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PRINCIPAL INVESTIGATOR: Steven F. Maier, Ph.D.

CONTRACTING ORGANIZATION: University of Colorado
Boulder, CO 80309

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Steven F. Maier
Matthew G. Frank

E-Mail: steven.maier@colordo.edu

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14. ABSTRACT

Drug abuse and misuse is a major health hazard in the military as well as in the population more generally. There have been major recent advances in our understanding of the alterations in the brain produced by drugs of abuse, and in how the "addicted brain" differs from the normal brain. However, many individuals who experience, or are exposed to a drug of abusedo not develop addiction, or abuse the drug. For example, the overwhelming majority of patients that receive opiates for painrelief while hospitalized do not develop opiate addiction. That is, use does not always develop into abuse, and the factors thmediate this transition are largely unknown. The development of an understanding of the factors and brain mechanisms that throw the balance towards the development of abuse from use would be a major step in the development of therapies that caameliorate addiction.The core hypothesis is that stressors, via their production of increased glucocorticoids (GCs), sensitize microglia so that these cells produce excessively high levels of inflammatory mediators such as IL-1 when acted upon by drugs of abuse, and that this process is responsible, in whole or in part, for the increased vulnerability to drug abuse produceby stressful experiences. This is a novel, and previously unexplored hypothesis. The work, if successful, could lead to a re-conceptualization of GCs as a sensitization factor that induces a vulnerability to neuroinflammatory processes and thereby open a new field of investigation into the role of stress and GCs in the etiology of substance abuse disorders.

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Introduction

Drug abuse and misuse is a major health hazard in the military as well as in the population more generally. There have been major recent advances in our understanding of the alterations in the brain produced by drugs of abuse, and in how the "addicted brain" differs from the normal brain. However, many individuals who experience, or are exposed to a drug of abuse neither develop addiction, nor abuse the drug. For example, the overwhelming majority of patients that receive opiates for pain relief while hospitalized do not develop opiate addiction. That is, use does not always develop into abuse, and the factors that mediate this transition are largely unknown. The development of an understanding of the factors and brain mechanisms that throw the balance towards the development of abuse from use would be a major step in the development of therapies that can ameliorate addiction.

Drug abuse is exacerbated by deployment in war zones, and particularly by exposure to trauma, resulting in high co-morbidity with PTSD. Although there is a wealth of human and animal data clearly demonstrating that exposure to stressful conditions potentiates drug taking, the development of addiction, and the reinstatement of extinguished drug self-administration, the mechanisms involved are poorly understood, and consequently there are few, if any, preventative or curative treatments. The hallmark of the stress response is an increase in adrenal glucocorticoids (GCs) (cortisol in the human, corticosterone in the rodent), and it is known that the GC response to stressors is involved in the facilitation of addictive processes produced by stress. Thus, for example, adrenalectomy prevents stressor-induced potentiation of drug self-administration, as well as the augmentation of drug-induced dopamine release in reward-related areas of the brain produced by prior stress. However, the mechanism(s) by which stress and GCs exaggerate behavioral and neurochemical responses to drugs of abuse are poorly understood, and so therapeutic targets have correspondingly not been identified. The goals of the present proposal are to further our understanding of how stress and/or GCs potentiate responses to drugs of abuse, and to identify therapeutic targets that would allow the blockade of stress effects on drug use and addiction.

In the first year of this project, we conducted an extensive transcriptional phenotyping of stress-induced sensitization of the neuroinflammatory response to several drugs of abuse including morphine, methamphetamine, and cocaine. In doing so, we have uncovered key experimental parameters (drug x time of drug exposure x brain region) in which stress potentiates the neuroinflammatory effects of drugs of abuse. Thus, the proposed work is off to a good start.

Body

Specific Aim I. Do stress and/or glucocorticoids potentiate neuroinflammatory responses to drugs of abuse?
IA. Acute stress and acute rises in glucocorticoids.
1A1. Stress.

