Re-examining extreme sleep duration in bats: implications for sleep phylogeny, ecology and function

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1   Abstract

Bats are quoted as sleeping for up to 20 hours a day, an example of extreme sleep duration amongst mammals. Given that duration has historically been one of the primary metrics featured in comparative studies of sleep, it is important that determinations of species-specific sleep duration are well founded. Here, we summarise the evidence for the characterisation of bats as extreme sleepers and discuss whether it provides a useful representation of the sleep behaviour of Chiroptera. Although there are a wealth of activity data to suggest that the diurnal cycle of bats is dominated by rest, citable evidence that they spend the vast majority of this time asleep is limited to a single species studied under conditions which may not be representative of a natural sleeping environment. Furthermore, extreme sleep durations are not expressed under all conditions or in all bat species. This calls into question the validity of using an extreme sleep duration to represent bats in comparative studies of sleep. We suggest best practices, which should be adopted, where possible, for sleep studies in Chiroptera and identify behavioural and physiological factors that make this group an interesting case study for researching sleep function.

2   Introduction

Sleep is increasingly being recognised as a state with cognitive and restorative functions that are of vital importance in the lives of all animals (Frank and Heller, 2019). In bats (Order: Chiroptera), a diverse group of mammals loosely separated into large frugivo-

rous and small insectivorous forms, sleep has received little attention. To date the sleep patterns of only four species of bats have been characterised using electrophysiological recordings (see Table 1). Notwithstanding, bats are well represented in the sleep literature due to the daily duration of their sleep. Research articles, textbooks and popular science books have propagated the view that bats are extreme sleepers, spending up to 20 hours a day in this state (Siegel, 2009, 2005, 2012; Herculano-Houzel, 2015; Zhao et al., 2010; Zepelin et al., 2005; Allison and Van Twyer, 1970; Burger et al., 2020; Linde and Savary, 1974; Braun, 1975). This is based on the sleep behaviour research of two species, the little brown bat (*Myotis lucifugus*) and the big brown bat (*Eptesicus fuscus*) (Brebbia and Pyne, 1972; Zepelin and Rechtschaffen, 1974). Despite the importance of these studies to the overall characterisation of bats as extreme sleepers, few have attempted to contextualise their findings. In particular, the sparse accounts of the conditions used in electrophysiological experiments and the lack of diversity of species studied has not been addressed.

Why some animals sleep longer than others remains a leading question in the sleep research field. Comparative studies use the variation in sleep duration across animal species to search for correlates that may give insight into the functions of sleep (E.g., Allison and Cicchetti, 1976; Capellini et al., 2008; Lesku et al., 2006). Because the number of species available for such studies is relatively small, extreme durations such as those documented in bats can have a strong influence on the results and subsequent conclusions. It is therefore important to understand the factors that explain the extreme sleep durations recorded in bats and whether they have been correctly compared

with recordings from other species.

Here, we first present the case for bats displaying an extreme daily sleep duration in comparison to other species. We then discuss challenges to this assessment posed by conflicting data and confounding factors in the original data. We review the scientific impact of bats having been considered extreme sleepers over the past half-century and the consequences of this changing. We end by discussing the potential contribution bats could make to the sleep field moving forward and suggest best practices for achieving this. This review reveals the complexity involved in answering as seemingly simple a question as "how long do bats sleep?" and identifies behavioural and physiological factors that make this group an interesting case study for researching sleep function.

3   Evidence of extreme sleep duration

Bats have long been recognised for their propensity for sleep. An early encyclopedia entry from 1797 documents how bats "inhabit dark places, which they quit only for nocturnal excursions" and may be found "for the greatest part of the day" suspended by their feet, concealed within their wings (Buffon and Barr, 1797). One of the first attempts to quantify the length of sleep in bats can be found in the writings of Moffat (1904) who described the "remarkably somnolent" lesser noctule (*Nyctalus leisleri*), quoting a daily sleep duration of 21.5 hours during its "season of activity", thus distinguishing the behaviour from hibernation.

Emergence profiles of bat colonies provide insight into the roosting behaviour and

therefore, indirectly, sleep behaviour of bats. The great majority of bats are nocturnal, emerging from their day roosts at dusk and returning before dawn (Erkert, 1982). For most species, this means movement outside the roost in restricted to between 6PM-6AM or less (Erkert, 1982; Swift, 1980). Following an initial peak in emergence activity at the beginning of the night, a secondary peak at dawn is also present for many species and is associated in particular with insectivory (Erkert, 1982). Roosting behaviour may be affected by seasonal changes in temperature and day length. The time pallid bats (*Antrozous pallidus*) spend in the day roost ranges from 14 hours in the summer to 19 hours in the spring (Vaughan and O'Shea, 1976). Furthermore, individuals may spend longer periods in the day roost than the colony emergence profile would suggest. Bats in *Asellia tridens* colonies continue to make foraging trips for up to 10 hours after sunset, yet individuals on average only spend around 4 hours foraging per night (Amichai et al., 2013).

In order to rest and decrease energy costs, bats spend as much as 75% of the time away from the day roost in night roosts (Lewis, 1994; O'Shea and Vaughan, 1977). Night-roosting may therefore make a meaningful contribution to the total sleep duration in some species. Kunz (1980) estimated that little brown bats (*Myotis lucifugus*) spend 15 hours in day roosts and 5 hours in night roosts per 24 hour period, with the remaining 4 hours taken up by foraging.

Whilst studies of wild activity patterns do not differentiate between roosting behaviours (e.g., sleeping, grooming, mating), and therefore cannot be used to assess how much of this time is dedicated to sleep, they do show that bats are often in an

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appropriate environment to sleep for 12-20 hours a day.

High temporal resolution laboratory recordings of activity facilitate finer-grain descriptions of bat rest-activity cycles. As predicted from wild observations, bats maintained under controlled 12:12 light-dark cycles largely limit their activity to the dark period (Erkert, 1982). This behaviour has been shown to be endogenously controlled in little brown bats (*Myotis lucifugus*) and tricoloured bats (*Pipistrellus subflavus*) , persisting over multiple days in constant darkness before beginning to free-run (Griffin and Welsh, 1937). In the same study, bats maintained under dim light conditions were inactive for up to 18.8-20.2 hours per day and exhibited the aforementioned bimodal activity pattern typical of some insectivorous species in the wild with dusk and dawn peaks and little activity in between (Supplementary 1). These results suggest individual bats may only be truly active for a portion of the night, providing additional time for sleep to occur.

