

Office of Research Services Funding Application (New Faculty)

DEADLINE: February 28

Please refer to

<http://www.ucdenver.edu/about/WhoWeAre/Chancellor/ViceChancellors/Research/OAVCRCA/Pages/Research%20Services.aspx> for other information.

**NOTE WELL: THIS APPLICATION MUST BE WRITTEN FOR AND ACCESSIBLE TO LAY REVIEWERS. (LINE SPACING NO LESS THAN 1.5, FONT SIZE NO LESS THAN 12, FONT MUST BE TIMES NEW ROMAN)
PLEASE ATTACH A 2-PAGE CV.**

Applicant Information

Name: Greenwood Benjamin Date: February 26 2016
Last First

Department: Psychology

Phone: 303-556-5899 Email Benjamin.greenwood@ucdenver.edu

Title of Project: Role of oxytocin in MDMA-induced elimination of traumatic memories

Amount of Request: \$25,000

Date of appointment to tenure track: August 2014

Required Match

A 20% match is required. **STARTUP FUNDS ARE NOT ALLOWED AS MATCHES.**

Please have the responsible party for the match send an email verification to carie.carroll@ucdenver.edu.

Laura Argys, Pater Kaplan, and a MAPS representative have all emailed Carie Carrol verifying their willingness to provide matching funds.

Project Abstract

Provide a project abstract (one-third page)

When memories are recalled they enter a state of reconsolidation during which they are susceptible to modification. Reconsolidation therefore represents a potential target for therapeutics aimed at erasing traumatic memories, which lay at the heart of anxiety and post-traumatic stress disorder. We observed that low-dose 3,4-methylenedioxy-methamphetamine (MDMA; ecstasy) administered immediately after recall of a traumatic memory weakened the memory and reduced fear in rats. The goal of this proposal is to further characterize the ability of MDMA to erase traumatic and non-traumatic memories, and to investigate the role of brain oxytocin neurons in this effect. These experiments could contribute to a breakthrough in treatment of anxiety disorders. The Multidisciplinary Association for Psychedelic Studies is willing to supplement this grant with additional funds, so this project has high likelihood of providing sufficient data for future manuscripts and grant proposals.

Project Description

Provide a project description indicating the significance of the creative activity (limited to three and a half pages)

Anxiety disorders including post-traumatic stress disorder (PTSD) are the most pervasive mental health disorders in the world, with an estimated lifetime prevalence of 23%. Traumatic events precipitate development of anxiety and PTSD, and memories of these events are at the heart of anxiety and PTSD symptoms. However, current treatment strategies for these disorders do not target the traumatic fear memories. Rather, selective serotonin reuptake inhibitors, currently the only drugs approved for PTSD despite the fact they weren't developed for this purpose, have no effect on trauma memories, but do have debilitating side effects and poor long-term efficacy. A common behavioral therapy for anxiety, exposure therapy, takes advantage of the phenomenon of fear extinction in order to form new memories that trauma cues won't lead to aversive outcomes. However, extinction memories are very weak relative to the original traumatic memories, and anxiety symptoms resurface in many patients even after exposure therapy. Alternative therapeutic options are needed for the successful treatment of anxiety disorders. Erasing or modifying traumatic memories could be effective in long-term treatment of anxiety and PTSD, but means to erase fear memories are largely unknown.

When it comes to memory, it is "use it or lose it." That is to say, if memories aren't recalled, they fade with time. This is because when memories are recalled they enter a labile state during which they are susceptible to modification. Typically, memory recall triggers the synthesis of new proteins in the neural circuits that underlie the memory; thereby strengthening it. This process is known as memory reconsolidation. One reason fear memories are so persistent is that individuals will ruminate over, or re-live, traumatic events; thus the fear memory undergoes constant recall and reconsolidation.

However, just as recalled memories are able to be strengthened during reconsolidation, they are also susceptible to interference. Memory recall therefore represents a brief moment in time when traumatic memories can be targeted for modification or erasure by potential therapeutics.

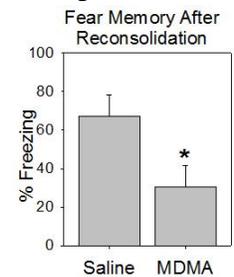
The drug propranolol provides an excellent example of the therapeutic potential of targeting reconsolidation for the treatment of PTSD. Propranolol is a norepinephrine receptor antagonist that is commonly used to treat high blood pressure. Propranolol, when administered immediately after recall of recent traumatic memories, can interfere with reconsolidation and weaken the fear memory. This makes sense considering that norepinephrine is critical for memory reconsolidation, and propranolol blocks the action of norepinephrine. The potential of propranolol to treat PTSD received much attention in the [lay press](#). However, new data indicate that propranolol is only effective at interfering with reconsolidation of *recent* fear memories, but not older traumatic memories already stored in long term memory [1]. This is an obvious limitation for the ability of propranolol to treat anxiety disorders, many of which stem from traumatic experiences in early life.

