MERIDIAN

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Institutional Animal Care and Use Committee

Animal Use Protocol Application

Neurovascular Coupling and Microglial Dynamics in the Somatosensory Cortex During Chronic Sleep Restriction

Protocol Identification

Principal Investigator:	Dr. Elena Lindqvist	**Department:**	Neuroscience
Submission Date:	14 January 2026	**Requested Duration:**	3 years
Funding Source(s):	Nexa Foundation for Brain Health (NFB-2026-0417); Vertex Neuroscience Innovation Award		
USDA Pain Category:	Category D (majority; see Sec. 6)		

Type of Submission

☒ New Protocol ☐ Renewal of MU-IACUC-_____ ☐ Amendment to MU-IACUC-_____ ☐ Annual Continuation

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Protocol Number Assigned: _____ Date Received: _____

Review Type: ☐ Full Committee ☐ Designated Member Date of Review: _____

Approval Category: ☐ Approved ☐ Approved with Modifications ☐ Deferred Pain Category Confirmed: ☐B ☐C ☐D ☐E

Approval Date: _____ Expiration Date: _____

IACUC Chair Signature: _____ Date: _____

1. Investigator and Personnel Information

1.1 Principal Investigator

Name:	Dr. Elena Lindqvist	**Rank/Title:**	Associate Professor
Department:	Neuroscience	**Office Phone:**	(415) 555-0142
Email:	e.lindqvist@meridian.edu	**Building/Room:**	Krieger Hall, Rm. 318
Emergency Contact:	K. Osei, Co-Investigator — (415) 555-0198 (24 hr)		

1.2 Co-Investigators and Study Personnel

Name	Role on Protocol	Training Status	Occ. Health Enrolled
Dr. Elena Lindqvist	Principal Investigator; survival surgery, stereotaxic implantation	☒ Current	☒ Yes
Dr. Kwame Osei	Co-Investigator; EEG/EMG recording, data analysis	☒ Current	☒ Yes
Priya Nair	Senior Research Technician; husbandry oversight, drug administration, monitoring	☒ Current	☒ Yes
Marcus Feld	Graduate Student; behavioral testing, tissue collection	☒ Current	☒ Yes
Dr. Sofia Alvarez	Attending Veterinarian (Meridian Comparative Medicine)	☒ Current	☒ Yes

All listed personnel have completed the Meridian University CITI "Working with the IACUC" and species-specific rodent handling modules (renewal cycle: 3 years) and are enrolled in the Occupational Health and Safety Program for Animal Handlers prior to any animal contact.

1.3 Funding Sources

Funding Agency	Award Number	Period of Support
Nexa Foundation for Brain Health	NFB-2026-0417	Feb 2026 – Jan 2029
Vertex Neuroscience Innovation Award	VNI-2025-118	Sep 2025 – Aug 2027

Grant and protocol congruency have been verified: the animal numbers, species, and procedures requested below match the vertebrate animals sections of both award applications on file with Meridian Sponsored Programs.

2. Lay Summary and Scientific Objectives

2.1 Lay Summary (non-technical, for public IACUC minutes)

Chronic sleep restriction is common among shift workers, caregivers, and students, and has been linked to impaired memory and slowed reflexes in humans, but the underlying biology in the brain is not well understood. This project asks whether the small blood vessels and immune cells of the brain change their behavior after repeated short nights of sleep, and whether those changes explain why thinking and sensation become sluggish. Mice will undergo a mild, well-tolerated sleep-restriction schedule while a small, permanently implanted window lets us watch blood flow and immune-cell movement in the living brain through a microscope, without repeated surgery. A smaller group of rats will wear lightweight head sensors to confirm that the sleep-restriction schedule produces the intended brain-wave changes. Findings will help explain a common human health problem and may point toward ways to protect the brain during periods of insufficient sleep.

2.2 Specific Scientific Objectives

1. Quantify changes in cortical capillary blood flow and neurovascular coupling latency in the primary somatosensory cortex (S1) of mice subjected to 14 days of chronic sleep restriction, using chronic cranial-window two-photon imaging.
2. Characterize microglial process motility and phagocytic engagement with synaptic elements in S1 across the sleep-restriction time course, using the Cx3cr1-GFP reporter line.
3. Determine whether pharmacological or environmental refinement (enriched recovery housing) accelerates normalization of neurovascular and microglial metrics after restriction ends.
4. Validate that the sleep-restriction paradigm produces the expected electrophysiological signature (reduced slow-wave activity, increased theta power in wake) in a separate rat cohort instrumented with epidural EEG/EMG, confirming paradigm fidelity independent of the imaging cohort.

