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Loss of *DmGluRA* exacerbates age-related sleep disruption and reduces lifespan

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ABSTRACT

Declines in sleep amount and quality—characterized by excessive daytime sleepiness and an inability to sleep at night—are common features of aging. Sleep dysfunction is also associated with age-related ailments and diseases, suggesting that sleep is functionally relevant to the aging process. Metabotropic glutamate receptors (mGluRs)—which are critical regulators of neurotransmission and synaptic plasticity—have been implicated in both age-related disease and sleep regulation. Therefore, in this study, we examined the sleep and aging effect of complete genetic loss of mGluR signaling in *Drosophila melanogaster*. Genetic knockdown of the sole *Drosophila* mGluR—known as *DmGluRA*—reduced daytime wakefulness and nighttime sleep, recapitulating age-related sleep changes that occur across species. Furthermore, loss of *DmGluRA* significantly reduced lifespan and exacerbated age-related sleep loss in older flies. Thus, we identify *DmGluRA* as a novel regulator of sleep whose loss results in an age-relevant sleep phenotype that is associated with shortened lifespan. This is the first evidence that mGluR signaling regulates sleep/wake in a manner that is relevant to the aging process.

1. Introduction

Sleep is a critical component of health and wellness, regulating a broad range of physiological processes such as cognition (Dinges et al., 1997; Van Dongen et al., 2003), metabolism (Spiegel et al., 2009), and immunity (Prather et al., 2015). Declines in sleep quality and impaired ability to sleep are common features of aging (reviewed in Cooke and Ancoli-Israel, 2011). In addition, sleep dysfunctions are associated with higher risk for age-related dementia (Lim et al., 2013; Pase et al., 2017) and neurodegeneration (Postuma et al., 2009; Singletary and Naidoo, 2011; Zhou et al., 2017). This suggests that sleep is functionally relevant to the aging process, although much remains unknown about what mechanisms may underlie such a relationship.

Metabotropic glutamate receptors (mGluRs) are g-protein–coupled receptors that activate intracellular signaling cascades to modulate synaptic plasticity and neurotransmission (Conn and Pin, 1997). In mammalian systems, mGluRs exist as 8 different subtypes that can be classified into 3 groups (type I, II, or III) based on their amino acid sequence homology and signal transduction mechanisms (Niswender and Conn, 2010). There has been some evidence that dysregulation of various mGluR groups is associated with age-related decline. For example, reduced hippocampal expression of the mGluR2 subtype is correlated with memory impairments in aged rats (Ménard and Quirion, 2012). In Alzheimer's disease subjects, both mGluR1 and mGluR2 downregulation has been observed in multiple disease-relevant brain regions (Albasanz et al., 2005; Richards et al., 2010), and the extent of mGluR1 downregulation in the cortex correlates with disease progression (Albasanz et al., 2005). These data suggest that mGluR signaling may be involved in aging processes in the brain. Furthermore, mGluRs have been implicated in sleep regulation from sleep studies in rodent animal models. Previous studies have examined the effects of subtype-specific knockdown of mGluRs and found that loss of different mGluR subtypes produces various changes in sleep and wake. Genetic loss of the group II mGluRs increases wakefulness and light-mediated shifts in circadian rhythms (Pritchett et al., 2015), whereas null mGluR5 mice exhibit altered sleep responses after sleep deprivation (Ahnaou et al., 2015). These results suggest that different mGluRs may regulate sleep, although further analysis is required to exclude redundancy or compensatory effects from other mGluR subtypes that might occur after loss of any single mGluR.

Here, we sought to examine the role of mGluR signaling in sleep and aging in an animal model containing a single isoform of mGluR. We investigated the effects of complete genetic knockdown of mGluR in *Drosophila melanogaster,* also known as the common fruit fly. In contrast to mammals, *Drosophila* carries just one functional

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gene for mGluR, known as *DmGluRA* (Parmentier et al., 1996). The *Drosophila* animal model is an ideal system in which to study the relationship between sleep and aging because *Drosophila* exhibits conserved features of sleep (Hendricks et al., 2000; Shaw et al., 2000) and also undergoes changes in sleep quality with age that are similar to those found in higher order species (Brown et al., 2014; Koh et al., 2006). We examined sleep behavior in flies that are null for the *DmGluRA* gene and found that loss of *DmGluRA* signaling in young flies reduces wakefulness during the day while reducing sleep at night, recapitulating the daytime sleepiness and nighttime insomnia effects of aged sleep (reviewed in Cooke and Ancoli-Israel, 2011). Interestingly, although young null *DmGluRA* mutants do not exhibit any differences in total sleep in a 24-hour day, examination of sleep in aged flies reveals that null *DmGluRA* mutants become short sleepers later in life. Aged *DmGluRA* mutants display much greater age-related sleep loss than wild-type flies, and we also find that this is accompanied by a significantly shortened lifespan. Therefore, we identify *DmGluRA* as a novel regulator of sleep and demonstrate that loss of *DmGluRA* signaling has negative consequences on aging and lifespan.

