Original Article

Early Life Stress as a Risk Factor for Substance use Disorders: Clinical and Neurobiological Substrates

Sajoy Purathumuriyil Varghese[1,2], Janitza L. Montalvo-Ortiz[3], John G. Csernansky[3], Rodney I. Eiger[4], Amy A. Herrold[3,5], Maju Mathew Koola[6], Hongxin Dong[3]

ABSTRACT

Background: Early Life Stress (ELS) can profoundly influence an individual's genotype and phenotype. Effects of ELS can manifest in the short-term, late life and even in subsequent generations. ELS activate corticotrophin releasing factor (CRF); CRF influences drug seeking and addiction. The aim of this study was to examine the effects of endogenous elevated levels of CRF on addiction. **Materials and Methods:** Inducible forebrain over-expression of CRF mice (tetop-CRH x CaMKII-tTA) was used for this study. Morphine (10 mg/kg) was administered every other day for 10 days or with increasing doses of morphine: 20, 40, 60, 80, 100, and 100 mg/kg. The behavioral trials including morphine sensitization, Somatic Opiate Withdrawal Symptoms (SOWS) were conducted in a single, open field, activity. After behavioral trial, animals were perfused for immunohistochemistry analysis. **Results:** CRF-over expressed (CRF-OE) mice showed increase in morphine sensitization and withdrawal symptoms after morphine administration compared to wild type (WT) mice. The two-way ANOVA in the morphine sensitization study showed a significant effect of treatment ($P<0.05$) and genotype for distance traveled ($P<0.01$). In the SOWS study, opiate withdrawal symptoms such as rearings, circling behavior, grooming, and jump in CRF-OE were amplified in parallel to WT mice. In the immunohistochemistry study, pro-dynorphine (PDYN) expression was increased after morphine administration in both amygdala and nucleus accumbens (NAcc). **Conclusions:** CRF-OE in the forebrain increases the sensitization and withdrawal symptoms in morphine treated mice. On exposure to morphine, in CRF-OE mice the PDYN protein expression was increased as compared to WT mice in the amygdala and NAcc.

Key words: *Corticotrophin-releasing factor, early life stress, opioid use disorder, substance use disorders, morphine*

Access this article online	
Website: www.ijpm.info	Quick Response Code
DOI: 10.4103/0253-7176.150816	

INTRODUCTION

Early life stress (ELS) consists of a wide spectrum of adverse experiences: Physical, sexual, and emotional abuse; marital discord; parental loss; parental neglect; witnessing domestic violence; parental alcoholism; or living with those who have substance use disorders (SUD), or mental illness.[1] According to Diagnostic and

[1]Department of Mental Health, Captain James A. Lovell Federal Health Care Center, Departments of Psychiatry and Behavioral Sciences, [2]Rosalind Franklin University of Medicine and Science, North Chicago, IL 60064, [3]Northwestern University, Feinberg School of Medicine, Chicago, IL 60611, [4]Jesse Brown Veterans Affairs Medical Center, University of Illinois at Chicago, College of Medicine, Chicago, IL 60612, [5]Center of Innovation for Complex Chronic Healthcare, Edward Hines Jr., Veteran Affairs Hospital, Hines, IL 60141, [6]Department of Psychiatry, Clinical Research Program, Sheppard Pratt Health System, University of Maryland School of Medicine, Baltimore, MD 21204, USA

Address for correspondence: Dr. Sajoy Purathumuriyil Varghese
Department of Mental Health, Captain James A. Lovell, Federal Health Care Center, 3001 Green Bay Road, North Chicago, IL, 60064 USA. Department of Psychiatry and Behavioral Sciences, Rosalind Franklin University of Medicine and Science, 3333 Green Bay Road, North Chicago, IL, 60064, USA. E-mail: sajoy.varghese@va.gov

Statistical Manual of Mental Disorders (Fifth Edition), SUD is a maladaptive pattern of substance use, leading to clinically significant impairment or distress. This relapse is accentuated under stress conditions.[2] There is strong evidence supporting the role of ELS as a risk factor of mental illness. ELS induces pathological brain circuits and cognitive changes that are related to drug addiction.[3] Acute/chronic stress is associated with the motivation to abuse addictive substances.[2]

