ROUTING AND ACTION
MEMORANDUM

ROUTING

TO: (1) Life Sciences Division (Gregory, Frederick)

Report is available for review

(2) Proposal Files Proposal No.: 66193-LS

DESCRIPTION OF MATERIAL

CONTRACT OR GRANT NUMBER: W911NF-17-1-0534

INSTITUTION: Medical College of Georgia Research Institute, Inc.

PRINCIPAL INVESTIGATOR: Joe Tsien

TYPE REPORT: Interim Progress Report

DATE RECEIVED: 10/4/19 11:24AM

PERIOD COVERED: 01-Aug-2018 through 31-July-2019

TITLE:

ACTION TAKEN BY DIVISION

(x) Report has been reviewed for technical sufficiency and IS [x] IS NOT [ ] satisfactory.

(x) Material has been given an OPSEC review and it has been determined to be non sensitive and, except for manuscripts and progress reports, suitable for public release.

Approved by SSL\FREDERICK.GREGORY on 3/2/20 9:02AM

ARO FORM 36-E

REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
04-10-2019	Interim Progress Report	1-Aug-2018 - 31-Jul-2019

4. TITLE AND SUBTITLE

A Novel Approach to Predicting Resilience to Post-Traumatic Stress Disorder

5a. CONTRACT NUMBER
W911NF-17-1-0534

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611102

6. AUTHORS

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES

Medical College of Georgia Research Institu
1120 15th Street

Augusta, GA 30912 -4810

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)

U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
66193-LS

12. DISTRIBUTION AVAILIBILITY STATEMENT

Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Joe Tsien
UU	UU	UU	UU		19b. TELEPHONE NUMBER 706-721-3760

RPPR Interim Progress Report
as of 02-Mar-2020

Agency Code:

Proposal Number: 66193LS **Agreement Number: W911NF-17-1-0534**
INVESTIGATOR(S):

Name: Joe Tsien
Email: jtsien@augusta.edu
Phone Number: 7067213760
Principal: Y

Organization: **Medical College of Georgia Research Institute, Inc.**
Address: 1120 15th Street, Augusta, GA 309124810
Country: USA
DUNS Number: 966668691 EIN: 581418202

Report Date: 31-Aug-2019 Date Received: 04-Oct-2019
Interim Progress Report for Period Beginning 01-Aug-2018 and Ending 31-Jul-2019

Title: A Novel Approach to Predicting Resilience to Post-Traumatic Stress Disorder
Begin Performance Period: 11-Sep-2017 **End Performance Period:** 10-Sep-2019
Report Term: 1-Annual

Submitted By: Joe Tsien Email: jtsien@augusta.edu
Phone: (706) 721-3760

STEM Degrees: **STEM Participants:**

Major Goals: Fear is a powerfully adaptive cognitive process essential for an individual's survival. But too much or inappropriate fear learning and fear generalization can lead to psychiatric disorders, such as anxiety and PTSD. Traditionally, a vast amount of research on the dopamine (DA) system focused on its role in reward and drug addiction. However, emerging studies suggest that dopamine circuits are also important for regulating fear behaviors. Our laboratory has uncovered that in response to fearful events, DA neurons in the ventral tegmental area (VTA) of freely behaving mice showed distinct tri-phasic firing patterns, namely "suppression during the event" -followed-by "rebound-excitation upon the termination of the event" before returning to the basal level (termed as suppression-and-off-set rebound-excitation, or rebound-excitation for short). This finding has led us to postulate a novel hypothesis that the DA neurons' rebound-excitation represents a key safety-signal essential for tapering down anxiety and fear overgeneralization, and the production of this safety-signal requires the NMDA receptor in the DA neurons.

The major goals of the project are to test this DA rebound excitation hypothesis. Specifically, we have generated dopamine neuron-specific NMDA receptor knockout mice, and our preliminary analyses suggest that these mutants indeed exhibited fear-induced anxiety phenotypes. Over the past two years, we have applied state-of-the-art technologies to examine two specific predictions of this hypothesis:
1) NMDA receptors in DA neurons are crucial for generating robust safety-learning signals at the termination of fearful experiences;
2) Restoration of DA neuron tri-phasic firing patterns can reduce anxiety phenotypes in the DA-NR1-KO mice, thereby offering new insights into how PTSD might be treated.

Accomplishments: We have established that the VTA DA neurons process fearful information in a freely behaving state, we have used a set of fearful stimuli such as earthquakes (A small mouse box placed on a vertex machine), a sudden freefall drop (inside a small elevator), or a sudden blast of air (air blast) to the animal's back which are known to produce robust fear memories. We implanted an adjustable bundle of 8 tetrodes (32 channels) into the VTA on the right brain hemisphere of mice. We recorded a total of 135 well-isolated units from the VTA region of 14 mice. Of these, 73 cells met previously established criteria to be putative dopamine (DA) neurons. These putative DA neurons typically exhibited broad, tri-phasic action potentials. They all showed low baseline firing rates (0.5-10 Hz) with regular and irregular firing patterns. We haved confirmed that they were DA neurons, we also injected the DA receptor agonist apomorphine (1 mg/kg, i.p.) at the end of the experiments. As expected, apomorphine suppressed VTA DA neuron firing activity. We found three distinct subtypes of putative DA neurons: 1) Type-1 DA units (61.6% or 45 out of 73 recorded units) decreased their firing, but had a strong 'offset rebound-excitation" right after the stimuli were terminated; 2) Type-2 DA units (15% or 11/73 cells) suppressed their firings

without rebound excitation; 3) Type-3 DA units (19% or 14/73 units) showed multi-phasic temporal dynamics, which increased their firings initially then followed by the second-stage suppression before turning to baseline. Therefore, DA neurons which responded to reward cues are generally tuned to fearful events (24). DA neurons' responses to fearful information was observed from the very first trial, thereby supporting the notion that the VTA neurons are well-positioned to process fear behavior.

Moreover, we have generated DA neuron-specific NR1 knockout mice by crossing the DAT-Cre line with the homozygous floxed NR1 mice. We revealed that these mutant mice exhibit normal basic anxiety, as indicated by open-field and elevated O-maze. Interestingly, upon exposures to fearful events, these mice showed a drastic increase in anxiety. These mutant mice also showed depression as indicated by their reduced struggling in the tail suspension test. These behavioral results suggest that the NMDA receptor in the DA neurons is crucial for preventing experience-dependent anxiety pathogenesis. In addition, our in vivo recordings showed that DA neurons in the KO mice showed blunted rebound-excitation in comparison to the wild-types. This provides strong evidence for our hypothesis that the NMDA receptor in the DA neurons is crucial for the generation of a safety-signal in the reward circuit, and the lack of this safety-signal makes such animals particularly vulnerable to the onset of PTSD.

Importantly, we have recorded neural acitivty of DA neurons in the VTA of both wild-type and knockout mice. We found that VTA DA neurons in the wild-type mice showed suppressed activity during struggle in the tail-suspension test, followed by rebound-excitation when animals stopped struggling. In contrast, VTA dopamine neurons in the mutant mice increased firing during the initiation of struggle but completely suppressed once struggle stopped.

In another set of experiments, we have used optogenetic stimulation of VTA DA neurons to verify the identity of DA neurons. We have used the subregion- and cell type-specific Cre/loxP-mediated recombination technique that we pioneered in the 1990s (Tsien et al. Cell 1996) to genetically tag DA neurons with channelrhodopsin-2 (ChR2) in the mouse VTA. We have crossed a transgenic mouse line (ChR2Ai32, Jackson Lab) with our DAT-Cre line. Cre/loxP-mediated deletion of the floxed STOP cassette in ChR2Ai32 mice permits the expression of ChR2 selectively in the DA neurons. Our PCR has confirmed the generation of DAT-Cre/ChR2Ai32 double transgenic mice. In addition, we also used adeno-associated viral (AAV) constructs containing lox-STOPlox-channelrhodopsin-2 (AAV-floxed STOP-ChR2). We found that light-stimulation triggered the firing of the putative VTA neurons identified by electrophysiological properties. This suggest the optogenetic stimulation can be used to restore offset rebound-excitation in the mutant VTA DA neurons.

