

DARPARA2402

Frequently Asked Questions

Somnus

Last Updated: **6/10/2024**

Updates **HIGHLIGHTED** below

General Questions

1. Is a DARPA representative available for a call to discuss our proposed approach?

In the interest of fairness to all potential proposers, as Dr. Pava will not have the bandwidth in his schedule to honor all requests, we will not be scheduling any program-related calls/meetings. All questions can be submitted to Somnus@darpa.mil and answers will be published in this FAQ.

2. Could you clarify if available funding of up to \$2m is for each project funded lasting up to 18 months or will this amount be split between several projects that are funded?

A: Each project.

Teaming Questions

3. Is DARPA looking for a team from several institutions, or whether the team can consist of scientists within the same university? Similarly, would you expect us to include commercial/industrial partners at this stage?

There are no limits on collaboration between institutions (number or type), but proposals must address all aspects of the work solicited for in the Research Announcement. To this end, if elements of the work are to be divided across institutions, proposals need to clearly explain how that is achievable without compromising results.

Program Structure

4. Regarding the recent Research Announcement, we were wondering if this is a continuation from the earlier Somnus BAA issued about a year ago? Were there any awards from the opportunity and are you aware of this info or those of performers posted anywhere? And has Somnus advanced beyond a BAA?

This is not a follow-on activity from the Somnus Special Notice (DARPA-SN-23-40) issued under the BTO Office-Wide BAA in March 2023 and no awards were made from that special notice. DARPA has decided to re-solicit this effort via a RA.

5. Based on the language listed in the “Funding Limitation” in the RA’s overview, we assume F&A indirects are not allowed – can you please confirm or clarify?

Facilities and Administrative costs (i.e., overhead) are permitted.

6. Would the Government confirm that fee is allowable on labor proposed for this effort?

Profit nor fee are permitted on this effort.

7. Is there a confirmed start date for the project?

As of right now, we do not have a firm start date for the project.

8. Whereas the focus of this solicitation is clearly on mammalian models of sleep deprivation, would there be scope for some preliminary work in humans as well?

Human subjects research is not in scope for Somnus as per the RA. Somnus is focused on assessing the impact of sleep deprivation on molecular pathways that may exist between the gut and the brain. Somnus is not focused on developing or testing interventions that alter the cognitive effects of sleep deprivation.

9. Is DARPA only interested in regions of the large intestine that would most closely reflect fecal samples (such as the cecum) or can we select regions of the small intestine? Microbial species in the ileum display a circadian rhythm in activity, interface directly with intestinal epithelial cells and are involved in immune function. Studies have also revealed that sleep deprivation could impair intestinal mucosal barrier integrity.

Proposers must include rationale for the intestinal region selected for collection in the context of its involvement in sleep and/or circadian rhythm, immune response, and host-microbiome interface. While analyses of the cecum are prominent in the published literature, DARPA is certainly open to other intestinal regions of interest, including the ileum which plays a significant role in immune regulation, inflammatory response, and potential sleep modulating stimuli.

Proposers should be cognizant of the tissue mass requirements for planned -omics analyses on terminal tissue biospecimens (100mg per planned -omics analysis for each biospecimen sample). If the intestinal region of interest does not meet the mass requirements, samples may need to be pooled across animals. In this case, proposers must provide clear rationale for choosing the selected region despite the lack of inter-individual resolution that the tissue biospecimens will provide and should describe an appropriate pooling strategy.

10. Will the “government resource partner” also be doing the bioinformatics/microbiome-association/biomarker identification, etc. described on page 5? Or will the performer team be getting the data back from the “government resource partner” to conduct these analyses?

A: As stated in the Somnus RA, the government resource team, identified by DARPA, will analyze biospecimens collected by the selected performer team(s) to characterize molecules and mechanisms of host interactions with the gut microbiome that are associated with the impact of acute sleep deprivation and restorative effect of sleep on cognitive performance, and will further conduct extended, system-level analyses of datasets obtained in the Base Period, with the goal of identifying putative molecular/biochemical pathways associated with host-microbiome interaction in the gut and resultant molecules in the blood that correspond to sleep pressure and its effects on cognitive function.

All government resource-generated data will be shared with the selected performer team(s). To that end, performer(s) will be expected to enter into a material transfer agreement and data sharing agreement with Somnus government resource team members to facilitate transfer of biospecimens and data generated under Somnus.

Full Proposals

11. Would you be able to provide the specific Task Description Document Template for this announcement?

The TDD can be found in the Model Research Other Transaction (OT) (see Attachment 6, “Model Research Other Transaction (OT)”).