Early life stress activates the corticotrophin-releasing factor (CRF) system. CRF is defined as a 41-amino acid polypeptide that mediates hormonal, autonomic and behavioral responses to stressors. Activation of CRF triggers the hypothalamic-pituitary-adrenal axis, the main mechanism that mediates stress response. Extra-hypothalamic actions of CRF can kindle the neuronal circuits responsible for stress-induced anxiety, dysphoria, and reinstatement of drug use behaviors.[4] The activation of brain stress systems by CRF may drive dependence and compulsivity in addiction.[2]

In mammals, CRF actions are mediated by two distinct receptor pathways that includes CRF receptor-1 (CRFR1) and -2 (CRFR2).[5] CRF has high affinities towards the mammalian CRFR1 in a nonselective manner. CRFR1 is the primary molecular site for stress activation.[6] CRFR1, a 415-amino acid protein, is the primary receptor subtype expressed in both brain and peripheral tissues.[7] In a genetically inactivated ($CRFR1^{-/-}$) animal model, dopamine-mediated incentive behavioral manifestation of addiction, such as anxiety, is significantly increased. Another study also found increased anxiety-like and somatic signs of opiate withdrawal in $CRFR1^{-/-}$ mice.[8] In addition, preclinical studies have found that CRFR1 antagonists can reverse both state - (protracted abstinence) and stressor-induced reinstatement of drug seeking behavior.[9]

Stress-like (negative emotional) state or stressor exposure is a contributing factor in a majority of relapses in humans. There is strong evidence for a role of amygdala and nucleus accumbens (NAcc) in the mediation of morphine reward behaviors, as well as the modulation of anxiety.[10] In human alcoholics it has been shown that the endogenous opioid system activates the kappa (κ)-opioid receptor by the upregulation of prodynorphin (PDYN). This may be involved in the development of neurocognitive dysfunction associated with drug addiction.[11] Repeated morphine administration leads to long-lasting upregulation of the PDYN expression in the amygdala and NAcc of mice.[12]

In this paper, we examine the morphine sensitization and withdrawal symptoms in CRF-over-expressing (CRF-OE) mice and evaluate the PDYN expression in the amygdala and NAcc of morphine-treated CRF-OE mice. To the best of our knowledge, this is the first study that examined the effects of increased endogenous levels of CRF in the forebrain on morphine sensitization and withdrawal and PDYN expression. We hypothesized that over-expression of CRF in the forebrain may play an important role in increasing the vulnerability to drug addiction by modulating PDYN expression in the brain.

MATERIALS AND METHODS

Animals

All protocols are in accordance with National Institutes of Health guidelines and Animal Studies Committee at Northwestern University Guidelines. C57BL/6 male mice were used in the study. CRF-OE mice were used to mimic the endogenous increased stress response and wild type (WT) mice were used as controls. Mice were housed on a 12 h/12 h light/dark cycle and were given access to rodent food and water *ad libitum*.

Corticotrophin-releasing factor-over-expressing mice were developed as per published literature.[13] Inducible forebrain over-expression of corticotropin-releasing hormone (CRH) mice were generated by crossing male tetop-CRH mice to female mice expressing the tetracycline transactivator under the control of calcium/calmodulin dependent kinase II (CaMKII) promotor (CaMKII-tTA mice from Jackson Laboratory, Bar Harbor, ME, USA). Control mice were mice positive for the tetop-CRH transgene or CaMKII-tTA transgene alone or WT littermates exposed to doxycycline in the same time frame as their respective OE groups.

Drugs

All drugs used were purchased from Sigma (St. Louis, MO, USA) and every dose was freshly prepared before administration. Morphine (10 mg/kg) was diluted by adding 1 ml of 0.9% saline. All compounds and vehicles (saline 0.9%) were administered via the intraperitoneal (i.p.) route in a constant volume of 1 μl/kg of body weight.

