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PRINCIPAL INVESTIGATOR: William A. Carlezon Jr., Ph.D.

CONTRACTING ORGANIZATION: McLean Hospital
Belmont, MA 02478

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**6. AUTHOR(S)**
William A.. Carlezon Jr., Ph.D.

E-Mail: bcarlezon@mclean.harvard.edu

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McLean Hospital
115 Mill Street
Belmont MA 02478
USA

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**14. ABSTRACT**

This final report describes progress and accomplishments in Years 1-2 of our 2-year award, which was designed to use animal models to understand how nicotine (ingested by Soldiers via smoking or chewing tobacco) affects vulnerability to develop post-traumatic stress disorder (PTSD). We had previously discovered studies in which rats voluntarily self-administer nicotine to the point of dependence, receive fear conditioning (training), and are tested for fear responses 10 days later with no additional access to nicotine have abnormally reduced responses to environments previously associated with the stressor, which we term "context-potentiated startle (CPS)". These previous findings suggest that self-administered nicotine is producing some anti-anxiety (beneficial) effects under these specific conditions. The current studies were designed to see if non-voluntary nicotine administration, modeling the nicotine patch, also produces the same outcomes. Aim 1 was designed to examine the long-term effects of nicotine infusion on fear behaviors when the nicotine is discontinued after the traumatic event. Aim 2 was designed to examine the long-term effects of nicotine infusion on fear behaviors when the nicotine is continued after the traumatic event. Aim 1 results are encouraging, in that it appears that nicotine may have some medically-useful effects in protecting the rats from the behavioral consequences of stress. The most therapeutic-like findings are found at an intermittent duration of pre-treatment (10 days), followed by termination of the nicotine exposure after the trauma. Shorter periods of nicotine have no effects, whereas longer periods of nicotine results in loss of efficacy (see below). Aim 2 results were more variable and thus less encouraging. *Projected to Soldiers*, our Aim 1 data suggest a potentially beneficial effect of using a nicotine patch-like approach to prevent key signs of stress-related illness, although dosing would need to be carefully managed, perhaps by tapering and/or discontinuing treatment for brief periods. These beneficial effects were not seen in Aim 2, when the drug was administered in the time period between the trauma and testing, suggesting the possibility of better outcomes if nicotine treatment is discontinued following the trauma, during periods of recovery.

**15. SUBJECT TERMS**

NONE LISTED

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## 1. INTRODUCTION

Tobacco use is prevalent in Soldiers. It is unknown whether this behavior decreases or increases vulnerability to stress-related illnesses such as PTSD. We have previously shown in rats that voluntary (self-administered) nicotine intake can have anti-anxiety effects in an animal model often used to study PTSD (fear conditioning in rats). Current studies are designed to see if non-voluntary nicotine administration, modeling medical assignment of the nicotine patch to Soldiers, also produces the same outcomes. Tests are designed to be sensitive to beneficial and deleterious effects: if nicotine administration reduces the impact of stress, then it should reduce fear conditioning, whereas if it increases the impact of stress, it should increase fear conditioning. This work may help to devise approaches that decrease new cases of stress-related illnesses in Soldiers via medical administration of nicotine.

