	[-0.0820, 0.6538]	0.1250	0.02227	[-0.4012, 0.4470]	0.9181

If sociality is treated as a numerical variable, with maximum group size as a proxy, as has been originally suggested by Dunbar (1992), absolute brain mass is negatively correlated with group size (PGLS: slope = -0.227832, p=0.0179), but this effect is largely due to the

naked mole-rat; after excluding it, the relationship is not significant anymore (PGLS: slope = -0.12054; p=0.3336). Relative brain size is not associated with group size either with the naked mole-rat (PGLS: p=0.1967) or without it (PGLS: p=0.4779).

When the absolute number of brain neurons is regressed on maximum group size, there is a significant negative relationship (PGLS: slope= -0.2228, p=0.0269) only if the naked mole-rat is included. However, the negative correlation of group size with the number cortical neurons remains significant even without this point (PGLS: slope =-0.2486, p=0.013) (Figure 22). The cortical/brain stem ratio again gets smaller with group size (PGLS: slope =-0.3262, p=0.0064), but this trend does not reach significance after removing the naked mole-rat. Mole-rats thus show a completely opposite situation to that predicted by Dunbar (1992) that neocortex size (presumably assumed equivalent to neocortex neurons) should put an upper limit on the maximum group size.

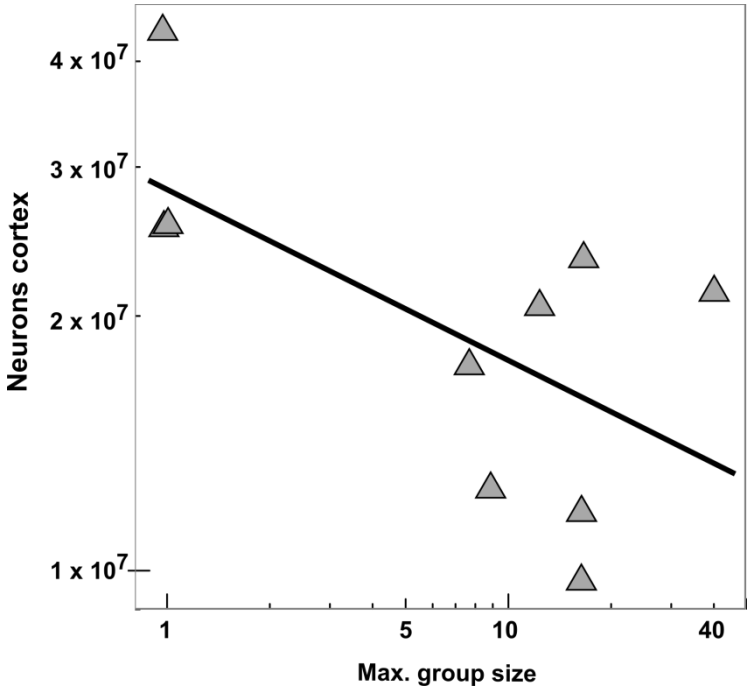

Figure 22. **Number of cortical neurons plotted against maximum group size**. Fitted line is from PGLS regression for the mole-rat species excluding HG. Data points represent species averages. Axes are log-transformed.

Summary of the main findings:

- **Solitary** mole-rats are generally **larger in body size** and have **larger absolute brain sizes**, while there are **no differences in encephalization** between the social grades.
- **Solitary** mole-rats tend to have a **higher absolute number of neurons** and have significantly higher numbers of neurons in the **cerebral cortex and diencephalon**.
- The social grades **do not differ in the ratio of neurons to brain stem neurons** in any of the brain structures examined.
- **Solitary** mole rats tend to have **higher relative number of cortical neurons** expressed as residuals from the relationship established in other rodents.
- There is **no relationship between group size and relative brain size** in mole-rats.
- There is a **negative relationship between group size and absolute brain size**, but that relationship is not robust and disappears after excluding the naked mole-rat.
- There is **a negative relationship between group size and number of cortical neurons** and a weaker relationship with the cortical/brain stem ratio.
- None of these results are consistent with the SBH in any of its versions and some are in the **opposite direction**, i. e. **solitary species having absolutely and relatively more cortical neurons**.