Apparatus

All behavioral trials were conducted in a single, open field, activity chamber (interior dimensions, 43 cm × 43 cm × 30 cm) obtained from Any-Maze (St. Albans, VT, USA). The chamber consisted of a polyvinyl chloride floor and acrylic sidewalls with aluminum corner supports. Any-Maze software was used to record the locomotor activity as distance traveled (cm).

Behavioral trials

Experiment 1: Morphine sensitization

Morphine sensitization is a commonly used trial to evaluate the locomotor activity after psychotropic drug administration in animals[14,15] [Figure 1]. Before

behavioral trialing; each mouse was habituated to the apparatus and trialing procedure for 5 min a day for 4 consecutive days [Figure 1]. On the third and final day of habituation, a saline control session was conducted in which each mouse received an injection of saline (1.0 ml/kg, i.p.) 15 min before being placed in the chamber. During drug treatment and locomotor activity trialing, all groups were administered morphine or saline every other day for 10 days. On days in which drugs were not administered, mice remained in the colony room and were left undisturbed.

Experiment 2: Morphine withdrawal symptoms

To study the effects of forebrain CRF-OE in the withdrawal stages of morphine addiction, we used somatic opiate withdrawal trial[16] with slight modifications [Figure 2]. Unlike constant morphine infusions or pellets, intermittent treatment with escalating morphine doses closely parallel the drug intake pattern of humans with opiate use disorder. During 6 consecutive days, WT and CRF-OE mice were treated every 12 h (8-10 a.m.; 8-10 p.m.) with increasing doses of morphine: 20, 40, 60, 80, 100, and 100 mg/kg. Eight hours after last injection, mice were individually placed into transparent Plexiglas cylinders (diameter, 23; height, 50 cm) and observed for the occurrence of opiate withdrawal signs during the following 30 min. Frequency of jump, circling behavior, rearings, and grooming were counted during 5 min intervals in which it occurred. Global score was calculated as the total number of opiate withdrawal symptoms examined.

Immunofluorescence

Thirty minutes after behavioral trialing, mice were anesthetized with pentobarbital (100 mg/kg, i.p.) and perfuse with 4% paraformaldehyde. Brains were quickly dissected and postfixed and coronal sections of 30 μM were collected. Selected sections were rinsed in phosphate-buffered saline and blocked with 5% normal goat serum. Incubation with primary antibody Guinea pig anti-PDYN (1:200, Millipore, CA, USA) was performed overnight at 4°C, followed by Cy3-conjugated goat anti-guinea pig IgG (1:500, Jackson ImmunoResearch, PA, USA) secondary antibody incubation for 2 h at room temperature. Images were captured using a Nikon microscope at a ×40 magnification. Cells with bright fluorescent red granules in the cytoplasm were regarded as positive. At least 10 sections of each animal were counted and averaged.

Data analysis

We used the two-way ANOVA to examine the distance traveled of the locomotor activity in Experiment 1 and Global score, rearings, circling behavior, grooming and jumps in Experiment 2. When genotype (i.e., CRF-OE vs. WT), drug condition (i.e., morphine vs. saline), genotype and drug interactions were found, Bonferonni's *post-hoc* analyses were performed. Significant differences were set at $P < 0.05$.

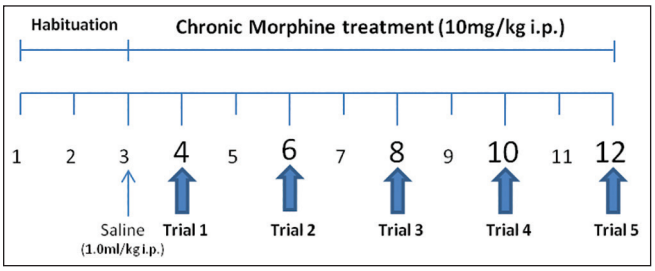

Figure 1: Experimental design of morphine sensitization. During habituation, mice were placed in the activity chamber for 5 min once a day, for 3 consecutive days. On day 3, saline (1.0 ml/kg intraperitoneal [i.p.]) was injected 15 min before habituation. During trialing, morphine (10 mg/kg, i.p.) or saline was injected every other day for 10 days (on days 4, 6, 8, 10, 12) for a total of 5 administrations. After 15 min of injection, mice were placed in activity chamber and locomotor activity was recorded for 5 min, measured as the distance traveled (cm)

