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14. ABSTRACT

This report describes progress in Year 1 of our 3-year award, which is 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 find that rats which voluntarily self-administer nicotine have increased responsiveness to a startle (white noise burst) stimulus. Projected to Soldiers, this effect could be beneficial under some circumstances and disruptive under others. Following a period of nicotine abstinence, these rats also have elevated levels of fear conditioning (a model of PTSD) that are persistent in repeated tests. Projected to Soldiers, this is an unequivocally negative effect. It is important to remember that nicotine may prove to have beneficial effects in experimental designs that will be tested in Year 2 (e.g., when nicotine self-administration is continued without a period of abstinence), and thus it is premature to make strong conclusions about whether the overall effects of nicotine on Solder behavior and fitness are helpful or harmful.

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INTRODUCTION

Tobacco use (smoking, chewing) is prevalent in Soldiers. Nicotine has two major effects that could influence Soldier behavior and fitness: anxiolytic effects that can have calming actions, and increases in alertness and cognitive function that can enhance aversive or traumatic memories. It is currently unknown whether nicotine use increases or decreases vulnerability to the development stress-related illnesses such as post-traumatic stress disorder (PTSD). It is known, however, that people with PTSD are more likely to smoke when experiencing symptoms. These people report that smoking relieves their symptoms even though objective measurements indicate that it produces increases in hallmark signs of PTSD, such as elevated responsiveness to a startle stimulus (typically a white noise burst). It should be emphasized that nicotine effects on the development of PTSD is a separate question from whether or not people with PTSD smoke, and an important one because it represents an issue for which a research-driven change in policy could affect Soldier health.

Animal models can offer insight on whether nicotine intake affects behavioral and molecular indicators of stress. Use of animal models enables standardization of numerous important factors, including genetics, past experiences, and levels of drug (including nicotine) intake. Perhaps most importantly, animal studies can be designed to be sensitive to beneficial or deleterious effects of nicotine. This is important because if nicotine is found to have beneficial effects, there may be safer ways to administer it to Soldiers (e.g., transdermal patch).

Our research involves a model of nicotine use (voluntary intravenous self-administration of nicotine in rats) and PTSD (fear conditioning, as reflected by fear-potentiated startle, in rats). There are several innovative elements. In addition to the fact that that our research fills a major gap in our understanding of how nicotine might affect the development of PTSD and related behaviors, our ability to use voluntary nicotine intake in rats enables insights not possible with experimenter-delivered nicotine. In general, experimenter-administered nicotine—which can be delivered by systemic injection, by placing an animal in a passive smoke box, or by adding it to the drinking water—produces aversive responses. Most importantly, however, there is good evidence that drugs produce fundamentally different physiological effects when taken voluntarily as opposed to when it is given by the experimenter. In addition, we are able to show that the amount of nicotine voluntarily taken by our animals produces physiological dependence, as defined by the emergence of withdrawal symptoms during periods of drug abstinence. We use fear-potentiated startle in rats because the same technique can be used to study PTSD in humans. Overall, this research is intended to facilitate efforts to devise approaches that decrease new cases of stress-related illnesses in Soldiers by determining how patterns of nicotine exposure affect resilience.

This research was designed to be particularly relevant to Soldiers and thus it has numerous implications for the military. For example, if we discover nicotine has detrimental effects, it may facilitate regulation of nicotine use. In contrast, should we discover that nicotine has beneficial effects, it may be possible to devise safer ways of delivering nicotine or develop new drugs that possess only the helpful effects of the drug. In the later years of our work (i.e., work scheduled to begin in Year 3), we may identify a biomarker of stress vulnerability that might facilitate the development of methods to that enable better ways to match Soldier duties with biological tendencies toward stress resilience or vulnerability. The outcome of our research may also be relevant to understanding how nicotine use in civilian populations affects vulnerability to developing PTSD, particularly among individuals who may routinely be exposed to stress (e.g., law enforcement, first responders).

BODY

Our work will provide insight on 3 basic questions of great relevance to the military. The first question is whether nicotine affects the development of conditioned fear under circumstances where nicotine self-administration is discontinued after exposure to the fear-inducing stressor. This question is addressed in Aim 1, which is currently near completion, and is intended to model Soldiers who are using nicotine during the time of the trauma but then remain abstinent until encountering a stressor that triggers a PTSD-related memory.

The second question is whether nicotine affects the development of conditioned fear under conditions where nicotine self-administration is continued after exposure to the fear-inducing stressor. This is intended to model Soldiers who are using nicotine during the time of the trauma and have continued to use nicotine when encountering a stressor that triggers a PTSD-related memory.

The third question is whether there a significant relationship between nicotine effects on stress-induced activation of the transcription factor CREB in the nucleus accumbens and nicotine effects on the development of conditioned fear. We will address this question in the final year of our 3-year award.