## 2. KEYWORDS

Nicotine, stress, nicotine patch, resilience, fear, PTSD, model, rat

## 3. ACCOMPLISHMENTS

**What were the major goals of the project?**

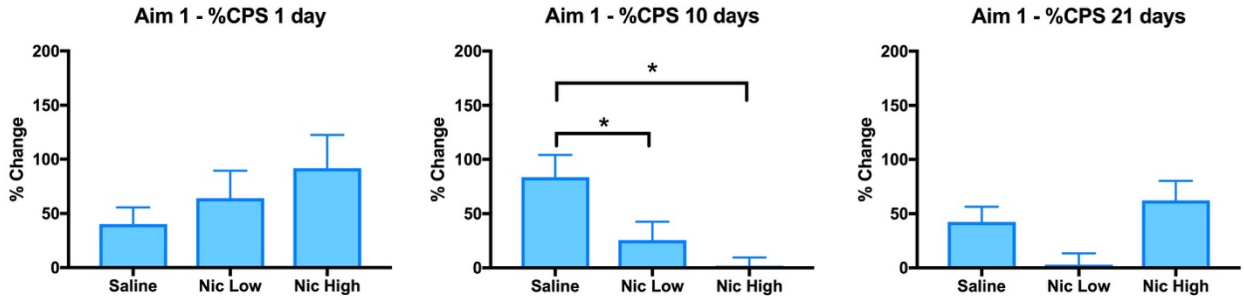
We have shown in rodent models that voluntary nicotine intake can have anti-anxiety effects. The current studies were designed to see if non-voluntary nicotine administration, modeling the nicotine patch, also produces the same outcomes. Tests were designed to be sensitive to beneficial and deleterious effects. There were 2 Specific Aims. Aim 1 was designed to examine the long-term effects of nicotine infusion on fear behaviors when the nicotine is discontinued after the traumatic event. Aim 2 was designed to examine the long-term effects of nicotine infusion on fear behaviors when the nicotine is continued after the traumatic event.

**What was accomplished under these goals?**

The proposed experiments were completed. To summarize, Aim 1 results are encouraging, in that it appears that nicotine may have some medically-useful effects in protecting the rats from the behavioral consequences of stress. The most therapeutic-like findings are found at an intermittent duration of pre-treatment (10 days), followed by termination of the nicotine exposure after the trauma. Shorter periods of nicotine have no effects, whereas longer periods of nicotine

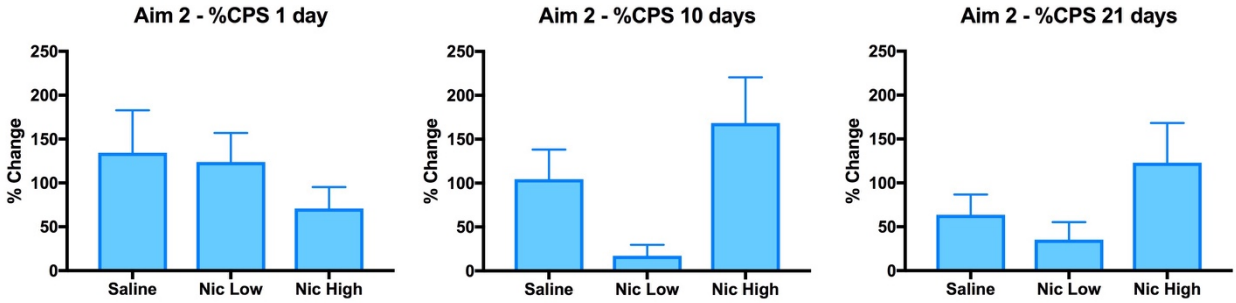
results in loss of efficacy (see below). Aim 2 results were more variable and thus less encouraging.

The premise of the work is that elevation in the acoustic startle response is a translationally-relevant endpoint (i.e., it can be measured in both rats and humans) that indicates hypervigilance, which is a diagnostic sign of PTSD. As such, lower startle responses following exposure to a trauma-related cue is a desirable outcome. As seen below, our Aim 1 data suggest that upon re-exposure to a trauma (footshock)-associated environment, rats given 1 day of subcutaneous nicotine before the trauma (**Left Panel**) tend to show enhancements in




acoustic startle responses (i.e., context-potentiated startle [CPS]), suggesting that acute (short-term) exposure to nicotine actually tends to enhance these fear-like responses. (Note that for clarity, we have combined three test sessions into a single bar, because there were no reliable differences among the tests.) Encouragingly, the response was reduced with 10 days of nicotine pre-exposure, with significant effects (*$P$<0.05) at both the low and high dose of nicotine (**Center Panel**), suggesting that a period of drug exposure is required to produce efficacy and/or tolerance to undesired side effects. However, efficacy is lost with long (21-day) periods of high-dose exposure (**Right Panel**). Together, these data suggest that moderate periods of high-dose exposure would be most effective, with both shorter and longer periods of exposure being ineffective and/or having counter-productive effects.