2. Materials and methods

2.1. Drosophila stocks and husbandry

Wild-type and mutant flies in the following study are in the white Canton-Special (wCS10) genetic background strain, which was originally a gift from Ronald Davis (Scripps Research Institute, Jupiter, FL). The *DmGluRA* null mutant carries the $DmGluRA^{112}$ null allele (Bogdanik et al., 2004) and was a gift from Tom Jongens (University of Pennsylvania, Philadelphia, PA). The null *DmGluRA* mutant was outcrossed into the wCS10 laboratory background strain for 10 generations before all molecular and behavioral experimentation. All flies were raised on standard dextrose media (University of Pennsylvania Cell Center, Philadelphia, PA) and maintained in a 12-hour light:dark cycle at 25 °C before and during all sleep recordings, except in those sleep recordings conducted in constant dark conditions (see Section 2.2). Female flies were used for all experiments to control for the effects of gender on behavioral outcomes.

2.2. Drosophila sleep assays

Flies were collected under CO_2 anesthesia after eclosion and allowed to grow to one week of age before recording. For all sleep assays, female flies were placed in glass locomotor tubes containing standard dextrose media and allowed to acclimate for one full day in the recording chamber before the start of data collection. Sleep and wake were recorded by video and analyzed as previously described (Zimmerman et al., 2008). Sleep is defined as 5 or more minutes of continuous inactivity (Shaw et al., 2000). For constant dark condition recordings, flies were placed in a recording chamber in which all light had been removed and sleep was recorded for 7 days and nights starting on the following day.

2.3. Negative geotaxis assay

Negative geotaxis was measured as previously described (Ali et al., 2011). Briefly, negative geotaxis was observed in groups of 10 flies at a time during which flies were placed inside 2 conjoined plastic vials (Genesee Scientific, San Diego, CA) and gently tapped to the bottom of the lower vial. An 8 cm demarcation was placed above the bottom of the plastic vial. For each trial, the number of flies passing the 8 cm mark after being tapped to the bottom of the vial was recorded. Climbing rate was recorded in each group for a total of 10 trials with 1 minute of rest provided between trials.

2.4. Lifespan assay

Female wCS10 wild-type and null *DmGluRA* female flies were collected under CO_2 anesthesia for the lifespan assay. At one week of age, 140 flies were collected per genotype and separated into groups of 20 flies placed in separate vials containing standard dextrose media. Survival was scored every 3–4 days when flies were switched to fresh vials of standard dextrose media.

2.5. Western blotting

Flies were sacrificed over dry ice. Pooled fly heads were homogenized in chilled standard lysis buffer (10 mM Tris-HCl, 1 mM EDTA, 10% Glycerol, 1% Triton-X, 150 mM NaCl) containing Halt Protease Inhibitor Cocktail (ThermoFisher Scientific). Head lysates were centrifuged to remove cellular debris, and the concentration of proteins was measured with the Pierce micro-BCA assay (ThermoFisher Scientific). Protein was run on sodium dodecyl sulfate polyacrylamide gels (10% Tris-HCl), transferred to nitrocellulose membranes (Bio-Rad), and incubated with DmGluRA 7G11 primary antibody (1:50, European Molecular Biology Laboratory, Heidelberg, Germany). Membranes were blocked with 5% milk and incubated with anti-mouse HRP secondary antibody (1:2500). Western blot analysis had been previously conducted in our laboratory to confirm the specificity of the DmGluRA antibody.

2.6. Statistical analysis

Comparisons of sleep and wake outcomes were performed using T-tests (for 2 groups) or one-way analysis of variance (for >2 groups) followed by pairwise T-tests with Holm-Sidak correction for multiple comparisons if the global null hypothesis of no differences among groups was rejected ($p < 0.05$). Unless otherwise noted, results are presented as mean and SEM. Categorical outcomes (e.g., distribution of bout lengths) were compared between groups using chi-squared tests. In analyses of lifespan, survival curves were compared between genotypes using the log-rank test. When relevant, analyses of daytime and nighttime sleep were performed separately.

3. Results

3.1. Loss of DmGluRA reduces daytime wakefulness and nighttime sleep

Drosophila sleep has a diurnal rhythm consisting of sleep bouts that occur across both the day and night. Sleep bouts range in length from a few minutes to approximately a couple hours and are longest during the night (Hendricks et al., 2000). Sleep during the day—which occurs primarily in the middle of the day—is often referred to as a midday “siesta” (Wijnen and Young, 2008). To assess the role of mGluR signaling in regulating sleep/wake behavior, we measured sleep/wake in *Drosophila* mutants lacking the *Drosophila* mGluR gene, *DmGluRA*. These mutants carry a null allele of *DmGluRA* and do not express DmGluRA protein (Bogdanik et al., 2004). After outcrossing the null *DmGluRA* mutant strain into the wild-type wCS10 genetic background, we reconfirmed that DmGluRA protein is not expressed in the null *DmGluRA* mutants (Fig. 1A). We measured sleep and wake using a video tracking system that records activity in individual flies (Zimmerman et al., 2008). Periods of inactivity lasting 5 minutes or more are recorded as sleep, based on previous studies that demonstrate that this period of inactivity is associated with reduced arousal response (Hendricks et al., 2000) and brain activity in *Drosophila* (van Alphen et al., 2013). We found that null *DmGluRA* mutants have