We addressed specific aim 1A1 by conducting a series of initial experiments in which animals were exposed to a single session of acute stress (100, 1.0 mA, 5 s tailshocks delivered via fixed electrodes while restrained in Plexiglas tubes, the standard acute stressor used in our laboratory), or served as home cage controls (HCC). A sample size of 4-6 animals per experimental group was used. 24 hours after stressor exposure, animals were treated with morphine (8 mg/kg ip), methamphetamine (10 mg/kg ip), cocaine (10 mg/kg ip) or vehicle (0.9% saline). 4 hours after drug or vehicle treatment, whole brain was flash frozen in liquid nitrogen and tissue micropunches of regions within brain reward pathways (nucleus accumbens, NAcc; prefrontal cortex, PFC; ventral tegmental area, VTA) were made. It should be noted that the highest proposed doses of drug were used initially to maximize the likelihood of observing stress-induced potentiation of the neuroinflammatory effects of drugs of abuse because prior findings suggest that the lowest doses as proposed in Specific Aim 1A1 may not have been sufficient to induce a neuroinflammatory response. RNA from tissue micropunches underwent transcriptional profiling utilizing real time RT-PCR. This profile consisted of 13 genes, which encompass key mediators of neuroinflammation and glial activation (See **Table 1** for descriptions).

Table 1. Transcriptional Profile of Neuroinflammatory Genes

Gene Name	Function
Interleukin-1 beta (IL-1b)	Pro-inflammatory mediator
Interleukin-6 (IL-6)	Pro-inflammatory mediator
Tumor necrosis factor-alpha (TNFa)	Pro-inflammatory mediator

Nuclear Factor kB inhibitor alpha (NFKBIA)	Induced by NF-kB
Interleukin-1 receptor antagonist (IL-1RA)	Anti-inflammatory mediator
Glial Fibrillary Acidic Protein (GFAP)	Astroglial activation marker
Major Histocompatibility Complex II (MHCII)	Microglial activation marker
Toll-like receptor 2 (TLR2)	Pattern recognition receptor for alarmins
Toll-like receptor 4 (TLR4)	Pattern recognition receptor for alarmins
CD14	TLR4 signaling adaptor
MD2	TLR4 signaling adaptor
Interleukin-1 type 1 receptor (IL-1R1)	Receptor mediating IL-1 beta signaling
NLRP3	Inflammasome mediating IL-1 processing
Beta actin	Structural housekeeping gene

Results

Effect of acute stress on the neuroinflammatory profile of drugs of abuse 4 h after drug treatment

It should be noted that in all descriptions of results, results that are claimed to have occurred are statistically significant. Transcriptional profiling of the NAcc, PFC, and VTA showed that stress potentiated the methamphetamine (METH)-induced gene expression of the pro-inflammatory cytokine IL-1b in all 3 brain regions (**Fig. 1**). Of note with regard to Figure 1, methamphetamine increased IL-1b in non-stressed animals (HCC) compared to vehicle treated HCC animals, whereas exposure to a stressor 24h previously synergized with methamphetamine to significantly increase the IL-1b response to METH. However, for all other neuroinflammatory genes, stress failed to potentiate the effects of cocaine and morphine at this time point in each of the brain regions examined. It is important to consider the caveat here that the kinetics of neuroinflammatory processes are highly dynamic, therefore examination of a single time point post-drug (4h here) may have simply failed to capture the effects of drug treatment, which may have occurred earlier and/or later than 4h. To address this design limitation, we then conducted time course experiments to examine the effects of each drug at earlier (2h) and later (6h) time points after drug treatment, drug exposure being 24 h after stress exposure or control treatment. Transcriptional profiling of neuroinflammatory genes (Table 1) was again conducted on RNA from PFC, NAcc, and VTA after 2h and 6h of drug exposure (Meth, 10 mg/kg; cocaine, 10 mg/kg; morphine, 8 mg/kg). From this time course experiment, transcriptional profiling generated 3744 data points.

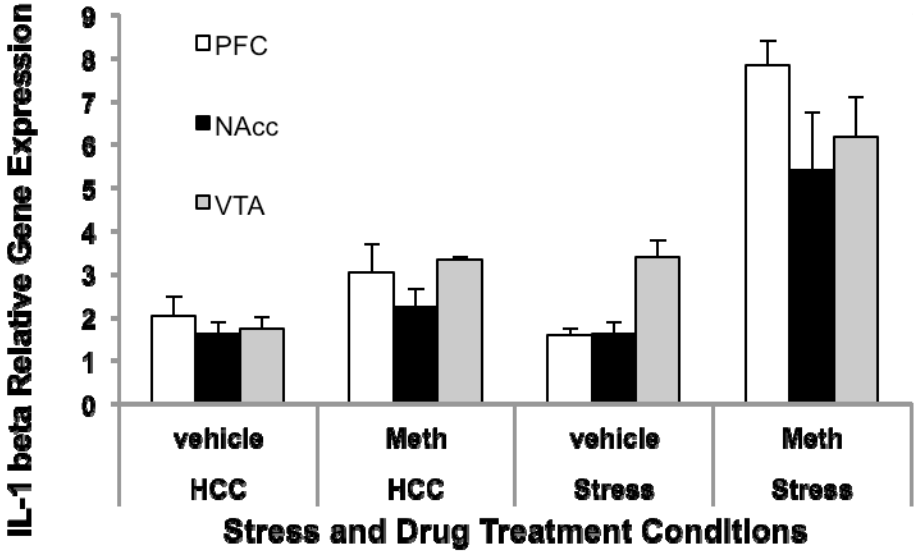

Figure 1. Stress potentiates the pro-inflammatory response to methamphetamine (METH).