Moderate doses of 3,4-methylenedioxy-methamphetamine (MDMA; ecstasy) have shown exciting promise in human clinical trials as an effective adjunct to psychotherapy for the treatment of anxiety. Two groups [2, 3] recently reported that MDMA-assisted psychotherapy resulted in a long-term reduction in PTSD symptoms for more than a year after treatment. General support for the use of MDMA as a supplement to psychotherapy is lacking, however, because of the neurotoxic effects of high-dose MDMA, and the stigma that surrounds its recreational use. *Moderate-dose* MDMA is safe in humans and devoid of neurotoxic effects. Elucidation of the mechanisms by which moderate dose MDMA can reduce anxiety symptoms could facilitate acceptance of the therapeutic use of MDMA by the medical community. Moreover, investigating these mechanisms could lead to the identification of neural circuits that could be targeted to eliminate persistent and debilitating traumatic memories.

In 2015 I received a small contract from the Multidisciplinary Association of Psychedelic Studies (MAPS) to begin characterizing the effects of MDMA on a rodent model of anxiety. We initially tested the hypothesis that MDMA would enhance the extinction of fear memories. We observed that fear extinction augmented by MDMA was not strong enough to resist the reappearance of the original fear memory. Rats that were given moderate-dose MDMA during the fear extinction still suffered from a relapse of anxiety in an environment different from where extinction was learned, a common means of promoting anxiety relapse in humans and rodents. These data, along with a discussion with our colleagues at MAPS which revealed that MDMA-assisted psychotherapy doesn't rely as much on exposure therapy as it does on free-recall of trauma, suggested that perhaps MDMA was reducing PTSD symptoms not by enhancing fear extinction, but by modifying reconsolidation of the traumatic memories after they were recalled during therapy.

In a preliminary study funded by MAPS, we exposed adult rats to a traumatic event which results in a strong fear memory. Two days later, the fear memory was reactivated by briefly exposing the rats to the context in which they initially received trauma. Immediately after memory recall, rats were given either saline or MDMA (3 mg/kg; the rat equivalent of the moderate-dose MDMA used in human trials). The idea was that MDMA administered immediately after fear memory recall would interfere with the reconsolidation of the fear memory. This hypothesis seems to have proven correct. When re-exposed to the trauma context the day after memory reactivation, we observed that rats given MDMA after recall of trauma memory displayed less fear (freezing; an innate fear response in rats that is used as an index of fear memory) than rats given saline (Figure 1),

indicating that MDMA interfered with the reconsolidation of the fear memory. Importantly, MDMA didn't reduce fear memory unless it was administered immediately after fear memory recall. These promising data suggest that low-dose MDMA could be effective at eliminating traumatic memories.



Several mechanisms could explain the observed effect of MDMA. MDMA could have modified the traumatic memory by interfering with reconsolidation of the memory of either 1) the *context* in which trauma occurred, or 2) the *association between the context and the traumatic event*. This distinction has important implications for the clinical use of MDMA. If the former is true, then MDMA could potentially weaken *any* contextual memory that is recalled during MDMA-paired therapy. These types of memories can be dissociated at the neuronal level. Context memory is critically dependent on a brain region called the hippocampus, and memory for fear associations depend on the amygdala. MDMA must therefore be acting on a neural circuit that can modify learning in one or both of these brain regions. High-dose MDMA is known to increase activity of many neuromodulators, but systems sensitive to moderate-dose MDMA are not well studied. One neuropeptide sensitive to MDMA is oxytocin, the factor responsible for the sense of well-being associated with MDMA. Interestingly, populations of oxytocin neurons originating in the hypothalamus project to the both the hippocampus and the amygdala. This anatomy puts oxytocin in a position to modulate memory in response to MDMA. The role of oxytocin in memory reconsolidation is understudied. The sole existing paper indicates that a single systemic administration of oxytocin impaired the reconsolidation of a contextual fear memory in rats [4], although the oxytocin circuits sensitive to MDMA were not identified. These data suggest that MDMA could interfere with reconsolidation of fear memory by increasing activity of discrete oxytocin neural circuits originating in the hypothalamus and terminating in the hippocampus or amygdala. **The goals of this proposal are to determine 1) whether MDMA can interfere with reconsolidation of long-term traumatic memories formed during early life, 2) whether MDMA interferes with reconsolidation of**

contextual memories involving the hippocampus, or memories of fear associations involving the amygdala, and 3) the specific oxytocin circuits recruited by MDMA during memory recall.

