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Editorial

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Sleep problems in old age: metabotropic glutamate receptor to the rescue

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Many biological processes undergo an age-associated progressive decline, and sleep is no exception. Aging leads to several changes in sleep patterns, such as increased sleep latency, reduced slow-wave sleep, and sleep fragmentation [1]. Consistent with sleep's critical role in learning and memory, disrupted sleep in old age is accompanied by memory impairments and cognitive decline [2]. Studies have revealed numerous molecular changes associated with aging [3–5], some of which are implicated in age-associated memory impairment [6, 7]. However, the molecular mechanisms underlying sleep disturbances in old age and their role in memory impairment are poorly understood. Due to a short lifespan and conserved molecular mechanisms underlying sleep, memory, and aging, *Drosophila* is a valuable model for discovering molecular pathways underlying age-dependent sleep and memory decline [8–16].

In this issue of *SLEEP*, Hou et al. [17] employed a small molecule screening approach to look for compounds that could enhance sleep in aged *Drosophila*. The authors first confirmed a previous finding that flies only 30 days old exhibited reduced and fragmented sleep [12], which are characteristics of age-associated sleep decline. This finding, along with an automated high-throughput sleep assay, made it feasible to screen 1280 small molecules to look for those that can rescue the age-dependent sleep decline. After multiple verification steps, the authors found that 3,3′-difluorobenzaldazine (DFB), an allosteric enhancer of the metabotropic glutamate receptor (mGluR)5, promoted longer and more consolidated sleep in young and old male and female flies. mGluR5 is one of the eight mGluRs, G-protein coupled receptors that modulate synaptic plasticity and neural network activity [18, 19]. *Drosophila* has only one mGluR, reflecting the general tendency for low genetic redundancy in flies compared with mammals. Using *mGluR* mutants, the authors showed that the sleep-promoting effect of DFB required functional mGluR.

Consistent with the sleep-promoting effects of DFB, transient pan-neuronal mGluR overexpression improved sleep in young and old flies. Furthermore, the authors found that transcript levels of *mGluR* and genes encoding its binding partners, *homer* and *shank* [20, 21], are reduced in aged flies, supporting the view that decreased mGluR activity contributes to age-dependent sleep decline. Given these genes' critical role in synaptic plasticity, learning, and memory [18–22], the authors next tested whether reduced mGluR expression in old age leads to memory impairment using the well-established aversive olfactory conditioning assay [23]. They found that pan-neuronal knockdown of mGluR significantly impaired memory in young flies but did not further impair the poor memory of old flies, consistent with mGluR playing a role in age-dependent memory decline. However, while DFB feeding and transient mGluR overexpression tended to improve memory in old flies, the improvement was not statistically significant. Together, these findings indicate that reduced mGluR expression mediates age-dependent sleep and memory decline and suggest mGluR as a potential therapeutic target for improving sleep and memory in old age.

A significant strength of this study is its use of a small-molecule screen to search for compounds that can improve sleep when administered to older animals, which differs from previous studies that focused on young animals. For instance, earlier small molecule screens identified genes and compounds that regulate sleep in young flies and zebrafish larvae [24, 25]. In contrast, the current study's screen using animals exhibiting signs of aging set a higher standard, as some age-related changes may be irreversible. Aging is associated with numerous transcriptomic, proteomic, and metabolomic alterations [3–5], and many genes and signaling pathways regulate sleep [8, 9, 26, 27]. Thus, it is encouraging and exciting that the authors were able to identify a compound, DFB, that could promote sleep in aged flies on its own.

The study's identification of a positive allosteric modulator of mGluR5 fits well with previous findings regarding the roles mGluRs play in memory and sleep. For example, a decline of mGluR2 expression in the hippocampus correlated with memory impairments in aged rats [28]. In addition, several studies in rodents and flies have implicated mGluRs in sleep regulation [12, 29, 30]. Interestingly, a study found that mGluR mutant flies displayed accelerated age-dependent sleep decline [12], consistent with the view that reduced mGluR contributes to sleep problems in old flies. However, there was no direct evidence that boosting mGluR activity alone would improve sleep in old age. Thus, the present study provides a significant advance toward understanding the

mechanisms underlying age-dependent sleep decline and finding potential therapeutic targets. Since patients with neurological diseases often suffer from sleep and memory problems, it will be interesting to test whether enhancing mGluR activity can rescue sleep and memory decline associated with diseases.