Finally, we examined the hypothesis that changes in the activity of the DA neurons in the VTA will manifest its effect at the level of altered HRV. Accordingly, we measured HR and HRV while optogenetically stimulating the DA neurons in the VTA in freely behaving mice. In our experimental protocol, we first introduced the mice into an open-field environment, which triggered a stressful response as evidenced from the rapid rise in HR and reduced HRV. Consistent with our hypothesis, we found that the elevated HR and HRV (in blue) induced by the open-field test can be readily reduced by the optogenetic stimulation of DA neurons in the VTA. The reduction in HR is most significant at the initial stage of light-stimulation (the first two-minute stimulation during which the phasic firing of DA neurons is most likely). This suggests that HR and HRV are indeed sensitively regulated by DA neuron activity in the VTA.

Training Opportunities: Nothing to Report

Results Dissemination: I originally planned to attend the Army/NASA conference held in Houston, but my travel request was denied due to the organized discrimination and retaliation by the Dean of Medical College of Georgia and other top administrators. I reported this situation to Dr. Frederick Gregory.

RPPR Interim Progress Report
as of 02-Mar-2020

Plans Next Period: Unfortunately, for the past year and a half, I have been subjected to a series of premeditated and racially motivated discrimination and hostilities, by Dr. David Hess, the dean of Medical College of Georgia and other top administrators at the Augusta University. For a detailed summary, please see the Chronologies that I have provided to Dr. Frederick Gregory on August 19, 2019. As a result, my administrative assistant Sandra Jackson was terminated in June 2018 by Dr. David Hess.

In the original grant application entitled “A Novel Approach to Predict resilience to PTSD”, you should find on Page 25 the Facilities, Equipment and Resources that Augusta University has guaranteed for this army grant project. The contract was signed off by Sara White, the executive officer at the time. These essential capacities and conditions are listed as follows:
1) Laboratory: "Our main laboratory (~3,600 sq. ft.) is located in the Hamilton Building. A total of 12 benches and eight designated rooms for in vivo recording in freely behaving mice while the animals perform various learning tests..."
2) Computer resources: "We have four dual-CPU workstations and twenty personal computers with various software connected to the campus-wide network with intern access".
3) Office: "I have a private office and have an administrative secretary."
4) Major Equipment and Additional Information: "We have eight 128-channel MAP neural recording systems (Plexon) as well as machine tools for constructing electrodes and microdrive head stages..."

The above equipment and facilities, highlighted in yellow, represent the essential core capacity that was all taken away from me since June 19, 2019. As a result, I have lost the basic capacity to carry out the army grant-funded project for which I have passionately committed and deeply appreciated.
Dr. Hess initiated a set of the premeditated hostile act under the cover of this witch-hunt investigation in his racially motivated retaliation when I voiced my concerns about his choices of candidates for the department chairman position. He first secretly took away my Start-up fund in February 2019. Dr. Hess then took away my endowed professor chair title (Georgia Research Alliance Eminent Scholar in Cognitive and Systems Neurobiology) and its endowment fund in April 2019. Both funds had been crucial for me to cover part of expensive animal costs as well as to hire extra personnel and buy supplies when the federal grants may not be sufficient for the proposed experiments. Loss of such valuable financial resources occurred at the most critical time-window when I was gearing up for progress reports and project renewal. Such hostile actions devastated me and our team emotionally, damaged my academic reputation and torpedoed our financial flexibility crucial for exploring new ideas and expanding the funded research.
On the evening of June 19, 2019, Dr. Michael Diamond, the Senior Vice President for Research, and the Dean Dr. David Hess inactivated my lab people’s electronic access cards to the lab and changed the locks on my office (located in the Hamilton Building). As a result, we abruptly found ourselves in the next morning with no access to the office and laboratory where two dozen of genetically modified mice had undergone surgery and were undergoing various in vivo recording and PTSD behavioral testing. Consequently, these live animals were all lost and, and our meticulously planned experiments were ruined.
Ever since June 19, 2019, we lost all of our eight in vivo recording rooms which were specially designed and renovated with a $3.6 million fund I obtained from the GRA foundation when I joined MCG in 2007. These special rooms provided sound-proof walls, equipped with temperature control, independent air ventilation system, fire-proof curtains for restricting visual cue during behavioral testing, and light/dark cycle control for chronic housing and recording. As you noted during the site visit, these precious in vivo recording rooms are now merely used for housing refrigerators and freezers from other laboratories.
In the subsequent weeks in July, we were further evicted from our Hamilton lab in a way designed to humiliate us publically. We were escorted and being watched over our shoulders as we tried to retrieve items. We were barred from our current lab notebooks, computers, my own Ph.D. thesis, my old lab notebooks used at Columbia and MIT years in the early 1990s, or my former students’ theses I supervised at Princeton.
Despite my repeated pleas to President Brooks Keel, the Senior Vice President Michael Diamond and the MCG dean Dr. David Hess, the University had chosen to disregard all my requests, nor to discuss the situation and alternative solution.
Without any explanation, they instructed people to disassemble and remove our Plexon 128-channel MAP neural recording systems and took away our computers we used to process and store experimental datasets, as well as those computers for running physiology rigs or behavioral instruments (i.e. fear conditioning apparatus, open-field, social interaction, elevated plus maze, etc.).
Moreover, during this eviction period, the University sent out the announcements, without notifying me or obtaining my consent, and invited other labs to take various items and equipment from my laboratory. This public looting of our equipment and lab items, --many of which I brought from Princeton and Boston University--, went on for many days and weeks. By the time it was over, many of my lab’s supplies and equipment, --including oscilloscopes,

stimulators, amplifiers, pipettes, microwave, brain slicer, scales and balances, heaters and incubators, surgery respirator and surgical tools, PCR machines, electrophoresis apparatus and power supplies, cameras and video recorders, etc.--, were stolen or cannibalized.
If we were not subjected to such premeditated sabotage and precision-guided destruction, we would have planned and performed a rich set of experiments for the army project. These would include the transfection of VTA dopamine neurons in the knockout mice with ChR2. It would have allowed us to label these neurons optogenetically and then stimulate them with blue light. Such experiments would enable us to address the question of whether the restoration of the DA neurons' bursting firing during or at the end of the fearful events can result in rescuing the PTSD phenotypes in the knockout mice. In another set of our originally planned experiments (as described in Specific Aim #3), we would have begun to perform surgery and measure heart rates and heart rate variability (HRV) while these mutant mice are subjected to PTSD-inducing events such as earthquakes or sudden free-fall. We could then further ask whether heart rate variability is altered in the PTSD models and correlated with changes in VTA dopamine neuron activity. These experiments would serve to achieve the goal of potentially developing a much-needed index for predicting the resilience to PTSD.
Sadly, institutional administrators had no regard for the laws and contracts. I am horrified and appalled by their discriminatory hostile acts which were apparently so well premeditated and then executed with such thoroughness and precision to strike where it hurts the most.

However, despite all the losses mentioned above, I have not lost my faith, my passion for science and knowledge, and my hopes for justice, generosity, human decency, and warmth. Once again, I would like to take this opportunity to express my deepest gratitude for the kind support I have received over the years from the Army Research Lab.