Aim 2, which examines the effects of including a period of continued nicotine exposure between the time of the trauma and testing—this is the *only* difference between Aim 1 and Aim 2—provide more insight on the relationship between dose and duration of treatment that would enable refinement of an approach that could be applied to humans. Our Aim 2 data suggest that continued nicotine exposure after the trauma may produce some similar effects, particularly at low doses and intermediate (**Middle Panel**) exposure. One area of concern is that the higher




nicotine dose appears to exacerbate CPS, which would be an undesirable outcome. This was not seen in Aim 1 when nicotine exposure was terminated after the trauma, as it might be if nicotine were used as part of a medical treatment regimen rather than when the drug is self-administered by the individual and intake/use is under their control. However, none of the differences were statistically significant, suggesting that the continued administration of nicotine in Aim 2 adds variability not seen when drug administration is terminated between the traumatic event and the time of testing.

**What opportunities for training and professional development has the project provided?**

The two individuals who served as Research Assistants on this project, Ridener and Holloway, gained training and thus critical experience in the lab that will help to guide them in their career choices. Both are going into medical related fields: Ridener is now a graduate student focusing on speech pathologies and rehabilitation, and Holloway will be going to medical school. Holloway had the opportunity to prepare the poster on this work that was presented at the 2018 Society for Neuroscience conference.

**How were the results disseminated to communities of interest?**

The data were presented to the scientific community at the 2018 Society for Neuroscience conference, which was held in San Diego CA in November, 2018. We are currently preparing a manuscript for publication. When we publish our findings, we generally highlight them via social media, which is seen by scientists as well as the lay public.

**What do you plan to do during the next reporting period to accomplish the goals?**

Nothing to Report; this is the final report.

4. **IMPACT**

**What was the impact on the development of the principal discipline(s) of the project?**

*Projected to Soldiers*, our Aim 1 data suggest a potentially beneficial effect of using a nicotine patch-like approach to prevent key signs of stress-related illness, although dosing would need to be carefully managed, perhaps by tapering and/or discontinuing treatment for brief periods to allow recovery from the apparent tolerance-inducing effects seen with the high-dose/long-exposure group. On the basis of Aim1 data, it would appear that there is an ideal treatment dose and duration, and not meeting it and/or exceeding it can each cause loss of the beneficial effects. In addition, these beneficial effects were not seen in Aim 2, when the drug was administered in the time period between exposure to the trauma and testing, suggesting the possibility of better outcomes if nicotine treatment is discontinued following the trauma, during periods of recovery.

**What was the impact on other disciplines?**

The same principles would apply to the use of "medicinal nicotine" in civilian populations to protect against the effects of stress or trauma, particularly when stress or trauma can be anticipated, such as in law enforcement, fire fighters, first responders, and individuals who respond to disasters or mass-casualty events.

**What was the impact on technology transfer?**

Nothing to Report.

**What was the impact on society beyond science and technology?**

As stated above, the ability to protect civilians who engage stressful or traumatic duties on behalf of their communities would have far-reaching significance, including reduction of monetary and human costs of stress- and trauma-related disabilities.

## 5. CHANGES/PROBLEMS

**Changes in approach and reasons for change**

There were no major changes to the experimental design compared to the original funded proposal.

**Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to Report; this is the final report.

**Changes that had a significant impact on expenditures**

Resources were expended on schedule, with no carry-over needed. Over the course of the studies, a small number of the rats developed infections or adverse responses to long-term nicotine administration. These rats were euthanized and replaced at no cost to the Army, via Dr. Carlezon's discretionary sources. All events were described in quarterly reports and requests for additional (replacement) rats were approved by both the McLean IACUC and ACURO.

**Significant changes in use or care of human subjects.**

Not applicable; no human subjects.

**Significant changes in use or care of vertebrate animals.**

Some replacement rats were required, and requests were approved by the McLean IACUC and ACURO.

**Significant changes in use of biohazards and/or select agents**

Not applicable; no biohazards or select agents.