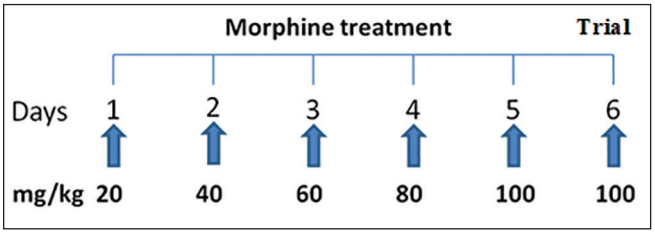

Figure 2: Experimental design of morphine withdrawal. Mice were treated with increasing doses of morphine: 20 mg/kg, 40 mg/ kg, 60 mg/kg, 80 mg/kg, 100 mg/kg, and 100 mg/kg during 6 consecutive days. Eight hours after last injection, mice were examined for the occurrence of opiate withdrawal signs during the following 30 min. Frequency of jump, circling behavior, rearings, and grooming were counted during 5 min intervals in which it occurred. Global score was calculated as the total number of opiate withdrawal symptoms examined

RESULTS

Morphine sensitization

Two-way ANOVA found a significant effect of treatment and genotype ($F_{3,30} = 8.56$, $P < 0.001$) for distance traveled [Figure 3]. *Post-hoc* Bonferroni's trial found a significant increase in distance traveled in mice treated with morphine (WT and CRF-OE) compared with saline-treated during Trial 4 ($P < 0.01$) and Trial 5 ($P < 0.01$). This increase was even higher in morphine treated CRF-OE mice compared with WT ($P < 0.05$) during Trial 5. No significant differences were found between WT and CRF-OE mice in Trial 1, Trial 2, and Trial 3. These data suggests that morphine increases locomotor activity. CRF-OE mice show higher morphine sensitivity as reflected by an increased distance traveled.

Morphine withdrawal

Opiate withdrawal was examined as the number of rearings, circling behavior, grooming, and jumps

[Figure 4]. The total number of morphine withdrawal symptoms was expressed as global score. Global score: Two-way ANOVA found a significant effect of treatment ($F_{1,20} = 15.14, P < 0.001$) on global score. Bonferroni's *post-hoc* trial found a significant increase of global score in morphine treated mice relative to saline ($P < 0.05$). Morphine treated CRF-OE showed a significantly higher global score than morphine treated WT mice ($P < 0.01$). Circling behavior: Two-way ANOVA found a significant effect of treatment ($F_{1,18} = 36.27, P < 0.001$) on circling behavior. Bonferroni's *post-hoc* trial found a significant increase of circling behavior in morphine treated mice relative to saline ($P < 0.01$) in both WT and CRF-OE mice. Jumps: Two-way ANOVA found a significant effect in genotype ($F_{1,15} = 4.90, P < 0.0428$), treatment ($F_{1,15} = 7.60, P < 0.0147$) and interaction of genotype and treatment ($F_{1,15} = 5.82, P < 0.0291$) of jumps. Bonferroni's *post-hoc* trial found a significant increase of jumps in morphine treated mice relative to saline of both WT ($P < 0.05$) and CRF-OE ($P < 0.01$) mice. Morphine treated Corticotrophin-releasing factor-OE mice showed a significant higher number of jumps than morphine treated WT mice ($P < 0.05$). Grooming: Two-way ANOVA found a significant effect in genotype ($F_{1,17} = 9.74, P < 0.0062$), treatment ($F_{1,17} = 27.79, P < 0.0001$) and interaction of genotype and treatment ($F_{1,17} = 21.93, P < 0.0002$) of grooming behavior. Bonferroni's *post-hoc* trial found a significant decrease of grooming in morphine treated mice relative to saline of WT mice ($P < 0.001$). Rearings: Two-way ANOVA found a significant effect in treatment ($F_{1,17} = 25.64, P < 0.0001$) and interaction of genotype and treatment ($F_{1,17} = 6.11, P < 0.0243$) of rearings. Bonferroni's *post-hoc* trial found a significant decrease of number of rearings in morphine treated mice relative to saline of both WT ($P < 0.05$) and CRF-OE ($P < 0.01$) mice. Morphine treated CRF-OE mice showed a significant lower number of rearings than morphine treated WT mice ($P < 0.05$).