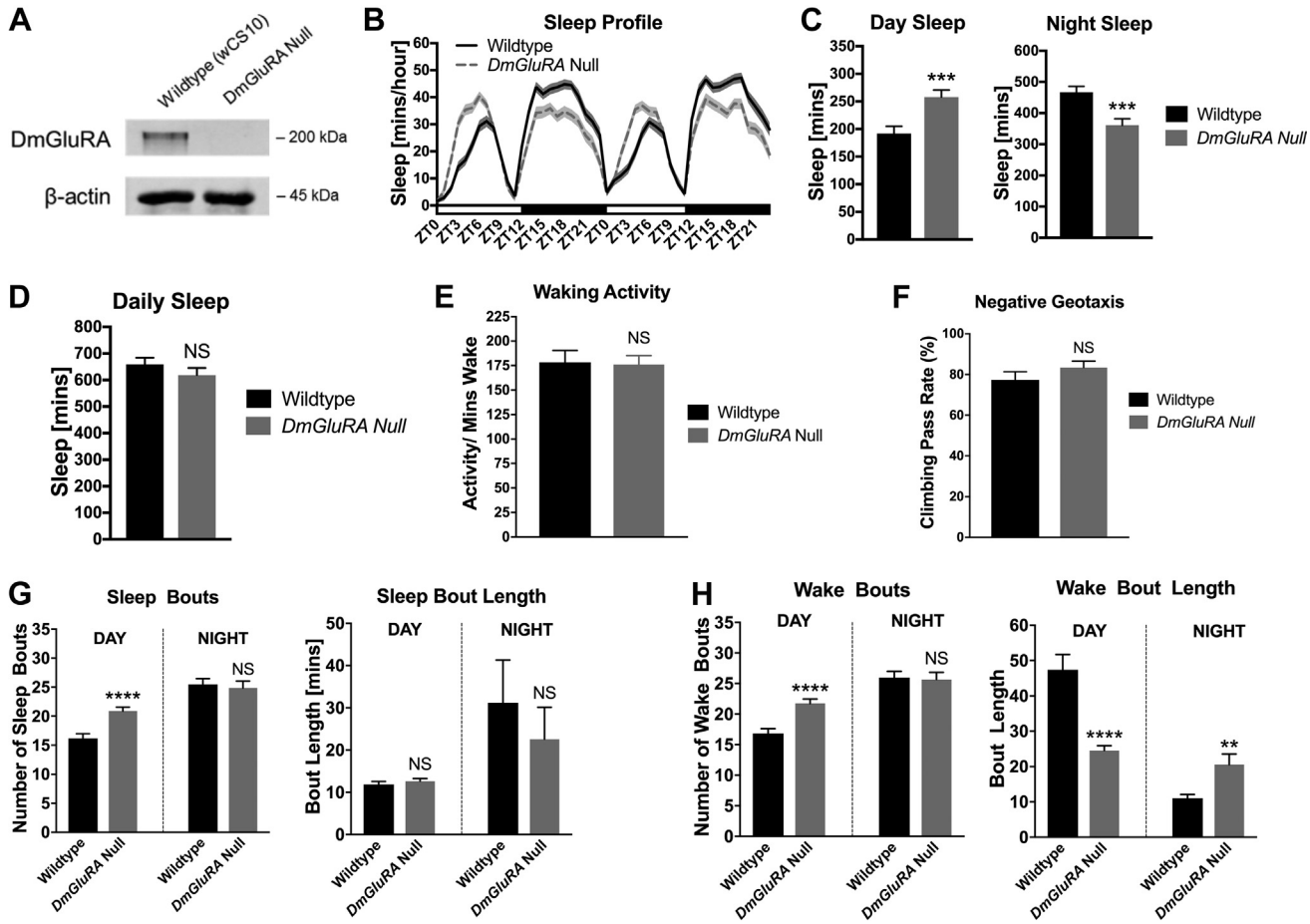

Fig. 1. Genetic loss of *DmGluRA* alters the distribution of sleep across the day and night. (A) Null *DmGluRA* mutants do not express DmGluRA protein, as seen from the expression of the ~200 kDa DmGluRA dimer that is expressed in wild-type flies and not in the null mutant. β-Actin is shown and was used as a loading control. (B) Daily sleep profile of wild-type white Canton-Special (wCS10) flies and null DmGluRA mutants over the course of 48 hours. Null *DmGluRA* mutants exhibit an altered distribution of sleep across the day and night compared with wild-type flies (N = 70 flies per group, shaded area represents SEM) (C) Quantification of total sleep amount during the day and night. Sleep levels are higher during the day and lower during the night in null *DmGluRA* mutants relative to wild-type flies (N = 70 flies per group, $^{***}p < 0.001$) (D) Quantification of total sleep amount during the 24-hour day. Total amount of daily sleep is unchanged in the null *DmGluRA* mutant relative to wild-type flies (N = 70 flies per group, NS = not significant, $p = 0.276$). (E) Rate of activity is unchanged in null *DmGluRA* mutants relative to wild-type flies (N = 70 flies per group, NS = not significant, $p = 0.2499$). (F) Null *DmGluRA* mutants do not have impaired locomotor ability as measured by the climbing pass rate in a negative geotaxis assay (N = 110 flies per group, NS = not significant, $p = 0.883$) (G) Null *DmGluRA* mutants exhibit more sleep bouts during the day. Average daytime sleep bout length is not different between null mutants and wild-type flies. At night, sleep bout number and sleep bout length are not significantly different between null *DmGluRA* mutants and wild-type flies (N = 70 flies per group, $^{**}p < 0.01$, $^{****}p < 0.0001$). (H) Daytime wake bouts are increased in null *DmGluRA* mutants, whereas average wake bout length is reduced. At night, wake bout number is not significantly changed in null *DmGluRA* mutants, whereas average wake bout length is increased compared with wild-type flies (N = 70 flies per group, $^{**}p < 0.01$, $^{****}p < 0.0001$).