DISCUSSION

Interindividual variation and potential sources of bias

While the intraspecific differences are substantially smaller than interspecific differences at large scales, they should not be downplayed unduly. After all, interindividual variation is the "raw material" for natural selection. Recently, a study of intraspecific variation (Herculano-Houzel et al., 2015b), using 19 male Swiss mice of the same age, showed relatively low coefficient of variation but a considerable range in neuronal numbers (1.63- to 4.53-fold depending on the structure). The careful reader cannot help but notice that the *maximum* reported number of brain neurons in this newer study is lower than the species average used in calculating the scaling relationship for rodents (Herculano-Houzel et al., 2011). Although in the grand scheme of things this does not matter much and the rules still seem to hold, inflated values for the lowest point do result in skewing the intercept higher and the slope lower.

In this context, it is interesting to compare our data for *H. glaber* that was identified as an outlier in the above mentioned study. Both ours and the previously published data were based on 3 individuals. Values for neurons in the rest of brain are virtually identical, we report slightly lower values for the cortex, but the most pronounced difference is in the cerebellum, where our average is 4.8 million higher which is about 30 % more than in the original dataset. This difference does not concern just neurons (which might imply a possible issue with staining) but also other cells and still within the range of individual variation in cerebellum reported for the mouse. Given our data, the naked mole-rat is still greatly below the expected number of neurons, but not quite so dramatically.

Sex can be one additional source of interindividual variation. It is not possible to properly evaluate sex differences in this data set, where only three individuals of each species were used and sexes could not be equally represented. However, from these limited data, it does not seem that there is a difference in relative or absolute brain size (although there might be in *Bathyergus*, where the males are substantially larger [Bennett et al., 2009]) or neuronal numbers and densities. Another, perhaps more serious, factor is age. In mammals, neurogenesis generally comes to a halt postnatally, although new nerve cells are being born

throughout the adult life in some parts of the central nervous system, neuronal cell death prevails (Ming & Song, 2005). A study of changes in neuronal numbers in rats during ontogeny (Morterá & Herculano-Houzel, 2012) reported increasing neuronal numbers at adolescence (2-3 months of age), with a subsequent decrease, up to 30 % decline from the maximum numbers at 22 months. Simultaneously with this neuronal decline the brain is still gaining in mass. Mammalian brains thus might lose neurons with age and densities might be higher in younger individuals. Unfortunately, the age of most of the animals used in this study was unknown (though all were adult), so there is no way to account for this potential source of bias.

Furthermore, the statistical treatment of these results gets complicated by some inherent characteristics of the data. There is only a 10-fold difference between the smallest and largest mole-rat brain and most brains are clustered around the same size, with *H. glaber* and *B. suillus* both alone at their end of the spectrum. These species at the extremes can have a large impact on the scaling relationships, especially in a modestly sized data set. Nevertheless, excluding these influential points does not substantially change the scaling rules in most cases, giving some assurance of their robustness.

Mole-rats compared with epigeic rodents

It is interesting and perhaps even surprising that mole-rats are generally in line with the brain cellular scaling rules for rodents. As it transpired that the naked mole-rat deviates from these rules by having substantially lower neuronal densities and thus neuronal numbers in the cortex and cerebellum, the authors offered an explanation that this is due the reduced visual system, associated with its strictly subterranean lifestyle, or possibly due the hypoxic conditions in its environment (Herculano-Houzel et al., 2011). Now, that we see this is not the case, a different explanation is needed. One such factor might be the nakedness and essential poikilothermy combined with a very low resting metabolic rate (Buffenstein & Yahav, 1991) and the fact that they usually occupy environments with low-quality food (Bennett & Faulkes, 2000). Since brain metabolism is almost a linear function of the number of neurons (Herculano-Houzel, 2011), reducing neuronal densities would be helpful in conserving energy. As an interesting side note, the naked mole-rat has a relatively larger

braincase compared to other bathyergids (Rodrigues et al., 2015), which highlights some caveats of using endocranial volume as an approximation of potential “brain power”.

Metabolic demands and reduction of visual cortex might also be the explanation for the generally lower, albeit not so severely (around 20% on average), neuronal densities in the cortex across the rest of mole-rat species which also have lower resting metabolic rates than epigeic rodents (Zelová et al., 2007). Incidentally, it has been hypothesised that sleep requirements across mammals should decrease with decreasing neuronal density per cortical area (Herculano-Houzel, 2015). This is in agreement with the finding that mole-rats actually sleep less than typical rodents (Bhagwandin et al., 2011; Kruger et al., 2016), but this might also be due to differences in brain physiology and adaptations for hypoxia. Indeed, Larson and Park (2009) observed that synaptic transmission in *H. glaber* is less sensitive to application of adenosine, a metabolite whose extracellular concentration in the brain plays a critical role in triggering sleep (Porkka-Heiskanen et al., 1997).