Whilst the length of time animals spend inactive can be used to estimate sleep time, this methodology often leads to overestimation as sleep can not be distinguished from other quiescent states such as torpor, though as we will see this issue also applies to more sophisticated sleep measures (Ancoli-Israel et al., 2003). Behavioural definitions of sleep typically also include the assumption of a species-specific posture, reversible perceptual disengagement from the environment and a homeostatic sleep rebound following deprivation (Campbell and Tobler, 1984; Tobler, 1985; Carskadon and Dement, 2017). An alternative approach used in most modern sleep studies is to

rely on well founded electrophysiological correlates of behavioural sleep (Campbell and Tobler, 1984). Electrical activity in the brain is detected by electrodes and converted into an electroencephalogram (EEG) from which signals characteristic of behaviourally defined sleep and wake can be identified. When performed in conjunction with electromyography (EMG), this approach allows researchers to divide the time course of sleep into two main stages, rapid eye movement (REM) and non-rapid eye movement (NREM) sleep, based on muscle tone and the frequency composition of the EEG signal.

An electrophysiological approach was first applied to the study of sleep behaviour in bats by D. R. Brebbia and associates in the 1960s and 70s (Brebbia and Paul, 1969; Brebbia and Pyne, 1972). Regional brain EEG and nuchal EMG were performed in tandem with measurements of brain temperature and heart rate in chronically implanted animals. In the first of two conference abstracts, the posture and diurnal distribution of sleep is described for two species of vespertilionids native to the Americas, the big brown bat (*Eptesicus fuscus*) and the little brown bat (*Myotis lucifugus*), followed by a detailed electrophysiological characterisation of different brain regions during sleep (Brebbia and Paul, 1969) (Box 1). As in other animals, sleep presented behaviourally as physical quiescence in a stereotyped postured and could be separated into two stages with distinct electrophysiological profiles. Namely, NREM cortical EEG was characterised by high voltage slow rhythms and spindle activity, and REM cortical EEG by a desynchronised trace similar to wakefulness. The second conference abstract describes the effects of ambient temperature manipulations on sleep in the little brown bat (Brebbia and Pyne, 1972). At 33°C, 83% of the recording time was occupied by

REM or NREM sleep, which equates to a total daily sleep duration of 19.9 hours, the figure most often quoted for this species. Interestingly, neither of these studies mention the duration of sleep for the big brown bat. The earliest record for this species is in a review by Zepelin & Rechtschaffen (1974), quoting a sleep duration of 19.7 hours. The citation for this data is a personal correspondence from Brebbia dated to the same period as the recordings in little brown bats which could suggest that sleep duration was also measured in big brown bats. However, as there is no mention of this in Brebbia's writings, the provenance of this measurement can not be verified.

The daily sleep durations measured using electrophysiological criteria appear to confirm that bats are indeed sleeping during the long periods of time they spend inactive. Whether such sleep durations should be considered extreme depends on comparison with other animals. In one review of sleep across mammalian orders, Brebbia et al's measures of daily sleep duration in Chiroptera were estimated to be two standard deviations above the mean (μ=10.46, σ=4.95) and more than 4 hours greater than the next longest order (Elgar et al., 1988). On this basis, a strong argument can be made for bats being considered extreme sleepers.

4 A re-examination of bat sleep duration

4.1 Variation in Chiroptera

In contrast to the narrative so far presented, not all measurements of sleep duration in bats may be classed as extreme by mammalian standards. 40 years after the first electrophysiological measurements of bat sleep duration, Zhao et al. (2010) recorded sleep electrophysiology in the lesser dawn fruit bat (*Eonycteris spelaea*) and the greater short-nosed fruit bat (*Cynopterus sphinx*). 5-6 mixed sex adult specimens of each species were captured from wild populations and transferred to a temperature and light regime controlled laboratory setting. As in the work of Brebbia et al., EEG and EMG electrodes were implanted to measure brain and muscle activity respectively and the sleep-wake cycle was scored using standard mammalian criteria. In stark contrast to the little brown bat (*Myotis lucifugus*), Zhao et al. reported sleep durations well below 20 hours in both species (see Table 1 for a comparison of sleep architecture). On average greater short-nosed fruit bats slept 14.8 hours per day, which was accumulated predominantly during the light period of the 12:12 hour light-dark regime employed in the experiment. The average daily sleep duration was slightly lower for lesser dawn fruit bats at 14.7 hours, and unlike greater short-nosed fruit bats was split evenly between the light and dark periods.

Zhoa et al's (2010) findings represent a marked departure from previous sleep duration measures. Based on their findings alone, bats would appear to have daily sleep durations comparable to species like the syrian hamster (14.4 hours) (Van Twyver,

1969) that do not generally enter discussions of extreme sleep. Furthermore, there is no obvious difference in the methodology of their experiment that may have contributed to sleep duration; they used wild-caught animals kept in laboratory conditions and scored sleep using standard electrophysiological criteria. Nonetheless, it may be possible to reconcile these differences in sleep duration between Zhoa et al's study and the work of Brebbia & Pyne if we consider the choice of species.

The species used in the two electrophysiological studies of chiropteran sleep duration have distinct evolutionary histories. Little brown bats (*Myotis lucifugus*) and big brown bats (*Eptesicus fuscus*) are vespertilionids belonging to the suborder Yangochiroptera, whereas lesser dawn fruit bats (*Eonycterus spelaea*) and greater short-nosed fruit bats (*Cynopterus sphinx*) are pteropodids belonging to the suborder Yinpterochiroptera. Molecular phylogenetics suggests the two chiropteran suborders diverged in the early Paleogene and have been evolving in tandem for 64 million years (Teeling et al., 2005). In addition to their phylogenetic separation, the species used in the studies also differ in their basic biology. The vespertilionids are a widespread family of small insectivores which typify the common chiropteran assemblage (Altringham, 1996). On the other hand, the predominantly frugivorous pteropodids (fruit bats) are larger and more reliant on vision for orientation than other bats as most lack the ability to echolocate (Pettigrew et al., 1989; Hutcheon and Garland, 2004).