an altered daily profile relative to wild-type flies that is characterized by an increase in sleep during the day and a decrease in sleep during the night (Fig. 1B and C). Genetic loss of *DmGluRA* did not change daily sleep amounts (Fig. 1D). To confirm that null *DmGluRA* mutants are not simply hypoactive or hyperactive, we measured the rate of activity of the null *DmGluRA* mutants and confirmed that *DmGluRA* mutants did not exhibit any significant changes in activity compared with wild-type flies during the sleep experiments (Fig. 1E). We also measured climbing ability of null *DmGluRA* mutants to confirm that the null mutants do not demonstrate any basal locomotor deficits compared with wild-type flies (Fig. 1F).

To further understand how sleep is altered in the null *DmGluRA* mutant, we examined sleep architecture in these flies by comparing the number and length of sleep and wake bouts to those parameters in wild-type flies. During the daytime, null mutants have a higher number of average sleep bouts than wild-type flies (Fig. 1G) while the average length of sleep bouts is not significantly changed (Fig. 1G). At night, despite the total reduction in nighttime sleep amount (Fig. 1C), there was no statistically significant difference in the average number of sleep bouts or in the average sleep bout length between null *DmGluRA* mutants and wild-type flies (Fig. 1G). We attribute this to a large variability in nighttime sleep bouts in both groups and on further analysis of the distribution of average bout lengths in individual flies, we observe that a greater proportion of null DmGluRA mutants have an average sleep bout length that is less than 15 minutes during the night compared with wild-type flies (Supplemental Figure 1).

Analysis of wake architecture reveals that daytime wakefulness is fragmented in null mutants (Fig. 1H). Null *DmGluRA* mutants have a larger number of wake bouts and the average wake bout length during the day is reduced in the mutant compared with wild-type flies. (Fig. 1H). In contrast, the average length of nighttime wake bouts in the null mutant is significantly longer compared with wild-type flies (Fig. 1H). This suggests that loss of *DmGluRA* causes an inability to maintain wake during the day (when wake is normally more consolidated) and sleep during the night (when sleep should be more consolidated).

3.2. DmGluRA *mediates light-dependent allocation of daytime and nighttime sleep*

Given the daytime- and nighttime-specific effect of changes in sleep after *DmGluRA* knockdown, we sought to determine how light entrainment might contribute to the *DmGluRA* mutant sleep phenotype. We therefore measured sleep in null *DmGluRA* mutants in the absence of external light cues. Although null *DmGluRA* mutants initially demonstrate reduced wake during active periods and less sleep during inactive periods in the first few days in the dark, after multiple days without light cues, the sleep and wake rhythms in null *DmGluRA* mutants become more similar to those in wild-type flies (Fig. 2A). After multiple days without light, the sleep amount is similar during the subjective day and night between wild-type flies and null *DmGluRA* mutants (Fig. 2B), and these changes are already observed after 2 days in constant dark (Supplemental Figure 2). This suggests that *DmGluRA* regulates behavioral state according to light onset and offset, maintaining wakefulness during light periods while promoting sleep during dark periods. In addition, sleep and wake architecture is similar between wild-type flies and null *DmGluRA* mutants in constant darkness (Fig. 2C and D), further demonstrating that the effects of *DmGluRA* knockdown on sleep and wake is dependent on light entrainment.

3.3. *Loss of* DmGluRA *reduces lifespan*

Because sleep/wake disruptions are known to negatively impact health, we sought to investigate the lifespan effects of *DmGluRA* knockdown in *Drosophila*. We conducted a survival assay of null *DmGluRA* mutants and wild-type flies and found that loss of *DmGluRA* is associated with a reduction in both median and maximum lifespan (Fig. 3A). When comparing the survival curves between genotypes, null *DmGluRA* flies had significantly worse survival compared with wild-type flies (log-rank test $p < 0.0001$). Average lifespan was reduced by more than 20% in null *DmGluRA* mutants compared with wild-type *Drosophila* (Fig. 3B). Thus, in addition to altering normal sleep/wake patterns, loss of *DmGluRA* appears to impact the aging process of the fly.

3.4. *Loss of* DmGluRA *exacerbates age-related sleep loss*

After the observation that loss of *DmGluRA* reduces lifespan, we sought to determine if sleep in null *DmGluRA* mutants undergoes any age-specific changes that are different from wild-type flies. We examined sleep behavior in aged, one-month-old wild-type flies and null *DmGluRA* mutants because it has been found that during this time, *Drosophila* exhibit behavioral changes that are indicative of a shift to senescence (Carey et al., 2006). Here, we discovered that at one month of age, both wild-type and null *DmGluRA* mutants exhibit both daytime and nighttime sleep loss relative to young flies (Fig. 4A). Notably, although young null *DmGluRA* mutants and wild-type flies exhibit no difference in daily sleep amount, aged null *DmGluRA* mutants sleep significantly less during the 24-hour day in comparison to wild-type flies (Fig. 4B). This difference in sleep appears to be because of a dramatic reduction in nighttime sleep because aged null *DmGluRA* mutants sleep more during the day than wild-type flies (Fig. 4C). Thus, aging is associated with both daytime and nighttime sleep loss as previously shown (Brown et al., 2014), but the loss of *DmGluRA* appears to exacerbate this effect, primarily because of a dramatic reduction in sleep during the night.