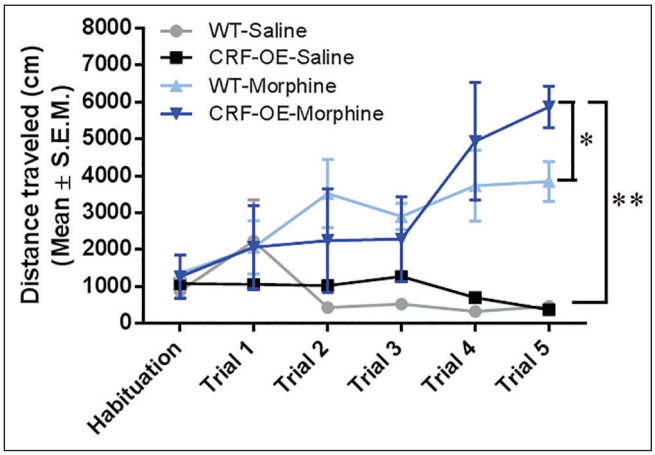

Figure 3: Morphine sensitization is increased in corticotrophin-releasing factor-over-expressing mice. Morphine sensitization is measured as the distance traveled (cm) during a 5 min trial. Data represent mean ± standard error of the mean (n = 5-6). *$P < 0.05$, ** $P < 0.01$

These data suggest that morphine induces a stereotyped behavior including increased circling behavior, increased number of jumps, decreased grooming, and decreased rearings in mice. The total number of morphine related effects (global score) is increased in CRF-OE mice relative to WT mice. This shows that morphine withdrawal symptoms are increased in CRF-OE mice.

Immunohistochemistry: Prodynorphin expression

Expression of PDYN is increased after morphine administration in both amygdala and NAcc ($P < 0.05$) [Figure 5]. Morphine treated CRF-OE showed higher number of PDYN positive cells in both amygdala and NAcc compared with morphine treated WT mice ($P < 0.01$). Our data suggest that CRF-OE mice have

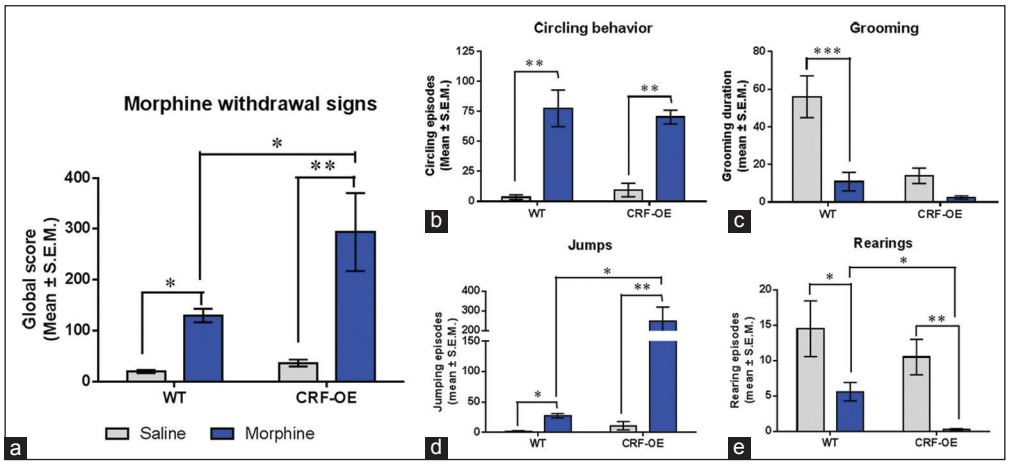

Figure 4: Morphine withdrawal symptoms are increased in corticotrophin-releasing factor-over-expressing (CRF-OE) mice. (a) Global score is expressed as the total number of opiate withdrawal symptoms examined in CRF-OE mice and wild type (WT) mice. Circling behavior (b), jumping frequency (c), grooming behavior (d) and number of rearings (e) are shown for WT and CRF-OE mice. Data represent mean ± standard error of the mean (n = 5-6). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

