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Title: The Sleep Nullifying Apparatus: a highly efficient method of sleep depriving Drosophila

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

Videographer: Film the screen for all SCREEN shots

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 10 (27) 19 in protocol and 8 in results.
Number of Shots: 19

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Krishna Melnattur:** Sleep homeostasis, the increase in sleep following sleep loss, is a defining characteristic of sleep. Sleep deprivation and sleep restriction are thus powerful tools to study sleep regulation and function. This protocol efficiently sleep-deprives and sleep-restricts flies while minimizing potential confounds.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Krishna Melnattur:** The SNAP deprives flies of >95% of sleep, even in flies with high sleep drive. Importantly, agitation with the SNAP does not harm flies and induces a rebound comparable to that obtained with hand deprivation, the standard for minimally disruptive sleep deprivation.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

1.3. **Krishna Melnattur:** Visualizing how the SNAP keeps flies awake will help investigators use and optimize this protocol.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Recording Sleep

2.1. Begin **[1]** by collecting eclosing flies into vials **[2]**, separating males and females **[3]**. House the flies in groups of less than 20 and keep them for 3 to 5 days in a light and humidity-controlled environment **[4]**.

Added shot: Establishing **NOTE: Use as 2.1.1.**

Added shot: Putting flies under before sorting **NOTE: Use as 2.1.2.**

2.1.1. Talent sorting flies into vials. **NOTE: Use as 2.1.3.**

2.1.2. Flies in their housing environment. **NOTE: Use as 2.1.4.**

2.2. Prepare an appropriate number of tubes with fly food at one end **[1]** and seal the end with wax **[2]**. Individually place awake, behaving flies into the tubes using an aspirator and plug the tubes with a foam stopper **[3]**. *Videographer: This step is important!*

2.2.1. Talent adding fly food to a tube.

2.2.2. Talent sealing the tube.

2.2.3. Talent placing flies into a tube.

2.2.4. Talent plugging the tube with a foam stopper. **NOTE CU/WIDE: Use combined with 2.2.3.**

2.3. Load the tubes into activity monitors to monitor sleep, making sure that the tubes are placed in the correct orientation **[1]**. The end of the tube with food should be at the top of the SNAP to ensure that flies do not get pushed into the food **[2]**. *Videographer: This step is important!*

2.3.1. Talent loading the tubes into the activity monitors.

2.3.2. Tubes loaded in the correct orientation.

2.4. Place activity monitors in the recording chamber and monitor sleep for at least two full days to estimate baseline sleep **[1]**. Save locomotor activity counts of flies in 1-minute bins from the time of lights on a given day to lights on the previous day using activity recording software **[2]**.

2.4.1. Talent placing the activity monitors in the recording chambers.

2.4.2. Talent at the computer, programming the locomotor activity counts.

2.5. Estimate sleep from the locomotor activity data with custom macros using 5 minutes of inactivity as the threshold for a bout of sleep **[1]**.

Added shot: Establishing **NOTE: Use as 2.5.1.**

Added shot: SCREEN **NOTE: Use as 2.5.2.**

Added shot: SCREEN **NOTE: Use as 2.5.3.**

2.5.1. Talent running the macros to estimate sleep. **NOTE: Don't use this.**

3. Sleep deprivation and recovery

3.1. If sleep is stable over the two baseline days, place activity monitors into the SNAP for overnight sleep deprivation on the third day **[1]**. Make sure activity monitors are secured in place with monitor holder pins, the monitor cords are plugged in, and the monitors oriented correctly **[2]**. *Videographer: This step is difficult and important!*

3.1.1. Talent placing the activity monitors.

3.1.2. Talent securing the activity monitor. **NOTE: Use this or combined with 3.1.1.**

3.2. Once the lights go on after overnight sleep deprivation, unplug activity monitors and take them out of the SNAP immediately **[1]**. Place the flies in a recording chamber where they will be undisturbed for two days to monitor recovery sleep **[2]**. *Videographer: This step is difficult and important!*

3.2.1. Talent unplugging the activity monitor.

3.2.2. Talent taking the monitor out of the SNAP. **NOTE: Use as combined with 3.2.1.**

3.2.3. Talent placing the flies in a recording chamber.

3.3. For each individual fly, calculate the hourly difference between sleep obtained during sleep deprivation and the corresponding hour during baseline, then sum the hourly differences to calculate total sleep lost **[1]**.