Analysis of sleep architecture in aged flies revealed that with age, both wild-type and null mutants have fewer daytime sleep bouts compared with young wild-type flies with no significant change in average length of sleep bouts during the day (Fig. 4D). In contrast, aged wild-type flies exhibit nighttime sleep fragmentation

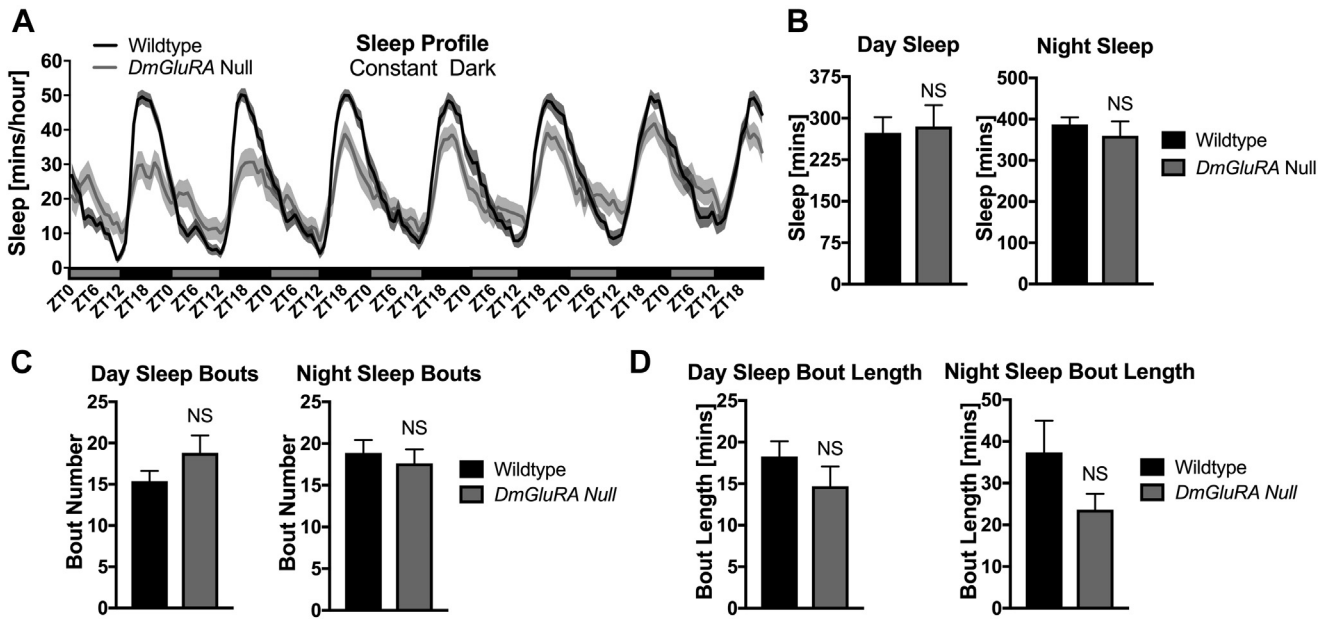

Fig. 2. Null *DmGluRA* mutants have a similar sleep profile to wild-type flies in constant conditions. (A) day sleep profile of wild-type white Canton-Special (wCS10) flies and null DmGluRA mutants in constant darkness (N ≥ 34 flies per group, shaded area represent SEM). (B) Quantification of total sleep (averaged over the last 3 days of recording) during the subjective day and night. Daytime and nighttime sleep amount is not significantly different between null *DmGluRA* mutants and wild-type flies in constant dark conditions (N ≥ 34 flies per group, NS = not significant, day: $p = 0.8063$, night: $p = 0.4607$). (C) Null *DmGluRA* mutants do not exhibit any changes in the average number of sleep bouts during the subjective day and subjective night (averaged over the last 3 days of recording) compared with wild-type flies in constant dark conditions (N≥ 34 flies per group, NS = not significant, day: $p = 0.1536$, night: $p = 0.5925$). (D) Null *DmGluRA* mutants do not exhibit any changes in the average length of sleep bouts during the subjective day and subjective night (averaged over the last 3 days of recording) compared with wild-type flies in constant dark conditions (N ≥ 34 flies per group, NS = not significant, day: $p = 0.2364$, night: $p = 0.1373$).

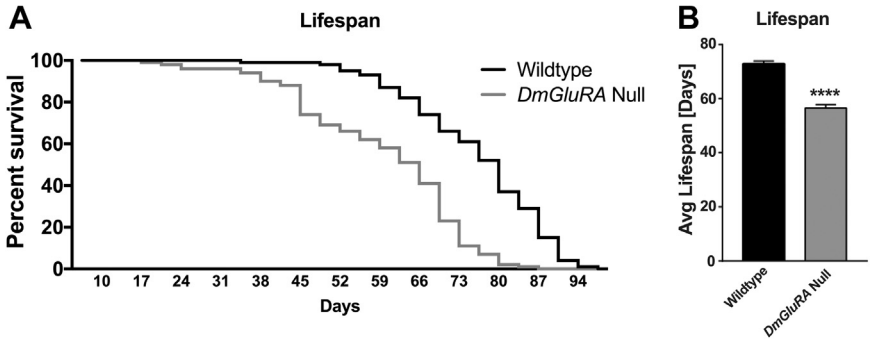

Fig. 3. Null *DmGluRA* mutants have reduced lifespan. (A) Survival curves of wild-type flies and null *DmGluRA* mutants. Null *DmGluRA* mutants exhibited a shorter median lifespan compared with wild-type controls (wild-type median age = 80 days; *DmGluRA* null median age = 66 days). Log-rank (Mantel-Cox) test was performed to determine statistical significance of difference between survival curves (N = 140 flies per group, $p < 0.0001$). (B) Average lifespan is significantly reduced in null *DmGluRA* mutants compared with wild-type flies (N = 140 flies per group, error bars represent SEM, $^{****}p < 0.0001$).