Drosophila is a valuable tool for discovering drugs to treat human diseases, as demonstrated in the study of Fragile X syndrome (FXS), a genetic disorder resulting from mutations in *Fragile X messenger ribonucleoprotein 1* (*FMR1*) [31, 32]. *Drosophila* has a highly conserved *FMR1* ortholog, and disrupting this gene in flies produces phenotypes resembling those observed in humans with FXS, including sleep and memory abnormalities [33, 34]. Building on research indicating hyperactive mGluR signaling in a mouse FXS model and reduced cyclic adenosine monophospate (cAMP) production in cells from human patients [35–37], McBride and colleagues [33] found that mGluR antagonists rescued several FXS-relevant phenotypes. Subsequent screening of 2000 drugs in the fly FXS model identified three compounds that promote Gamma-aminobutryric acid (GABA) receptor activity and rescue memory defects [38]. Together with the findings that GABA receptor signaling is deficient in the fly and mouse FXS models [39], these data suggest an appropriate balance between mGluR and GABA signaling is essential for optimal memory. The rescue of FXS-relevant phenotypes by mGluR antagonists and GABA agonists was validated in mouse models [33, 40, 41] and led to clinical trials in human patients [42]. An exciting recent development is a phase II clinical trial showing that an inhibitor of phosphodiesterase-4D, a key modulator of cAMP levels, significantly improved cognitive function in FXS patients [43]. Earlier studies found that phosphodiesterase-4 inhibition successfully rescued memory impairments in the fly FXS model [44, 45], highlighting the power of *Drosophila* as a tool for drug discovery.

In the present study, enhancing mGluR expression and activity significantly improved sleep but had a small, nonsignificant effect on memory. These findings may appear somewhat unexpected given the well-documented effects of sleep on memory consolidation [46, 47]. However, since both too much and too little mGluR activity can lead to poor memory, as mentioned above, multiple doses of DFB may have to be tested to find the appropriate dose to enhance memory. Additionally, determining whether manipulating the balance between mGluR and GABA signaling and their downstream effectors, such as cAMP and cAMP-response element binding protein (CREB), which are known to regulate sleep and memory [48–50], can improve sleep and memory in old age will be interesting.

Sleep and memory decline in old age likely result from a complex interplay of multiple molecular changes. Further research is needed to understand the mechanisms mediating age-dependent sleep and memory impairments and develop effective interventions to improve sleep and memory in older adults. The present study in aged flies provides a good entry point for future studies.

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Disclosure Statement

None declared.