Generally, the allocation of neurons to the examined brain regions corresponds to that of other rodents, but there is one notable exception. Interestingly, the olfactory bulbs account for 7-9 % of total neurons in the brains of mole-rats. The range reported for other rodents is 2 % in the capybara to 6 % in the hamster and the agouti. This is not reflected in olfactory bulb volumes which have the same range in the data other rodent species included (2-6 %) and mole-rats fall well within this range (4-6 %). Correspondingly, all the species except for *H. glaber* show higher than expected neuronal densities in the olfactory bulbs, in most cases quite substantial (30-50 %). This might constitute an adaptation to subterranean lifestyle, where the sense of smell becomes more important in the absence of visual cues. It has been shown that mole-rats indeed use olfaction to locate food resources, which are otherwise undetectable to them and would have to be searched for blindly (Heth et al., 2002; Lange et al., 2005). It has been suggested that in insectivores neuronal densities in the olfactory bulb increase with increasing mass (Sarko et al., 2009; Ribeiro et al., 2014) and this could be due to the larger species being moles, suggesting a more universal principle across subterranean mammals. But the dataset is too limited and the relationship rather contrived (after removing one outlier, it is based on just four observations). Because the sense of smell plays a critical role in colony members recognition (e.g. Heth et al., 2004), one might speculate

that it might have evolved under that pressure as well. However, the social categories are quite uniform in this respect. This is also in line with recent genetic evidence which suggests that there was a positive selection for increased olfactory receptor diversity in mole-rats but not related to the different social systems (Stathopoulos et al., 2014).

Pirlot (1989) suggested that subterranean rodents should have relatively large cerebella, because they need fine locomotory control for digging their tunnels and manipulating with food in darkness. Contrary to this conclusion, which was based on the comparison of volumetric proportions with epigeic rodents, mole-rats have about the expected numbers of neurons in their cerebella, with the notable exception of *H. glaber*, mentioned above.

Conservativeness of neuronal scaling rules

The inclusion of the 11 mole-rats species does not result in changing the previously published scaling rules for rodents significantly. But the naked mole-rat attests to the fact that large differences can be found even among closely related species. It has been proposed that distinct evolutionary shifts in the neuronal scaling rules occurred during the mammalian evolution and led to the modification of brain composition in high taxonomic units (Herculano-Houzel et al., 2014b). How these shifts arise and whether less dramatic changes at lower levels occur by the same or another mechanism is not known. Based on the available data it seems that the most evolutionary plasticity is shown in the olfactory bulbs and the cortex, i.e. in the telencephalon. This makes sense from the ontogenetic point of view, since this is the part of the brain that gets generated late during development (Finlay et al., 1998).

Good proxy for cognitive abilities?

While this is not the main subject of this thesis, the question of appropriate proxy for cognitive abilities is important for testing the SBH. The arguments have been made much more eloquently elsewhere (e.g. Dicke and Roth, 2016), so I limit myself to a short commentary.

The use of absolute number of brain (or cortical) neurons and neuronal densities would seem ideal, but it might take a while until sufficient data sets are available. Absolute brain size correlates reasonably well with the number of neurons at low taxonomic levels,

although the two values are not necessarily equally comparable. For example, in our dataset, *F. damarensis* has about the same brain size as *F. darlingi* but about 50 % more neurons. Nevertheless, absolute brain size is usable in closely related species and can be adopted if more detailed information is not available.

At the same time, I would also argue to maintain some balance and not to completely disregard relative brain size either. While only absolute brain size gives information about the actual processing capacity, relative brain size might tell us something about the evolutionary process leading to this state. But there is a big catch. Even with relative brain size, assessing the current situation gives us limited information. In might reveal some useful patterns, but unless we consider the evolutionary history, there is little to be inferred about the underlying selective pressures. It seems that proponents of the importance of relative brain size assume that higher relative brain size is indicative of brain enlargement. This may not always be the case, as pointed out by Safi et al. (2005). Theoretically all the combinations of increased/decreased brain/body size are possible. Nevertheless, reconstruction of ancestral states (Pagel, 1997) can be helpful in assessing if the increase in relative brain size was accompanied by a simultaneous increase in absolute brain size. In my opinion, only these cases should be considered as potentially enhancing cognitive abilities. Without such analysis, it is almost impossible to distinguish between selection for body size and brain size.