Given the variation between the species used in these studies, it is perhaps not surprising that sleep duration measures also differed. In fact, there is evidence to support the hypothesis that sleep duration differs between pteropodids and other bat species. In

their investigation into daily temperature changes of bats, Burbank and Young (1934) noted the peculiarity that attendants at two captive fruit bats colonies in the UK had never observed them "fully asleep". This may be an exaggeration of the observation that fruit bats are more active during the day than their counterparts. For example, using behavioural criteria Downs et al. (2015) found that nocturnal Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*) slept for only 28.8% of the 12 hour light phase in the laboratory and were usually awake when observed in the wild during the same period. Using similar criteria, the large flying fox (*Pteropus vampyrus*) was estimated to sleep up to 71% of the 12 hour light phase on average between the sexes, however this still represents a decrease compared to the little brown bat and does not take into account the lower sleep propensity expected of nocturnal animals in the dark phase (Hengjan et al., 2017).

If sleep duration is lower in the Pteropodidae, this would be consistent with comparative studies of sleep in mammals that show large, herbivorous species tend to sleep less than small, carnivorous species (Zepelin and Rechtschaffen, 1974; Allison and Cicchetti, 1976; Elgar et al., 1988; Siegel, 2005; Capellini et al., 2008). One theory to explain this relationship, the energy conservation hypothesis, posits that if the function of sleep is energy conservation, small mammals with a high metabolic rate may have evolved to sleep longer than large mammals with lower metabolic rates (Walker and Berger, 1980). In support of this hypothesis, pteropodids are almost exclusively homeothermic and only a few species are known to exhibit daily torpor, suggesting a lower requirement for energy conservation (Altringham, 1996).

An alternative to the energy conservation hypothesis is the foraging time hypothesis which posits that because sleep requires immobility, sleep duration should be negatively correlated with foraging time (Elgar et al., 1988). As a result, larger animals with higher energetic requirements and animals that consume low energy foods should sleep less. This could explain why extreme sleep durations have not been recorded in the larger, frugivorous pteropodids. However, both frugivores and insectivores spend a majority of their active phase roosting (O'Shea and Vaughan, 1977; Morrison, 1978) and have comparable foraging behaviour in terms of duration and distance travelled (Egert-Berg et al., 2018) which suggests foraging time may not be an explanatory factor for differences between pteropodids and other bats.

One final explanation for pteropodids sleeping for shorter durations than other bats could be related to sociality. Although the influence of the social environment on sleep duration in laboratory studies of bats has not yet been addressed, mammals that sleep socially have been found to have reduced sleep durations compared to species that sleep alone, perhaps as a result of needing to fulfill social interaction quotas (Capellini et al., 2008). A similar relationship may exist within Chiroptera, in which the highly social pteropodids (Harten et al., 2018; Prat et al., 2015; Hengjan et al., 2017) devote more time to interacting with conspecifics and less time to sleep than species such as the little brown bat.

Evidence from pteropodids points to there being considerable inter-specific variation in sleep duration between species of bat. Whilst this in itself does not challenge the idea that some bats are capable of extreme sleep duration, it does make clear the fact that,

as for any group of animals, the characterisation of chiropteran sleep behaviour should be informed by a range of species that encompass the order's diversity in morphology, ecology and evolutionary history.

4.2   Caveats to recordings of extreme sleep duration in bats

Whilst multiple sources indicate that bats may be inactive for up to 20 hours a day (Griffin and Welsh, 1937; Kunz, 1980), only Brebbia & Pyne's (1972) work with little brown bats (*Myotis lucifugus*) suggests this time is spent sleeping. Thus, the extreme sleep duration characterisation depends on a single study using an unknown number of specimens. Furthermore, descriptions of most of the experimental conditions under which little brown bats were studied are limited or missing from the cited source material (Brebbia and Pyne, 1972). Due to the age of the study, the use of invasive EEG implants and reference to temperature manipulations, we can assume that Brebbia & Pyne's sleep experiments were conducted under laboratory conditions. In general, laboratories are simple environments that fail to recreate the natural challenges to which animals in the wild have adapted and can introduce artificial challenges of their own (Rattenborg et al., 2017). Factors such as photoperiod (Borbély, 1978; Hubbard et al., 2013), light intensity (Borbély, 1978), ambient temperature (Harding et al., 2019), diet (St-Onge et al., 2016), social environment (Lendrem, 1984) and predation risk (Lima et al., 2005) have all been identified as capable of influencing sleep but can be difficult to reproduce in captivity. Two of these factors in particular, ambient

temperature and social environment, may have influenced the sleep behaviour of bats in Brebbia & Pyne's experiments.

Temperature dependency and torpor

A notable caveat to the 19.9 hours daily sleep duration reported for little brown bats, which citing studies usually ignore, is that it is temperature dependent. Brebbia & Pyne (1972) characterised the sleep-wake cycle of this species when exposed to a range of ambient temperatures (Figure 1). Total sleep duration was found to vary from a maximum of 19.9 hours at 33°C to a minimum of 11.0 hours at 26°C. Below 19°C, EEG signals lacked discernible sleep rhythms and at 5°C became isotonic. In addition to temperature, exposure time also had an effect on sleep duration, most notably the duration of REM sleep which decreased from 2.5 to 0.5 hours following "chronic" exposure to a temperature range of 19°C-21°C for multiple days. This means that depending on ambient temperature, total sleep duration and REM duration can be longer or shorter than the lesser dawn fruit bat (Figure 1). Whilst it is not unusual for sleep duration to fluctuate with ambient temperature (E.g., Rosenthal and Vogel, 1993), the magnitude of change recorded in little brown bats and the finding that they do not exhibit an extreme sleep duration across all temperatures raises two questions. First, for which temperature condition should sleep duration be reported? Second, are durations derived from temperature dependent sleep recordings comparable with other animals?