2.3 Background and Significance

Sleep loss is associated with measurable deficits in sensory processing and attention in humans, yet the vascular and immune contributions to these deficits remain poorly resolved at cellular resolution [8]. Prior work from our laboratory established that brief (48-hour) sleep restriction is sufficient to alter resting-state capillary diameter in mouse barrel cortex, but whether this reflects a transient hemodynamic adjustment or an early step toward sustained neurovascular dysfunction was not resolved [3]. Independently, microglia have been shown to survey and prune synapses in an activity-dependent manner, and altered microglial dynamics have been implicated in several models of cognitive impairment [6]. No study to date has simultaneously tracked neurovascular and microglial dynamics in the same living cortical volume across a chronic (14-day) sleep-restriction paradigm. The chronic cranial-window approach proposed here minimizes the number of surgical procedures per animal (one implantation, versus repeated acute preparations) and allows each animal to serve as its own longitudinal control, substantially reducing the total number of animals needed relative to cross-sectional endpoint designs [4].

3. Justification: The Three Rs

3.1 Replacement — Why non-animal or lower-order alternatives cannot answer this question

The specific aims require simultaneous, longitudinal, single-vessel-resolution imaging of cerebral blood flow and microglial process dynamics in an intact, sleep-cycling brain. Organotypic slice cultures and organoid models lack a functioning vasculature and cannot reproduce a physiological sleep/wake cycle. In-silico neurovascular models were evaluated (see Meridian Computational Neuroscience Core consultation, 09 Dec 2025) and can generate testable hypotheses but cannot yet predict cell-type-specific microglial engagement with sufficient fidelity to replace in vivo measurement. No validated non-animal model of sleep-dependent neurovascular coupling currently exists; a literature search of PubMed, Web of Science, and the NORINA database (search terms: "neurovascular coupling," "sleep restriction," "in vitro alternative," 2015–2026, last run 05 Jan 2026) returned no suitable substitute methodology.

3.2 Reduction — Minimizing animal numbers without compromising validity

- **Longitudinal within-subject design:** each imaging-cohort mouse serves as its own baseline, restriction, and recovery control, removing the need for separate cross-sectional cohorts at each time point.
- **Shared control cohort:** sham-restricted (gentle-handling) controls are shared across Aims 1 and 2 rather than duplicated per aim.
- **Power-based, not convention-based, group sizes:** sample sizes (Sec. 3.3) are derived from an a priori power analysis using effect sizes from our own pilot data [3], not round-number convention.
- **Pilot-validated exclusion criteria:** cranial-window clarity criteria are applied at surgery (Day 0) rather than after data collection, so under-powered animals are not carried through the full protocol.

3.3 Refinement — Minimizing pain, distress, and adverse impact

- Chronic cranial-window implantation replaces repeated acute craniotomies, reducing surgical events per animal from an estimated 4–5 (endpoint design) to 1.
- The sleep-restriction method uses a slowly rotating, low-torque platform ("gentle stimulation" paradigm) rather than forced locomotion or water-immersion methods, and is limited to 18 h/day with an 6 h undisturbed recovery window, consistent with published refinements of this model [8].
- Post-surgical analgesia follows a fixed multimodal schedule (Sec. 6.2) rather than PRN dosing, and body-condition scoring is performed twice daily during the first 72 h post-surgery.
- Home-cage enrichment (nestlets, gnawing block, red acrylic shelter) is maintained throughout, including during the restriction period, to reduce baseline stress unrelated to the experimental manipulation.

3.4 Statistical Justification of Animal Numbers

A power analysis was performed for the primary endpoint (neurovascular coupling latency, Aim 1) using pilot variance from n = 8 animals [3]. Assuming a two-sided repeated-measures ANOVA, $\alpha = 0.05$, and a target effect size of $d = 0.9$ (observed pilot effect $d = 1.1$), G*Power 3.1 indicates n = 12 animals per group achieves power > 0.85. Group sizes below add a 20% attrition allowance (surgical failure, window fogging, or humane endpoint) informed by our pilot attrition rate of 17%.