Interestingly, preliminary data from such analyses (Pavelková et al., in prep.) suggest that evolutionary decreases in absolute brain size in mole-rats are associated with substantially lower neuronal densities. The three mole-rat species with the lowest overall neuronal density and drastically low cortical neuronal density underwent a reduction in absolute brain size. This is quite unexpected, as one would reason that, if density changes at all, the decrease in brain volume might be accompanied by an *increase* in neuronal density, such as has probably occurred in birds in connection with active flight (Olkowicz et al., under review).

At the moment, this is not formally testable but it will be interesting to see if it is a more general mammalian pattern or a peculiarity of mole-rats, perhaps associated with the urgent need to conserve energy and oxygen consumption and thus “shed” unnecessary neurons.

The neuronal index has the theoretical advantage of taking into account both encephalization and the number of neurons. However, it is also subject to the same bias as any measure involving body mass, which is not a reliable characteristic and can vary dramatically both within species and even in the same individual. In fact, the very scaling rules for rodents used here as reference (Herculano-Houzel et al., 2011) use some suspicious body weights. In fact, the fit can be improved by using species averages taken from the PanTHERIA database (Jones et al., 2009). Even though this might be controversial, I would actually recommend this, as brain mass and neuronal numbers do not correlate with body mass intraspecifically (Herculano-Houzel et al., 2015b). Ideally, another less variable proxy for body size can be adopted, but body mass is the one that is most readily available.

A final note on limiting the comparison of neuronal numbers to the cortex: While the cortex is taken to be the seat of "intelligence", it does not work in isolation from the rest of the brain. Notably, the cerebellum is responsible for some higher cognitive functions (reviewed in Ramnani, 2006) and hominid evolution is characterized by an accelerated expansion of the cerebellum relative to the cortex (Barton, R. A., & Venditti, 2014). In my opinion, restricting comparisons to the pallium should be carefully considered and, conservatively, neurons in the whole brain should be included as well.

Mole-rats and the social brain hypothesis

The data presented here offer no support for the social brain hypothesis. Solitary and social mole-rats do not show any pronounced differences in the neuronal scaling rules or allocation of neurons to individual brain compartments. Where differences exist, they are in the opposite direction to that predicted by the hypothesis. The most striking distinction between the groups is in absolute body mass, which is larger in solitary species. This is an important point that has implications for most of the other studied variables. Brain mass (and the number of brain neurons) is tightly correlated with body mass. Accordingly, solitary species tend to have generally larger brains with more neurons than social ones. They also have relatively more neurons in the cortex. There is no difference or any discernible trend concerning relative brain size, which seems to be well conserved across mole-rats. Similar relationships hold when sociality is treated as a numerical variable expressed as maximum group size.

This adds to the recently accumulating body of evidence against the general validity of the hypothesis across vertebrates (Benson-Amram et al., 2016; Chojnacka et al., 2015; Matějů et. al, 2016; Reddon et al, 2016; Weisbecker et al., 2015). To examine these results critically, we may ask the following questions: How do the specific claims of the SBH and their implicated mechanisms apply in mole-rats and are there any particular cognitive challenges associated with the different mole-rat social systems?

First, there is social organization, either qualitative (solitary vs. social) or quantified as group size. Here, the findings are clearly in disagreement with the SBH. Of course, the prediction concerning group size was formulated for primates, not mole-rats (Dunbar, 1992). But here we must pause for thought. To human standards, mole-rats are clearly not the brightest creatures. A logical objection arises that primates are much more intelligent than mole-rats and, consequently, their relationships much more complicated. But this already assumes they are smart (for whatever reason) and that is why they need more brain power for managing life in large groups. If it really was the social environment that brought about this effect in the first place (that is, primates were "dumb" before adopting the social way of living), then there is nothing in general to preclude the same prediction from being applied to any animal group. To avoid circular reasoning, factors that are considered cognitively demanding in one group, cannot be arbitrarily decided not to be in another. Especially in light of the apparent corroboration of this prediction in "insectivores", a group that would not be expected to be socially or cognitively more sophisticated than mole-rats (Dunbar & Bever, 1998).

