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14. ABSTRACT: This final report describes progress and accomplishments in Years 1-3 of our 3-year award, which was designed to use animal models to understand how nicotine (ingested by warfighters via smoking or chewing tobacco) affects vulnerability to develop post-traumatic stress disorder (PTSD). We completed 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. This experimental design is intended to model warfighters who use nicotine during service but later quit. We find that rats which voluntarily self-administer nicotine and are exposed to a stressor (footshock) soon after intake have abnormally reduced responses to environments previously associated with the stressor, which we term "context-potentiated startle (CPS)", but no differences in the ability to learn the association between a discrete cue (a light) and the stressor, which we term "fear-potentiated startle (FPS)". Projected to warfighters, this suggests that self-administered nicotine is producing some anti-anxiety (beneficial) effects under these specific conditions. We also find that rats which voluntarily self-administer nicotine and are exposed to a stressor during nicotine withdrawal (i.e., caused by a missed dose of nicotine) have abnormally persistent CPS, but no differences in FPS. Projected to warfighters, this suggests that nicotine withdrawal is unambiguously detrimental. We also examined other permutations of our experimental design, including those in which access to nicotine is sustained for long periods of time between training and testing. Our findings with this design were conceptually similar to those when nicotine self-administration ceased: nicotine may have some beneficial effects, but nicotine withdrawal is unambiguously detrimental. Our funding period ended on August 31, 2015, and we received a no-cost extension through December 31, 2015 to enable us to finish the experiments. Our findings were presented at the Substance Abuse IPR (Fort Detrick) on 3 occasions: 2013, 2014, and 2015. We have presented abstracts on our work at the Society for Neuroscience meeting in 2013, 2014, and 2015, and are currently preparing a manuscript. We have a pending application (BA150236) requesting support to enable us to examine if the nicotine patch, as modeled in rodents, would have beneficial effects.					
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INTRODUCTION

Tobacco use (smoking, chewing) is prevalent in warfighters. Nicotine has two major effects that could influence warfighter behavior and fitness: anti-anxiety (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 if 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 metrics indicate that it produces increases in hallmark signs of PTSD, such as elevated responsiveness to a startle stimulus (white noise bursts). 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 policy change could affect warfighter health.

Animal models can offer insight on whether nicotine intake affects behavioral 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. 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 [FPS] in rats). We use FPS in rats because the same technique can be used to study PTSD in humans. It is important to emphasize that FPS in rodents is not a complete model of PTSD in humans, but it is often used to study the disorder and it does recapitulate numerous key domains—including an index trauma, persistent fearful memories, and persistent hyperarousal. Our studies have 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. 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. Overall, this research is intended to facilitate efforts to devise approaches that decrease new cases of stress-related illnesses in warfighters by determining how patterns of nicotine exposure affect resilience.

This research has numerous implications for the military. If we discover nicotine has detrimental effects, it may provide a compelling scientific justification to regulate nicotine use in the military. 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. 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., first responders).

Our work provides insight on 2 basic questions. The first question is whether nicotine or nicotine withdrawal affect the development of conditioned fear under circumstances where nicotine self-administration is discontinued after exposure to the fear-inducing stressor. These studies were intended to model warfighters who are using nicotine during the time of the trauma but then remain abstinent leading up to a time where they encounter a stressor that triggers a stress-related memory. The second question is whether nicotine or nicotine withdrawal affect the development of conditioned fear under conditions where nicotine self-administration is continued after exposure to the fear-inducing stressor. These studies were intended to model warfighters 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. We report on our results below.

While our funding period ended on August 31, 2015, we requested and received a no-cost extension. We also used our own discretionary resources to continue the work, at no additional cost to the Army, through December 31, 2015.

KEYWORDS

Nicotine, self-administration, withdrawal, stress, trauma, model, rat

ACCOMPLISHMENTS

By the end of the no-coast extension, we had accomplished the main goals of the work: nicotine or nicotine withdrawal affects the long-term behavioral consequences of exposure to a stressful (traumatic) event. Our