6. **PRODUCTS**

**Publications, conference papers, and presentations**

Society for Neuroscience 2018

Session Type: Poster
Session Number: 414
Session Title: Fear and Aversive Learning and Memory: Modulation
Date and Time: Monday Nov 5, 2018 1:00 PM - 5:00 PM
Location: San Diego Convention Center: SDCC Halls B-H
Abstract Control Number: 13092

Chronic nicotine exposure attenuates fear conditioning: a therapeutic model of the nicotine patch

I. HOLLOWAY JR, M. ROBBLE, E. G. MELONI, R. DESAI, W. A. CARLEZON JR

Department of Psychiatry, Harvard Medical School, McLean Hospital, Belmont MA, USA

**Journal publications**

Manuscript in preparation: we will cite W81XWH-17-1-0001 for support.

**Books or other non-periodical, one-time publications**

Nothing to Report**.**

**Other publications, conference papers, and presentations**

Nothing to Report; we were not invited to Fort Detrick this round.

**Website(s) or other Internet site(s)**

Nothing to Report.

**Technologies or techniques**

Nothing to Report.

**Inventions, patent applications, and/or licenses**

Nothing to Report; invention report (no inventions) submitted separately.

**Other Products**

Nothing to Report**.**

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

**What individuals have worked on the project?**

William A. Carlezon Jr., Ph.D. (PI): 24 months

Rajeev I. Desai, Ph.D. (Co-I): 24 months

Edward G. Meloni, Ph.D. (Co-I): 24 months

Elysia Ridener (Research Assistant): 6 months

Isaiah Holloway (Research Assistant): 16 months

**What other organizations were involved as partners?**

None.

8. **SPECIAL REPORTING REQUIREMENTS**

Not applicable.

9. **APPENDICES**

Poster presented at 2018 Society for Neuroscience conference, San Diego, CA (Poster 414.13, 11/06/2018); attached below

# Chronic intermittent nicotine exposure attenuates conditioned fear: A therapeutic model of the nicotine patch

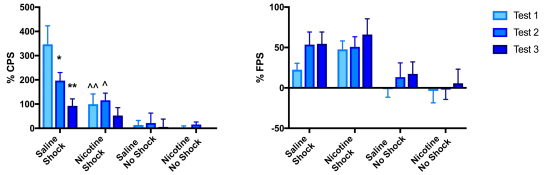
*Isaiah Holloway Jr., Mykel Robble, Edward Meloni, Rajeev I. Desai, and William A. Carlezon Jr.*
Harvard Medical School, Department of Psychiatry, McLean Hospital, Belmont, MA, 02478

414.13

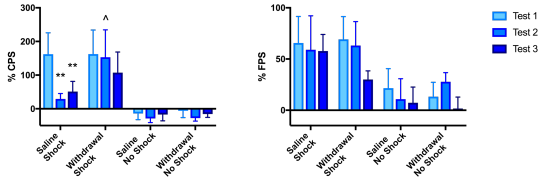
## Background

The use of nicotine products such as cigarettes and smokeless (chewing) tobacco in the military is highly prevalent, but it is not known how nicotine affects vulnerability to stress and stress-related conditions including post-traumatic stress disorder (PTSD). Previous work has demonstrated that nicotine can relieve stress while also enhancing cognitive function. These two broad actions may have opposing effects on vulnerability to stress-related illness such as PTSD, which is thought to involve learning and memory components. We have previously shown that intravenous self-administration (IVSA) of nicotine in rats can reduce the impact of a traumatic event, as reflected by decreased responsivity to a context previously associated with footshock in the fear-potentiated startle (FPS) paradigm. These findings suggest that nicotine use in soldiers might reduce pathological responses that occur in contexts that have similarities with those in which a trauma was experienced, whether in combat settings or after returning home. The present study examined whether the putative beneficial effects of nicotine IVSA on contextual fear learning are retained when the drug is delivered subcutaneously via a iPRECIO$_{TM}$ infusion minipump to more realistically model a safer route of nicotine delivery that is typically achieved by a nicotinic patch.

