SUMMARY STATEMENT

(Privileged Communication)

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Application Number: **1 F32 MH115431-01**

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Review Group:	**ZMH1 ERB-S (04) National Institute of Mental Health Special Emphasis Panel BRAIN Initiative: Kirschstein NRSA Individual Postdoctoral Fellowship (F32)**
Meeting Date:	**06/29/2017**
Council:	**AUG 2017**
PCC:	**7K-TGRM**
Requested Start:	**10/01/2017**
Dual IC(s):	**AA, AG, AT, DA, DC, EB, EY, HD, NS**
Project Title:	**Investigating the hypocretin to VTA circuit in memory consolidation during sleep**
	***Requested*: 3 Years**
Sponsor:	**de Lecea, Luis**
Department:	**Psychiatry**
Organization:	**STANFORD UNIVERSITY**
City, State:	**PALO ALTO CALIFORNIA**
SRG Action:	**Impact Score:33**
Next Steps:	**Visit https://grants.nih.gov/grants/next_steps.htm**
Human Subjects:	**10-No human subjects involved**
Animal Subjects:	**30-Vertebrate animals involved - no SRG concerns noted**

1F32MH115431-01 BORNIGER, JEREMY

RESUME AND SUMMARY OF DISCUSSION: This is an F32 application received in response to the BRAIN Initiative Fellows: Ruth L. Kirschstein National Research Service Award (NRSA) Individual Postdoctoral Fellowship RFA (MH-17-250) from an exceptional applicant who proposes to investigate hypothalamic and ventral tegmental circuits that may play a role in sleep-dependent memory formation. This impressive candidate has a stellar academic and research record, has been extremely productive (>10 first author publications), and received glowing letters of reference. The sponsor has an outstanding record of mentoring previous trainees and is a leader in the use of optogenetics in the study of sleep. The additional co-sponsor will further broaden the technical expertise of the mentors. The candidate will acquire valuable methodologies and techniques including optogenetics, fMRI, fiber photometry and complex data analysis that will be essential for success in his career. The broader research environment at Stanford is excellent and a significant strength of the application. In contrast to these strengths, while the research plan addresses an interesting topic, it appears somewhat underdeveloped and Aim 3 is not well-connected to Aims 1 and 2. The application lacks a clear description of how specific changes in VTA activity either promote or inhibit memory consolidation during sleep and if any such changes would be due to direct effects on limbic circuits, or an indirect effect of altering sleep architecture. The fMRI data (from Aim 3) may be confounded by the use of isoflurane, and the anesthetized state will not allow informative comparison to the results obtained from Aims 1 and 2. Overall, this is an excellent postdoctoral fellowship application from an exceptional candidate and outstanding mentors, but with some weaknesses identified in the research plan.

DESCRIPTION (provided by applicant): Chronic sleep disturbance affects 10-20% of the population in the developed world, representing a substantial public health problem. Given the ubiquitous nature of sleep across the animal kingdom, intense investigation is underway into the biological functions of sleep. A primary hypothesis is that sleep facilitates memory consolidation following learning, as sleep restriction or fragmentation impairs memory performance across species. The circuitry coupling sleep and memory remains undefined. Recently, ventral tegmental area (VTA) dopaminergic neurons have been demonstrated to control motivational gating of arousal. These cells are well known players in ‘reward and salience’ circuitry, and send projections to brain centers critical for memory formation and recall (i.e., hippocampus, amygdala, and prefrontal cortex). How these (or local VTA-GABAergic) neurons contribute to sleep-dependent memory consolidation is unknown. Wake-stabilizing hypocretin (Hcrt) neurons in the lateral hypothalamus send dense projections to the VTA, however, it is unknown how (or if) this circuit contributes to Hcrt-mediated arousal or memory function. This proposal will integrate in vivo optogenetics, calcium imaging, and EEG techniques along with behavioral assays to establish (or refute) a role for these cells in sleep-dependent memory consolidation. fMRI technology will be integrated with optogenetic manipulations and quantitative approaches (dynamic causal modeling) to delineate brain-wide responses to Hcrt stimulation. These findings will establish the Hcrt-to-VTA circuit as a node coupling vigilance states to memory consolidation, with the ultimate goal of providing comprehensive insight into disorders of sleep-wake dynamics and memory dysfunction.

PUBLIC HEALTH RELEVANCE: Chronic sleep disruption is common throughout the developed world, contributing to cognitive problems including memory disruption. In addition to providing structured opportunities for my professional development, the experiments described herein will advance our fundamental knowledge of neural circuits coupling arousal and memory. Future studies will build on these findings to provide a comprehensive understanding of disorders of sleep and waking as well as memory impairment.