We are close to where we anticipated we would be at the end of our first year (see Timeline, extracted from the proposal): the end of Aim 1. Specifically, we estimate that Experiment 1a is approximately 90% complete, and that Experiment 1b is 25% complete. Now that the studies are ongoing, we are adding animals each week and estimate that we are 4-6 weeks behind where we had hoped to be at this time, despite some minor challenges (explained in detail in quarterly reports, such as transitions in personnel, and equipment failures) we encountered while bringing this work on-line. Currently we do not anticipate any major challenges.

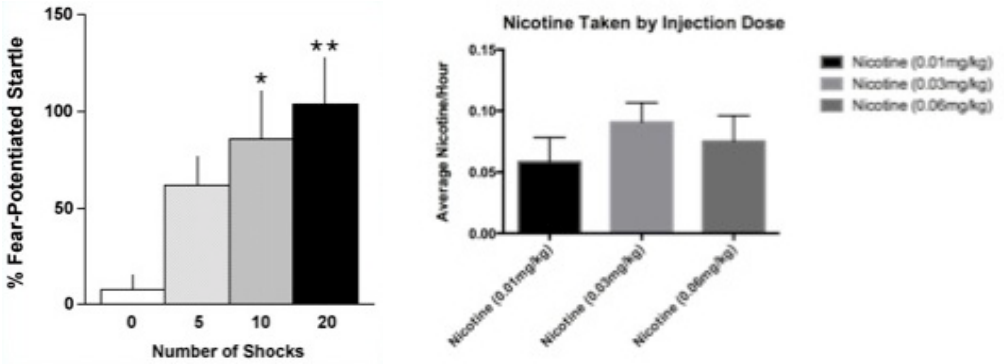
<table>
<tr><th colspan="4">Year 1</th><th colspan="4">Year 2</th><th colspan="4">Year 3</th></tr>
<tr><th>Q1</th><th>Q2</th><th>Q3</th><th>Q4</th><th>Q1</th><th>Q2</th><th>Q3</th><th>Q4</th><th>Q1</th><th>Q2</th><th>Q3</th><th>Q4</th></tr>
<tr><td>IACUC</td><td colspan="3" rowspan="2">Aim 1</td><td colspan="4" rowspan="2">Aim 2</td><td colspan="3" rowspan="2">Aim 3</td><td rowspan="2">Manuscript Preparation</td></tr>
<tr><td>Set up FPS Equipment</td></tr>
</table>

KEY RESEARCH ACCOMPLISHMENTS

We have accomplished virtually all of the Year 1 goals that we described in our proposal. Notable administrative accomplishments include (1) securing approval from McLean's IACUC and Army ACURO to perform the studies, (2) purchasing, setting up, and validating new fear conditioning equipment, and (3) advertizing, hiring, and training new personnel (twice). Scientifically, we have (4) established workable methods for the safe and effective use of extended access to nicotine, (5) validated the normal fear conditioning response in the strain of rats that we are using for our work (Long-Evans), and (6) collected enough data to enable us to submit a scientific abstract for the 2013 Society for Neuroscience (SfN) conference, which will be held in San Diego CA on November 9-13.

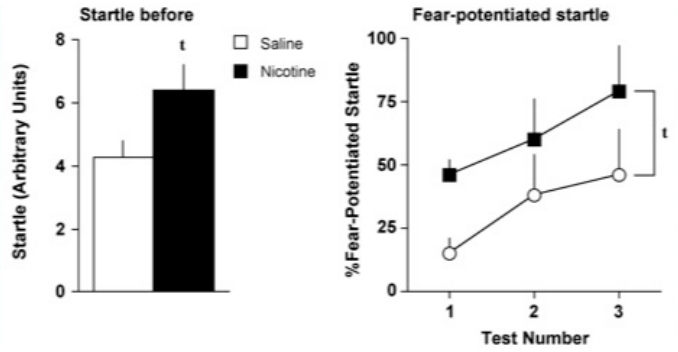
REPORTABLE OUTCOMES

Even at these early stages, with fewer than the final number of rats we need in each group, we already have several outcomes that should be reportable when we complete the studies as proposed. As can be seen in the Figure below (left panel), we first performed a study to confirm that different amounts of fear conditioning (as reflected by the number of footshocks delivered) would produce an orderly amount of fear conditioning (as reflected by % Fear-potentiated startle). This work provided the rationale for using an intermediate number of footshocks (N=10) for fear conditioning, since it is clear that this leaves room to see nicotine-induced increases or decreases in the effectiveness of the footshock. We then established a method of extended-access to nicotine-self administration that produces reliable levels of intake in rats. As can be seen in the Figure below (right panel), even though individual rats have preferences for specific unit doses of nicotine, they self-administer approximately the same amount of nicotine per hour (0.07 mg/kg) regardless of the amount of nicotine they receive per response (lever press; see legend for unit dose).