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: Staff Scientist (doctoral level)
Participant: Kun Xie
Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

A Novel Approach to Predicting Resilience to PTSD

Joe Z. Tsien

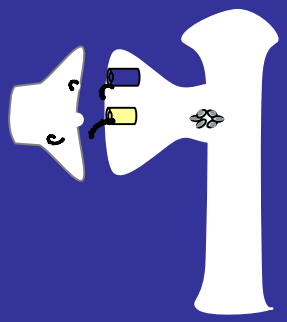
Department of Neuroscience and Regenerative Medicine

Medical College of Georgia

Augusta University

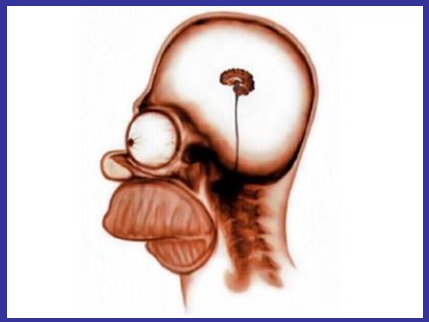
Integrated Analysis of PTSD Mechanisms

Molecules

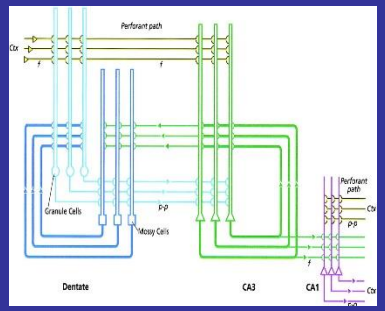


Synaptic Plasticity

Genetically modified mice



Mind



Circuits/systems

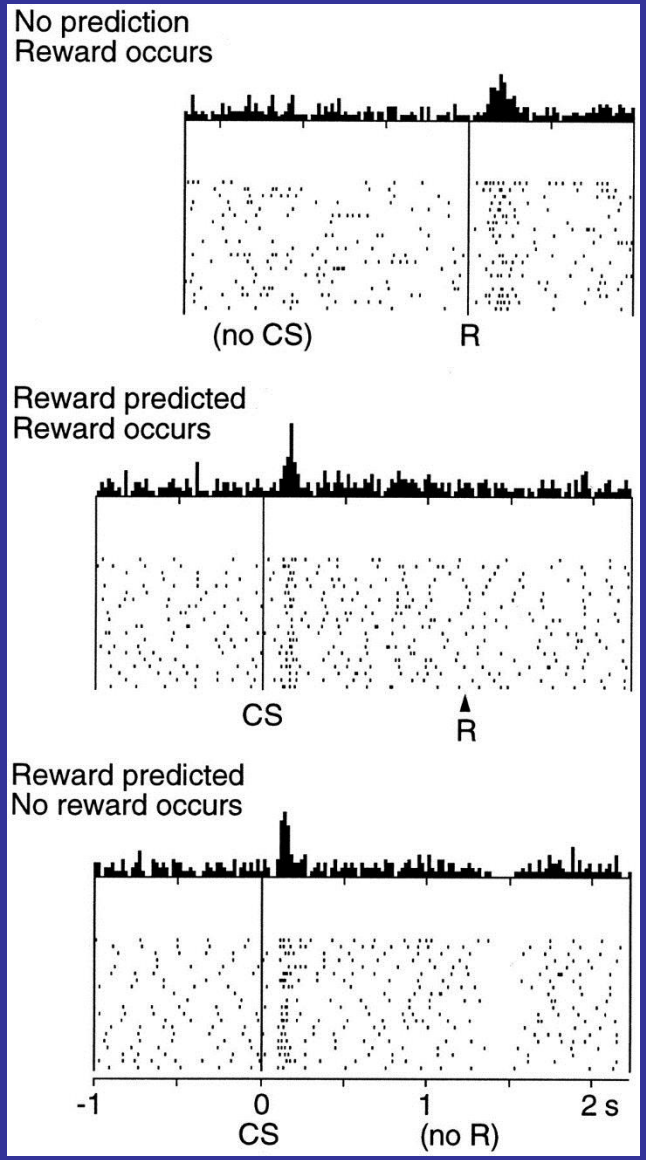
Dopamine hypotheses of motivation vs. reward

1. Dopamine is important for instrumental responding, but not consumption of food reward (Ikemoto & Panksepp, 1996)

2. Dopamine lesion rodents show sucrose preference and normal ‘liking’ facial response (Berridge & Robinson, 1998)

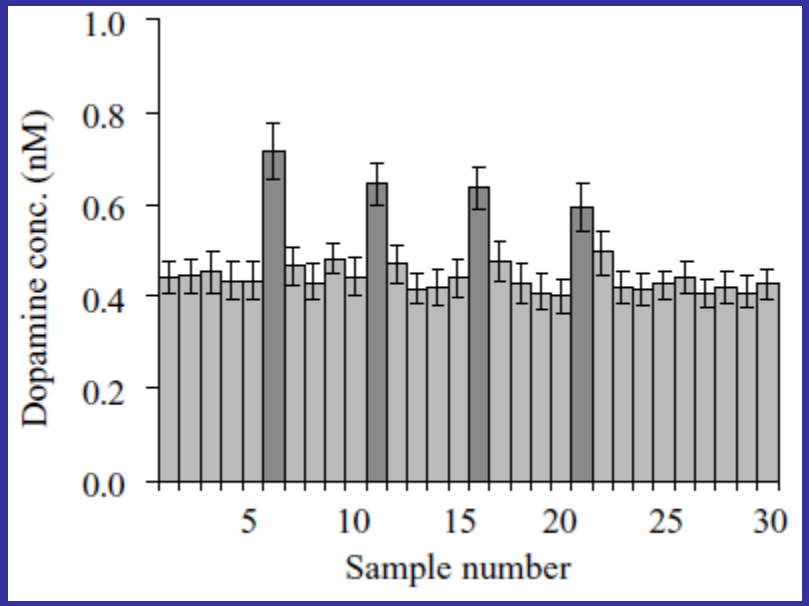
3. Dopamine-deficient mice (tyrosine hydroxylase deficient) show sucrose preference (Cannon & Palmiter, 2004)

Predictive reward signal of dopamine neurons



Schultz, 1998

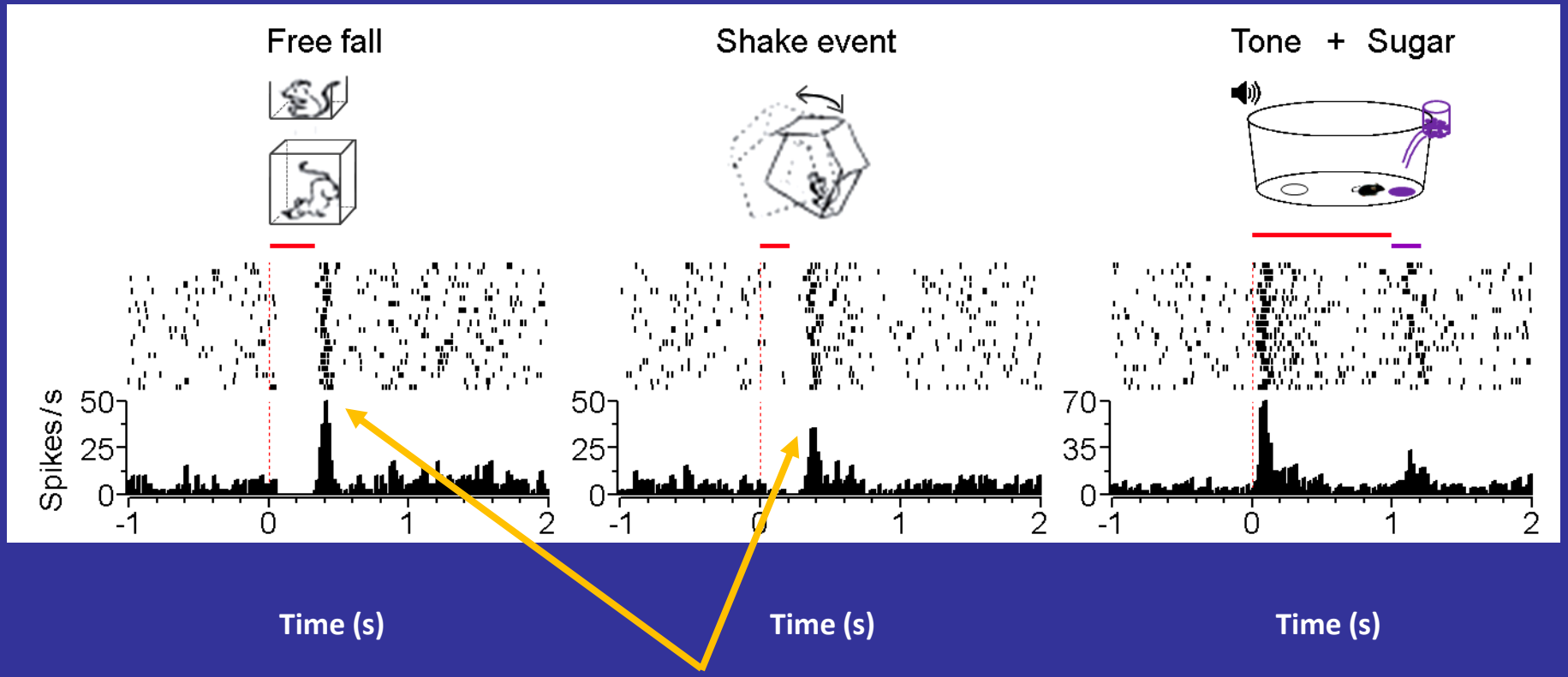
Do dopamine level change in reacting to fearful or aversive events?