CRITIQUE 1

Fellowship Applicant: 1

Sponsors, Collaborators, and Consultants: 1
Research Training Plan: 5
Training Potential: 2
Institutional Environment & Commitment to Training: 1

Overall Impact/Merit:

This is an impressive application from an outstanding applicant. The applicant proposes to interrogate hypothalamic and ventral tegmental circuits that he hypothesizes play a role in sleep-dependent memory formation. In Aim 1, he will use GCamp6f fiber photometry to measure activity of VTA neurons across different brain states and use optogenetics to inhibit or activate these cells in different brain states combined with memory tasks. In Aim 2, he will use optogenetics to determine how Hcrt neurons modulate the activity of VTA neurons and then determine if the effects of Hcrt activation on sleep-dependent memory are mediated by VTA circuits. Aim 3 will use a combination of fMRI and optogenetics (ofMRI) and modeling algorithms to map out the areas of the brain that are influenced by Hcrt circuitry. The applicant thus brings to bear an impressive arsenal of techniques to the problem. The mentorship team is very strong, the institution where the work will be conducted is exceptional, and the training potential is excellent. While the combination of techniques is impressive, the underlying hypotheses are a bit vague and not completely reasoned out. The application lacks a clear description of how specific changes in VTA activity either promote or inhibit memory consolidation during sleep. It is also unclear if any such changes would be due to direct effects on limbic circuits, or an indirect effect of altering sleep architecture. The former would be very novel and intriguing, but the latter is not that illuminating. In addition, the third aim is not well-integrated with the first two.

1. Fellowship Applicant
Strengths

- Impressive publication record. 15 first author papers and two book chapters.
- Very solid training in relevant areas of sleep biology. Good preparation for current post-doctoral work.
- Excellent academic record.
- Six academic awards and/or fellowships. Including the highest award at Ohio State (the Presidential Fellowship for graduate studies).
- Uniformly impressive letters of support.

Weaknesses

- Nothing significant.

2. Sponsors, Collaborators, and Consultants:
Strengths

- Dr. de Lecea is a world leader in the use of optogenetics in the study of sleep.
- He has an impressive track record of training students and post-doctoral fellows.
- Dr. Lee has deep experience in the ofMRI technique, and is very well-funded through 2020.
- The optogenetic techniques and related technologies are new to the applicant and will provide valuable breadth to his skill set.

Weaknesses

- Some concerns about funding for Dr. DeLecea as most grants will end in 2019.
- Dr. Lee is an assistant professor with less experience successfully mentoring post-doctoral fellows.

3. Research Training Plan:
Strengths

- Impressive combination of behavioral measures, optogenetics and imaging techniques.

Weaknesses

- There are no clear predictions about how changes in VTA or Hcrt-VTA activity would lead to increases or decreases in sleep-dependent memory consolidation. The applicant hypothesizes that VTA activity “changes across specific memory tasks” but the specific changes that would impact memory are not discussed. This is too vague. What exactly does the applicant think will happen to VTA DA or GABAergic neurons during sleep that directly influences memory consolidation? If the result of Hcrt-VTA activation is simply to arouse the animal and thereby impair sleep, that would not be very illuminating. It would only mean that the circuit’s contribution to sleep-dependent memory consolidation is indirect.
- While there is some evidence that VTA GABAergic neurons change their activity across the sleep-wake cycle, there is little evidence that this is true for DA neurons, based on the cited literature. Therefore, it is not clear what would be learned by measuring activity of DA neurons using calcium fluorescence during sleep. Why would this be more informative then past electrophysiological measures? More importantly, if VTA DA neuron firing is independent of vigilance state and activation of the VTA leads to arousal, it is not clear how DA signaling could contribute to something going on in sleep.
- It is not clear that any changes in VTA cells that might accompany a learning task would be detected with this approach. Past studies have shown that VTA spiking activity becomes coordinated with ‘replaying’ patterns during sleep in the hippocampus, but this required an ensemble approach which may not be feasible with fiber optometry (Gomperts et al., 2015). In any case changes in the VTA associated with learning may not be a simple increase or decrease in activity, and current evidence is that there is no clear relationship between what happens during learning and subsequent sleep.
- The VTA DA neurons differ in their projections, as might be true for the GABAergic cells. In Aim 1, how will the applicant know that the VTA region to be transfected projects to limbic/memory processing regions and not arousal circuits?
- If Hcrt neurons are generally silent during REM sleep and VTA DA neurons fire pretty much the same across sleep and wake, then what role could they play in VTA activity during sleep? Decreasing their activity would have no effect and increasing their activity would likely wake the animal or impact the “spectral components” of sleep? The latter is a non-trivial issue as altering ongoing EEG activity in sleep can itself impair sleep-dependent memory consolidation. The precise ideas here need to be worked out more.
- The applicant proposes a complex set of rodent memory paradigms to test. However, it does not appear that the mentors or applicant has in depth experience in these paradigms. A consultant/collaborator with such experience is needed.
- The project with Dr. Lee is not well-integrated with the overarching theme of investigating how VTA and Hcrt-VTA circuits contribute to sleep-dependent memory consolidation. It seems to be a mix between investigating that idea and also how activation of the LH circuits more generally map to other brain regions. The experiments described in Aim 3 also will be conducted in anesthetized mice (not in natural sleep or wake states), so there are no direct links between this and Aims 1 and 2.
- These issues are not addressed in the alternative outcomes in Aims 1-3.