Experimental Group	Power-Based n	Attrition (20%)	Requested n
Sleep-restricted, imaging cohort	12	+2	14
Sham-restricted control, imaging cohort	12	+2	14
Sleep-restricted, Cx3cr1-GFP cohort	12	+2	14
Sham-restricted, Cx3cr1-GFP cohort	12	+2	14
Refinement (enriched recovery) sub-study	10	+2	12

3.5 Species Justification

Mice (*Mus musculus*, C57BL/6J) are the lowest phylogenetic species with validated chronic cranial-window methodology, published sleep-restriction paradigms, and the Cx3cr1-GFP microglial reporter line required for Aim 2; no invertebrate or lower-vertebrate model provides an equivalent cortical microvascular and microglial architecture. A small rat cohort (Sprague Dawley) is justified solely for Aim 4 because reliable, artifact-free chronic EEG/EMG headcap recording across a 14-day restriction paradigm requires a cranial surface area not achievable in mice without confounding the imaging aims.

4. Species, Numbers, and Source

Species / Strain	Source	Sex	Age at Use	Total (3 yr)	Pain Cat.
Mouse, C57BL/6J	Jackson Laboratory	M/F	8–12 wk	180	D
Mouse, Cx3cr1$^{GFP/+}$ (B6 background)	Meridian in-house breeding colony	M/F	8–12 wk	96	D
Rat, Sprague Dawley	Charles River Laboratories	M	10–14 wk	24	C
	Total animals requested, all species:			**300**	

The Cx3cr1$^{GFP/+}$ line is maintained in-house under Meridian breeding protocol MU-IACUC-2023-0119 (approved, separate submission); animals are transferred to this protocol only after genotype confirmation.

4.1 Housing and Husbandry

Housing Facility:	Meridian Comparative Medicine Vivarium, Building D, Rooms D-210 (mice), D-214 (rats)
Caging:	Individually ventilated cages; mice group-housed 3–4/cage pre-surgery, singly housed post-cranial-window (to protect the implant); rats pair-housed
Environment:	12:12 h light/dark cycle (lights on 0700), 22±1 °C, 40–60% relative humidity
Enrichment:	Nestlets, cardboard gnawing block, red acrylic shelter (mice); PVC tunnel and chew block (rats); enrichment maintained during sleep restriction where compatible with the restriction apparatus
Diet:	Standard irradiated rodent chow and reverse-osmosis water, *ad libitum* except where noted for behavioral testing
Sentinel Health Monitoring:	Quarterly per Meridian Comparative Medicine SOP CM-014; colony is specific-pathogen-free

4.2 Identification

Mice are identified by ear notch applied at weaning (routine colony management, not a protocol procedure) and by cage card; rats by tail marking and cage card. No additional identification procedure is requested under this protocol.

5. Experimental Procedures

Procedure	Description	Frequency	Personnel
Cranial window implantation	Survival surgery; 3 mm circular craniotomy over S1, sealed with a glass coverslip and head-fixation bar	Once, Day 0	Lindqvist, Alvarez (DVM oversight)
Recovery & habituation	Post-surgical recovery (10 d min.) then head-fixation habituation (5 sessions, 15–30 min, increasing)	Days 1–15	Nair, Feld
Baseline imaging	Two-photon imaging of cortical blood flow and microglial morphology under light isoflurane	2 sessions	Osei, Nair
Sleep restriction	Rotating platform, gentle stimulation, 18 h/day with 6 h undisturbed recovery	Days 20–33 (14 d)	Nair, Feld
Repeated imaging	Two-photon imaging at Days 20, 27, 33 (restriction) and 40 (recovery)	4 sessions	Osei, Nair
EEG/EMG headcap implant (rat cohort only)	Survival surgery; epidural screw electrodes + nuchal EMG leads	Once, Day 0	Lindqvist, Alvarez
Terminal perfusion & tissue collection	Deep anesthesia, transcardial perfusion, brain extraction for histology	Once, endpoint	Lindqvist, Osei, Nair

5.1 Anesthesia and Analgesia

Agent	Dose / Route	Purpose	Monitored Parameter
Isoflurane	3–4% induction, 1–2% maintenance, inhalation	Surgical anesthesia, imaging sedation	Respiratory rate, pedal reflex, temperature
Buprenorphine SR	1.0 mg/kg, SC, once	Post-surgical analgesia (72 h coverage)	Body condition score, activity, incision appearance
Meloxicam	5 mg/kg, SC, once daily × 3 d	Adjunct post-surgical analgesia	As above
Bupivacaine	2 mg/kg, local infiltration	Incision-line local anesthesia at closure	N/A (single use)

All surgical procedures are performed on a warmed platform under aseptic technique (sterile instruments, gloves, and drapes) by personnel trained in rodent survival surgery. Body temperature is maintained at $37 \pm 0.5\,^{\circ}\mathrm{C}$ via feedback-controlled heating pad throughout anesthesia.