### Effects of IVSA of nicotine and withdrawal on context- and fear-potentiated startle




Rats with a history of saline IVSA had large increases in %CPS (relative to CPS measured immediately prior to fear conditioning). In contrast, rats with a history of nicotine IVSA showed a significant reduction in %CPS throughout the 3 test sessions. %FPS did not differ between saline and nicotine groups. Rats that did not receive foot shocks during the fear conditioning session showed negligible effects of CPS and FPS.




Rats that were in nicotine withdrawal during fear conditioning had a similar increase in %CPS compared to saline controls, however the nicotine withdrawal group did not show extinction across test sessions. %FPS did not differ between groups. Rats that did not receive foot shocks during the fear conditioning session showed negligible amounts of CPS and FPS. *P<0.05, **P<0.01, within group comparisons to Test 1; ^P<0.05, ^^P<0.01, daily between group comparisons to nicotine withdrawal-shock group, N's=8-10.

## Overall Aims

**Aim 1:** Determine the long-term effects of nicotine on fear when nicotine is discontinued after traumatic event.

**Aim 2:** Determine the long-term effects of nicotine on fear when nicotine is continued after traumatic event.

## Design

**Nicotine or Saline Delivery:** iPRECIO$_{TM}$ infusion minipump programmed to deliver nicotine or saline subcutaneously with a 12hr on and 12 hr off period.

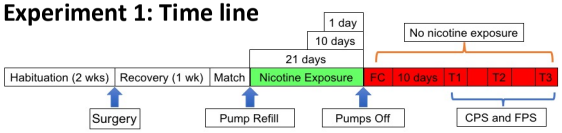
**Nicotine Dose:** 0.3 mg/kg (Low) or 1.0 mg/kg (High)

**Exposure Duration:** 1, 10 or 21 days

**Aim 1:** 10-day withdrawal period before behavioral tests.

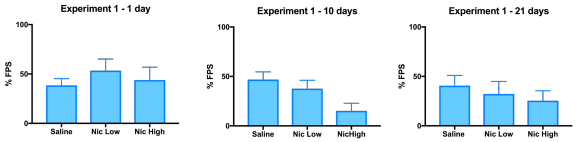
**Aim 2:** Continued nicotine exposure following fear conditioning.

## Experiment 1: Time line




## Experiment 2: Time line


## Experiment 1: The effect of chronic intermittent nicotine exposure on fear when nicotine is discontinued following trauma

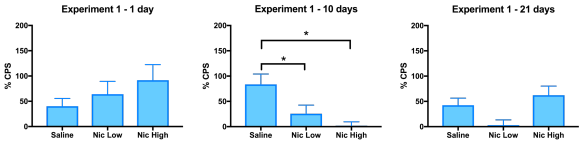



**Saline n = 19**
**Nic Low n = 12**
**Nic High n = 13**

**Saline n = 18**
**Nic Low n = 14**
**Nic High n = 13**

**Saline n = 17**
**Nic Low n = 13**
**Nic High n = 15**

Rats were exposed to chronic intermittent nicotine (0.3 mg/kg/day, 1.0 mg/kg/day, or saline) for either 1, 10, or 21 days prior to fear conditioning. Following fear conditioning, nicotine was immediately discontinued for the remainder of the experiment. After a 10-day withdrawal period rats were tested for fear- and context-potentiated startle in 3 separate sessions, 48 hours apart. Since extinction was not observed across sessions the data were combined for presentation. No significant main or interactive effects on FPS were found.

