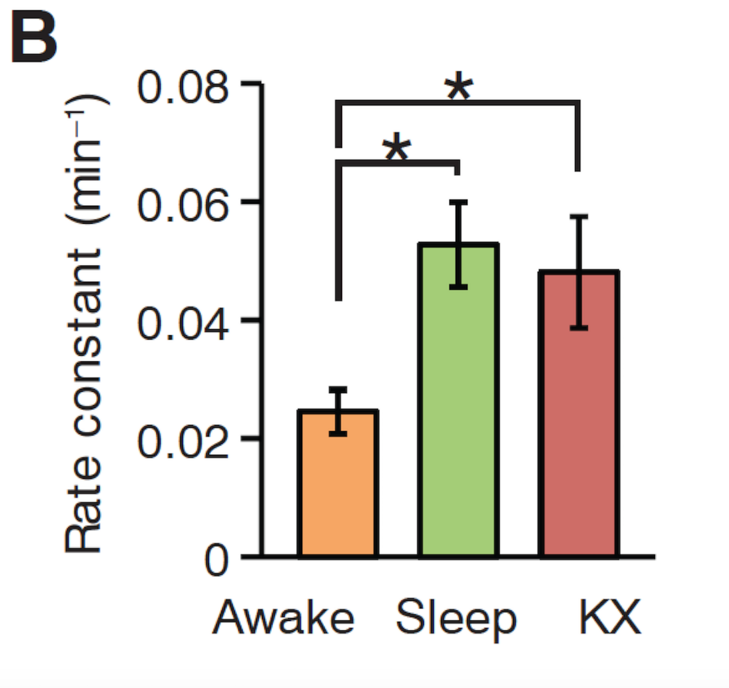
HOW TO USE THIS RESOURCE

Show the figure below to your students along with the caption and background information. The "Interpreting the Graph" and "Discussion Questions" sections provide additional information and suggested questions that you can use to guide a class discussion about the characteristics of the graph and what it shows.



*Caption: The different rates at which β-amyloid (Aβ) was cleared from the brains of mice that were awake, asleep, or in an induced sleep state using the anesthetic ketamine/xylazine (KX). The * indicates a P value < 0.05, meaning that the differences in clearance rates are statistically significant in this study. The error bars represent standard error of the mean.*

BACKGROUND INFORMATION

Nearly all animals require sleep, though the reasons for this are not fully understood. However, the fact that sleep has been evolutionarily conserved suggests that it serves vital biological functions. Lack of sleep impairs brain function and, in prolonged cases, can cause dementia or death. Surprisingly, most of the mechanisms by which sleep preserves brain function remain a mystery. In this study, scientists set out to investigate whether sleep plays a role in removing metabolites (the molecules produced during normal metabolism) from the brain. Metabolites can impair neurological function when they accumulate at abnormally high levels. One such metabolite is a protein called β-amyloid (Aβ). During cell metabolism, it is deposited in the spaces between brain cells (called interstitial spaces) and then removed by circulating cerebrospinal fluid. The buildup of Aβ proteins in the brain is linked to neurodegenerative diseases like Alzheimer's disease (AD).

Previous research has shown that levels of Aβ are higher in the brains of animals that are awake than in those that are asleep, so the researchers in this study tested whether the rate of Aβ removal is higher during sleep. The scientists injected radioactively labeled Aβ into the brains of 25 awake mice, 29 that were sleeping naturally, and 23 that were anesthetized via a thin tube previously installed in the skull. The anesthetized mouse group was used to determine whether differences in Aβ clearance rates that may be seen between asleep and awake states are due to circadian rhythms, which do not occur under anesthesia. At several time points between 10 and 240 minutes post-injection, three to six mice per time point were humanely euthanized in order to measure the levels of labeled Aβ in their brains and determine the clearance rate.

INTERPRETING THE GRAPH

Each bar represents a group of mice and their rate of Aβ clearance from the brain (per minute). The error bars are standard error of the mean (SEM), which represent roughly half of the 95% confidence interval.

The *y*-axis is the elimination rate constant, which is the rate at which a substance is removed from a system. It describes the fraction of the original amount that is removed per unit time. So if the rate constant is 0.1 per minute, then 10% of the substance is removed per minute. The rate constants in this figure were derived from the time-disappearance curve in Figure 3A (not shown). The amount of Aβ remaining in the brain at each time point was plotted to create the curve. The rate constant can be calculated by fitting an exponential decay function to the curve, which can be done using statistical software, but can also be calculated from the slope if the data is plotted as a natural log of concentration against time. If your students are curious about rate constants, you may consider showing them Figure 3A.

The starred (*) brackets show that, for this study, there was a statistically significant difference (at a P value of < 0.05) in clearance rates between both sleep states ("Sleep" and "KX") when compared with the "Awake" group. However, there was no statistically significant difference between the "Sleep" and "KX" groups when compared to each other. ANOVA was used in these comparisons because it is designed to compare the means of more than two groups simultaneously. For students familiar with the t-test, remind them that a t-test can only compare two means at a time.

These results indicate that the metabolites that build up in the brain during normal metabolism are removed more quickly during sleep than when awake. The lack of a significant rate difference between the two sleep states ("Asleep" and "KX") provides evidence to support the hypothesis that Aβ clearance rate is influenced by the sleep state itself, regardless of whether it is natural sleep or anesthesia-induced.

Teacher Tip: Prompt your students to explain the parts of the graph as applicable:

- Graph Type: Bar graph with SEM error bars
- *X*-axis: States of consciousness of laboratory mice: awake, asleep, or anesthetized by KX
- *Y*-axis: Rate of Aβ clearance from the brain per minute

DISCUSSION QUESTIONS

- Rate is expressed as change over time. Describe what change is being studied in this experiment.
- How would you describe the difference between awake and sleeping mice in terms of Aβ clearance rate?
- Does the data in this figure support the researchers' hypothesis? In what ways?
- Is there a significant difference in the rate of Aβ clearance between sleeping and anesthetized mice?
- What is the purpose of including anesthetized (induced sleep) mice in this experiment?
- Why did the scientists use radioactively labeled Aβ instead of unlabeled Aβ to measure Aβ clearance in the mouse brains? What drawbacks/limitations might there be in using injected, radiolabeled Aβ as a proxy for natural sources of Aβ in the brain?
- How would you interpret the error bars in this figure?

- Based on the data in this figure, why do you think that people with insomnia have decreased brain function?
- Why do you think the scientists chose to use mice as a model for brain function and sleep?

SOURCE

Figure 3B from:

L. Xie *et al*. 2013. Sleep drives metabolite clearance from the adult brain. *Science* 342 (6156), 373-377.
View article:
http://science.sciencemag.org/content/342/6156/373

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