Similarly, the potential objection that mole-rat sociality is kin-based and thus does not require much brain power is not justified, in my opinion. Many, if not most, animal societies are kin-based to some extent, even those of primates (e.g. Lee, 2001; Silk, 2002) and close relatedness certainly does not prevent conflicts of interest, although conflict may be attenuated. Sibling competition and parent-offspring conflict is witnessed across the animal kingdom (e.g. Trivers, 1974; Harper, 1986). All is not peaceful even in the highly inbred naked mole-rat colonies, as evidenced by the queen shoving subordinates to assert her dominance and monopolize breeding, targeting specifically those that would pose the greatest threat or those that are "lazy" workers, or the power struggle among high ranking individuals after queen removal (Reeve, 1992; Clarke & Faulkes, 1997, 2001).

Let us examine the factors proposed to be cognitively demanding/promoting brain enlargement. Higher group size potentially requires better memory, but only if group members are distinguished as individuals, which is demanding on its own (Dunbar, 1998). In large mole-rat colonies it might the case that recognition happens only at the level of kin/non-kin distinction. That does not seem to be the case in *F. anselli* and *F. damarensis*, as experimental studies concluded that recognition is not based on colony membership or genetic relatedness (Burda, 1995; Jacobs, & Kuiper, 2000), but individuals are forgotten after a separation of about 18 days. This "forgetting" might actually be adaptive, because in the wild, if a member is not in contact with the colony for such a long time, it is probably either dead or dispersed and has thus become competition. Mixed evidence exists for *H. glaber*. Indirect signs point to individual discrimination (Judd & Sherman, 1996), but in a study by O'Riain and Jarvis (1997), when family members were removed from the colony shortly (12 hours), they were not recognized anymore.

Another factor proposed as cognitively demanding by Dunbar is keeping track of other group members and their relationships. This does not seem to be applicable in mole-rats, although the dominant female in *H. glaber* might be keeping tabs on the level of work performed by the subordinates (Reeve, 1992), but it seems rather unlikely. Maintaining group cohesion, mentioned by Shultz and Dunbar (2007), may take a number of forms and they do not elaborate why it would require much brain power. In the case of mole-rats, group cohesion is probably achieved by increased social tolerance towards kin which affects some parts of the brain, such as the pattern of expression of oxytocin and vasopressin receptors in the telencephalon, differing between social and solitary species (Kalamatianos et al., 2010; Valesky et al., 2012). But there is little reason to expect it to be affecting brain size.

However, there can be other factors specific to mole-rats. The extensive burrow systems of social mole-rats might potentially select for better spatial orientation and memory and also promote learning, as these are continually rearranged by other colony members and must be learned. This might be expected to result in a relatively large hippocampus, a structure involved in spatial processing and memory (reviewed in Burgess et al., 2002). Hippocampal size has not been tested at the cellular level, but in our volumetric data there is again no difference between the groups (Kverková et al., in prep.). Cooperative foraging may also be considered cognitively demanding, not in the same way as cooperative hunting, but because it requires potentially some sort of communication and learning about the location of a

newly found food source and remembering it. There is evidence of such behavioural coordination. Judd and Sherman (1996) reported that in *H. glaber*, workers are recruited to a new resource by a special vocalization and they locate it by following the individual scent of the recruiter.

In summary, not all, but some proposed underlying factors connecting group size to brain size are present in mole-rats and there are potentially others, putting more cognitive demands on the social species. Even if we conservatively exclude *H. glaber*, there is a negative correlation between group size and relative number of cortical neurons and therefore a discrepancy with the SBH.

Then there is the association with bondedness, focused on the quality of the social relationships. According to Shultz & Dunbar (2007), in non-primates, social monogamy (pairbondedness) should promote brain enlargement. This criterion corresponds to the solitary/social factor. Solitary mole-rats are socially intolerant and do not show bondedness of any kind. In the social species, there is both social monogamy and "extension of the pairbond to non-reproductive group members" in the sense Shultz and Dunbar proposed to explain the evolutionary transition to large social groups. This is intimately related to the notion of cooperative breeding, so we might treat them together.