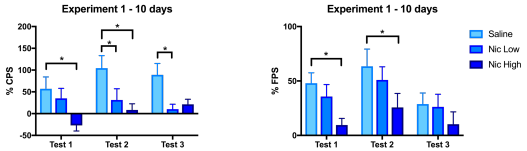



**Saline n = 19**
**Nic Low n = 12**
**Nic High n = 13**

**Saline n = 18**
**Nic Low n = 14**
**Nic High n = 13**

**Saline n = 17**
**Nic Low n = 13**
**Nic High n = 15**

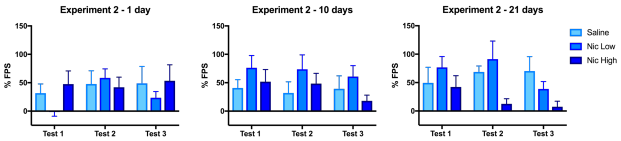
Data were analyzed using a two-way ANOVA (drug dose X treatment duration). There was a nonsignificant trend towards a main effect of treatment duration ($p = 0.0993$) and a significant drug dose x treatment duration interaction ($p = 0.0031$). Follow up post hoc tests revealed that, in the 10-day exposure condition, both the low ($p = 0.047$) and high dose ($p = 0.0038$) of nicotine significantly reduced %CPS relative to saline controls.




**Saline n = 18**
**Nic Low n = 14**
**Nic High n = 13**

To further assess the effect of a 10-day nicotine exposure on FPS and CPS, data analysis was expanded to include all test sessions. The low dose of nicotine significantly reduced %CPS compared to saline controls at Test 2 ($p = 0.0424$) and Test 3 ($p = 0.0269$). The high dose of nicotine significantly reduced %CPS compared to saline controls at Test 1 ($p = 0.0204$) and Test 2 ($p = 0.07$). In addition, the high dose of nicotine also reduced %FPS compared to saline controls at Test 1 ($p = 0.0407$) and Test 2 ($p = 0.0454$).

## Experiment 2: The effect of chronic intermittent nicotine exposure on fear when nicotine is continued following trauma

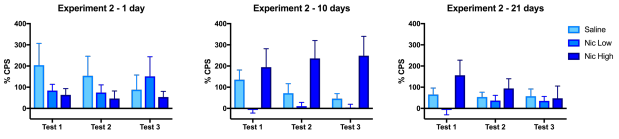



**Sal n = 7**
**Nic Low n = 8**
**Nic High n = 9**

**Sal n = 7**
**Nic Low n = 8**
**Nic High n = 7**

**Sal n = 7**
**Nic Low n = 9**
**Nic High n = 9**

Rats were exposed to chronic intermittent nicotine (0.3 mg/kg/day, 1.0 mg/kg/day,or saline) for either 1, 10, or 21 days prior to fear conditioning. Following fear conditioning, nicotine delivery continued for the duration of the experiment. Ten days after fear conditioning rats were tested for fear- and context-potentiated startle in 3 separate sessions, 48 hours apart. Data from all test sessions are displayed. Data collection is on going, but these results suggest that low dose nicotine suppresses FPS when exposure begins 1 day prior to fear conditioning. In addition, these results suggest that high dose nicotine enhances extinction of FPS when exposure beings 21 days prior to fear conditioning.




**Sal n = 7**
**Nic Low n = 8**
**Nic High n = 9**

**Sal n = 7**
**Nic Low n = 8**
**Nic High n = 7**

**Sal n = 7**
**Nic Low n = 9**
**Nic High n = 9**

Though data collection is on going, results thus far suggest that a 1 day exposure to nicotine prior to fear conditioning reduces CPS. Interestingly, in the 10 day exposure condition the doses of nicotine tested appear to be having opposite effects on CPS; low dose nicotine reduces CPS while high dose nicotine enhances it. A similar, though less stark pattern is apparent in the 21 day exposure condition.

## Conclusions

- Following nicotine IVSA, nicotine reduces contextual fear if trauma is experienced while under the influence of the drug. Experiencing trauma during nicotine withdrawal enhances contextual fear.
- A 10 day passive exposure to chronic intermittent nicotine prior to fear conditioning produces a reduction in contextual fear.
- Under the same conditions, but with nicotine delivery continued following fear conditioning, the nicotine doses examined here produce opposite effects; low dose nicotine reduces, while high dose nicotine enhances contextual fear.
- These studies demonstrate that the protective effect of active nicotine intake on contextual fear remains when using a passive method of drug delivery.
- This insight highlights the potential for moderate durations of nicotine exposure to be used as a preventative therapeutic for PTSD and other stress-related disorders in populations, such as war fighters, who are likely to experience trauma.

Funded by DOD Grant W81XWH-17-1-0001 (to WC)