findings, and our interpretations, are as follows. Adult male Long-Evans rats were allowed to self-administer nicotine (0.03 mg/kg/inj) in 12-hr (overnight) extended access sessions in 2-level standard operant conditioning chambers for a minimum of 14 sessions. Criteria for inclusion included IVSA of >0.7 mg/session for 4 out of 5 consecutive sessions and observable signs of spontaneous withdrawal 11.5 hrs after nicotine intake. Each rat in the nicotine exposure condition had a control counterpart that self-administered saline for an equivalent number of sessions. Rats required 21.4 ± 1.9 days (Mean $\pm$ SEM) to meet criteria for stable nicotine IVSA and received a total of 0.99 ± 0.06 mg/kg/session, which corresponds well with the range of nicotine doses known to have rewarding effects in rats; during the session that immediately preceded fear conditioning, nicotine-reinforced responding (reflected by lever-presses at the active lever) was reliably higher than saline-reinforced responding when examined at individual time points over the 12-hr time course of the last session or as an overall mean. Rats were then fear conditioned at one of two time points: either (1) immediately after or (2) 11.5 hrs after their last SA session, a time at which they were experiencing spontaneous nicotine withdrawal. Rats that had self-administered nicotine showed dependence, as indicated by increases in well-characterized spontaneous withdrawal behaviors such as ptosis and shakes/tremors. Fear conditioning consisted of 10 pairings of a 4-sec light (conditioned stimulus; CS) co-terminating with a 0.5-sec 0.6 mA footshock. Two different patterns of post-training nicotine intake were examined: for some rats, nicotine exposure was discontinued between fear conditioning and testing, whereas for others nicotine SA continued. The first pattern is intended to model military personnel who are using tobacco at the time of a traumatic event but then discontinue use (e.g., upon returning home/stateside), whereas the second pattern is intended to model military personnel who are using tobacco at the time of a traumatic event and then continue to use it. At the beginning of the fear conditioning session, match data were collected to guide group assignment such that all groups have equivalent (matched) pre-training startle responsiveness. These sessions also enabled us to confirm that neither nicotine nor nicotine withdrawal caused changes in reactivity (sensitivity) to the footshock during fear conditioning that could explain subsequent behavioral differences seen during the test sessions. At 10 days after fear conditioning, rats were tested three times, at 48-hr intervals; during these intervals, nicotine availability was the same as used in the intervening 10 days between training and testing. We examined two metrics: %CPS (Context-potentiated startle, which reflects responsivity to the general context previously associated with the trauma); and %FPS (fear-potentiated startle, which reflects responsivity to the combination of the context plus the very specific cue that predicted the trauma).

For rats that received fear conditioning immediately after IVSA sessions plus no further nicotine exposure, nicotine has no effect on startle responsivity during match sessions. However, upon re-testing 10 days later with no further nicotine access, rats that had received fear conditioning training immediately after nicotine IVSA showed reduced %CPS and normal %FPS. This 10-day period was designed to allow nicotine dependence to resolve, as described in the literature; indeed, no signs of nicotine withdrawal were observed at the time of Tests 1-3. We interpret these data to indicate that rats that had been self-administering nicotine shortly before a traumatic event later show much less evidence of hyper-vigilance (exaggerated startle responses) than controls when placed back into the general context where they had received the trauma (i.e., CPS data). This effect is independent of the ability to learn about the trauma, which other tests show is intact (i.e., FPS data). Hyper-vigilance and elevated startle responses are disruptive to normal behavior and diagnostic criteria for PTSD in humans, and should not be confused with a healthy effect, heightened cognitive function, or enhanced readiness. Extrapolating these results to warfighters, our findings suggest that nicotine might reduce pathological responses that occur in contexts that have broad similarities with those in which a trauma was experienced, whether in theater or after returning home. ***This would be a beneficial effect of nicotine.***

In contrast, the pattern of results was much different for rats that received fear conditioning during nicotine withdrawal plus no further nicotine exposure. Nicotine withdrawal had no effect on startle responsivity during match sessions. However, upon re-testing 10 days later with no further nicotine access, rats that had received fear conditioning training during nicotine withdrawal showed poor extinction of %CPS and normal %FPS. We interpret these findings to indicate that when rats with reliable nicotine self-administration habits are exposed to footshock during nicotine withdrawal, they later show much more evidence of hyper-vigilance than controls when placed back into the shock-related context. Extrapolating these results to warfighters, this suggests that experiencing a trauma during nicotine withdrawal enhances the pathological responses that occur in contexts with similarities to those in which the trauma was experienced. ***This would be a detrimental effect of nicotine withdrawal (a missed dose of nicotine in nicotine users).***

A broadly similar pattern of results was seen when nicotine self-administration continued during the 10 intervening days between fear conditioning and testing. Again, nicotine had no effect on startle responsivity

during match sessions. However, upon re-testing 10 days later after continued nicotine access, rats that had received fear conditioning training after nicotine self-administration showed brief elevation of %CPS and a brief reduction of %FPS. ***This would be a mixed effect of nicotine***, with improvements in some (FPS) but not other (CPS) domains.

The pattern of results was much different for rats that received fear conditioning during nicotine withdrawal. Again, nicotine withdrawal had no effect on startle responsivity during match sessions. Upon re-testing 10 days later with continued nicotine access, rats that received fear conditioning during nicotine withdrawal showed sustained elevation of %CPS but reduced %FPS as well as enhanced extinction. When comparing the data between experiments, it appears that the negative effect of experiencing a trauma during nicotine withdrawal on %CPS is retained regardless of whether nicotine access continues after fear conditioning. However, it appears that continued nicotine self-administration facilitates extinction of FPS; considering that extinction is a form of new learning that weakens the expression of old memories, this represents a potential pro-cognitive (enhanced learning) effect of continued nicotine access. ***This would be a detrimental effect of nicotine withdrawal together with a beneficial effect of continued nicotine exposure.***