relative to young wild-type flies as measured by an increase in the number of sleep bouts and a decrease in average sleep bout length (Fig. 4E), whereas aged *DmGluRA* null mutants exhibit decreases in the average sleep bout length at night (Fig. 4E). Analysis of wake architecture in aged wild-type and *DmGluRA* null flies relative to young wild-type flies shows that wake is consolidated during the daytime (Fig. 4F). At night, aged null *DmGluRA* mutants exhibit a significant increase in average wake bout length relative to both young and aged wild-type flies (Fig. 4G).

4. Discussion

In this study, we demonstrate that loss of the *Drosophila* mGluR *DmGluRA* confers an aged-sleep phenotype and accelerates age-related sleep loss in the fly. Increased sleepiness during the day and an inability to sleep at night are common features of sleep in the elderly (Reviewed in Cooke and Ancoli-Israel, 2011) and it has been previously reported that aged *Drosophila* have sleep patterns that are less restricted to the night and more evenly dispersed across the 24-hour day/night cycle (Koh et al., 2006). Here, we demonstrate that genetic loss of *DmGluRA* leads to a redistribution of daily sleep; null *DmGluRA* mutants have higher amounts of daytime sleep and lower amounts of nighttime sleep compared with wild-type *Drosophila.* Thus, the sleep profile of young null *DmGluRA* mutants resembles an aged-sleep phenotype. The effect of *DmGluRA* on sleep in young flies does not persist in constant conditions, suggesting that *DmGluRA* regulates daytime wakefulness and nighttime somnolence in a light-dependent manner. Previous work has demonstrated that cell-specific genetic knockdown of *DmGluRA* in light-sensitive pigment dispersing factor clock cells in the *Drosophila* brain increases locomotor activity after light offset and before light onset (Hamasaka et al., 2007). Because we observed more wakefulness during these times in our experiments, it suggests that *DmGluRA* signaling from these clock cells are likely contributing to the observed sleep phenotype in the null mutant. Because DmGluRA protein is also broadly expressed throughout the *Drosophila* brain (Devaud et al., 2008), it is likely that mGluR signaling in other brain regions may also regulate sleep/wake, and future investigation will be necessary to address this.

An interesting finding in this study is that null *DmGluRA* mutants are short sleepers, but only with age. In young flies, loss of *DmGluRA* did not alter the total sleep over the 24-hour day. However, aged flies without *DmGluRA* sleep significantly less than aged wild-type flies. In this study, we found that aged wild-type and *DmGluRA* mutant flies displayed more consolidated wake than young flies during the daytime, in accordance with our prior observations previously reported in aged flies (Brown et al., 2014). Although wake fragmentation rather than consolidation has been previously reported in aged animals, variations in our behavioral results compared with other literature may be because of our use of video analysis, which has been shown to be more accurate than *Drosophila* activity monitoring system measurements of sleep and wake, particularly during the day where wake may be underestimated by *Drosophila* activity monitoring system (Zimmerman et al., 2008). The overall significant decrease in sleep in the aged mutant flies compared with the aged wild-type flies suggests that loss of *DmGluRA* accelerates age-related sleep loss rather than directly regulating sleep amount at all ages of development. Thus, it may not merely be sleep fragmentation or short sleep in early life that determines lifespan but also the proper timing and distribution of sleep across the day that may be important for the aging process. The association between short sleep in aged null *DmGluRA* flies and a reduction in the average lifespan is supported by evidence across species. In humans, short sleep duration has been strongly associated with increased mortality (although it should also be noted that abnormally long sleep duration produces the same association) (reviewed in Grandner et al., 2010) and a previous study on short-sleeping *Drosophila* mutants also found an association between higher wake amounts and increased mortality (Bushey et al., 2010). Certainly, what remains to be determined is whether sleep can be causally linked to aging outcomes and lifespan. For example, in the context of our current findings, it will be important to determine whether manipulations that increase sleep in aged null *DmGluRA* mutants could rescue the negative effects on lifespan.

In this study, aged flies were defined as flies that were one month old. Previous studies examining the aging effect on *Drosophila* sleep reported reduced sleep time and increased sleep fragmentation in flies that were 2 months of age (Brown et al., 2014; Koh et al., 2006), or twice the age of the aged group in our study. For the purposes of this study, we chose to examine sleep in one-month-old flies to measure sleep as close to the onset of age-related changes as possible. At one month of age, behavioral senescence may be just beginning (Carey et al., 2006) and the rhythm strength of sleep has not diminished to the extent that is observed at 2 months of age (Koh et al., 2006). An analysis of sleep across the lifespan of *Drosophila ananassae* recently found 30–35 days to be the age when changes in sleep efficiency are first observed (Kaladchibachi et al., 2019), further supporting this notion. Because aging-induced reductions in locomotor behavior (Carey et al., 2006; Koh et al., 2006) may mask sleep differences between groups at more advanced ages, we believe that one month may represent a critical midlife time point where aged-related changes in *Drosophila* behavior should be observed in future studies.