To answer the first question, we must consider the thermal conditions faced by bats

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in their natural habitats. Bats are found in all environments apart from certain deserts and high latitudes (Altringham, 1996) and have been recorded at environmental temperatures as low as -17°C during the winter and as high as 55°C during the summer ((Davis, 1963; Mislin and Vischer, 1942), reviewed in (Stones and Wiebers, 1965)). The temperature at which bats sleep is influenced by their roosting behaviour. Roosts provide stable microclimates relative to outside air temperatures and have been shown to increase in temperature by as much as 7°C when occupied by clusters of bats due to social thermoregulation (Willis and Brigham, 2007). As a result, even in temperate climates during the summer with an average ambient air temperature of 18°C, average day roosts temperatures can exceed 35°C (Burnett and August, 1981). These observations suggest that the 5°C-33°C temperature range used by Brebbia and Pyne (1972) is biologically relevant. Furthermore, it seems logical to report sleep time from the higher ambient temperatures used in this study considering that sleep rhythms were only clear above 19°C. However, there is no information available to inform the choice between the 26°C or 33°C conditions despite a c.9 hour difference. Using a single figure to characterise daily sleep duration in this species is not empirically supported.

Answering the second question is more difficult as there are many reasons why sleep duration may be dependent on temperature. One possibility is that sleep duration in these experiments was influenced by torpor. Like many small endotherms, bats are able to alleviate the high energetic demands of maintaining a constant body temperature through the use of torpor. Torpor is an energy conserving physiological

state in which metabolic rate is decreased and body temperature is depressed as a result of the animal becoming poikilothermic (Lyman, 1982). Conditions believed to promote torpor include low environmental temperatures, limited food availability and drought (Ruf and Geiser, 2015). The temperature threshold at which torpor becomes favourable seems to be particularly high among bats compared to other mammals, with some reported to become poikilothermic at temperatures of $>30°C$) (Amichai et al., 2013), and is likely as a result of their high rates of thermal conductance (Bradley and Deavers, 1980) combined with a more metabolically costly form of locomotion (Thomas and Suthers, 1972). Thus although torpor is often associated with winter hibernation (seasonal torpor) in response to low environmental temperatures, many bats in all climes also employ shorter bouts of torpor (daily torpor) throughout the year (Stawski et al., 2014). In the case of the little brown bat, individuals frequently enter torpor, with bouts lasting 2-3 weeks during the winter and 1-24 hours during the summer (Matheson et al., 2010).

As in other animals, the relationship between sleep and torpor in bats is unknown (Ruczynski et al., 2014). Using currently accepted criteria, it is possible for these states to coincide (Strijkstra et al., 1999; Royo et al., 2019; Huang et al., 2021, see Box 2). If sleep and torpor did coincide in Brebbia and Pyne's recordings, this may render their findings incompatible with sleep recordings from other animals for two reasons. First, sleep in a state of torpor may not be comparable with non-torpid sleep. An increase in slow-wave activity has been observed during the period following a torpor bout similar to that seen after sleep deprivation, suggesting that sleep pressure increases during

torpor as in wake, albeit it at a decreased rate (Deboer and Tobler, 2003; Vyazovskiy et al., 2017). Second, sleep duration may be decoupled from sleep need in species capable of torpor. As torpor is typically entered through NREM sleep (Deboer and Tobler, 1994), animals may sleep to fulfill their torpor requirement. In studies where torpor can not be distinguished from sleep, the occurrence of torpor will therefore result in the elongation of recorded sleep duration.

As body temperature or metabolic measures were not reported, we can only infer whether bats entered torpor in Brebbia and Pyne's (1972) study. Sleep at 19-21°C was characterised by shivering, an increase in the ratio of NREM:REM sleep and a reduction in the voltage of electrical activity in the brain which could suggest shallow torpor. Below 19°C an isoelectric EEG lacking sleep rhythms suggests a deepening of torpor, which the authors recognised as a "unique state of consciousness...at hypothermic extreme" (Brebbia and Pyne, 1972). Although the sleep EEG was described as "typically mammalian" above 26°C and little brown bats usually defend a homeothermic above 32°C (Stones, 1964), the possibility that bats were in torpor can not be ruled out at these temperatures either given that normal sleep rhythms are present during high temperature torpor bouts in other animals and that bats have been reported to be torpid above 30°C in the wild (Royo et al., 2019; Amichai et al., 2013).

In summary, the 19.9 hour sleep duration recorded at a temperature of 33°C is not representative of the sleep behaviour of the little brown bat (*Myotis lucifugus*) in Brebbia and Pyne's study. Sleep duration varied considerably across a naturalistic range of temperatures for this species. Furthermore, the influence of torpor on sleep

duration could not be accounted for. In fact this holds for any study of sleep in bats that lacks information regarding the temperature or metabolic state of the subjects, including the study by Zhao et al. (2010). Therefore, whilst little brown bats are capable of extreme sleep durations, such lengths are not the common condition and their sleep may not be directly comparable with other animals in which sleep has been studied.

Social environment

Of the laboratory conditions that are thought to have an effect on sleep, one stands out as having the potential to influence bats more than many other mammalian orders: the social environment. Most bats are gregarious, which has been attributed in part to a limited availability of suitable roost sites, and are found in groups ranging in size from several individuals to some of the largest aggregations of mammals in the world (Kerth, 2008; Stepanian and Wainwright, 2018). Social roosting can provide benefits such as reduced thermoregulatory costs (social thermoregulation) and reduced evaporative water loss simply as a result of aggregation (Willis and Brigham, 2007). Furthermore, many groups of bats contain complex social systems in which animals engage in a diversity of social behaviours (Kerth, 2008; Harten et al., 2019; Carter and Wilkinson, 2013; Knörnschild et al., 2012). Little brown bats (*Myotis lucifugus*) form stable colonies (i.e., that persist throughout the reproductive season) of many thousands of individuals usually in permanent roosts such as caves and mines (Fenton and Barclay, 1980; Langwig et al., 2012; Sagot, 2016). Although it has been suggested

that social systems of little brown bat colonies are relatively simple in comparison to some bat species, evidence of social behaviours such as vocal communication during interactions, swarming and even social learning indicate that the social environment plays an important role in the lives of these animals (Barclay et al., 1979; Schowalter, 1980; Gaudet and Brock fenton, 1984). Unfortunately, we do not know the specific housing conditions used by Brebbia & Pyne (1972). However, most animals, whether social or solitary in the wild, are isolated during sleep studies (Rattenborg et al., 2017). Thus it is important to consider the possibility that the sleep data for the little brown bat were not collected under naturalistic social conditions.