Dopamine level in the rat nucleus accumbens increases during repeated footshocks

Young, 2004

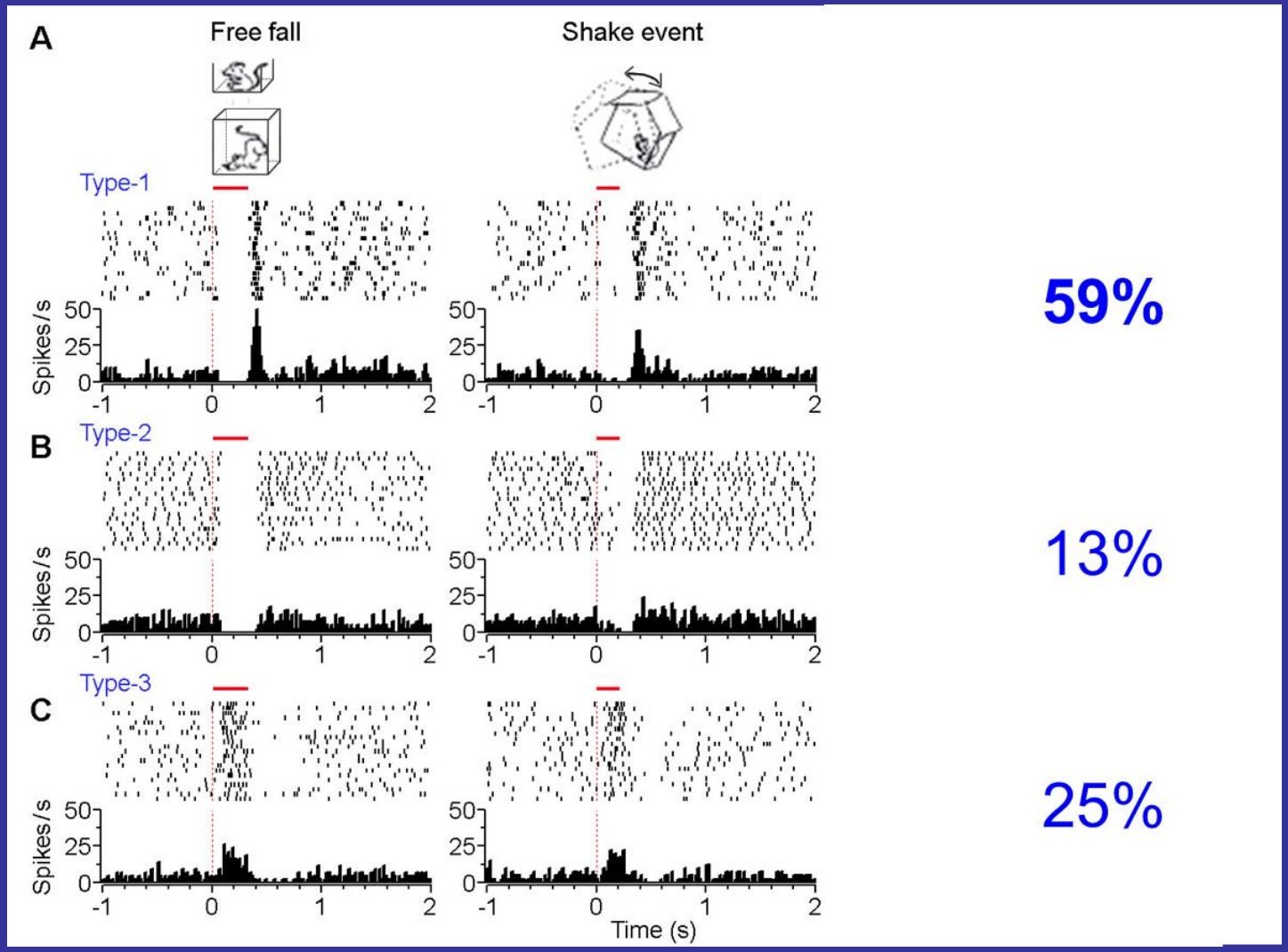
We discovered that DA neurons in the VTA reacted strongly to various fearful events (in addition to reward signals)



DA neurons exhibited rebound-excitation at the end of fearful stimulation

Wang DV and Tsien JZ. (2011) Convergent processing of both positive and negative motivational signals by the VTA dopamine neuronal populations. PLoS One, Feb 15;6(2):e17047. doi: 10.1371/journal.pone.0017047.