Effect of acute stress on the neuroinflammatory profile of drugs of abuse: time course experiments

Cocaine

Transcriptional profiling of the NAcc, PFC, and VTA showed that stress failed to potentiate the neuroinflammatory response to cocaine at 2h or 6h post-cocaine treatment. Further, cocaine, independent of stress treatment, failed to induce changes in the neuroinflammatory profile. In a follow-up experiment, we expanded the time course to include 30 min post-cocaine treatment to determine whether cocaine induced rapid changes in neuroinflammatory response genes. Transcriptional profiling resulted in 672 additional data points. Stress failed to potentiate the effects of cocaine in all 3 brain regions. However, cocaine, independent of stress treatment, profoundly altered the neuroinflammatory response profile in the VTA. Cocaine increased expression of IL-1b, IL-6, TNFa, GFAP, MHCII, TLR2, TLR4, CD14, MD2, IL1R1, and NLRP3. The pattern of data was similar across experimental groups. As one example, please see **Fig. 2**, which shows the cocaine induction of IL-1b only in HCC animals. However, in the NAcc and VTA cocaine failed to alter the neuroinflammatory response profile. The time course experiments clearly show that, while cocaine is neuroinflammatory in the VTA, prior stress exposure does not potentiate these neuroinflammatory effects.

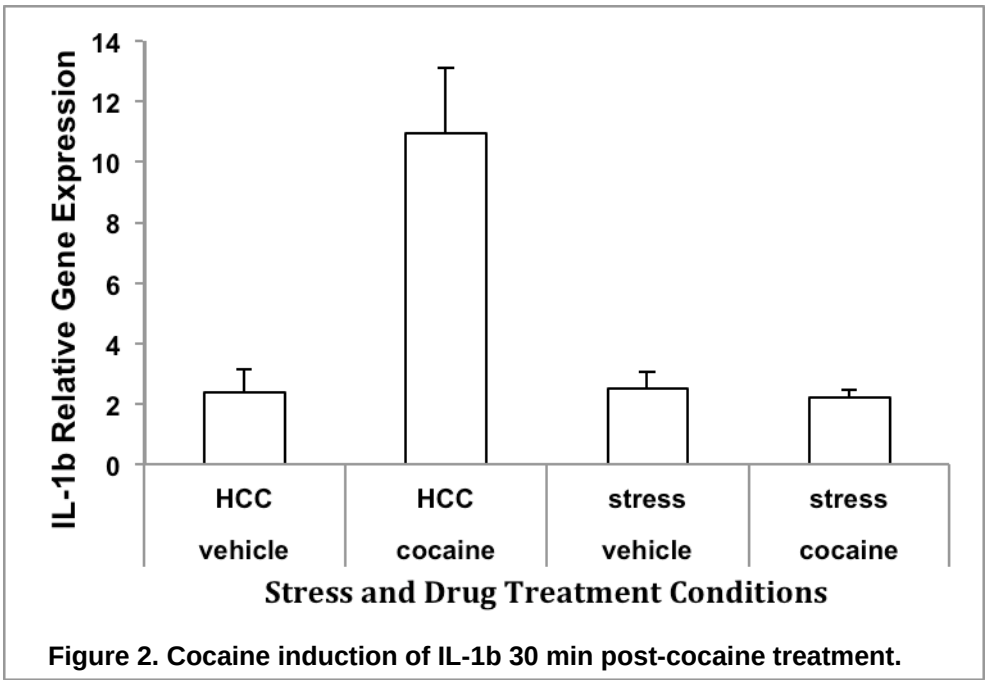

Figure 2. Cocaine induction of IL-1b 30 min post-cocaine treatment.