3.3.1. SCREEN: Talent calculating sleep lost. *Videographer: Film the screen as author performs this step.*

3.4. Next, calculate the hourly difference between sleep obtained during recovery and the corresponding hour during baseline, then sum the hourly differences to calculate total sleep gained **[1]**.

3.4.1. SCREEN: Talent calculating sleep recovered. *Videographer: Film the screen as author performs this step.*

3.5. Calculate the average percentage of sleep recovered over 12, 24, and 48 hours of the recovery period for each genotype. Finally, compute the average and maximum daytime sleep bout length on baseline and recovery days for each genotype **[1]**.

3.5.1. SCREEN: Talent calculating the average percentage of sleep recovered and the average and maximum daytime sleep bout length. *Videographer: Film the screen as author performs this step.*

Results

4. Results: Sleep Deprivation using the Sleep Nullifying APparatus (SNAP)

4.1. Flies were sleep deprived in the SNAP and recovered sleep during the day **[1]**. The effectiveness of SNAP in keeping flies awake was demonstrated with the high activity exhibited by flies during sleep deprivation **[2]**.

4.1.1. LAB MEDIA: Figure 2 A.

4.1.2. LAB MEDIA: Figure 2 B.

4.2. To quantitatively estimate the effectiveness of sleep deprivation and of recovery, sleep lost during deprivation and then regained in the recovery days was calculated for each individual fly **[1]**.

4.2.1. LAB MEDIA: Figure 2 C.

4.3. Importantly, there was no significant change in baseline sleep between the deprivation day and the baseline day, indicating that sleep is stable in these flies **[1]**.

4.3.1. LAB MEDIA: Figure 2 C. *Video Editor: Emphasize 0 – 12 h.*

4.4. The SNAP effectively deprived flies of over 98% of their night-time sleep. Flies recovered approximately 20% of their sleep in the first 12 hours and did not recover additional sleep during the night **[1]**. They began to recover sleep the following day and recovered 36% of their sleep over 48 hours **[2]**.

4.4.1. LAB MEDIA: Figure 2 D.

4.4.2. LAB MEDIA: Figure 2 D. *Video Editor: Emphasize the yellow bar.*

4.5. Sleep homeostasis is characterized both by increased sleep duration and by increased sleep depth during the recovery period. Daytime sleep consolidation is commonly used as a readout of sleep depth. Sleep consolidation can be assessed as the average sleep bout duration over the entire day **[1]**.

4.5.1. LAB MEDIA: Figure 2 E.

4.6. However, as sleep pressure is dissipated during recovery, the average sleep bout duration is reduced. Changes in the maximum sleep bout duration can provide a more sensitive metric **[1]**.

4.6.1. LAB MEDIA: Figure 2 F.

Conclusion

5. Conclusion Interview Statements

5.1. **Krishna Melnattur:** This procedure can be easily modified to minimize sleep loss and thus control for non-specific effects of the stimulus. The SNAP can be configured to restrict sleep, mimicking chronic sleep loss in humans. It can also be used to measure arousal thresholds.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

5.2. **Krishna Melnattur:** Sleep deprivation using the SNAP has yielded important insights into sleep function through studies that examined the negative consequences of sleep loss. By identifying manipulations that interfere with the expression of rebound sleep, sleep deprivation with the SNAP has also helped elucidate homeostatic mechanisms that regulate sleep.

5.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.