References

1. Mander BA, *et al*. Sleep and human aging. *Neuron*. 2017;**94**(1):19–36. doi: 10.1016/j.neuron.2017.02.004
2. Dahan L, *et al*. Age-related memory decline, dysfunction of the hippocampus and therapeutic opportunities. *Prog Neuro-Psychopharmacol Biol Psychiatry*. 2020;**102**(April):109943. doi: 10.1016/j.pnpbp.2020.109943
3. Moaddel R, *et al*. Proteomics in aging research: a roadmap to clinical, translational research. *Aging Cell*. 2021;**20**(4):1–14. doi: 10.1111/acel.13325
4. Panyard DJ, *et al*. The metabolomics of human aging: advances, challenges, and opportunities. *Sci Adv*. 2022;**8**(42):1–14. doi: 10.1126/sciadv.add6155
5. Johnson FB, *et al*. Molecular biology of aging. *Cell*. 1999;**96**(2):291–302. doi: 10.1016/s0092-8674(00)80567-x
6. Bishop NA, *et al*. Neural mechanisms of ageing and cognitive decline. *Nature*. 2010;**464**(7288):529–535. doi: 10.1038/nature08983
7. Gupta VK, *et al*. Restoring polyamines protects from age-induced memory impairment in an autophagy-dependent manner. *Nat Neurosci*. 2013;**16**(10):1453–1460. doi: 10.1038/nn.3512
8. Ly S, *et al*. The neurobiological basis of sleep: insights from Drosophila. *Neurosci Biobehav Rev*. 2018;**87**(December 2017):67–86. doi: 10.1016/j.neubiorev.2018.01.015
9. Joiner WJ. Unraveling the evolutionary determinants of sleep. *Curr Biol*. 2016;**26**(20):R1073–R1087. doi: 10.1016/j.cub.2016.08.068
10. Piper MDW, *et al*. Drosophila as a model for ageing. *Biochim Biophys Acta Mol Basis Dis*. 2018;**1864**(9):2707–2717. doi: 10.1016/j.bbadis.2017.09.016
11. Mariano V, *et al*. Modelling learning and memory in drosophila to understand intellectual disabilities. *Neuroscience*. 2020;**445**:12–30. doi: 10.1016/j.neuroscience.2020.07.034
12. Ly S, *et al*. Loss of DmGluRA exacerbates age-related sleep disruption and reduces lifespan. *Neurobiol Aging*. 2019;**80**:83–90. doi: 10.1016/j.neurobiolaging.2019.04.004
13. Vienne J, *et al*. Age-related reduction of recovery sleep and arousal threshold in drosophila. *Sleep*. 2016;**39**(8):1613–1624. doi: 10.5665/sleep.6032
14. Koh K, *et al*. A Drosophila model for age-associated changes in sleep:wake cycles. *Proc Natl Acad Sci U S A*. 2006;**103**(37):13843–13847. doi: 10.1073/pnas.0605903103
15. Yamazaki D, *et al*. The Drosophila DCO mutation suppresses age-related memory impairment without affecting lifespan. *Nat Neurosci*. 2007;**10**(4):478–484. doi: 10.1038/nn1863
16. Tamura T, *et al*. Aging specifically impairs amnesiac-dependent memory in drosophila. *Neuron*. 2003;**40**(5):1003–1011. doi: 10.1016/s0896-6273(03)00732-3
17. Hou X, *et al*. Small-molecule screening in aged Drosophila identifies mGluR as a regulator of age-related sleep impairment. *Sleep*. 2023;**46**(5):zsad018. doi.org/1093/sleep/zsad018
18. Nakanishi S. Metabotropic glutamate receptors: synaptic transmission, modulation, and plasticity. *Neuron*. 1994;**13**(5):1031–1037. doi: 10.1016/0896-6273(94)90043-4
19. Conn PJ, *et al*. Pharmacology and functions of metabotropic glutamate receptors. *Annu Rev Pharmacol Toxicol*. 1997;**37**:205–237. doi: 10.1146/annurev.pharmtox.37.1.205
20. Clifton NE, *et al*. Regulation and function of activity-dependent homer in synaptic plasticity. *Complex Psychiatry*. 2019;**5**(3):147–161. doi: 10.1159/000500267
21. Monteiro P, *et al*. SHANK proteins: roles at the synapse and in autism spectrum disorder. *Nat Rev Neurosci*. 2017;**18**(3):147–157. doi: 10.1038/nrn.2016.183