Morphine

Transcriptional profiling of the NAcc, PFC, and VTA showed that stress potentiated the morphine-induced increase in NFKBIA at 2h post-morphine treatment in the NAcc, but not in the VTA or PFC (**Fig. 3**). Morphine treatment, independent of stress, increased NFKBIA, TNFa, and NLRP3 expression in the VTA 2h post-treatment. NFKBIA is induced by the pro-inflammatory transcription factor NFkB, which is considered a master regulator of pro-inflammatory gene induction such IL-1b. These data suggest that morphine's peak effects on neuroinflammtory genes may occur earlier than 2h. We are currently undertaking studies to explore the effects of morphine 30 min after drug treatment.

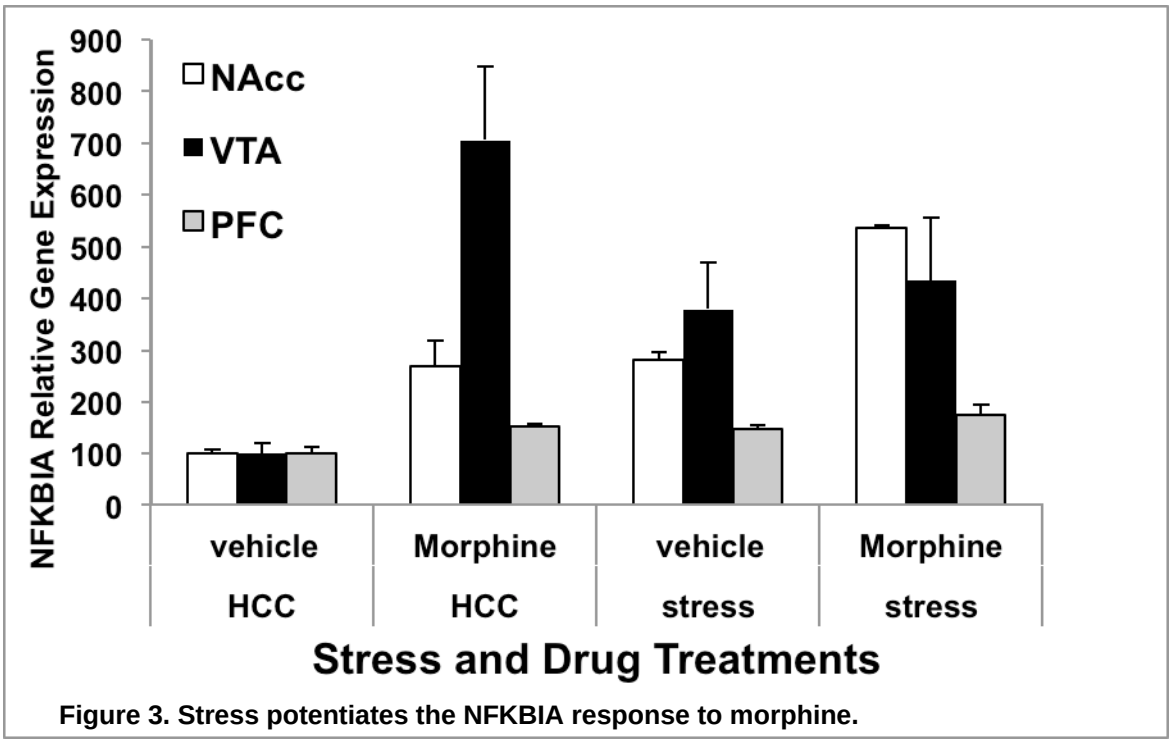

Figure 3. Stress potentiates the NFKBIA response to morphine.

Methamphetamine (METH)

Transcriptional profiling of the NAcc, PFC, and VTA showed that stress potentiated the neuroinflammatory response to METH in NAcc (IL-1b and CD14), VTA (TNFa), and PFC (IL-1b) 2h post-METH treatment. Of the neuroinflammatory response profile, stress induced the greatest potentiation in IL-1b of the NAcc (**Fig. 4**). It should be noted that stress potentiated the METH induced increase in IL-1b greater than 5 fold compared to HCC animals treated with MEH. Further, the data shown in Fig. 4 represents data from the time course study as well as a