Three types of putative DA neurons in VTA

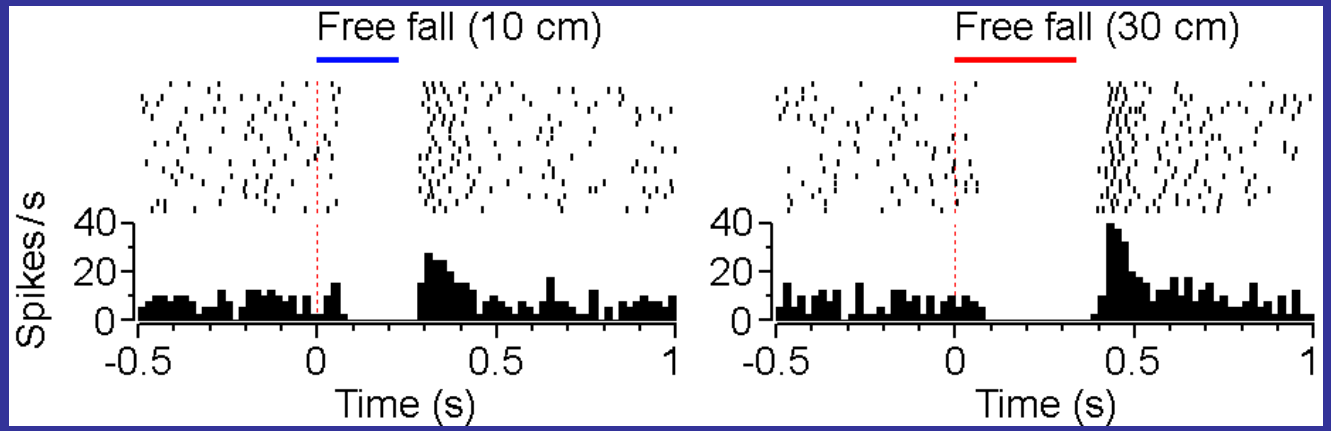


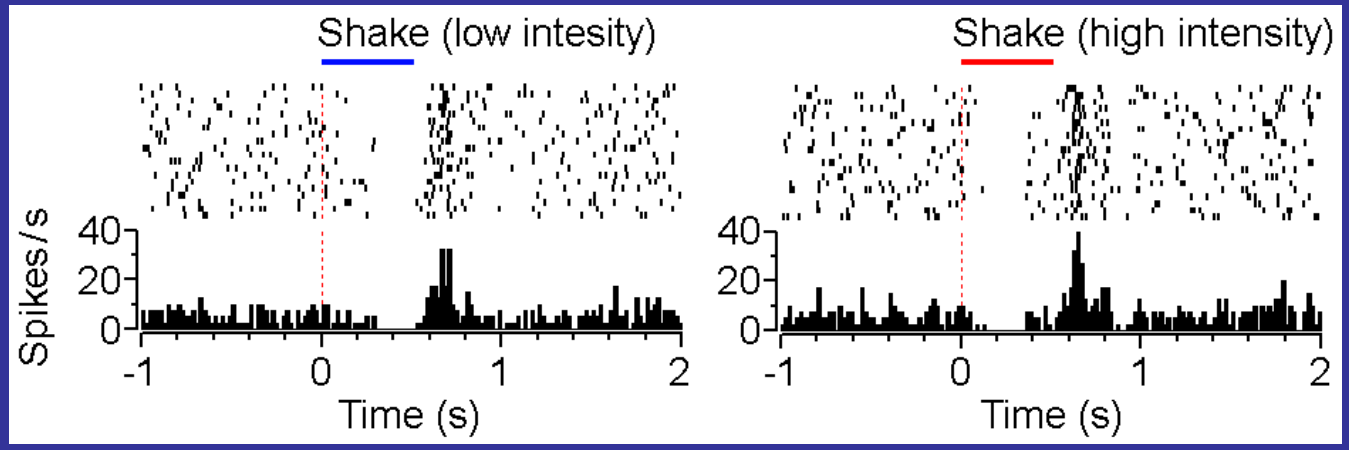
59%

13%

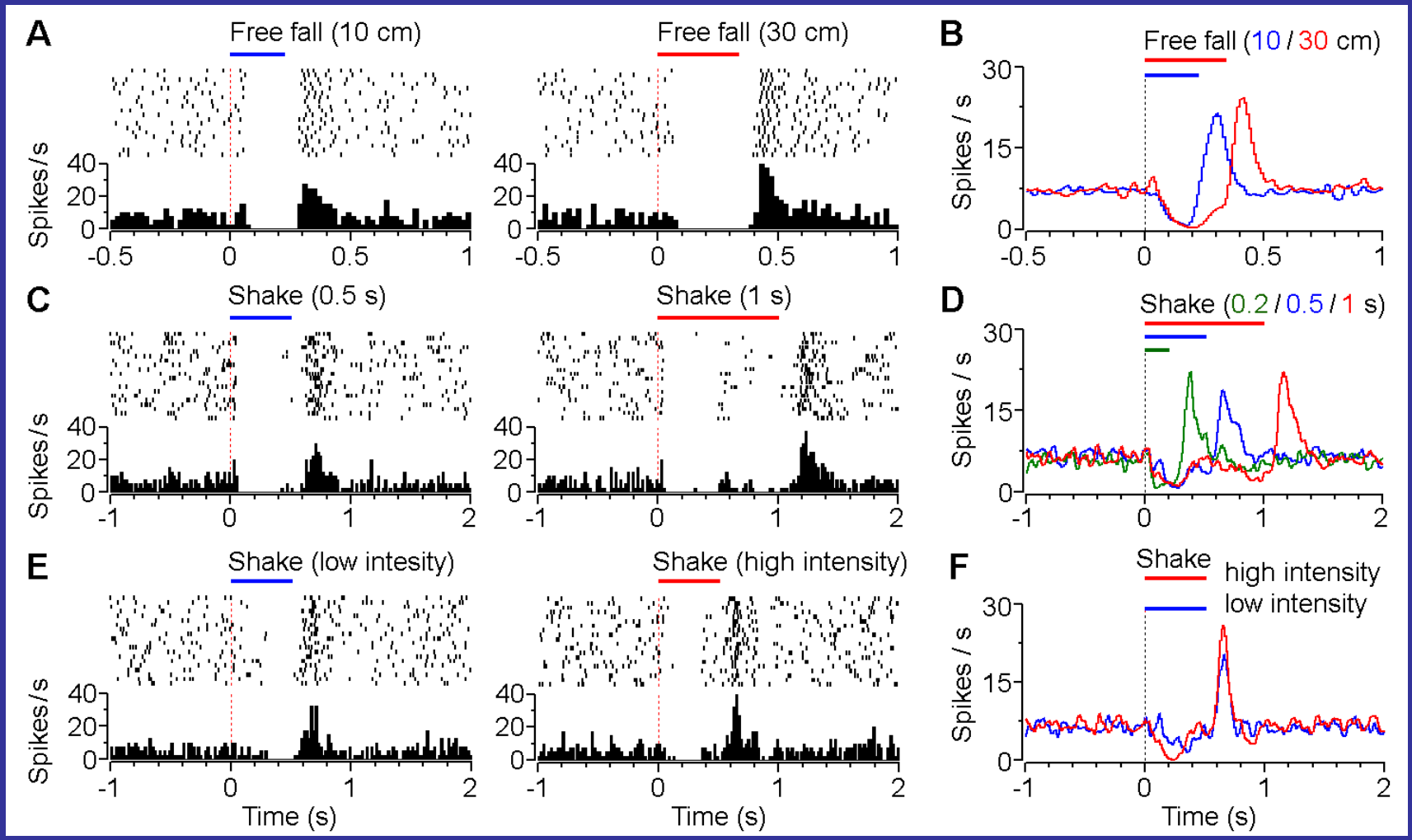
25%

DA neurons' rebound-excitation was correlated with intensities of free fall or earthquakes

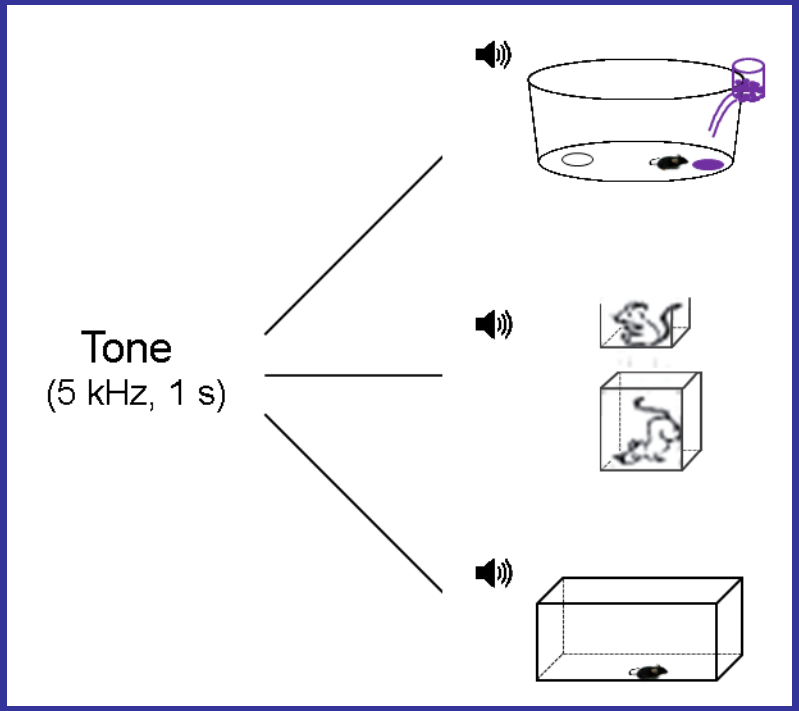




DA neurons' rebound-excitation in relations to intensity or duration of fearful events