4. Training Potential:
Strengths

- Applicant will attend an excellent grant writing workshop (Stanford Grant Writing Academy).
- Will apply for membership in the Center for Mind, Brain and Computation, which provides intensive courses in computational neuroscience. Will attend courses regardless.
- Stanford University is a premier scientific institution. A world leader in scientific innovation and training.
- The optogenetic techniques and related technologies are new to the applicant and will provide valuable breadth to his skill set.

Weaknesses

- Dr. de Lecea has a fairly large lab. It is not clear how frequently he will meet personally with the applicant.

5. Institutional Environment & Commitment to Training:
Strengths

- Stanford University is a premier scientific institution; a world leader in scientific innovation and training.
- The applicant will have access to world-class seminars and workshops.

Weaknesses

- [None noted]

Protections for Human Subjects:
Not Applicable (No Human Subjects)

Vertebrate Animals:

- YES, all four points addressed

Biohazards:
Not Applicable (No Biohazards)

Training in the Responsible Conduct of Research:
Acceptable
Comments on Format (Required):

- Acceptable

Comments on Subject Matter (Required):

- Acceptable

Comments on Faculty Participation (Required):

- Acceptable

Comments on Duration (Required):

- Acceptable

Comments on Frequency (Required):

- Acceptable

Applications from Foreign Organizations:

- Not Applicable

Select Agents:

- Not Applicable (No Select Agents)

Resource Sharing Plans:

- Acceptable

Budget and Period of Support:
Recommend as Requested

CRITIQUE 2

Fellowship Applicant: 1
Sponsors, Collaborators, and Consultants: 1

Research Training Plan: 2
Training Potential: 1
Institutional Environment & Commitment to Training: 1

Overall Impact/Merit:

This is an exciting proposed project that investigates the contribution of VTA to memory consolidation during sleep. The research proposal uses an outstanding combination of techniques including optogenetics, calcium imaging, fMRI imaging of multiple brain areas, behavioral assay, and computational modeling. This is an ambitious and thoughtful project that will have a large impact on a very important question: mechanisms of memory consolidation during sleep. Although ambitious, it appears likely to succeed, given the stellar CV of the applicant and the postdoc mentors.

1. Fellowship Applicant:
Strengths

- Investigates the contribution of VTA circuit to sleep-wake cycle and memory consolidation.
- Uses great combination of techniques to address important questions.
- Applicant's CV is stellar.

Weaknesses

- None.

2. Sponsors, Collaborators, and Consultants:
Strengths

- Sponsors are excellent.

Weaknesses

- None

3. Research Training Plan:
Strengths

- Research plan is excellent.

Weaknesses

- Many aspects of the research plan are not fully developed. This is perhaps mitigated by the vast scope of the proposal, combined with very limited space available. Hence, this is not a major concern.

4. Training Potential:
Strengths

- Excellent.

Weaknesses

- None.

5. Institutional Environment & Commitment to Training:
Strengths

- Excellent.

Weaknesses

- None.

Vertebrate Animals:

- YES, all four points addressed.