Multiple relationships between sleep duration and sociality have been suggested. A key benefit of grouping which is thought to influence sleep is decreased risk of predation. For example, theoretical and empirical evidence suggests that the collective vigilance of groups increases with group size even if individual vigilance is reduced (Bertram, 1980; Pulliam, 1973). As predation risk is negatively correlated with sleep duration across mammals, this could suggest that grouping could increase sleep duration (Capellini et al., 2008). Commensurate with this, Lendrem (1983) found that as corporate vigilance in Barbary doves (*Streptopelia risoria*) increased with group size, so too did the time individuals spent with their eyes closed which could suggest an increase in sleep duration. In fact, the opposite trend has been observed in comparative studies of sleep which find greater sleep durations in solitary versus social species (Capellini et al., 2008). To explain this trend, it has been suggested that sleep may be disrupted in social environments, either due to individuals spending more time un-

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dertaking social interactions (Capellini et al., 2008) or being disturbed by conspecifics (Zhao et al., 2010). It should be noted that as most "normative" sleep duration data used in comparative studies has been acquired from isolated animals regardless of their grouping behaviour in the wild, differences in sleep between species associated with sociality may have been masked, making it difficult to predict the effects of the social environment.

One final possibility which has received little attention is that sleep duration is increased by grouping but this effect is not observed in comparative studies due to the isolation of subjects in sleep experiments. Without a group to sleep in, social animals may be extra vigilant in sleep experiments, causing a negative bias in the sleep duration data for these species.

Although the effect of the social environment on sleep has not been studied directly in bats, there is indirect evidence to support the theory that sociality may influence sleep through predation risk. Klose et al. (2009) recorded vigilance behavior in a colony of adult tree-roosting flying foxes (*Pteropus poliocephalus*) and found that bats at the periphery of the colony engaged in more environmental vigilance events (vigilance directed towards the surroundings rather than conspecifics) and were more vigilant in general than centrally positioned bats, though only the first result was significant. Although predation is likely to be more relevant to open roosting bats, even cave dwelling species such as little brown bats (*Myotis lucifugus*) experience predation in their roosting environment, meaning the social environment may still influence predation risk (Fenton and Barclay, 1980; Lima and O'Keefe, 2013). Indeed, activity levels have been

found to differ significantly with position in clusters of little brown bats, with central individuals spending close to 4% more of the day roosting period at rest than peripheral individuals (Burnett and August, 1981). Whether these group position effects are related to corporate vigilance or other factors such as social thermoregulation has yet to be determined.

On the other hand, there is also indirect evidence in bats to suggest sleep may be disrupted by conspecifics. Individual actions such as grooming have been reported to disturb neighbours and aggressive interactions with physical and auditory components have been documented between roosting bats (Porter, 1978; Fenton and Barclay, 1980). Such interactions may be concentrated at the onset of the roosting period when arriving bats compete for positions within clusters (Burnett and August, 1981). Groups of little brown bats have been observed exiting torpor canonically in "arousal cascades" (Czenze et al., 2013; Turner et al., 2014). Because the social thermoregulation benefits of arousing in a cascade are less than arousing synchronously, it has been suggested that such events result from maladaptive disturbances of torpid individuals by normothermic individuals (Turner et al., 2014). This is supported by evidence that a torpor arousal cascade initiated by researchers at a cave-dwelling colony of *Myotis* bats continued long after the researchers had left (Thomas, 1995). If bats do respond to social arousal cues, this could explain how bats trapped inside caves lacking diurnal environmental rhythms are able to maintain their circadian phase relative to free-moving bats in the same roost (Marimuthu et al., 1978, 1981).

Both extension and reduction of sleep duration may be predicted outcomes of sleep-

ing socially in bats. The opposing forces of reduced predation risk and increased disruption risk associated with grouping may even act in tandem, with the overall effect on sleep depending on the balance between the two. For example, grouping may promote sleep in species that form small clusters and use exposed roost site, whilst the opposite may occur in species that form large aggregations in protected roost sites. It is therefore difficult to predict the effect of the social environment in recordings of extreme sleep duration in little brown bats. However, it seems likely that the natural sleep behaviour of bats in the wild is influenced by the social environment meaning characterisations of their sleep behaviour should reflect this.

5 Implications for comparative analyses of sleep duration

Arguably the ultimate reason we are interested in measuring parameters of sleep in different species is to gain insight into the functions of this phenomenon, and one of the most important parameters historically has been sleep duration (Campbell and Tobler, 1984; Lesku et al., 2009). It is usually assumed that sleep confers some form of benefit, the magnitude of which is a function of the time spent in this state. However, there are also significant costs associated with sleep, such as the inability to access

sustenance and reproduce (Siegel, 2009). As sleep and wake are mutually exclusive, we would expect natural selection to act on the trade-off between the benefits and costs of each state in order to optimize sleep duration (Schmidt, 2014). The existence of inter-specific variation in sleep duration is important as it suggests that differences between species alter the optimal trade-off between time spent sleeping and awake. Thus, if we can identify the features that are important in predicting differences in sleep duration between species, we might be able to infer something about the functions of sleep (Lesku et al., 2009). This has been the aim of cross-species comparative studies which correlate sleep parameters and other potentially relevant features of morphology (e.g., body mass), physiology (e.g., metabolic rate) and ecology (e.g., predation risk). A number of theories of sleep function have subsequently been developed from this methodology. For example, the energy conservation hypothesis for sleep was first posited by Allison & Van Twyver (1970) based on comparisons of NREM sleep duration in endotherms and ectotherms.

Overall, however, efforts to correlate inter-specific variation in sleep duration with features thought to be associated with sleep function, such as basal metabolic rate (BMR), encephalization and body mass, have met with mixed success, often producing weak or inconsistent results (Lesku et al., 2008; Capellini et al., 2008). For example, many studies have sought to correlate sleep time with BMR. If sleep, as is often hypothesised, has an energy conservation function, the sign of this correlation is of great significance. Yet depending on the method used to control for body mass, BMR is either positively (Zepelin and Rechtschaffen, 1974) or negatively (Lesku et al., 2009)

correlated with sleep time. From our re-examination of extreme sleep duration in bats, we can infer issues with the sleep duration dataset that may explain it's sensitivity to different applications of the comparative method.