I believe that am a strong applicant for this New Faculty funding mechanism. My lab is very **productive**. Since starting at UCD, I have appeared as primary (first or last) author on 4 published or in press manuscripts, 3 of which include UCD graduate or undergraduate co-authors. In fact, an undergraduate in my lab is first author on 1 of these papers. I was successful at obtaining external funding my first year at UCD, and applied for 6 other grants for external funding, including 3 NIH grants. I just submitted a revision of one of these NIH grants, marking the 4th NIH grant submitted. We are also very **active**. Six of my lab members attended the Society for Neuroscience conference in October, where they co-presented 3 posters containing new data from my lab. Three of my students successfully obtained UROP grants last year, and 6 more students are applying this year. I will be hosting a visiting researcher from Japan in my lab from 3/16-3/17, who I expect will increase our productivity even further. My lab's achievements are impressive considering that we operate under a tight budget and have **need for additional funds**. In addition to wet lab equipment needs resembling those of a typical molecular biology lab, we need to purchase our rats (\$50-\$270 each) and their housing (\$1.85/cage/day), as well as all the associated behavioral testing equipment. Despite these substantial costs, new behavioral neuroscience faculty at UCD receive about half of the startup funds as their counterparts in Biology and Physics at UCD. Finally, the proposed studies are a logical **extension** of the work I am doing with MAPS funding. I submitted a proposal to MAPS to fund similar studies and others, but their primary investment is in clinical effects of MDMA, so they did not fund my proposal. However, **MAPS wishes to motivate support for this project and is generously willing to supply the MDMA free of charge, and to supplement my existing contract with an additional \$5,000 should this grant be funded**. Thus, this New Faculty grant will have a **large impact** on my research program beyond the requested funds alone.

References:

1. Giustino, T.F., et al., *Revisiting propranolol and PTSD: Memory erasure or extinction enhancement?* Neurobiol Learn Mem, 2016. **130**: p. 26-33.
2. Mithoefer, M.C., et al., *Durability of improvement in PTSD symptoms and absence of harmful effects or drug dependency after MDMA-assisted psychotherapy: a prospective long-term follow-up study.* J Psychopharmacol, 2013. **27**(1): p. 28-39.
3. Oehen, P., et al., *A randomized, controlled pilot study of MDMA-assisted psychotherapy for treatment of resistant, chronic PTSD.* J Psychopharmacol, 2013. **27**(1): p. 40-52.
4. Hou, Y., et al., *Effects of oxytocin on the fear memory reconsolidation.* Neurosci Lett, 2015. **594**: p. 1-5.

Methodology Description

Provide a description of the methodology to be used in approaching the project (one-half page)

We will expose rats to traumatic foot shocks either during early life or during adulthood. Rats will be re-exposed to the trauma context during adulthood in order to reactivate the long- or short-term traumatic memory. Saline or MDMA will be given immediately after memory recall, and ability of MDMA to interfere with reconsolidation will be assessed by re-exposing rats to the trauma context the next day and observing freezing. In different rats, oxytocin neurons in the hypothalamus that project to the hippocampus (context memory) or amygdala (fear associations) will be identified by using viral-mediated neural tract tracing combined with *in situ* hybridization for oxytocin mRNA. The ability of MDMA to activate these oxytocin circuits after fear memory recall will be revealed by labeling for *cfos* mRNA, a marker of recent neural activation, within these identified neurons in rats given saline or MDMA immediately after fear memory recall. If MDMA enables oxytocin to interfere with reconsolidation of context memory, then hippocampal-projecting oxytocin neurons will be activated by MDMA, and MDMA should eliminate other contextual memories in follow-up studies even if they aren't associated with fear. In contrast, if MDMA enables oxytocin to interfere with reconsolidation of memory of context-shock associations, then amygdala-projecting oxytocin neurons will be activated by MDMA, and in follow up studies, MDMA should eliminate memory for auditory cue-fear associations which involve the amygdala but not the hippocampus.