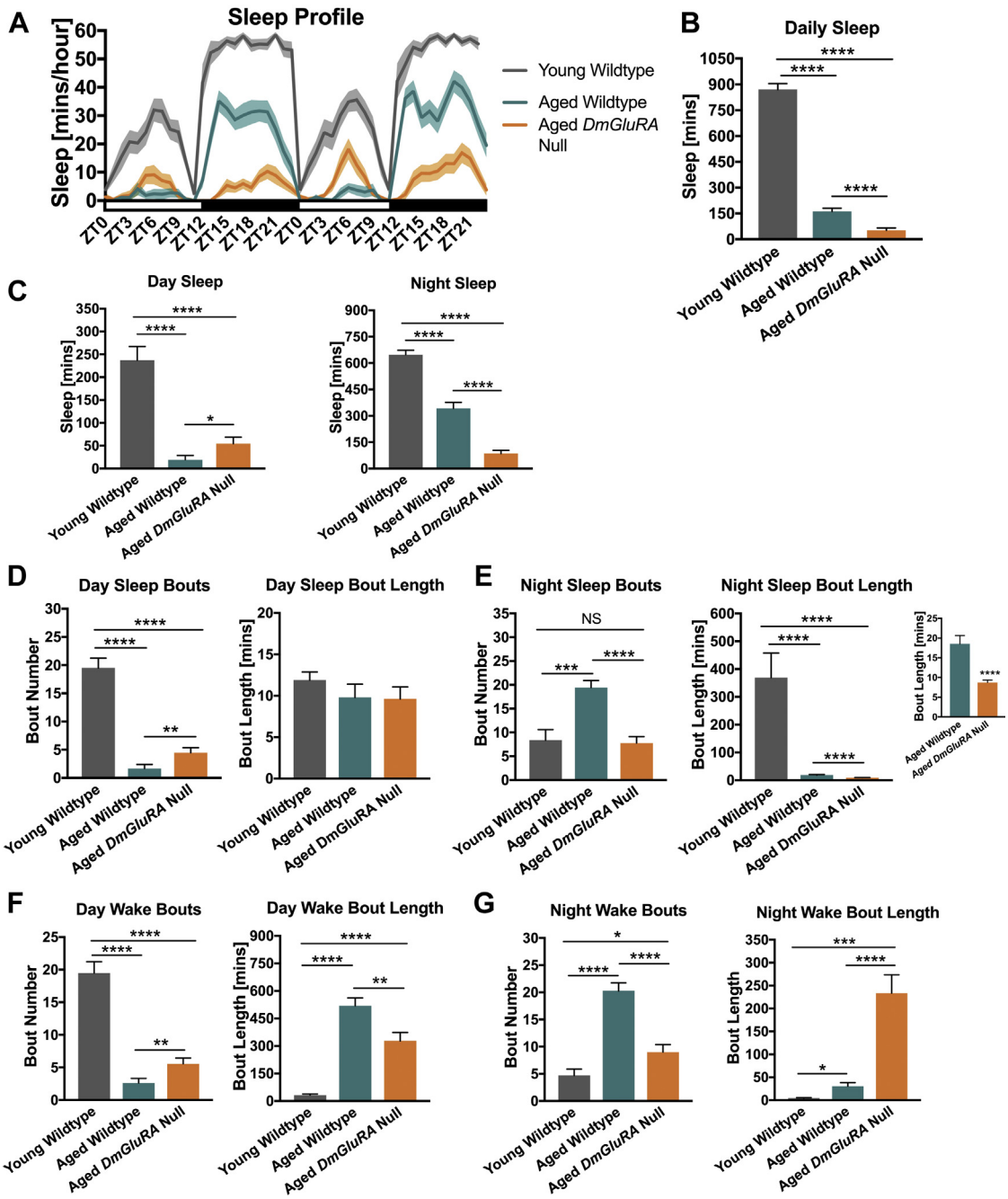

Fig. 4. Age-related sleep loss is exacerbated in null *DmGluRA* mutants. (A) Sleep profile of young wild-type flies at one week of age compared with aged wild-type and null *DmGluRA* mutants at one month of age (N ≥ 15 flies per group, shaded area represents SEM). (B) Sleep per 24 hours day. Both aged wild-type and aged *DmGluRA* mutants exhibit significant reductions in daily sleep amount compared with young wild-type flies. Aged *DmGluRA* mutants also exhibit significantly less daily sleep compared with aged wild-type flies. (N ≥ 15 flies per group, $^{****}p < 0.0001$). (C) Total daytime sleep and nighttime sleep are reduced with age. Both wild-type and null *DmGluRA* mutants display significant daytime and nighttime sleep loss at one month of age compared with one-week-old wild-type flies. At one month of age, null *DmGluRA* mutants sleep more during the day and less at night than one-month-old wild-type flies (N≥15 flies per group, $^{*}p < 0.05$, $^{****}p < 0.0001$). (D) Daytime sleep bouts are reduced in both aged wild-type and aged null *DmGluRA* mutants compared with young wild-type flies (N ≥ 15 flies per group, $^{**}p < 0.01$, $^{****}p < 0.0001$). Average sleep bout length during the day is not significantly changed (N ≥ 15 flies per group, $p = 0.4874$). (E) Nighttime sleep bouts are not significantly different between young wild-type flies and aged null *DmGluRA* mutants, although they are reduced relative to aged wild-type flies, which exhibit an increase in nighttime sleep bout number relative to young wild-type flies. Nighttime sleep bout length is significantly reduced in aged null *DmGluRA* mutants relative to young wild-type (N ≥ 15 flies per group, $^{**}p < 0.01$, $^{****}p < 0.0001$). (F) With age, daytime wake becomes more consolidated in both wild-type flies and null *DmGluRA* mutants. Daytime wake bouts are reduced, whereas average wake bout length is increased in aged wild-type and null *DmGluRA* mutants compared with young wild-type flies (N ≥ 15 flies per group,$^{**}p < 0.01$,$^{****}p < 0.0001$). (G) At one month of age, both wild-type and null *DmGluRA* mutants have increased nighttime wake bouts and average wake bout length than young wild-type flies. Null *DmGluRA* mutants exhibit more frequent and longer wake bouts compared with aged wild-type flies (N ≥ 15 flies per group, $^{*}p < 0.05$, $^{***}p < 0.001$, $^{****}p < 0.0001$).

How might mGluR signaling modulate the aging process? Like all cells in the body, neurons are vulnerable to the effects of age, as many homeostatic processes in the cell degrade with time (Nikoletopoulou and Tavernarakis, 2012; Vayndorf et al., 2016). In young animals, glutamatergic receptor expression and calcium signaling in neurons change across sleep and wake states (Bushey et al., 2015; Lanté et al., 2011), which suggests that sleep may be important for regulating neural excitability and maintaining

synaptic homeostasis. In addition to mGluRs, glutamate binds ionotropic receptors present on many different cell types (Meldrum, 2000). Ionotropic glutamate receptors such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and N-methyl-D-aspartate receptors are transmembrane ligand-gated ion channels that mediate fast synaptic transmission (Traynelis et al., 2010). Analysis of mGluRs in cultured suprachiasmatic nucleus neurons has demonstrated that mGluR activation inhibits ionotropic glutamate receptor–mediated calcium increases in the cell (Haak, 1999). Furthermore, mGluR activation leads to a stable reduction in the expression of AMPARs at the cell surface (Sanderson et al., 2011). Interestingly, a reduction in AMPARs at the membrane has been found to be a correlate of sleep (Lanté et al., 2011), and during sleep, mGluR signaling is necessary for memory consolidation (Diering et al., 2017). Thus, one interpretation for why loss of mGluR would exacerbate sleep loss is that without mGluR signaling, modulation of intracellular calcium and receptor trafficking is lost, leading to increased cellular excitability during periods when inhibitory modulation is required, such as during sleep.