IMPACT

To summarize, the effects of nicotine depend greatly upon the precise testing conditions but in general are beneficial, including reduced anxiety-like responses when encountering contexts or cues associated with a previous trauma and reductions in anxiety-like responses that would normally be expected when a trauma is experienced during nicotine withdrawal. In contrast, the consequences of experiencing a trauma during nicotine withdrawal are unequivocally negative. The variability in our findings—the unique patterns associated with each specific procedure—likely reflect the fact that these rats (like warfighters) all have unique individual histories of drug intake leading up to fulfilling our inclusion criteria for testing, as well as the fact that the experimental design required that some groups receive interaction with the investigator after the trauma (which might reduce anxiety-like responses) whereas others did not. **Despite these differences, our findings suggest that nicotine has some beneficial effects that could be harnessed to improve warfighter health, performance, and safety, but also that nicotine withdrawal should be avoided at all costs.**

On the basis of these findings, we applied for a new grant (BA150236, submitted on June 15, 2015) to examine whether nicotine could be used therapeutically to protect warfighters. Smoking cigarettes and chewing tobacco are not safe methods to deliver nicotine. These “nicotine self-administration behaviors” are linked to decreases in fitness and increases in the risk of diseases such as cancer, both of which are major concerns for warfighters during and after their service. We proposed to determine if the putative beneficial effects of nicotine on contextual fear conditioning are retained when the drug is given by a different (safer) route of administration. Nicotine patches are approved by the Food and Drug Administration (FDA) and could be easily utilized in a military context. Animal models are well-suited for answering the types of research questions described in this proposal, which are intended to provide a compelling scientific justification for studies that could be conducted in humans. Because rats are intolerant of having the patches on their skin, and their fur can interfere with drug absorption, we will approximate the nicotine patch by using subcutaneous minipumps placed underneath the skin. These minipumps can be programmed to deliver nicotine at the same rate as a patch for sustained periods of time. The pumps can also be deactivated to terminate nicotine delivery. Our studies will be designed to ask the question of whether nicotine delivered non-contingently (i.e., not by voluntary self-administration) can produce the same beneficial effects on contextual fear conditioning as when delivered contingently (i.e., by voluntary self-administration). To control these studies properly and obtain information that will be maximally useful to the military, we will examine numerous permutations, including variable degrees of nicotine exposure before and after the trauma. Our hypothesis is that we will be able to identify conditions under which nicotine can have beneficial effects when given by this safer route of administration. Our grant has been reviewed and is currently being considered for funding.

We have presented our findings at the 2013, 2014, and 2015 Society for Neuroscience (SfN) conferences. In addition, the PI (Dr. Carlezon) has presented these findings in-person to the Army at the Substance Abuse IPR meetings in September 2013, 2014, and 2015. We are currently working on a manuscript to submit for peer-reviewed publication.

CHANGES/PROBLEMS

Changes and problems slowed the pace of our work to a much greater extent than we could have anticipated when writing the application. The primary changes involved transitions in personnel. The project required

work in two separate buildings and occasional off-hour (night, weekend, holiday) responsibilities. The first two research assistants (Finn, Lamont) had difficulties managing these responsibilities. Our next research assistant (Webber) worked on the project for 18 months before leaving to become a physician's assistant. The next research assistant (Ridener) worked on the project through the end of the award period. Each transition slowed progress by creating gaps in personnel coverage and the need for training and re-training. Other issues that arose over the course of the project were general difficulties training the rats to stably acquire nicotine self-administration behavior, infections at the site of the intra-jugular self-administration catheter that required euthanizing affected rats, as well as infrastructural (facilities) challenges related to historic snowfall levels during the winter of 2014-2015. We addressed all of these challenges as they arose and, despite them, collected important data that we hope will ultimately contribute to improved health and resilience in the military.

PRODUCTS

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. Presented at the Society for Neuroscience Conference, San Diego, CA, November 9-13, 2013.

Webber CJ, Adam CW, Meloni EG, Caine SB, Carlezon WA Jr (2014) Effects of self-administered nicotine on fear conditioning in rats. Presented at the Society for Neuroscience Conference, Washington DC, November 15-19, 2014.

Ridener E, Adam CW, Stoll K, Webber CJ, Meloni EG, Caine SB, Carlezon WA JR (2015) Effects of voluntary nicotine self-administration on fear conditioning in rats. Presented at the Society for Neuroscience Conference, Chicago IL, October 17-21, 2015.

PARTICIPANTS

William A. Carlezon Jr., Ph.D. (PI): duration of the award period

S. Barak Caine, Ph.D.: duration of the award period

Edward G. Meloni, Ph.D.: duration of the award period

Christopher Adam: duration of the award period

Kristen Finn: initial 2 months of the award period

Evan Lamont: 1 month of the award period

Chelsea Webber: 18 months of the award period

Elysia Ridener: final 17 months of the award period (including no-cost extension).