Additional Project Information

(i) How does this project advance the applicant's career

Research: This project will lead to publications, research seminars, and follow-up external funding.

Teaching: These funds will have a big impact on the number of independent study, honors, and graduate students trained in my lab and on their productivity. Dissemination of resulting data will attract attention directed toward my research from the media and scientific community.

(ii) Provide future funding activities, agency, program name, program officer, and deadlines identified

1) Dopamine Circuits Controlling Voluntary Exercise. New R21. BRLE review group, NIMH.

Program officer: Dr. Lois Winsky. Deadline: 6/16/16. **2) Targeting Reconsolidation for the**

Elimination of Fear. New R03. NMB review group, NIMH. Program officer: Dr. Aleksandra Vicentic. Deadline: 2/16/2017.

(iii) What specific outlets for the work accomplished in the project are likely (exhibits, journal articles, etc.). I anticipate this work resulting in at least 1 high-impact paper, 3 - 6 conference abstracts and accompanying presentations, and 1 - 3 seminars. Follow-up funding enabled by this project will result in similar outlets for years to come. My work has a history of media attention (NY Times Magazine, Glamour, Outside Mag, etc.) and I anticipate this project will receive similar attention. Funds from my 2014 ORS large grant led successfully to similar dissemination. My graduate student, Courtney Bouchet, is current writing up those data for submission to the Journal of Neuroscience.

(iv) What other support might arise upon successful completion of the project (matching funds, in-kind support, etc.)

MAPS is willing to supplement my existing contract with another \$5,000 should this grant be funded.

Data from this project will contribute to a planned NIH R03 new submission (see grant #2 above).

Applicant's Pending and Current Funding

(a) Pending proposals: (title, agency, amount, date submitted, when will decisions be made)

Exercise and Dopamine Modulation of Fear Extinction. NIH R15 *Revision*. \$466,500 total costs.

Submitted 2/25/2016. Review date not set, but likely late May 2016.

(b) Current funding: (title, agency, amount, duration)

Modulation of Fear Extinction and Reconsolidation by MDMA. Multidisciplinary Association for Psychedelic Studies. \$20,866. 7/1/2015 – 4/30/2016. This contract funded the preliminary data shown in Figure 1.

Detailed Budget

Provide a detailed budget

Undergraduate research assistant 1 year plus fringe= \$15,102
Professional research assistant (PRA; 30% time) = \$11,118
96 rats (Charles River Labs); 48 @ \$34 each (\$1,632) and 48 @ \$54 each (\$2,592) = \$4,224
Rat housing @ \$1.85 / cage / day = \$2,931
Neural tract tracing virus (provided by Montpellier Institute of Molecular Genetics, France) = \$1,200
Contribution to *in situ* hybridization (DNA cloning, slides, enzymes, reagents, disposables): \$425
Student travel to conferences will be paid for by student UROPs and Dr. Greenwood's startup funds
MDMA generously provided by MAPS = \$0.00 additional
Total: \$35,000. Total ORS contribution: \$25,000. CLAS and department matching: \$5,000. MAPS supplement: \$5,000.

Detailed Budget Justification

Provide a short detailed budget justification

The time consuming nature of these experiments requires skilled assistants. Megan Miner (undergraduate) and Esteban Loetz (PRA) are already skilled at the required techniques, including behavioral testing, stereotaxic injection of virus and *in situ* hybridization. Megan is volunteering more than 20 h / week in the lab, and Esteban is a part time PRA in my lab and I pay 30% of his salary (Dr. Sondra Bland pays the remainder). With help from additional undergraduate volunteers, this project can be completed in a year. I will supervise all aspects of the work, but my time is not listed in the budget because I do not feel using research funds to supplement my salary is appropriate until I have a larger external research grant. 96 rats are required to complete the experiments with all appropriate control groups included. Cost of rat housing is fixed by OLAR. The virus for tract tracing is a Biosafety Level 1 canine adenovirus encoding green fluorescent protein and will be used to identify hypothalamic neurons projecting to the hippocampus and amygdala. My lab has had recent success using this virus and we can vouch for its effectiveness. *In situ* hybridization is required to identify the oxytocin neurons and their activity after MDMA. Costs will exceed the \$425, but I will pay for the remainder out of startup. My limited remaining startup funds are being used on many other productive projects and I cannot afford to complete these studies on startup funds alone. Thank you.

Pledge to Report Signature

I pledge to report to the Office of Research Services the projects outcomes at its conclusion and to update ORS on future developments related to the initial funding.

Signature: 

Date: **2/26/2016**