increased PDYN expression in brain regions related to morphine addiction. PDYN protein activated κ-opioid receptor causing increased locomotor activity and severe opioid withdrawal symptoms. The data indicates that CRF-OE induces PDYN protein in the amygdala and NAcc under morphine conditions.

DISCUSSION

The principle finding of this study is that over-expression of CRF in the forebrain increases the sensitization and withdrawal symptoms in morphine treated mice. In addition, PDYN protein expression is increased in the amygdala and NAcc of morphine treated mice, which is further increased in CRF-OE mice.

Early life stress and psychopathology

Early life stress may affect neurodevelopment through mechanisms such as gene-environment interactions and epigenetic regulation, thus leading to diseases in adulthood. Brain imaging studies have shown that exposure to trauma at an early age can result in several neurostructural changes, such as the reduction of the hippocampus and corpus callosum.[3] Research has shown that brain stress systems can play a role in the compulsivity and persistence of drug taking behavior. There are three stages of neurobiological changes associated with drug dependence, which includes binge/intoxication, withdrawal/negative effects and preoccupation/anticipation related neurobiological circuits. Brain stress systems contribute to the three stages of addiction cycle that endorse the compulsivity of drug-taking in addiction.[17]

Early life stress can alter the normal regulation of CRF. In a study done on precocial species that combines behavioral, endocrinological, and transcriptomic measurements, ELS resulted in a dampened corticosterone response to restraint stress in affected birds and in their male offspring. Stress-specific genes, such as early growth response-1 (EGR1) and CRFR1, were increased immediately after restraint stress, but not under baseline conditions.[18] In animal models, researchers have shown changes in structural plasticity in the mesolimbic reward system that is affected by chronic stress to increase drug self-administration. As a result, medium spiny neurons in the NAcc show altered dendritic spine formation.[19,20]

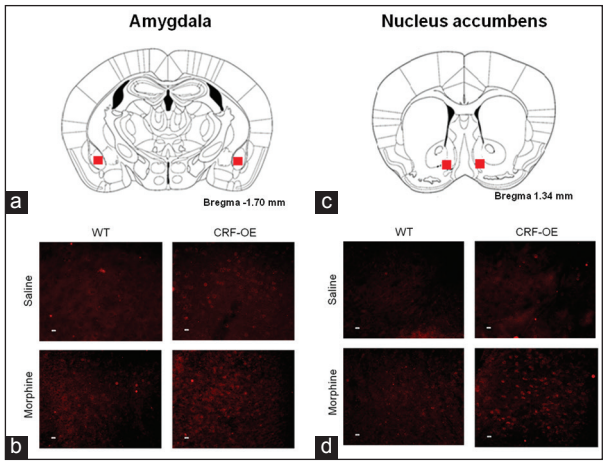

Figure 5: Prodynoprhine (PDYN) expression is increased in the amygdala and nucleus accumbens (NAcc) of corticotrophin-releasing factor-over-expressing (CRF-OE) mice. Coronal sections diagram for amygdala (a) and NAcc (c) are shown. PDYN positive cells in the amygdala (b) and NAcc (d) of saline and morphine treated wild type and CRF-OE mice ($n = 4$)

Experiment 1: Morphine sensitization

Corticotrophin-releasing factor-over-expressing produced increased sensitization to the locomotor effects of morphine. These effects were acutely elevated during the initial administration of morphine. It is interesting to note that that the degree of sensitization observed with morphine was greater in CRF-OE mice compared to WT mice. This suggests a higher sensitization effect to morphine when CRF levels are elevated in the forebrain.