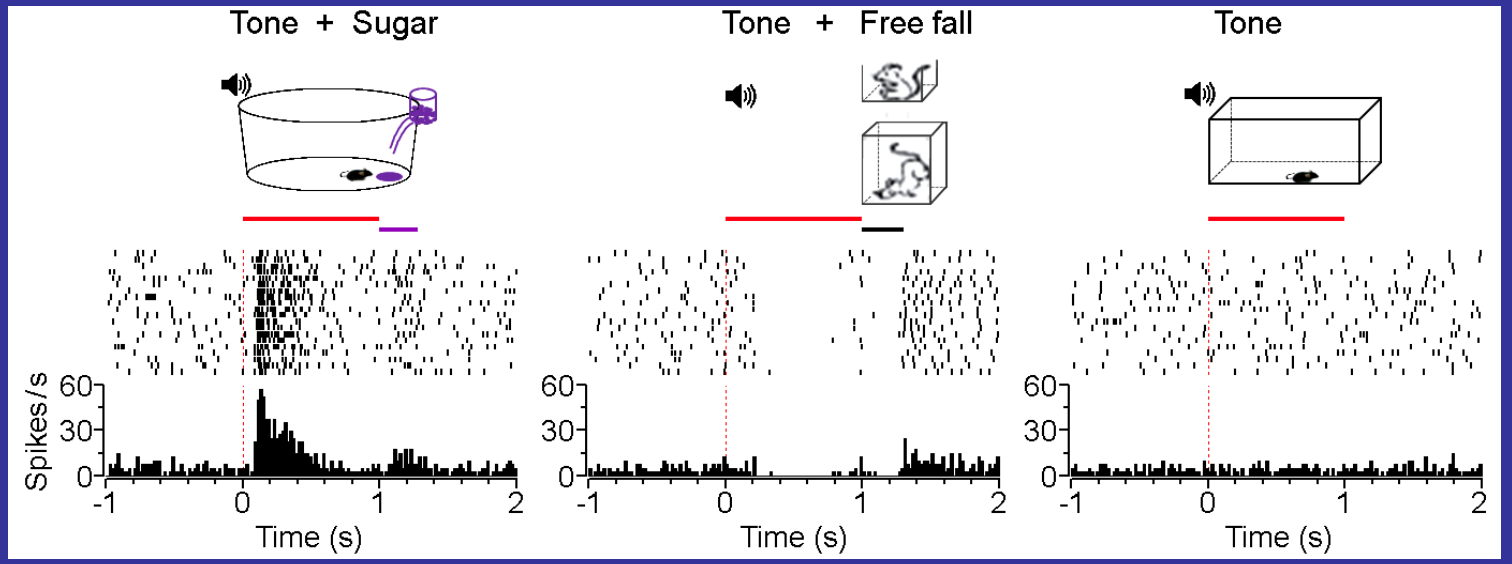


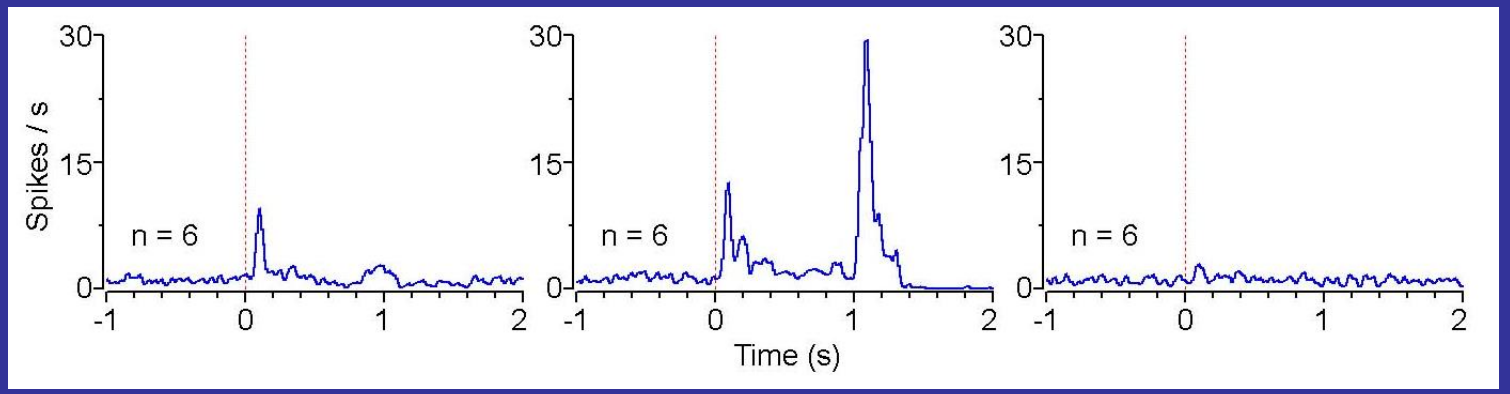
VTA DA neurons encode and integrate context information with motivational signals



Different contexts can allow the identical tone to be used as a predictor for either reward or free-fall event.

Integral encoding of events and contexts (same tone, different contexts) by DA neurons





We postulated the Dopamine Rebound-Excitation Hypothesis: Putting brakes on PTSD.

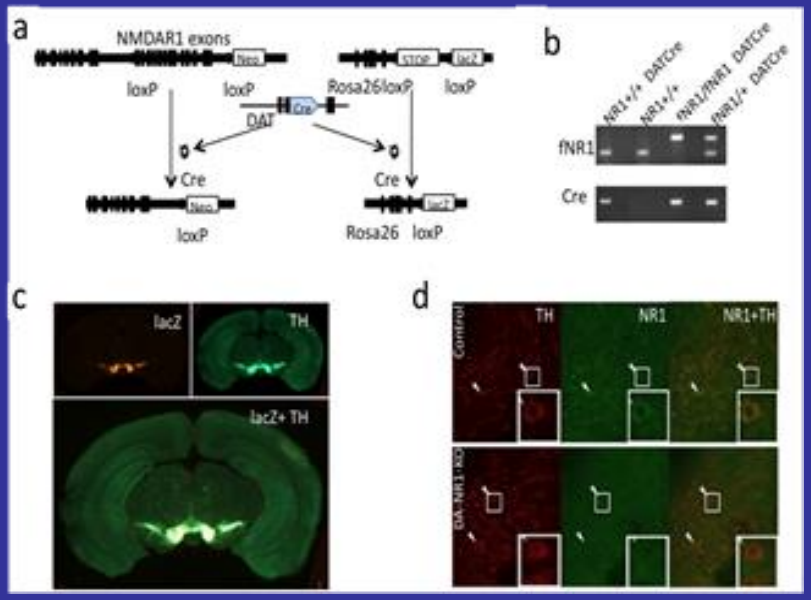
DA neurons' rebound-excitation signal in the VTA at the termination of fearful events serves as a safety-learning signals crucial for gating resilience to PTSD (Lee, Wang & Tsien, *Front Psychiatry*. 2016 Sep 27;7:163)

1) Specific Aim #1: NMDA receptors in DA neurons are crucial for generating this rebound-excitation (safety-learning) signal, and impairment of this safety signal resulted in PTSD-like phenotypes.

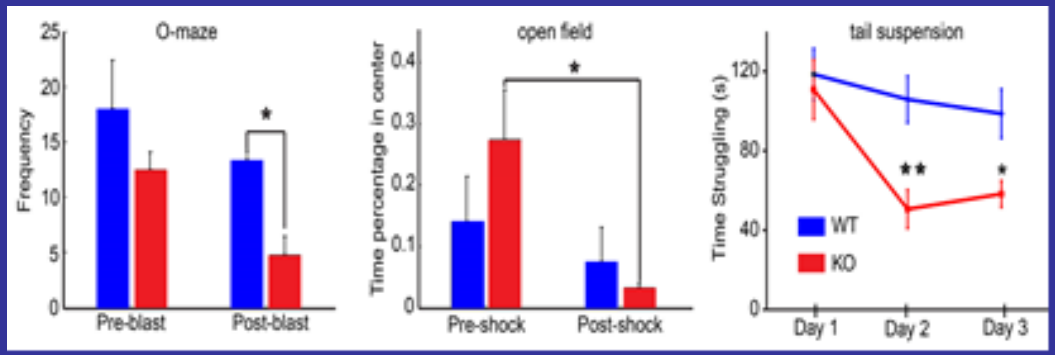
2) Specific Aim #2: Restoration of NMDA receptor in the DA neurons can reduce or rescue anxiety phenotypes in the DA-NR1-KO mice.

3) Specific Aim #3: Abnormal DA neuron activities in the VTA of KO mice can be potentially detected through changes in hear-rate variability for predicting stress-resilience before individuals face new fearful traumatic situations.