Most importantly, we find that, under the experimental design used for Aim 1 (a period of nicotine abstinence following the fear conditioning) that voluntary nicotine self-administration has two important effects. First,

during a brief period before fear conditioning commences, we find that nicotine increases sensitivity to a 105 dB startle (noise burst) stimulus (below, left panel, t=trend, P<0.1). Depending on the circumstances, this could be beneficial or deleterious: increased sensitivity to low-level stimuli (e.g., quiet noises) may be beneficial with respect to vigilance, whereas hypersensitivity to loud noises (e.g., explosions) may be disruptive or dangerous. Second, we found that extended nicotine self-administration followed by nicotine abstinence increases fear conditioning in rats (below, right panel; t=trend, P<0.1). Importantly, this finding does not diminish with repeated testing. Projected to Soldiers, this is envisioned as an unequivocally negative effect of nicotine, although it is important to remember that nicotine may prove to have beneficial effects in other experimental designs (e.g., those used in Aim 2).



We describe these findings in our SfN abstract. Going forward, we will report all findings (including "non-effects") each year at the annual Society for Neuroscience conference (see Appendix). We would like to try to package all experiments within a single report for a high-impact journal; however, we will prepare manuscripts for smaller data sets if requested by the Army. The need to disseminate the research in a timely manner was emphasized at the Substance Abuse Research IPR meeting, held at Fort Detrick on September 24-25, 2013.

CONCLUSIONS

Rats that voluntarily self-administer nicotine have increased responsiveness to a startle stimulus. Projected to Soldiers, this effect could be beneficial under some circumstances and disruptive under others. These rats also have elevated levels of fear conditioning that are persistent in repeated tests. Projected to Soldiers, this is an unequivocally negative effect. It is important to remember that nicotine may prove to have beneficial effects in other experimental designs (e.g., those used in Aim 2), and thus it is premature to make strong conclusions about whether the overall effects of nicotine on Solder behavior and fitness are helpful or harmful.

REFERENCES

Webber CJ, Adam CW, Meloni EG, Caine SB, Carlezon WA Jr (2013) Examining the effects of self-administered nicotine in an animal model of post-traumatic stress disorder. To be presented at the Society for Neuroscience Conference, San Diego, CA, November 9-13.

APPENDIX

Webber CJ, Adam CW, Meloni EG, Caine SB, Carlezon WA Jr (2013) Examining the effects of self-administered nicotine in an animal model of post-traumatic stress disorder. 2013 Society for Neuroscience Abstracts, in press

Tobacco use (smoking, chewing) is prevalent in the military. Nicotine can reduce stress and improve coping. It can also enhance cognitive performance and alertness, and facilitate certain forms of learning. These two actions can be conceptualized as having opposite effects on vulnerability to develop post-traumatic stress disorder (PTSD). We designed experiments to examine how nicotine self-administration followed by a period of abstinence affected the development, expression and persistence of PSTD-like symptoms as assessed in the fear-potentiated startle (FPS) paradigm. FPS is a well-validated method of studying stress and memory extinction; exaggerated startle is observed in humans with PTSD as well as in animal models. As a background for these larger experiments, we first examined the development and extinction of fear conditioning in experimentally naïve adult male Long-Evan rats. Rats (N=8 per group) were given 0, 5, 10, or 20 pairings of a 3 sec light cue that co-terminated with a 0.6 mA footshock (fear conditioning). Two days later, FPS was assessed by light cue. As expected, there was a positive linear relationship between number of stimulus pairings and FPS, with 10 pairings causing an intermediate response. All groups demonstrated extinction one week after initial fear conditioning. Control rats, which received only light cue, did not exhibit FPS. We then examined intravenous self-administration in separate rats. In a preliminary study, rats (N=6 per group) were allowed to self-administer either 0.01 mg/kg/inj of IV nicotine (base) or saline for at least 10 daily 3-hr sessions. Criteria for stable self-administration were $\geq$30 injections earned for 3 consecutive sessions with <25% variability across sessions: 100% of nicotine rats and 33% of saline rats met those criteria, earning on average 65.3 ($\pm$11.9) and 30.3 ($\pm$6.9) injections, respectively. In 12-hr overnight sessions, rates varied considerably but peak numbers of injections were 177.5 ($\pm$30.3) and 50.2 ($\pm$5.2), respectively. These data suggest that these conditions support acquisition of nicotine self-administration in consecutive 12-hr sessions, and intake is significantly different from saline. This work provides the basis for larger studies, exploring the effects of intravenously self-administered nicotine on the development and persistence of fear conditioning in rats when nicotine is continued or discontinued following footshock training. These studies will help to determine whether the anxiolytic or pro-mnemonic effects of nicotine prevail in animal models, and thus may provide insight on how nicotine might affect vulnerability to PTSD in Soldiers.

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