Experiment 2: Morphine withdrawal symptoms

Elevated doses of morphine induce a higher frequency of events associated with morphine withdrawal symptoms behavior, including increased circling behavior, increased number of jumps, decreased grooming, and decreased rearings in mice. Our data indicates a dramatic increase in major somatic signs of opiate withdrawal in CRF-OE mice, suggesting that CRF-OE mice are more vulnerable to somatic opiate withdrawal. This effect could be modulated by the increased PDYN expression in the amygdala and NAcc shell.

Strength and limitations

This study has several strengths that include the detailed examination of morphine effects, including sensitization and withdrawal symptoms. It also examines the effects on PDYN protein expression, modulated by morphine administration, in different brain regions relevant to addiction processes in the brain. Additional studies are needed to confirm our studies, including the examination of a different animal model of morphine addiction such as conditioned place preference.

Clinical implications

Psychiatric patients with a history of ELS may be particularly at high risk to develop SUD and a special screening for abuse is warranted in patients with these histories for personalized management, which is a National Institute of Mental Health Mission.[21] In 603 patients admitted to a psychiatric ward during a period of 1 year at the Atlanta Veterans Affairs Medical Center showed an association between SUDs and childhood

physical and sexual abuse. In addition, the multivariate analyses showed that males with substance-induced mood disorder were more likely (odds ratios 2.3) to report childhood physical abuse compared to males without substance-induced mood disorders ($P = 0.01$).[1]

More research is needed on how to best treat psychiatric patients with histories of ELS to prevent symptoms and to further our understanding of the potential effects of ELS such as abuse on the clinical course of psychiatric illnesses.

CONCLUSION

Our preliminary data suggest that CRF-OE morphine-treated mice exhibited a higher sensitization and were more prone to opioid withdrawal symptoms compared to WT morphine-treated mice. These animal models of ELS may be more sensitive to morphine effects of withdrawal and behavioral/motor sensitization. Therefore, children experiencing ELS may be more vulnerable to developing opiate addiction. Early-stage interventions should be developed to prevent the elevation of CRF levels, thus facilitating the reduction of morphine-induced motor sensitization and withdrawal effects in rodent models of ELS.

ACKNOWLEDGMENTS

The research activities were conducted during General Psychiatry Residency, PGY-4 (2010-2011) and Addiction Psychiatry fellowship (2011-2012) of Dr. Varghese. Dr. Varghese and Janitza L. Montalvo-Ortiz Ph.D. candidate contributed equally with the manuscript preparation and are joint first authors. Dr. Varghese and Janitza L. Montalvo-Ortiz were supervised by Dr. Dong (2010-12). Drs. Csernansky, Eiger, Herrold and Koola contributed with manuscript preparation. A poster abstract was accepted at the 69th Annual Scientific Meeting of Society of Biological Psychiatry (SOBP) in New York, New York on May 8th to 10th, 2014.