The first inferred issue is sample size. Comparative studies in mammals typically sample only around 50-80 species or c.1% of the total number of species (Zepelin and Rechtschaffen, 1974; Lesku et al., 2008). Previous studies have identified a phylogenetic signal in sleep traits (Capellini et al., 2008), suggesting this level of sampling is sufficient to capture the variation in sleep architecture in animals. However, as evidenced by the c.26% difference in sleep time between the little brown bat and both species of fruit bat, it is certainly not sufficient to capture the variation in Chiroptera (Brebbia and Pyne, 1972; Zhao et al., 2010). Furthermore, undersampling is directly responsible for bats being considered extreme sleepers, for the little brown and big brown bat species were the only chiropterans available to the initial comparisons of sleep duration amongst animals which birthed this idea. The case of Chiroptera suggests that some of the observed variation in sleep architecture between taxa could be the result of chance sampling of extreme representatives, leading to a false phylogenetic signal. If true, this would have significant implications for use of the comparative method and could explain some of it's shortcomings.

The second inferred issue is the age of studies measuring sleep duration. Many studies used in comparative analyses date back more than 40 years when electrophysiological sleep research was in its nascency. Although many of the fundamental aspects of such studies have not changed since, some that can influence the measurement of

sleep duration have. The paramount example of this from bats is the treatment of torpor. In extreme sleep recordings from bats, no mention is made of the possible occurrence of torpor despite indicative changes in physiology being reported. This is problematic, for sleep duration may be affected by torpor and torpid sleep may not be comparable with non-torpid sleep in terms of function. As our understanding of this state and its relationship with sleep develops, the lack of detail in the reporting of experimental variables in early experiments such as Brebbia and Pyne (1972) may cause us to reassess whether they should be included in comparative analyses.

The third inferred issue is the use of laboratory conditions. Almost all sleep data are laboratory recordings, meaning the results of comparative analyses are predicated on the assumption that animals sleep the same in the laboratory as in the wild (Lesku et al., 2006). There is some evidence to suggest that the sleep behaviour of wild bats is recapitulated faithfully in a laboratory setting, such as the finding that insectivorous species exhibit bimodal activity pattern in both environments (Erkert, 1982; Griffin and Welsh, 1937). However there is also evidence to suggest that bats alter their sleep behaviour in response to laboratory conditions, such as the marked differences in sleep duration of little brown bats at different ambient temperatures (Brebbia and Pyne, 1972). Furthermore, the influence of many standard laboratory conditions in sleep experiments, such as social isolation, have not been explored at all despite being known modulators of sleep duration. Variation between studies in how much laboratory conditions differ from those in which species evolved, and in the sensitivity of species to these diferences, may therefore constitute an important sources of variation in the

sleep duration dataset with unknown affects on comparative analyses.

6   Best practices for future experiments

The purpose of this review is not to discourage the comparative approach or as others have to advise against the use of bats in such analyses (Zepelin and Rechtschaffen, 1974). Rather the aim is to highlight the need for studies that measure sleep duration in a way that allows for meaningful comparison. In fact, Chiroptera may be an ideal group in which to employ the comparative approach. Bats are one of the most speciose orders of mammals, second only to rodents (Wilson and Reeder, 2005). Furthermore, they are a diverse group, with many features important to sleep differing both within and between the two major evolutionary lineages; the Yangochiroptera and the Yinpterochiroptera. Unlike previous cross-taxa comparative analyses that suffer from the effects of confounding variables, the Chiroptera potentially presents us with the opportunity to identify factors associated with specific examples of the evolution of sleep duration between species within the same order. For example, by investigating sleep in frugivorous relatives of the little brown bat, it could be determined whether the shorter sleep duration of pteropodids is related to diet or evolutionary history. However, to achieve this aim, sleep duration must be measured in a way that is accurate, reproducible and capable of capturing natural differences in sleep between species.

We suggest the following as good practices for designing experiments to characterise natural sleep behaviour in bats. These should not be interpreted as a checklist of

necessary criteria but as a framework to help guide researchers and highlight potential factors they may not have considered which should be addressed when interpreting the results of an experiment.

In an ideal scenario, such experiments should be carried out in the natural environment in which that bat's sleep behaviour has evolved (Rattenborg et al., 2017). Given the high risk, high reward nature of these studies, researchers may wish to sacrifice some elements of optimal experimental design (E.g., control of environmental conditions, large sample sizes) in order to acquire these data. In such cases, detailed accounts of the methods and conditions under which experiments were performed are paramount. Where experiments must be carried out in a laboratory, all attempts should be made to replicate the conditions in which that bat would sleep in the wild. This includes both abiotic (e.g., temperature, humidity, light levels and regimes) and biotic (e.g., group size, food type and availability) conditions. Studies should be appropriately powered by recording multiple individuals over multiple sleep-wake cycles. Recordings should only commence after subjects have been habituated to the experimental conditions and if using invasive monitoring techniques, after the effects of surgical procedures have dissipated. To assess sleep duration, both behavioural and electrophysiological correlates of vigilance states should be measured and validated through assessment of homeostatic sleep regulation and state-specific arousal thresholds, particularly when studying a species for the first time. Sleep rhythms should be detected using cranial EEG electrode positions (e.g., frontal and occipital cortex, cerebellar reference) informed by the underlying brain structure and should be measured in tandem with

EMG and EOG to facilitate sleep scoring. Some measurement of the metabolic state of the bats during sleep experiments such as body temperature, heartrate or O^2 consumption should be recorded to assess potential crossover with periods of torpidity. Behavioural features that correlate strongly with electrophysiological sleep state or duration in a species should be noted as these may be useful for measuring sleep in subsequent studies where more invasive techniques are difficult to employ (e.g., wild studies).

We also suggest the following considerations be made when analysing and reporting data from sleep experiments in bats. When scoring vigilance states in a species of bat for the first time, researchers should not assume that states will present in the same way as other mammals and carry the burden of proof to show that their scoring is justified through statistical means or by presenting appropriate examples of the features associated with each state. Additionally, researchers should comment on any occurrences of marked metabolic depression that could constitute torpor and whether they coincided with sleep. A clear record of all conditions used in sleep experiments should be reported to ensure that readers can contextualise the results and if needed reproduce them. Finally, researchers should report both the variability in daily sleep duration of bats in the study as well as the average used to characterise their sleep behaviour.