Reasons behind the purported cognitive demands of monogamy are not very clearly explained. Shultz & Dunbar (2007) mention that making the right mate choice is important, as well as mate guarding to prevent cuckoldry. This would not be readily extensible to social mole-rats, since they neither have much choice of potential mates and probably such opt for the first available mate, nor do they have to worry too much about ensuring paternity. Although there are documented cases of extra-pair paternity, especially in the genus *Cryptomys* (Bishop et al., 2004), the opportunities for cheating are much lower than in, say, monogamous birds. However, the pair-bond seems to be unexpectedly strong on the part of males. In experiments with the eusocial *F. anselli*, bonded males did not take the opportunity to copulate with unfamiliar females and even behaved aggressively towards them (Bappert et al., 2012). As for the need to cooperate and display social tolerance, this is obviously highly developed in social mole-rats.

Burkart and van Schaik (2016) call for comparisons controlled for phylogenetic relatedness and as many other factors as possible in testing the cooperative breeding hypothesis, so bathyergids provide the ideal opportunity. Unfortunately, mole-rats are not very amenable

to cognitive tests and there is absolutely no evidence of cooperative breeding leading to higher neuronal numbers in the brains or cortices of mole-rats.

What about the differences in social complexity between the social and eusocial categories? Group stability is stressed by Shultz & Dunbar (2007) as another important factor contributing to social complexity, with the implication that more stable groups are more cognitively demanding. In mole-rats, stability largely depends on the tendency of young to stay in the natal group. Potentially, there is a decision to be made. If conditions are favourable and odds of successfully dispersing and reproducing high, the individual should take the chance. In the naked mole-rat, where most detailed information is available, the situation is as follows: To disperse successfully, subordinates need to attain a certain body mass. Individuals that work less grow at a higher pace and accumulate more fat reserves. Thus, they improve their chances of dispersal or attaining reproductive status within the colony. Moreover, the queen directs aggressive behaviour towards the largest subordinates (Reeve, 1992; O'Riain, 1996). The pattern is probably similar in *Fukomys*, where inbreeding is not common (Hazell et al., 2000). There is a potential conflict then: more intensive help increases inclusive fitness but limits the odds of successful breeding; slacking off can pay if favourable conditions arise for dispersal but can also incur punishment. Individuals should thus weigh the risk of dispersal against the gain in inclusive fitness if they stay. In a theoretical system, this could certainly favour the evolution of intelligence (although it is a very anthropomorphic assumption). But that clearly did not happen in mole-rats. Not even slight differences in any index of cognitive potential are evident between the social (where most individuals eventually disperse) and eusocial groups.

The emergence and stability of hierarchy is another proxy for social complexity (Bergman & Beehner, 2015). Eusocial mole-rats tend to have linear dominance hierarchies according to Landau's linearity index (Landau, 1951). This has been reported in *F. damarensis* and *H. glaber* (Jabobs et al., 1991; Clarke & Faulkes 1997). In social species (*C. pretoriae*, *F. darlingi*) less linear hierarchies with Landau's index 0.3 – 0.77 have been found (Gabathuler et al., 1996; Moolman et al., 1998). Thus stricter hierarchies seem to exist in eusocial species. However, these are not necessarily cognitively demanding, as would be the case in primates (MacLean et al., 2008). It has been demonstrated that dominance hierarchies can result from

following a few simple rules (Sakai et al., 2016). Sophisticated communication can also reflect social complexity (Freeberg et al., 2012). Among mole-rats, comparison between social and eusocial species is unfortunately not possible, because vocal repertoire has been examined only in three eusocial (Pepper et al., 1991; Credner et al., 1997; Yosida & Okanoya, 2009; Bednářová et al., 2013) and one solitary species (Knotková et al., 2009). Not surprisingly, the eusocial species have a rich variety of vocal signals, whereas vocalizations in *H. argenteocinereus* are limited to mating context.

To conclude, solitary mole-rats seem to have a higher cognitive potential based on their absolutely larger brains and numbers of brain and cortical neurons. The difference in overall body size can seem trivial but it might hold the key to the explanation of the findings that are incongruent with the SBH. The importance of absolute size in evolution should not be disregarded. Even Jerison (1973), in his seminal work where he popularized the encephalization quotient, wrote: "Absolute size may not be ignored in the analysis of organ relationships and in the study of the evolution of body and organ systems." Large animals are not just blown out copies of smaller ones. Size impacts everything from simple physical constraints to physiology, life-history, ecology and even has implications for sociality (e.g. Peters, 1986). Even in the original data set used in the influential paper by Dunbar (1992), body mass is significantly positively associated with group size (OLS: $p = 0.0001$). When the interaction with body mass is included in the linear regression of neocortex ratio against group size, the model performs somewhat better based on Akaike's information criterion (AIC; Burnham et al., 2011) (Δ AIC = 3.732), but the relationship with either variable is no longer significant, in line with the fact that these predictors are collinear. Schillaci (2008) found that after controlling for body size neocortex ratio is no longer correlated with group size in primates, with the same data set used by Kudo and Dunbar (2001) to affirm such a relationship. This strongly suggests that even in primates, body size plays a large role in the increasing brain size with group size. Similarly, the recent study in ground squirrels (Matějů et al., 2016) reported a relationship between absolute, but not relative, brain size and social complexity. This effect is even stronger in body mass, suggesting that it is mediated by selection on overall size, not the brain. In this respect, mole-rats seem to go against the general pattern. To understand this, we need to consider how body size is connected with sociality.