Biohazards:
Acceptable

Training in the Responsible Conduct of Research:

- Appropriate

Comments on Format (Required):

- Appropriate

Comments on Subject Matter (Required):

- Appropriate

Comments on Faculty Participation (Required):

- Excellent

Comments on Duration (Required):

- Appropriate

Comments on Frequency (Required):

- Appropriate

Applications from Foreign Organizations:
Not Applicable

Select Agents:
Acceptable

Budget and Period of Support:
Recommend as Requested

CRITIQUE 3

Fellowship Applicant: 1
Sponsors, Collaborators, and Consultants: 2
Research Training Plan: 4
Training Potential: 3
Institutional Environment & Commitment to Training: 1

Overall Impact/Merit:

This F32 grant application asks for three years of support to investigate the role of hypocretin (Hcrt) neurons in the lateral hypothalamus in sleep-dependent memory consolidation by combining in vivo optogenetics-fMRI, calcium imaging, and EEG techniques along with behavioral assays.

The PI obtained his undergraduate training at Indiana University and PhD training at Ohio State University. He just started his postdoc position at Stanford University. He has 14 first-author publications.

The primary sponsor is a professor of Psychiatry and Behavioral Sciences at Stanford. He is a world-renown leader in the study of the molecular and cellular basis of arousal and sleep. The sponsor has substantial mentoring experience. Although the sponsor has substantial external funds, there seems to be a one-year gap to the ending date of this proposed F32 grant (09/2020).

The candidate's long-term career goal is to obtain a faculty position at an academic research institution so that he can contribute to the understanding of the neural mechanisms underlying sleep-wake control and cognitive disturbance. Through this training opportunity, he will acquire multiple valuable methodologies and techniques that will be essential for the success of his career.

The proposed project is interesting, timely and potentially can impact the understanding of the relationship between sleep-wake control and memory. However, enthusiasm is somewhat dampened by an overly ambitious plan and several technical issues in experimental designs.

1. **Fellowship Applicant**

Strengths

- Stellar education and research record.
- All 'A' student as an undergraduate student.
- 14 first-author publications.
- Received many awards during undergraduate and graduate trainings.

Weaknesses

- None.

2. Sponsors, Collaborators, and Consultants:

Strengths

- A world leader in the study of the molecular and cellular basis of arousal and sleep.
- The sponsor has a very successful mentoring record.
- Three trainees received Young Investigator Awards from NARSAD.
- Two received Young Investigator Awards from the Sleep Research Society.
- Three received NIH K99 pathway to independence awards.
- The co-sponsor (Prof. Lee) will provide expertise in ofMRI and DCM.

Weaknesses

- There is a one-year gap in the sponsor's funding to the ending date of this proposed F32 grant (09/2020).

3. Research Training Plan:

Strengths

- Scientific premise for each aim is well articulated.
- The proposed research could answer important questions including: **(1)** how do VTA neurons contribute to memory consolidation during sleep? **(2)** How do Hcrt neurons drive VTA activity to promote arousal and alter memory consolidation?

Weaknesses

- The research plan is overly ambitious. Extending the circuit-level manipulation and measurement to the network level analysis might be a distraction to the PI.
- Laser light-related hemodynamic artifacts in ofMRI measurement is not discussed.
- Isoflurane is a potent vasodilator and can be a confounder to the ofMRI signal.
- It is not clear how reliable the 'close-loop' system is.
- It is unclear how the proposed study in Aim 3 can answer the question that the information of brain-wide response to Hcrt neuron stimulation be can be harnessed to understand relationships between arousal and memory. fMRI data were collected separately in anesthetized animals and it is difficult to link these data to any behavioral assessment related to arousal and memory.

4. Training Potential:

Strengths

- The candidate's long-term career goal is to obtain a faculty position at an academic research institution so that he can contribute to the understanding of the neural mechanisms underlying sleep-wake control and cognitive disturbance.
- The candidate will acquire multiple valuable methodologies and techniques like optogenetics, fMRI, fiber photometry and complex data analysis that will be essential for success of his career.

Weaknesses

- The PI will heavily rely on the co-sponsor (Prof. Lee) for the training in ofMRI and DCM; Prof. Lee will only meet with the candidate once a month.

5. Institutional Environment & Commitment to Training:
Strengths

- Excellent.

Weaknesses

- None.

Protections for Human Subjects:
Not Applicable (No Human Subjects)

Vertebrate Animals:

- YES, all four points addressed.

Training in the Responsible Conduct of Research:
Acceptable

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

VERTEBRATE ANIMALS: Acceptable. All required assurances, protections, and justifications are provided.

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

Footnotes for 1 F32 MH115431-01; PI Name: Borniger, Jeremy

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER

National Institute of Mental Health Special Emphasis Panel
NATIONAL INSTITUTE OF MENTAL HEALTH
BRAIN Initiative: Kirschstein NRSA Individual Postdoctoral Fellowship (F32)
ZMH1 ERB-S (04)
06/29/2017

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