The results of this study have important implications for our understanding of processes that are mediated by mGluR signaling. For example, mGluRs—including *DmGluRA*—are critical for learning and memory (Diering et al., 2017; Schoenfeld et al., 2013). Thus, mGluR dysregulation may underlie both sleep and cognitive impairments in old age, which is supported by data that higher expression of mGluRs is associated with better cognitive outcomes in aged rodents (Ménard and Quirion, 2012). In the periphery, *Drosophila DmGluRA* signaling is required for development of the neuromuscular junction (NMJ). Null *DmGluRA* mutants have been previously shown to exhibit altered NMJ morphology and changes in cellular excitability at the NMJ (Bogdanik et al., 2004). A limitation of the experiments described in this study is that examination of sleep in a genetic null does not allow us to rule out changes in development or in peripheral signaling as contributors to the observed sleep phenotype. Although we did not identify any changes in baseline locomotor ability or activity that would indicate a behavioral contribution of peripheral mGluR signaling in null flies, we cannot definitively attribute the sleep effects to brain-specific mGluR signaling. In addition, we must also consider whether changes in development of glutamatergic synapses as a result of *DmGluRA* knockdown might mediate sleep behavior. Future directions will be to examine the effects of conditional genetic knockdown of mGluR signaling in the brain. Furthermore, as previously discussed, mammalian systems express multiple mGluR subtypes, and different mGluR subtypes may have distinct roles in regulating sleep and aging. Future investigation will be necessary to address this.

Identifying molecular regulators of sleep regulation may have important clinical implications for the treatment of age-related disease. There is a great deal of evidence linking poor sleep to negative health outcomes and better sleep quality to increased longevity and improved health in the elderly (reviewed in Grandner et al., 2010). If sleep has functional consequences for aging, it is possible that therapies that improve sleep might concurrently improve other outcomes for age-related diseases such as Alzheimer's or Parkinson's disease, where sleep disturbances are common symptoms (Knie et al., 2011; Musiek et al., 2015). Furthermore, addressing sleep dysfunction in early life or midlife might even prevent later decline. mGluRs have long been considered for their therapeutic potential (reviewed in Vaidya et al., 2013) and thus may represent an avenue for sleep therapy development in the future.

Disclosure

The authors state that there are no actual or potential conflicts of interest.

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Authors' contributions: SL designed and performed the experiments, analyzed and interpreted data, and wrote the article. NN designed the experiments, interpreted data, and wrote the article.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2019.04.004.

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