7 Conclusion

The extreme sleep durations reported in some bat species have potentially important implications for our understanding of sleep function. However, there is a weak body of available evidence to support the characterisation of bats as extreme sleepers. We have identified multiple methodological and theoretical caveats to existing sleep measurements, such as the unknown influence of torpor and social environment. Furthermore, reports of shorter sleep durations in other species of bat could represent a challenge to this characterisation. These points may reflect general issues with the quantity and quality of sleep duration data available to comparative analyses of sleep. If we are to answer the question of whether bats are extreme sleepers, further recordings of sleep duration are needed. If extreme sleep durations can not be replicated, this would suggest that sleep duration in bats has been exaggerated. If extreme durations can be replicated, this would confirm that inter-specific differences in sleep duration exist between bats and would identify Chiroptera as a group in which to explore questions about the factors responsible for the variation in sleep duration amongst animals.

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9   Supplementary materials

9.1   Kymography analysis

Inactivity times were estimated from the published graphical representations of the kymograph recordings made by Griffin & Welsh (1937). Image processing software was used to calculate the cumulative proportion of inactive periods in each 24 hour kymography plot. Only continuous activity periods were included (i.e., single movements were ignored). Bats in this experiment were hand-fed once daily, during which time the kymography record is void. We calculated a sleep time range to account for the possibility that bats would have been sleeping (maximum) or awake (minimum) during void periods had they not been interrupted for feeding. *Myotis lucifugus* sleep duration was measured from the kymography plot of a single bat over 3 days (3PM-2:59PM, 12-15th June 1936) under subdued daylight conditions (Sunrise=7:20PM,Sunset=4:10AM). *Pipistrellus subflavus* sleep duration was measured from the kymography plot of a single bat over 10 days (12PM-11:59AM, 16-21;22-25;26-28th October 1936) in constant darkness (excluding feeding periods). We ignored days where Griffin & Welsh attributed high activity to dehydration. Kymography plots for a second pipistrelle are also available, however the recording periods are not contiguous and therefore can not be directly compared to the other recordings.
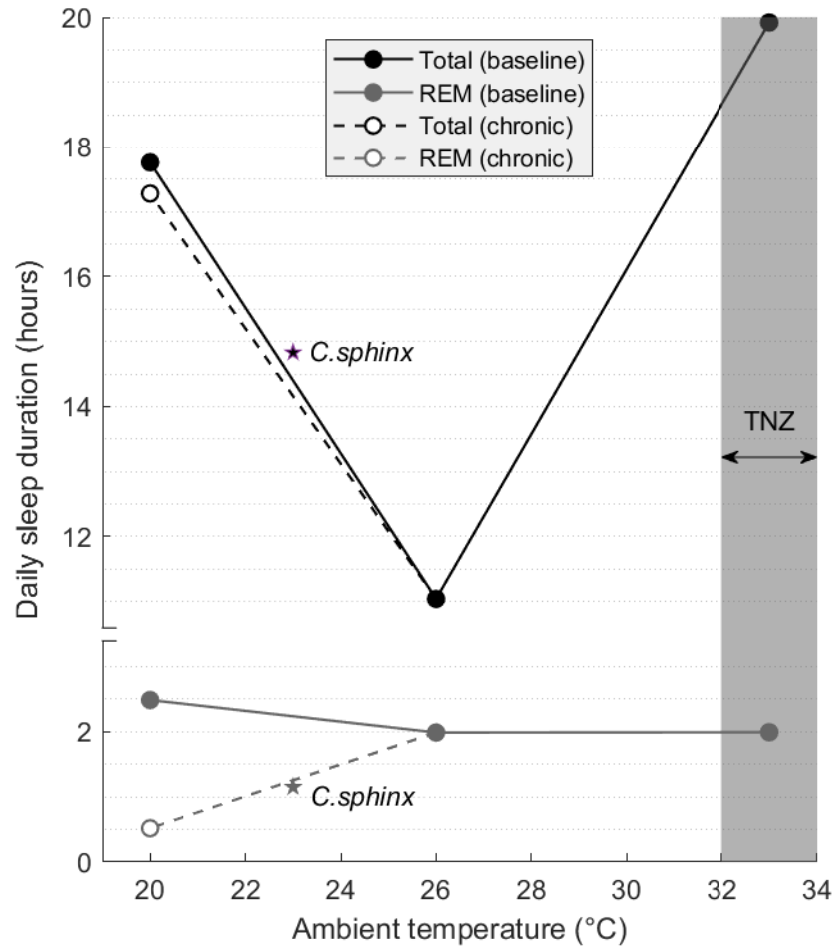
Figure 1: Daily sleep duration as a function of ambient temperature in the little brown bat (*Myotis lucifugus*). The 19°C-21°C temperature condition is represented by the mean (20°C). Hours of total sleep and REM sleep are shown both for baseline exposure lengths and multi-day chronic exposure at 19°C-21°C. Overlap with the species specific thermoneutral zone (TNZ) at which captive bats were reported by Stones (1964) to defend a homeothermic body temperature is highlighted in grey. Sleep durations for *Cynopterus sphinx* are overlayed. Data adapted from Brebbia & Pyne (1972) and Zhao et al. (2010)

Box 1: Features of bat sleep

Setting

Bats spend their resting period in day roosts. Sheltered environments such as caves, rock crevices, tree hollows and suitable man-made structures are common roost sites (Altringham, 1996). Exposed roost sites such as tree trunk exteriors and foliage are used predominantly by tropical species (Altringham, 1996). The typical sleeping posture adopted by bats is to hang vertically by their feet from a perch with their wings tucked into their bodies, sometimes obscuring their faces (Brebbia and Paul, 1969; Downs et al., 2015) [a,b,c]. Social conditions during sleep are likely to vary between sexes as in many bat species females form colonies whereas males can be either colonial or solitary (Kerth, 2008). In group roosting populations, bats can be sparsely distributed or huddled together to form clusters which provide thermal benefits (Downs et al., 2015; Martínková et al., 2020) . Conflict within clusters of sleeping bats has been documented and has even been associated with a specific call type (Prat et al., 2016) [d].