5.2 Survival Surgery

Cranial window implantation is performed once per animal under isoflurane anesthesia with the multimodal analgesia regimen above. The procedure (skull thinning, craniotomy, coverslip placement, head-bar cementing) takes approximately 60–75 minutes. Post-operative monitoring follows Meridian Comparative Medicine SOP CM-031: checks every 2 h for the first 6 h, then twice daily for 3 days, recording body condition score (1–5 scale), incision status, and general activity on a standardized post-op sheet. Animals not regaining normal grooming and ambulation within 48 h are evaluated by the attending veterinarian.

5.3 Non-Survival / Terminal Procedures

At the conclusion of imaging (Day 42), animals are deeply anesthetized with isoflurane (5% to surgical plane, confirmed by absence of pedal and corneal reflex) and undergo transcardial perfusion with heparinized saline followed by 4% paraformaldehyde. Death is confirmed by cessation of heartbeat and respiration prior to any further tissue procedure, consistent with AVMA guidelines [2]. No animal regains consciousness following induction for this procedure.

6. USDA Pain and Distress Category

Cat.	USDA Definition	Applies to This Protocol
B	Animals bred, conditioned, or held, but not used in an invasive procedure or one causing more than momentary pain or distress.	☐ N/A
C	Involves no pain, distress, or use of pain-relieving drugs (e.g. routine blood draw, non-invasive imaging under brief sedation).	☒ Rat EEG headcap: routine post-op monitoring only
D	Involves procedures causing pain or distress that is appropriately relieved with anesthesia and/or analgesia.	☒ Cranial window & headcap surgery, all imaging cohorts
E	Involves pain or distress that cannot be relieved and is not medicated, because relief would interfere with study objectives.	☐ N/A — not requested

⚠ **Category E is not requested under this protocol.** All procedures causing more than momentary pain are performed under general anesthesia with a scheduled multimodal analgesia regimen (Sec. 5.1). The sleep-restriction paradigm itself is classified Category D on a precautionary basis (potential distress from platform motion) even though pilot data show no elevation in fecal corticosterone metabolites relative to cage-control handling [3].

6.1 Humane Endpoints

Animals are removed from the study and euthanized (Sec. 8) if *any* of the following criteria are met, assessed at every monitoring check:

- Body weight loss $> 20\%$ of pre-procedure baseline, or $> 15\%$ within 48 h.
- Body condition score ≤ 2 on the 1–5 scale (Ullman-Culléré system).
- Cranial window opacity, infection, or implant failure precluding data collection and causing visible discomfort (persistent head-scratching, guarding).
- Failure to right within 60 seconds when placed on its back, or absence of grooming for > 24 h post-surgery.
- Seizure activity, persistent vocalization at rest, or self-mutilation at the surgical site.
- Any condition that, in the judgment of the attending veterinarian, warrants early euthanasia on welfare grounds, independent of the criteria above.

6.2 Monitoring and Adverse Event Reporting

Post-surgical animals are monitored per the schedule in Sec. 5.2. Any animal meeting a humane endpoint, or any unanticipated adverse event (e.g. unexpected mortality, procedure-related injury), is reported to the IACUC office within 1 business day using Form MU-IACUC-AE-01, and to the attending veterinarian immediately. The PI maintains a running adverse-event log, reviewed at each annual continuation.

7. Euthanasia and Occupational Health

Species	Method	AVMA-Consistent
Mouse (both strains)	Deep isoflurane anesthesia to surgical plane, confirmed absent pedal/corneal reflex, followed by transcardial perfusion (primary method) or bilateral thoracotomy as secondary confirmation	☒ Yes
Rat	Deep isoflurane anesthesia to surgical plane, followed by exsanguination via cardiac puncture (secondary confirmation)	☒ Yes

Both methods are consistent with the current *AVMA Guidelines for the Euthanasia of Animals* [2]. Death is confirmed by a second, physical method (perfusion or exsanguination) in every case before disposal.