REFERENCES

1. Koola MM, Qualls C, Kelly DL, Skelton K, Bradley B, Amar R, *et al.* Prevalence of childhood physical and sexual abuse in veterans with psychiatric diagnoses. J Nerv Ment Dis 2013;201:348-52.
2. Koob GF, Zorrilla EP. Neurobiological mechanisms of addiction: focus on corticotropin-releasing factor. Curr Opin Investig Drugs 2010;11:63-71.
3. Brietzke E, Kauer Sant'anna M, Jackowski A, Grassi-Oliveira R, Bucker J, Zugman A, *et al.* Impact of childhood stress on psychopathology. Rev Bras Psiquiatr 2012;34:480-8.
4. Koob GF. The role of CRF and CRF-related peptides in the dark side of addiction. Brain Res 2010;1314:3-14.
5. Hauger RL, Olivares-Reyes JA, Braun S, Catt KJ, Dautzenberg FM. Mediation of corticotropin releasing factor type 1 receptor phosphorylation and desensitization by protein kinase C: a possible role in stress adaptation. J Pharmacol Exp Ther 2003;306:794-803.
6. Contoreggi C, Lee MR, Chrousos G. Addiction and corticotropin-releasing hormone type 1 receptor antagonist medications. Ann N Y Acad Sci 2013;1282:107-18.
7. Parham KL, Zervou S, Karteris E, Catalano RD, Old RW, Hillhouse EW. Promoter analysis of human corticotropin-releasing factor (CRF) type 1 receptor and regulation by CRF and urocortin. Endocrinology 2004;145:3971-83.
8. Papaleo F, Kitchener P, Contarino A. Disruption of the CRF/CRF1 receptor stress system exacerbates the somatic signs of opiate withdrawal. Neuron 2007;53:577-89.
9. Lu L, Liu D, Ceng X, Ma L. Differential roles of corticotropin-releasing factor receptor subtypes 1 and 2 in opiate withdrawal and in relapse to opiate dependence. Eur J Neurosci 2000;12:4398-404.
10. Gadd CA, Murtra P, De Felipe C, Hunt SP. Neurokinin-1 receptor-expressing neurons in the amygdala modulate morphine reward and anxiety behaviors in the mouse. J Neurosci 2003;23:8271-80.
11. Bazov I, Kononenko O, Watanabe H, Kuntic V, Sarkisyan D, Taqi MM, *et al.* The endogenous opioid system in human alcoholics: molecular adaptations in brain areas involved in cognitive control of addiction. Addict Biol 2013;18:161-9.
12. Turchan J, Lason W, Budziszewska B, Przewlocka B. Effects of single and repeated morphine administration on the prodynorphin, proenkephalin and dopamine D2 receptor gene expression in the mouse brain. Neuropeptides 1997;31:24-8.
13. Dong H, Murphy KM, Meng L, Montalvo-Ortiz J, Zeng Z, Kolber BJ, *et al.* Corticotrophin releasing factor accelerates neuropathology and cognitive decline in a mouse model of Alzheimer's disease. J Alzheimers Dis 2012;28:579-92.
14. Smith MA, Greene-Naples JL, Felder JN, Iordanou JC, Lyle MA, Walker KL. The effects of repeated opioid administration on locomotor activity: II. Unidirectional cross-sensitization to cocaine. J Pharmacol Exp Ther 2009;330:476-86.
15. Stewart J, Badiani A. Tolerance and sensitization to the behavioral effects of drugs. Behav Pharmacol 1993;4:289-312.
16. Núñez C, Martín F, Földes A, Luisa Laorden M, Kovács KJ, Victoria Milanés M. Induction of FosB/DeltaFosB in the brain stress system-related structures during morphine dependence and withdrawal. J Neurochem 2010;114:475-87.
17. Koob GF, Volkow ND. Neurocircuitry of addiction. Neuropsychopharmacology 2010;35:217-38.
18. Goerlich VC, Nätt D, Elfwing M, Macdonald B, Jensen P. Transgenerational effects of early experience on behavioral, hormonal and gene expression responses to acute stress in the precocial chicken. Horm Behav 2012;61:711-8.
19. Li Y, Acerbo MJ, Robinson TE. The induction of behavioural sensitization is associated with cocaine-induced structural plasticity in the core (but not shell) of the nucleus accumbens. Eur J Neurosci 2004;20:1647-54.
20. Robinson TE, Kolb B. Structural plasticity associated with exposure to drugs of abuse. Neuropharmacology 2004;47 Suppl 1:33-46.
21. Insel TR. Translating scientific opportunity into public health impact: a strategic plan for research on mental illness. Arch Gen Psychiatry 2009;66:128-33.

How to cite this article: Varghese SP, Montalvo-Ortiz JL, Csernansky JG, Eiger RI, Herrold AA, Koola MM, *et al*. Early life stress as a risk factor for substance use disorders: Clinical and neurobiological substrates. Indian J Psychol Med 2015;37:36-41.

Source of Support: The manuscript preparation of Maju Mathew Koola was supported by the NIMH T32 grant MH067533-07 (PI: William T. Carpenter, MD) and the American Psychiatric Association/Kempf Fund Award for Research Development in Psychobiological Psychiatry (PI: Koola), **Conflict of Interest:** None declared.