It is not known if bats are able to sleep when away from their roost during flight like birds (Rattenborg et al., 2016), however it seems unlikely given that their wings are less well designed for controlled gliding necessary for aerial sleep (Norberg, 1990). There is one report of a bat species sleeping with one eye open, which could suggests bats are capable of unihemispheric slow-wave sleep (USWS) similar to birds (Rattenborg et al., 2016), however electrophysiological data to support this claim is lacking (Downs et al., 2015) [c].

Physiology

The physiological changes reported to occur during sleep in bats largely resemble the standard mammalian condition. NREM sleep is typified by decreases in muscle tone, brain temperature and heart rate, and a cortical EEG trace containing high amplitude slow waves interspersed with spindles (Brebbia and Paul, 1969; Zhao et al., 2010) [a,b,f,g]. Cortical and collicular spiking, similar to the hippocampal spiking that characterises NREM sleep in cats, has also been observed (Brebbia and Paul, 1969; Flanigan et al., 1973) [a,b]. REM sleep is typified by an increase in brain temperature, a variable heart rate, collicular spiking and a low amplitude desynchronised cortical EEG trace with concomitant ear, wing and forelimb twitches (Brebbia and Paul, 1969; Zhao et al., 2010) [a,b,f,g]. Nuchal muscle tone is lost during REM sleep in the Pteropodidae (Zhao et al., 2010) [f,g] but sustained in the Vespertilionidae (Brebbia and Paul, 1969) [a,b].

A prominent theta peak in the EEG during REM sleep has been reported in some bat species (Zhao et al., 2010) [f,g], similar to the condition observed in rodents, but not in others (Brebbia and Paul, 1969) [a,b], similar to the condition observed in primates. In one pteropo- did, the activity of the hippocampus during sleep sessions [i] shows similarities with both groups (Ulanovsky and Moss, 2007; Yartsev et al., 2011; Las and Ulanovsky, 2014) [d]. The higher frequency of sharp-wave ripples detected in the local field potential (LFP) and the presence of behaviourally silent CA1 pyramidal cells (cells that fire during sleep sessions but not during behavioural tasks) indicate a resemblance to rodents (Thompson and Best, 1989; Buzsáki et al., 2003), whereas the discontinuous nature of their theta activity more closely resembles primates (Stewart and Fox, 1991).

Timing and architecture

Most bats are considered to be nocturnal except for some of the Pteropodidae species that have been reported to be active between dawn and dusk [e], suggesting a crepuscular or diurnal activity profile (Erkert, 1982; Speakman, 1995; Trewhella et al., 2001). Under laboratory conditions, most species studied sleep predominantly in the light phase (Brebbia and Paul, 1969; Zhao et al., 2010) [a,b,f], but in one pteropodid sleep time was evenly divided between light and dark phases (Zhao et al., 2010) [g]. Daily sleep duration estimates range from 6.9-21.5 hours (Downs et al., 2015; Moffat, 1904) [e,h] based on behavioural observations and from 11.0-19.9 hours (Brebbia and Paul, 1969) [b] based on electrophysiological recordings.

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a=Eptesicus fuscus, b=Myotis lucifugus, c=Epomophorus wahlbergi, d=Rousettus aegyptiacus, e=Pteropus livingstonii, f=Cynopterus sphinx, g=Eonycteris spelaea, h=Nyctalus leisleri

[i] A period of rest introduced before, between and after experimental manipulations in electrophysiological studies to provide a negative control. Sessions typically consist of an animal being placed in a bag or cage for between 5-20 minutes during which sleep is expected to occur but is not confirmed via scoring

Box 2: Defining torpor and sleep

Torpor is usually defined using metabolic criteria whilst sleep tends to be defined using behavioural and electrophysiological criteria. Metabolic, behavioural and electrophysiological states often coincide such that animals may be classified as either torpid or asleep. Whilst displaying neural oscillations characteristic of NREM and REM sleep, metabolic rates in most animals are not reduced to levels commonly considered to represent torpor. Likewise, the EEG of animals during periods of metabolic depression characteristic of torpor is often isoelectric and lacks discernible sleep rhythms. There is even evidence to suggest that torpid bats can move during "cold arousals", violating the sleep criterion of behavioural quiescence (Mayberry et al., 2018).

However, using non-complementary criteria leads to instances in which both torpor and sleep apply. Gray mouse lemurs (Royo et al., 2019), European ground squirrels (Strijkstra et al., 1999) and laboratory mice (Huang et al., 2021) have been shown to enter torpor through a state of electrophysiological euthermic NREM. Electrophysiological REM has also been detected during shallow torpor at relatively high ambient temperatures (Royo et al., 2019), although this state is usually abolished as animals become hypothermic and EEG power decreases (Strijkstra et al., 1999; Huang et al., 2021). It is therefore possible for torpor and sleep to coincide using the commonly accepted criteria for these states.

Table 1: Sleep architecture and constitutive variables for bat species in which electrophysiology has been performed

Suborder	Family	Species	Common name	Habitat	Diet	Lifespan (years)	Adult Mass (g)	Total sleep time (h)	SWS time (h)	REM time (h)	NREM-REM cycle time (min)
Yang.[i]	Vesp.[ii]	*Myotis lucifugus*	Little brown bat	Temperate	Insectivorous	34	8	19.92	17.93(.90[iii])	1.99(.10)	12.0
Yang.	Vesp.	*Eptesicus fuscus*	Big brown bat	Temperate / Tropical	Insectivorous	19	16	19.70	15.80(.80)	3.90(.20)	7.5
Yin.[iv]	Ptero.[v]	*Cynopterus sphinx*	Greater short-nosed fruit bat	Tropical	Frugivorous	10	44	14.83	13.68(.92)	1.15(.08)	6.1
Yin.	Ptero.	*Eonycteris spelaea*	Cave nectar bat	Tropical	Frugivorous / Nectarivorous	-	57	14.73	13.94(.95)	0.86(.06)	2.2

[i] Yangochiroptera   [ii] Vespertilionidae   [iii] Proportion of total sleep   [iv] Yinpterochiroptera   [v] Pteropodidae

Sleep data from (Zhao et al., 2010; Brebbia and Pyne, 1972; Zepelin and Rechtschaffen, 1974). Mass data from (Jones et al., 2003). Lifespan data from AnAge (Tacutu et al., 2018).