7.1 Occupational Health and Safety

Personnel with animal contact are enrolled in the Meridian Occupational Health and Safety Program for Animal Handlers, which includes an initial health questionnaire, offer of respiratory clearance for N95 use, and annual re-enrollment. Rodent allergen exposure is mitigated by ventilated caging, PPE (gloves, lab coat, N95 respirator during cage changes), and biosafety cabinet use for any procedure generating aerosols. No hazardous biological agents, radioisotopes, or human-derived materials are used in this protocol. Sharps (scalpel blades, needles) are disposed of in approved sharps containers per Meridian EHS policy.

7.2 Carcass and Tissue Disposal

Perfused carcasses and post-fixation tissue not retained for histology are disposed of as biohazardous waste via the Meridian Comparative Medicine incineration contract, logged per animal on Form MU-CM-DISP-02. Tissue retained for histology is stored in 4% paraformaldehyde at 4 °C in the Lindqvist laboratory, Krieger Hall Rm. 322, and logged in the laboratory sample inventory.

8. Investigator Assurances

Certifications (all required)

☒ I have read the Meridian University Animal Welfare Assurance and the *Guide for the Care and Use of Laboratory Animals*, 8th edition [4].

☒ The procedures described avoid or minimize discomfort, distress, and pain to the animals, consistent with sound research design.

☒ A literature search for alternatives to painful/distressful procedures was conducted within the past 12 months (Sec. 3.1) and found no suitable alternative.

☒ This protocol does not unnecessarily duplicate previous experiments, to the best of my knowledge.

☒ Animals will not be used in more than one major survival surgical procedure, except as scientifically justified in writing (none requested here).

☒ All personnel listed in Sec. 1.2 are qualified and trained for the procedures they will perform.

8.1 Investigator and Reviewer Signatures

Principal Investigator	Attending Veterinarian	Department Chair
Dr. Elena Lindqvist	Dr. Sofia Alvarez	Prof. Harold Ngata
Date: _____	*Date:* _____	*Date:* _____

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Final Determination: ☐ Approved ☐ Approved with Required Modifications ☐ Withheld Protocol Expiration: _____

Post-Approval Monitor Assigned: _____

IACUC Chair Signature: _____ Date: _____

8.2 Regulatory References

- National Research Council. *Guide for the Care and Use of Laboratory Animals*, 8th ed. National Academies Press, 2011. [4]
- American Veterinary Medical Association. *AVMA Guidelines for the Euthanasia of Animals*, 2020 ed. [2]
- Animal Welfare Act and Animal Welfare Regulations, 9 CFR Parts 1–3. [1]
- Public Health Service. *Policy on Humane Care and Use of Laboratory Animals*, 2015. [5]
- Percie du Sert, N. et al. The ARRIVE Guidelines 2.0. *PLOS Biology*, 2020. [7]

Next Scheduled Continuation

Annual continuation review due 14 January 2027; full triennial re-review due 14 January 2029. Responsibility: Dr. Elena Lindqvist. Reminder issued automatically by the Meridian IACUC eProtocol system 60 days prior.

References

[1] Animal Welfare Act and Animal Welfare Regulations, 2019. 9 CFR Parts 1–3. United States Department of Agriculture.

[2] American Veterinary Medical Association. AVMA Guidelines for the Euthanasia of Animals. Technical report, American Veterinary Medical Association, Schaumburg, IL, 2020.

[3] Elena Lindqvist and Marcus Feld. Cortical Microvascular Remodeling under Acute Sleep Restriction in Mice. *Journal of Comparative Neurophysiology*, 58(3):211–226, 2024.

[4] National Research Council. *Guide for the Care and Use of Laboratory Animals*. National Academies Press, Washington, DC, 8th edition, 2011.

[5] Office of Laboratory Animal Welfare. Public Health Service Policy on Humane Care and Use of Laboratory Animals. Technical report, National Institutes of Health, Bethesda, MD, 2015.

[6] Kwame Osei and Priya Nair. Activity-Dependent Microglial Synaptic Engagement in the Adult Somatosensory Cortex. *Glial Cell Biology Reports*, 12(4):455–470, 2023.

[7] Nathalie Percie du Sert, Viki Hurst, Amrita Ahluwalia, Sabina Alam, Marc T. Avey, Monya Baker, William J. Browne, Alejandra Clark, Innes C. Cuthill, Ulrich Dirnagl, et al. Reporting Animal Research: Explanation and Elaboration for the ARRIVE Guidelines 2.0. *PLOS Biology*, 18(7):e3000411, 2020.

[8] Bethany M. Walker. Sleep Loss and the Sensory Cortex: A Review of Attentional and Perceptual Deficits. *Annual Review of Sleep Science*, 9:88–114, 2017.