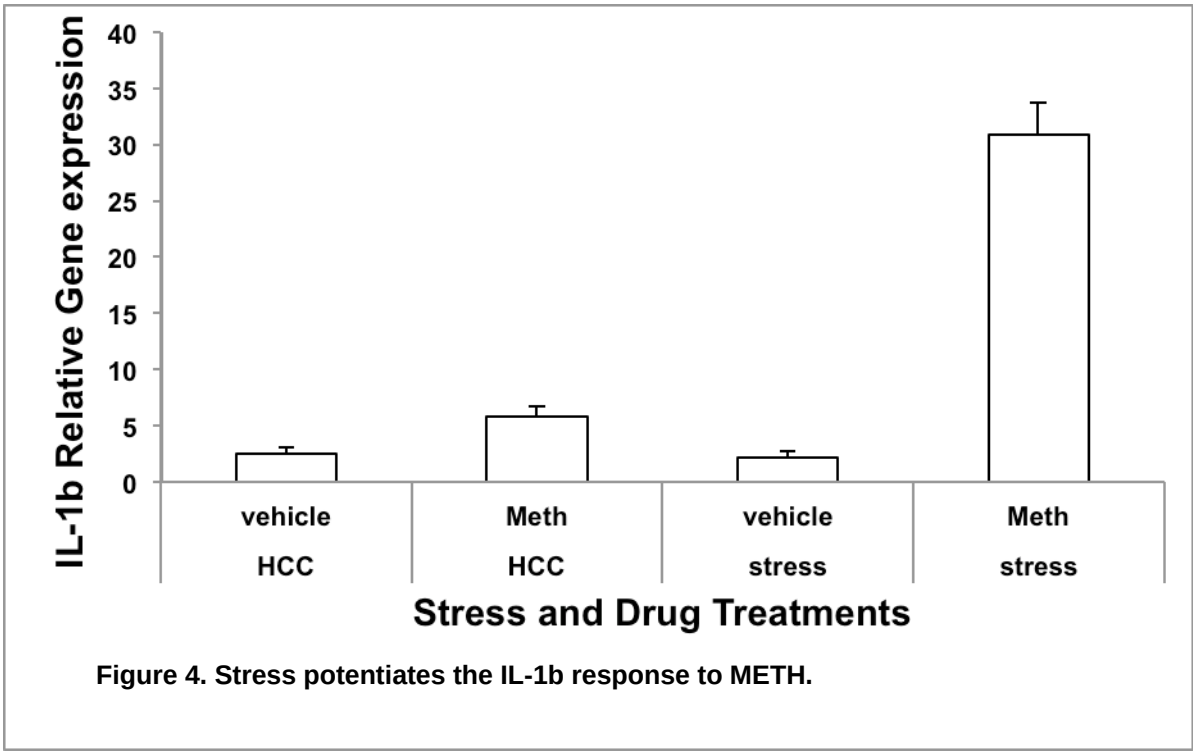

Figure 4. Stress potentiates the IL-1b response to METH.

replication of the 2h time point. METH treatment, independent of stress treatment, resulted in profound increases in the neuroinflammatory profile of the NAcc (CD14, IL-1b, IL1R1, NFKBIA, and TNFa), VTA (CD14, IL-1b, and NFKBIA) and PFC (CD14, IL1R1, MHCII, NFKBIA, and TNFa) 2h post-METH treatment.

Stress

The methamphetamine and morphine data suggest that stress must be sensitizing or priming the CNS neuroimmune microenvironment to the pro-inflammatory effects of methamphetamine. Indeed, exposure to stress resulted in profound increases in several genes of the neuroinflammatory profile 24h after stress in the NAcc (CD14, IL-6, MD2, NFKBIA, and TNFa), VTA (CD14, IL-1b, IL1R1, IL-6, MHCII, NFKBIA, TLR4, and TNFa), and PFC (CD14, IL-6, MD2, MHCII, and NFKBIA). Most notable about these effects of stress is that there is a high degree of overlap between stress modulated genes and drug modulated genes. For example, comparing stress-induced changes in NAcc (CD14, IL-6, MD2, NFKBIA, and TNFa) with METH-induced changes in NAcc (CD14, IL-1b, IL1R1, NFKBIA, and TNFa) reveals several of the same genes (CD14, NFKBIA, TNFa) modulated in both treatment conditions.

Key Research Accomplishments

- We have delineated the conditions (drug x brain region x time) under which stress potentiates the neuroinflammatory response to drugs of abuse.
- The foregoing allows us to focus the research plan and accelerate our understanding of the neuroimmune mechanisms of stress-induced sensitization effects.
- We are currently verifying the effects detailed above at the protein level.

- The findings here of stress-induced potentiation of the neuroinflammatory response to drugs of abuse are novel findings, which we are currently in the process of preparing for publication.

Reportable outcomes

Conclusions

The present findings clearly show that stress sensitizes the neuroinflammatory response to drugs of abuse, and that this effect is most pronounced with methamphetamine. Therefore, the present findings provide a solid basis for exploring the neuroimmune mechanism(s) whereby stress primes the neuroinflammatory response. Clearly, the focus will be on methamphetamine.

References