22. Riedel G, *et al*. Glutamate receptor function in learning and memory. *Behav Brain Res*. 2003;**140**(1–2):1–47. doi: 10.1016/s0166-4328(02)00272-3
23. Davis RL. Olfactory learning. *Neuron*. 2004;**44**(1):31–48. doi: 10.1016/j.neuron.2004.09.008
24. Nall AH, *et al*. Small-molecule screen in adult Drosophila identifies VMAT as a regulator of sleep. *J Neurosci*. 2013;**33**(19):8534–8540. doi: 10.1523/JNEUROSCI.0253-13.2013
25. Rihel J, *et al*. Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. *Science*. 2010;**327**(January):348–351. doi:10.1126/science.1183090
26. Shi G, *et al*. Human genetics and sleep behavior. *Curr Opin Neurobiol*. 2017;**44**:43–49. doi: 10.1016/j.conb.2017.02.015
27. Shafer OT, *et al*. The regulation of drosophila sleep. *Curr Biol*. 2021;**31**(1):R38–R49. doi: 10.1016/j.cub.2020.10.082
28. Ménard C, *et al*. Group 1 metabotropic glutamate receptor function and its regulation of learning and memory in the aging brain. *Front Pharmacol*. 2012;**3**(October):1–12. doi: 10.3389/fphar.2012.00182
29. Ahnaou A, *et al*. Relevance of the metabotropic glutamate receptor (mGluR5) in the regulation of NREM-REM sleep cycle and homeostasis: evidence from mGluR5 (-/-) mice. *Behav Brain Res*. 2015;**282**:218–226. doi: 10.1016/j.bbr.2015.01.009
30. Pritchett D, *et al*. Deletion of metabotropic glutamate receptors 2 and 3 (mGlu2 & mGlu3) in mice disrupts sleep and wheel-running activity, and increases the sensitivity of the circadian system to light. *PLoS One*. 2015;**10**(5):e01255231–e01255221. doi: 10.1371/journal.pone.0125523
31. Tsiouris JA, *et al*. Neuropsychiatric symptoms of fragile X syndrome: pathophysiology and pharmacotherapy. *CNS Drugs*. 2004;**18**(11):687–703. doi: 10.2165/00023210-200418110-00001
32. McBride SMJ, *et al*. Using Drosophila as a tool to identify pharmacological therapies for fragile X syndrome. *Drug Discov Today Technol*. 2013;**10**(1):e129–e136. doi: 10.1016/j.ddtec.2012.09.005
33. McBride SMJ, *et al*. Pharmacological rescue of synaptic plasticity, courtship behavior, and mushroom body defects in a Drosophila model of Fragile X syndrome. *Neuron*. 2005;**45**(5):753–764. doi: 10.1016/j.neuron.2005.01.038
34. Bushey D, *et al*. The drosophila fragile x mental retardation gene regulates sleep need. *J Neurosci*. 2009;**29**(7):1948–1961. doi: 10.1523/JNEUROSCI.4830-08.2009
35. Huber KM, *et al*. Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proc Natl Acad Sci U S A*. 2002;**99**(11):7746–7750. doi: 10.1073/pnas.122205699
36. Berry-Kravis E, *et al*. Reduced cyclic AMP production in fragile X syndrome: Cytogenetic and molecular correlations. *Pediatr Res*. 1995;**38**(5):638–643. doi: 10.1203/00006450-199511000-00002
37. Bear MF, *et al*. The mGluR theory of fragile X mental retardation. *Trends Neurosci*. 2004;**27**(7):370–377. doi: 10.1016/j.tins.2004.04.009
38. Chang S, *et al*. Identification of small molecules rescuing fragile X syndrome phenotypes in Drosophila. *Nat Chem Biol*. 2008;**4**(4):256–263. doi: 10.1038/nchembio.78
39. Gantois I, *et al*. Expression profiling suggests underexpression of the GABA(A) receptor subunit delta in the fragile X knockout mouse model. *Neurobiol Dis*. 2006;**21**(2):346–357. doi: 10.1016/j.nbd.2005.07.017
40. Michalon A, *et al*. Chronic pharmacological mGlu5 inhibition corrects fragile X in adult mice. *Neuron*. 2012;**74**(1):49–56. doi: 10.1016/j.neuron.2012.03.009
41. Henderson C, *et al*. Reversal of disease-related pathologies in the fragile X mouse model by selective activation of GABAB receptors with arbaclofen. *Sci Transl Med*. 2012;**4**(152):1–12. doi: 10.1126/scitranslmed.3004218
42. Berry-Kravis EM, *et al*. Drug development for neurodevelopmental disorders: lessons learned from fragile X syndrome. *Nat Rev Drug Discov*. 2018;**17**(4):280–299. doi: 10.1038/nrd.2017.221
43. Berry-Kravis EM, *et al*. Inhibition of phosphodiesterase-4D in adults with fragile X syndrome: a randomized, placebo-controlled, phase 2 clinical trial. *Nat Med*. 2021;**27**(5):862–870. doi: 10.1038/s41591-021-01321-w
44. Choi CH, *et al*. Pde-4 inhibition rescues aberrant synaptic plasticity in drosophila and mouse models of fragile x syndrome. *J Neurosci*. 2015;**35**(1):396–408. doi: 10.1523/JNEUROSCI.1356-12.2015
45. Choi CH, *et al*. Multiple drug treatments thatincrease cAMP signaling restorelong-term memory and aberrantsignaling in fragile X syndromemodels. *Front Behav Neurosci*. 2016;**10**(June):1–21. doi: 10.3389/fnbeh.2016.00136
46. Diekelmann S, *et al*. The memory function of sleep. *Nat Rev Neurosci*. 2010;**11**(2):114–126. doi: 10.1038/nrn2762
47. Stickgold R. Sleep-dependent memory consolidation. *Nature*. 2005;**437**(7063):1272–1278. doi: 10.1038/nature04286
48. Feany MB, *et al*. A neuropeptide gene defined by the Drosophila memory mutant amnesiac. *Science*. 1995;**268**(5212):869–873. doi: 10.1126/science.7754370
49. Hendricks JC, *et al*. A non-circadian role for cAMP signaling and CREB activity in Drosophila rest homeostasis. *Nat Neurosci*. 2001;**4**(11):1108–1115. doi: 10.1038/nn743
50. Vecsey CG, *et al*. Sleep deprivation impairs cAMP signalling in the hippocampus. *Nature*. 2009;**461**(7267):1122–1125. doi: 10.1038/nature08488