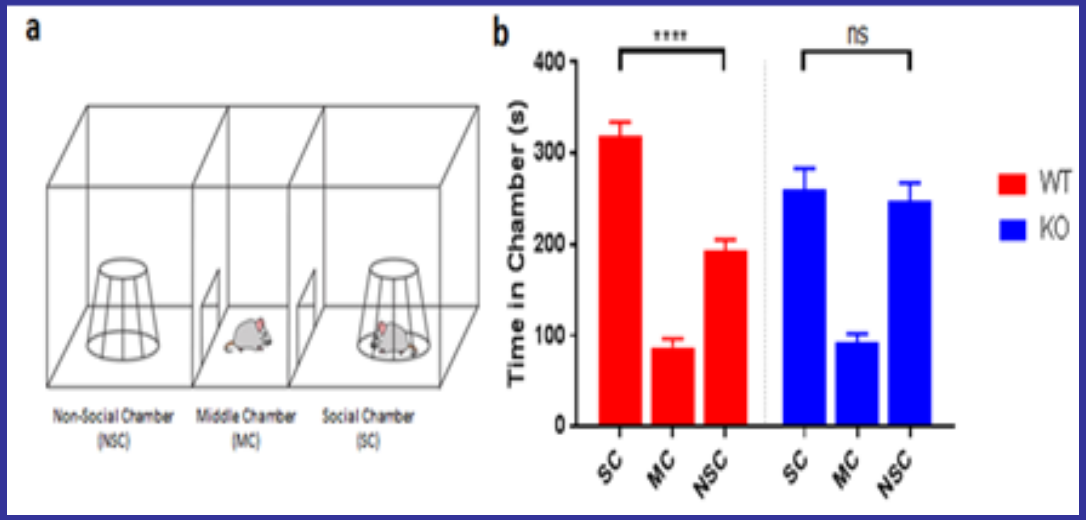
Generation of DA-neuron specific NR1 knockouts



The KO mice exhibited PTSD-like phenotypes

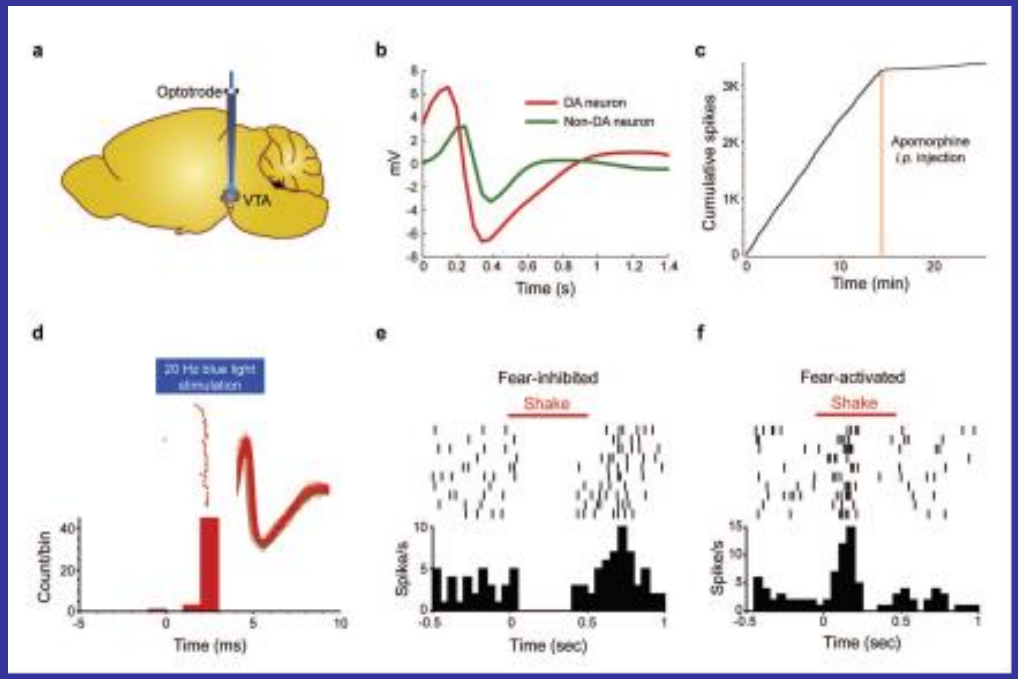


KO mice exhibited deficits in social motivation



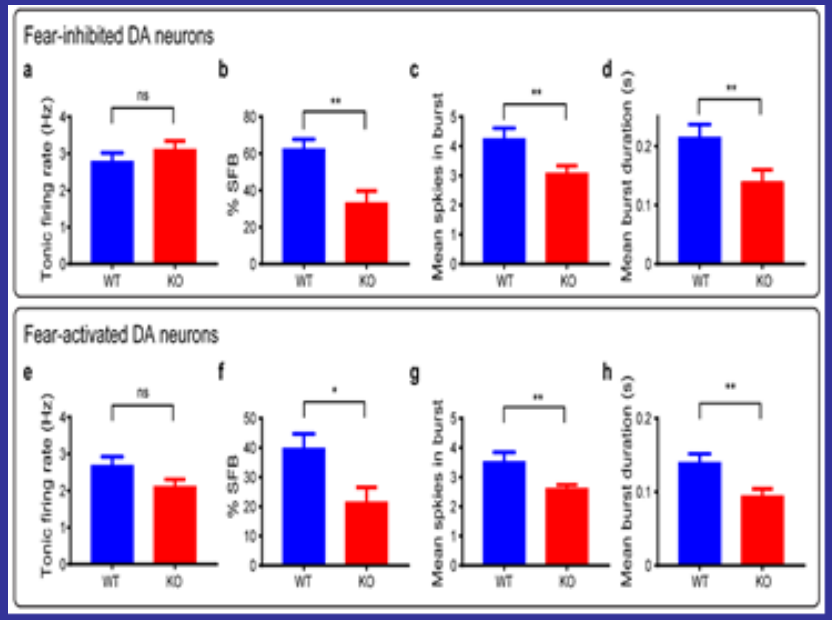
(a) Sociability paradigm. Mouse starting in the middle chamber (MC) can freely explore the social chamber (SC) vs. non-social chamber (NSC). (b) Control mice (blue) spent significantly more time in the social chamber, whereas KO mice (red) had no preference for either chamber. All data shows mean ± s.e.m.*P<0.05; ** P<0.01; *** P<0.001. **** P<0.0001.

Optogenetic verification of DA neurons in the VTA



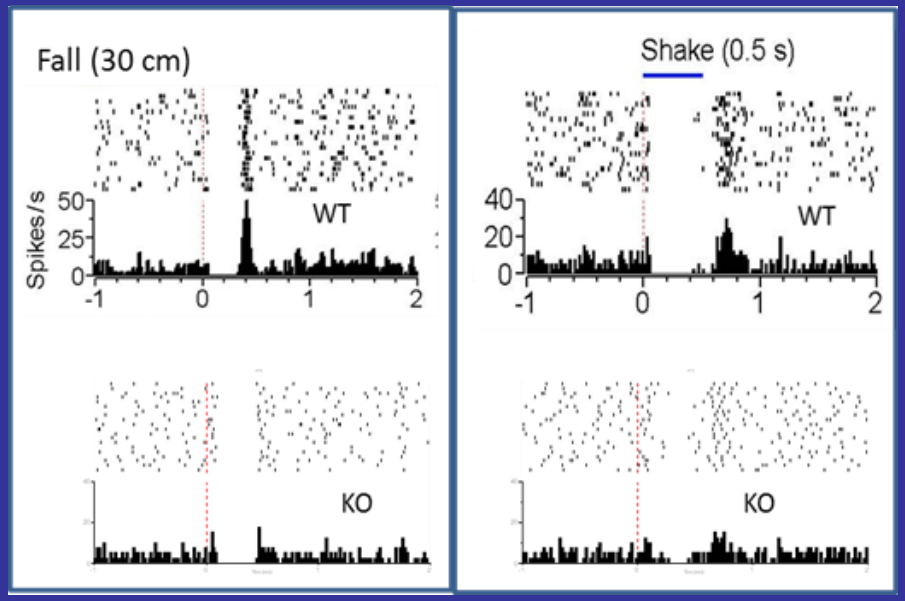
In vivo recordings in the VTA of freely behaving mice. (b) DA neuron exhibiting broad, triphasic waveform, in comparison with simultaneously recorded non-DA neuron on the same tetrode recordings. (c) DA neuron firing was suppressed after apomorphine injection. (d) DA neuron responding to 20Hz blue light-stimulation in the mice transfected with ChR2 in the VTA. The Inserted sublet showed light-induced waveforms (red) overlapping with waveforms from native firing. (e) Optogenetically identified DA neurons (type-1, fear-inhibited subtype) exhibited rebound-excitation in response to earthquake. (f) Fear-activated subtype of DA neurons.