Technical Questions

12. Can DARPA provide the number of specimens to be collected or analyzed?

As the complexity of an experimental design and its corresponding analyses increases, the number of animal subjects required to achieve statistical significance also increases. Proposers are encouraged to minimize the complexity of their experimental design where possible, to achieve statistically robust results that address the goals of Somnus. Moreover, proposers must specify the number of animals included in their proposed experimental and control groups, along with a power analysis to justify the proposed group size. Proposers should anticipate collecting blood and fecal samples at 2 key timepoints (at minimum) as well as at terminal timepoints, and brain and intestinal tissues at terminal timepoints. If several cohorts are being proposed, with a group size of 12 animals per cohort, proposers should plan on collecting around 200 samples of each, blood and fecal (total, non-terminal + terminal) biospecimens, and around 100 for each of the following terminal tissues: brain region 1, brain region 2, and intestinal tissue.

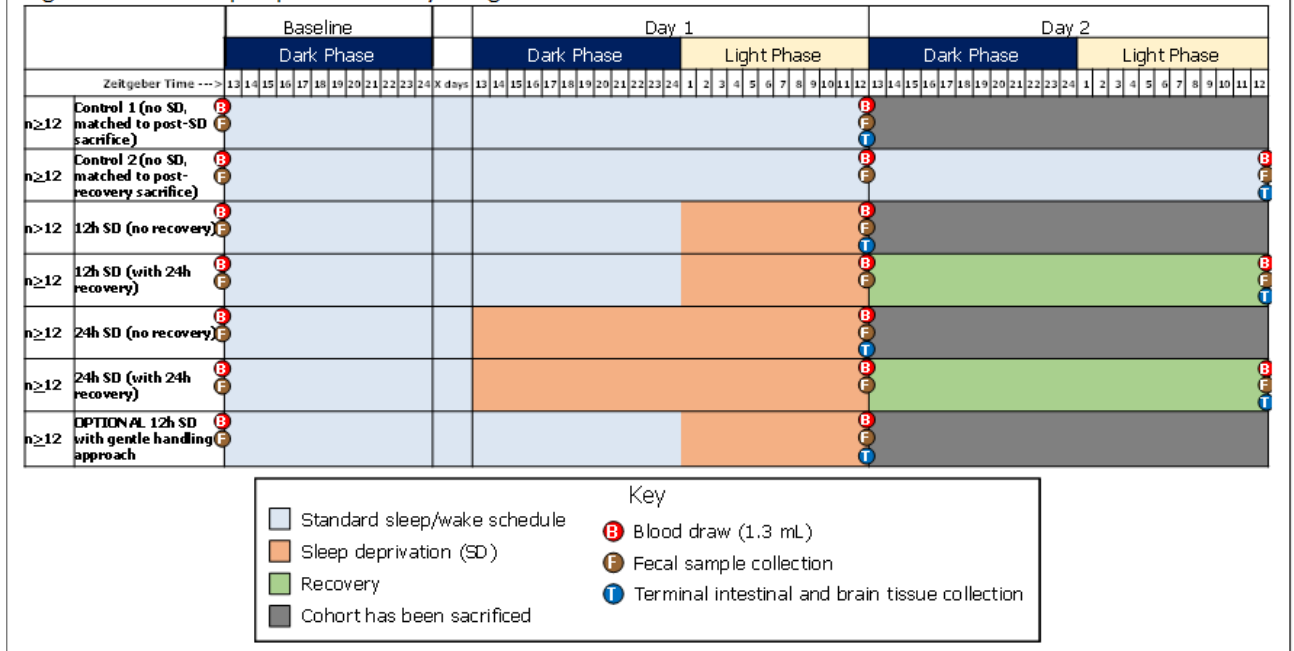
13. DARPA is requesting the collection of biospecimens that are only obtainable at terminal timepoints (brain and intestinal tissues), while also collecting those same biospecimens at key timepoints (e.g., baseline, sleep-deprived, and recovered states). Are proposers expected to design experiments to include novel methods of tissue collection on live animals or is it acceptable to design the experiment with several cohorts to collect the required biospecimens?

DARPA welcomes innovative methods of biospecimen collection; however, proposers are highly encouraged to include different cohorts to enable collection of brain and intestinal biospecimens at key timepoints. While data collection at terminal time points will necessitate between-group comparisons across experimental conditions (i.e., following periods of sleep deprivation and recovery) and matched control groups, the proposed experimental design must also allow for within-subjects comparisons of cognitive performance and non-terminal biospecimens across sleep-deprived and non-sleep-deprived states, such that inter-individual effects of sleep deprivation and recovery on both cognitive performance and corresponding host-microbiome interactions may be assessed.