It is generally accepted that sociality evolved under different pressures in different taxa and the brain is simultaneously subject to these pressures as well. Of course, there is never a single factor acting in isolation, but the relative importance of the different components varies. Primates probably adopted group living mainly to minimize predation risk (e.g. Van Schaik, 1983), while ecological factors, such as resource availability, predominate in the rise of sociality in ground squirrels (Blumstein, 2013) and hystricognath rodents (Hayes et al., 2011; Maher & Burger, 2011). It is also the prevalent explanation for the evolution of sociality in mole-rats (Jarvis et al., 1994), but here the prediction is that limited and patchily distributed resources combined with hard-to-work soils should lead to the emergence of cooperative breeding. This explains the concurrent limitation on body size as well, because while there is a higher probability of finding a geophyte by a group, it also has to be shared among a larger number of individuals. Based on the data of Matějů et al. (2006), it is not possible to decide if the absolutely larger brains are the product of selection for brain size or coordinated response of the brain to selection for body size. In light of the present results, it seems that cooperative breeding in rodents does not require larger brains. Moreover, the same process can happen in the opposite direction: if sociality is associated with decreased body size, brains get smaller as well. Thus the interpretation of larger brains evolving as a consequence of social living is weakened.

So while there is evidence for real social complexity in the cooperatively breeding mole-rats, it does not manifest in larger brains or more neurons. There might be several explanations. One is that selection would indeed favour enhanced cognition, but the social species are facing an energetic constraint that is not offset by the potential benefits. Brains are metabolically expensive (Mink, 1981) and, simultaneously, excavating the burrow systems carries an enormous energetic cost (Vleck, 1979). However, if the observed brain size is the result of a tight balance between energetic demands restricting it and social environment expanding it, we should observe a decrease once the selective pressure for cognition relaxes. Therefore solitary species should have smaller brains, if this pressure is exerted by social living, but quite the opposite is observed. Conveniently, these constraints are relaxed in the aptly named giant mole-rat *F. mechowii* that occurs in mesic areas of central Africa and unlike others mole-rats might supplement its diet with invertebrates (Kawalika & Burda, 2007). The response is indeed a concerted increase in both body and brain, with the brain

lagging behind, not indicating selection for larger brain size (Pavelková et al., in prep.). Another possibility is that this selective pressure does not require bulky adjustments or quantitative changes in the brain. In fact, there is evidence that seemingly major cognitive leaps in behavioural complexity can evolve with little change in the neural substrate (Chittka & Skorupski, 2011). And, of course, it is possible that social complexity in mole-rats is simply not cognitively demanding and no special neural substrate is needed.

Having said that, there is another finding that is worth exploring: That the higher relative number of cortical neurons in solitary mole-rats is mostly independent of body size, since neither the cortex proportion in the brain nor cortical neuronal density changes much with brain mass in mole-rats and cannot thus be attributed to the differences in overall brain size. We should look for another factor that can be responsible for this difference.

Under some circumstances, it might be more cognitively demanding for an animal to "fend on its own" than to rely on a social group. But there is no clear indication as to why that should be so in the case of subterranean lifestyle. Foraging for tubers or digging burrows does not require much innovation or flexibility that would drive cognitive power (Lefebvre et al., 2004). An interesting parallel from the insect world presents itself. For instance, sociality in wasps (Vespidae) is associated with reduction of mushroom bodies, the analogue of vertebrate higher brain centres (Farris, 2008), which is interpreted as "distributed cognition" (O'Donnell et al., 2015). In a similar manner, empirical data and computer modelling suggest that in resource-poor environments, ants tend to have smaller colony sizes where individual workers have larger brains, whereas in resource-rich environments, large numbers of small-brained workers can still accomplish the same tasks effectively by the means of "collective intelligence" (Feinerman & Traniello, 2015). Maybe the eusocial mammals resemble eusocial insects in this respect?