NMDA receptor knockout did not affect the basal tonic firing, yet selectively reduiced burst firing in the DA neurons



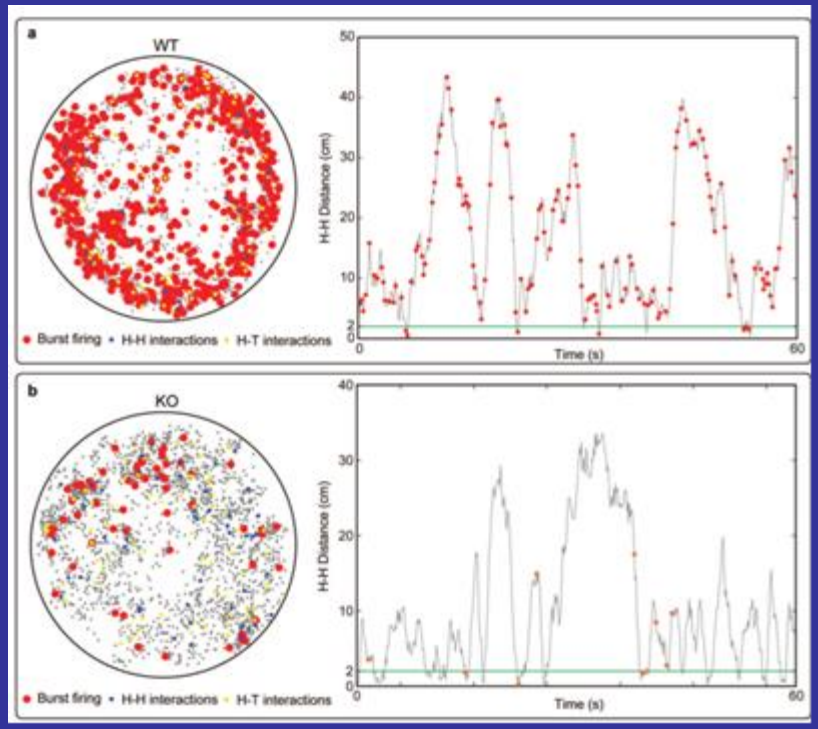
(a) Tonic firing did not differ significantly between WT and KO mice in aversive-inhibited DA neurons. (b) Percentage of burst spikes was significantly reduced in KO mice. (c) Burst duration was significantly shorter in KO mice. (d) Numbers of spikes within a burst was also reduced in KO mice. (e) Similar tonic firing between WT and KO mice in fear-activated DA neurons. (f) Burst spikes were reduced in KO mice. (g) Burst duration was shorter in KO mice. (h) Reduction in mean number of spikes occurring within a burst was observed in KO mice. All data analysis using unpaired two-tailed t test, Mann Whitney U. All data shows mean ± s.e.m.*P<0.05; ** P<0.01.

NMDA receptor knockout selectively impaired DA neurons' rebound-excitation signals



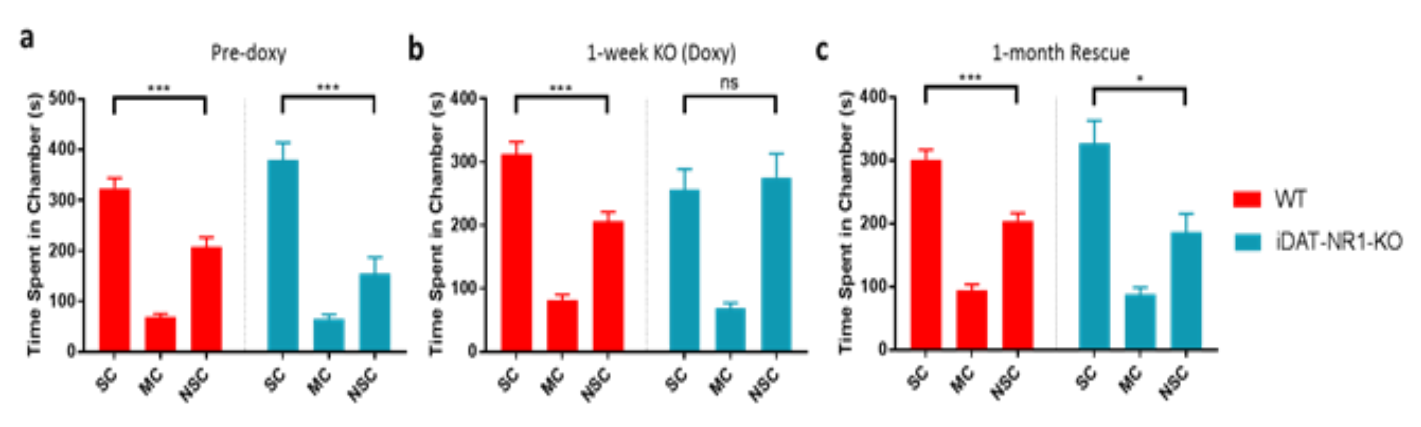
Blunted rebound excitation in type-1 VTA DA neurons in the knockout mice. Top row subpanels show typical suppression-rebound-excitation responses of type-1 DA neurons in wild-type (WT) mice to 30 cm free fall (left) and 0.5 sec earthquake (shake). Type-1 DA neurons in the KO mice showed greatly reduced rebound excitation (bottom panel).

Social interaction increased bursting firing in the wild-type mice, but not in the knockout mice



(a) Left: An open chamber plotted with five minutes of dyadic social interactions in a control mouse: red, DA burst firing events; blue, head-head (H-H) interactions; yellow, head-tail (H-T) interactions. The right plot shows the same DA neuron with burst firing events plotted against the distance between two mice. (b) Left, an open chamber plotted with five minutes of dyadic social interactions in a KO mice (red, DA burst firing events; blue, head-head interactions; yellow, head-tail interactions). Right, a plot showing the same DA neuron's burst firing events plotted against the distance changes between the subject KO mouse and the stimulus mouse.

Impaired social motivation was readily rescued in inducible DA-NR1-KO (iKO) by doxycycline



(a) Under normal drinking water conditions, control littermate and iKO mice showed greater preference for the social chamber than the non-social chamber. (b) Following 1 week of doxycycline treatment in drinking water (which induced NMDA receptor knockout by suppressing transgenic NR1 rescue-expression), the iKO mice no longer showed preference for the social chamber, while strong social chamber preference remained intact in control mice. (c) One month of normal drinking water immediately after doxycycline treatment (which restored NMDA receptor expression) rescued the sociability in iKO mice. All data analysis was done with one-way ANOVA with Bonferroni Post-hoc analysis. All data shows mean ± s.e.m.*P<0.05; *** P<0.001.

Conclusions up-to-date:

1) We showed that dopamine (DA) neurons in the ventral tegmental area (VTA) exhibited rebound-excitation at the termination of various fearful events, leading to a novel hypothesis that DA neurons' rebound-excitation signal serves as a safety-learning signals crucial for gating resilience to PTSD (Specific Aim #1).

2) NMDA receptors in DA neurons are crucial for generating this rebound-excitation (safety-learning) signal, and impairment of this safety signal resulted in PTSD-like phenotypes (Specific Aim #1).

3) Restoration of NMDA receptor in the DA neurons can reduce or rescue anxiety phenotypes in the DA-NR1-KO mice (Specific Aim #2).

To be investigated in Year 3:

To examine whether abnormal DA neuron activities in the VTA of KO mice can be potentially detected through changes in hear-rate variability for predicting stress-resilience before individuals face new fearful traumatic situations (Specific Aim #3).