Proposers should clearly describe their planned timeframe for data collection from the cohorts and must address potential confounds such as batch effects and seasonal effects, should cohorts be tested sequentially.

The experimental plan depicted in Fig. 1 is provided as an example of cohort groupings for biospecimen acquisition and other physiological measures across sleep deprived and non-sleep deprived states.

Fig. 1: Somnus sleep deprivation study design



14. Automated methods of sleep deprivation enable substantially higher throughput and reduce the workload of laboratory staff, but they also are more stressful than gentle handling methods. Does DARPA prefer the use of automated methods of sleep deprivation or manually induced sleep deprivation approaches (like gentle handling)?

Proposers are free to offer any sleep deprivation approach based on validated and published methods. For any proposals that include an automated method of sleep deprivation, respondents must include one additional experimental group in which sleep deprivation is induced through a gold standard gentle handling approach. Proposers that aim to pursue a gentle handling approach to induce sleep deprivation in all cohorts do not need to include this optional group in their proposed study design.

Additionally, in accordance with the quality assurance and quality control (QA/QC) plan, proposers should verify quality and accuracy of the raw physiological data and the results derived from these automated sleep deprivation methods. Quality assurance (QA) measures include human oversight of data streams from automated recording systems, and quality control (QC) protocols include an independent review of either the entire dataset or a randomized subset of at least 10% of the data.

15. . DARPA is requesting the collection of two brain regions for analysis, based on their involvement in sleep-wake regulation, cognitive function, and any known sensitivity to circulating factors, particularly those originating from the gut. Studies have shown bidirectional communication between the gut microbiota and the hypothalamus, which is part of a major stress response system in the body. Would the hypothalamus be of interest to this study?

Proposers must include rationale for the two brain regions selected for collection based on their involvement in sleep-wake regulation as well as correlation with the proposed cognitive task(s). The hypothalamus and cortex regions are good examples.

Proposers should be cognizant of the tissue mass requirements for planned -omics analyses on terminal tissue biospecimens (100mg per planned -omics analysis for each biospecimen sample). If brain regions of interest do not meet the mass requirements, samples may need to be pooled across animals. In this

case, proposers must provide clear rationale for choosing the selected region despite the lack of inter-individual resolution that the tissue biospecimens will provide. Moreover, proposers must clearly describe how samples will be pooled across animals in the context of the experimental design and analysis of sleep pressure and cognitive performance.

16. Operant conditioning often uses reward-based motivation, most commonly in the form of sucrose pellets. Knowing that sugar intake can significantly alter the gut microbiome composition and metabolic function, is DARPA open to alternative reinforcement approaches in proposed behavioral assays?

Proposers must provide rationale for use of the proposed cognitive task(s) within the overall context of analyzing host-microbiome interactions across conditions of varying sleep pressure. Any task that produces confounding effects on gut microbiome content due to variable ingestion of food or other sugar-based rewards across animals is not acceptable. DARPA encourages proposers to consider behavioral tasks implemented without delivery of a food or other sugar-based reward.

17. We note that fecal specimens for metaproteomics are absent from the government-supplied analyses slated for this proposal. May we include this vital analysis in our work?

While the planned -omics analyses by the Somnus government resource team includes untargeted proteomics analyses for terminal blood biospecimens, as well as brain and intestinal tissue specimens, it does not include metaproteomics on fecal samples. As we appreciate the additional information this data could provide, you may consider collecting additional fecal specimens beyond the volume required for the Somnus government partner analysis (i.e., 1-2 fecal pellets per subject, per timepoint, as specified on p. 9 of the Somnus Research Announcement [RA]). Should you choose to propose additional data collection and/or analyses beyond the specifications in the Somnus RA, you may do so as per the "Additional data collection and analysis" section on p. 9 of the Somnus RA, as long as the total proposed cost does not exceed \$2M. Alternatively, you may propose to collect additional biospecimens to analyze using non-DARPA funding source(s); in this case, no additional costs should be included in your Somnus budget for collection of those biospecimens.

18. A draw of 1.3 mL (per the requirements in the RA) from an adult mouse is much too great a volume. How stringent is this requirement?

As stated in the Somnus RA (pp. 8-9), due to volume requirements for government partner -omics analyses of the blood biospecimens, a minimum volume of 1.3 ml blood must be collected per animal, per collection timepoint. Proposers must demonstrate that the proposed timing and volume of blood draws adhere to their organization's IACUC guidelines regarding the maximum volume of blood collection that may take place per animal across a given amount of time. To facilitate necessary spacing of blood draws between baseline and experimental time points, proposers are encouraged to consider strategies for spacing the collection of baseline blood biospecimens prior to the onset of the proposed sleep deprivation protocol.