Admittedly, the analyses performed here have limited explanatory power, since it is possible that sociality is an ancestral trait in mole-rats (Burda, 1999). But this is offset by the fact that the loss of sociality was accompanied with absolute brain enlargement, while a potential regain was not. In the case of mole-rats, it is more about showing how vertebrate social systems can work *without* being accompanied by the evolution of advanced cognitive abilities. Also the groups are unfortunately small, which restricts statistical power and allows

for uncovering only rather substantial and unambiguous effects. However, that might not be a problem, because biological significance of tiny differences in relative brain size is doubtful, anyway.

Furthermore, it is possible that reproductive and non-reproductive individuals in the eusocial mole-rats differ. Brain morphology might be affected by levels of activity and social stress (reviewed in Tamashiro et al., 2005; Hillman et al., 2008). It is unlikely these differences would contribute to evolution, as they are environmentally induced, but potentially could put the social species at a disadvantage because only non-reproductives were examined. In the literature, there is evidence of such subtle differences. In *H. glaber* and *F. damarensis*, breeders have larger volumes of several brain regions associated with reproduction and social behaviour, but no differences were found in cortical thickness (Holmes et al., 2007; Anyan et al., 2011). In the same species, workers actually have increased neurogenesis in the hippocampus compared with reproductive individuals (Peragine et al., 2014; Oosthuizen & Amrein, 2016). In any case, these differences are so small as not to be detectable by the isotropic fractionator and no differences between the social categories were found even in volumetric data on these regions (Kverková et al., in prep.).

How can the apparent absence of positive selection on brain size in the social mole-rats be reconciled with a rather sound support of the SBH in primates? It seems that behavioural modification can happen in the form of fine-tuning requiring little quantitative changes in brain composition. The mechanisms underlying substantial cognitive enhancements are probably different. In some cases, the initial factors responsible for the evolution of large brains may just be the consequence of a concerted response of brain and body to selection for body size. In this view, the brain simply "tags along" , but not without functional implications. The runaway process of increasing intelligence envisioned by Humphrey (1976) can only be realized in animals with already large brains, whether these are the result of adaptive processes or simply the passive consequence of selection for body size. Another aspect is that given the differences in neuronal scaling rules, primates potentially benefit from brain enlargement more than rodents and this might set a positive feedback in motion. As Hofman (1989) phrased it: "It is evident that internal factors of brain design, bearing no relation to the selective reasons of initial enlargement, may be the primary determinants directing the evolution of brain size." We do not know what increase in the number of

neurons or neuronal packing density is needed to confer a substantial cognitive advantage. Perhaps the selection for body size is not effectively coupled to selection on cognition when neuronal density is decreasing with increasing brain size, such as is the case in rodents. Thus the overarching general patterns in brain organization might give rise to some characteristics particular to the evolution of intelligence in each phylogenetic lineage.

CONCLUSIONS

Using the isotropic fractionator, it was shown that African mole-rats generally conform to the brain cellular scaling rules in rodents and to the mammalian pattern governing the scaling of glia and glia/neuron ratios, but still show some possible adaptations to their underground environments, such as a reduction and stability of neuronal densities in the cortex and an increase of neuronal densities in the olfactory bulbs. Using the data on brain size, neuronal numbers and their allocations to different brain parts, it was demonstrated that no version of the SBH hypothesis is supported in mole-rats, a group containing the only "eusocial" mammals. Potential benefits of cognitive enhancements are probably outweighed by the metabolic demands of more neurons needed to confer such an improvement.

In the future, it would be interesting to acquire neuronal level data for other groups of subterranean rodents and see if their brains show patterns similar to the bathyergids and perhaps if fewer neurons in the cortex are related to the degree of reduction of the visual system. This type of "quantitative neuroanatomy" is still in its beginnings and not many data are available for deducing evolutionary patterns. Of course, one must not resort to reducing the brain to a bag of neurons and still bear in mind the intricate net of synaptic connections and physiological processes regulating brain function. The isotropic fractionator should be employed not instead, but in conjunction with more qualitative approaches. And such application is sure to reveal more unexpected aspects of brain evolution.

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