



University of Kentucky
UKnowledge

Oswald Research and Creativity Competition

The Office of Undergraduate Research

2018

Effects of Environmental Conditions on c-fos Expression in Rat Nucleus Accumbens After Remifentanyl

Usman Z. Hamid

University of Kentucky, usman.hamid@uky.edu

Rebecca S. Hofford

University of Kentucky

Michael T. Bardo


University of Kentucky, mbardo@uky.edu

Notes:

Usman Z. Hamid won the first place in the Biological Sciences category.

[Right click to open a feedback form in a new tab to let us know how this document benefits you.](#)

Follow this and additional works at: <https://uknowledge.uky.edu/oswald>

 Part of the [Neuroscience and Neurobiology Commons](#)

[Right click to open a feedback form in a new tab to let us know how this document benefits you.](#)

Recommended Citation

Hamid, Usman Z.; Hofford, Rebecca S.; and Bardo, Michael T., "Effects of Environmental Conditions on c-fos Expression in Rat Nucleus Accumbens After Remifentanyl" (2018). *Oswald Research and Creativity Competition*. 10.

<https://uknowledge.uky.edu/oswald/10>

This Article is brought to you for free and open access by the The Office of Undergraduate Research at UKnowledge. It has been accepted for inclusion in Oswald Research and Creativity Competition by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.

Effects of Environmental Conditions on c-fos Expression in Rat Nucleus Accumbens After
Remifentanyl

Usman Z. Hamid, Rebecca S. Hofford, Ph.D., Michael T. Bardo, Ph.D.

Department of Psychology
University of Kentucky
Lexington, Kentucky 40506

Abstract

Previous studies have shown that adolescents raised in impoverished conditions are more likely to develop drug abuse in adulthood. In addition, both stress-inducing living conditions (impoverishment/isolation) and drugs of abuse may lead to an increase in the c-fos transcription factor in the reward circuit of the brain, particularly in the nucleus accumbens. The aim of the current study was to quantify the number of c-fos positive cells in the nucleus accumbens of enriched and isolated rats exposed to the opioid remifentanil. Thirty-two male Sprague-Dawley rats were raised in either enriched or isolated conditions for one month, after which they received 10 i.v. infusions of 3 $\mu\text{g}/\text{kg}$ remifentanil or saline through the jugular vein. Eighty-five minutes after the last infusion, rats underwent perfusions. After immunohistochemistry was performed on tissue containing the nucleus accumbens, the average number of c-fos positive cells per slice was obtained using ImageJ. Using a 2x2 between subjects ANOVA, with drug and environment as factors, this research demonstrated a main effect of environment on c-fos expression in the nucleus accumbens, with isolated rats expressing more c-fos positive cells than enriched rats. However, there was no significant effect of drug treatment, suggesting that remifentanil did not increase total c-fos as expected. This study demonstrated the cellular consequences of being raised in different living conditions, as it showed that individuals raised under high levels of stress may be at risk of altered cell signaling and gene expression in the reward system of the brain.

Introduction

Over the past decade, human stress levels have been on the rise (Chaby *et al*, 2015). Adolescents suffering from chronic stress may be especially vulnerable to developing mental health problems, such as depression (Young *et al*, 2015). In addition, early life stress may lead to the development of drug abuse in adulthood (Bali *et al*, 2015). Furthermore, there is a positive association between chronic stress and addiction vulnerability in adulthood (Sinha, 2009). Thus, adolescents raised in impoverished conditions have a large chance of developing drug addiction. Current research focuses on determining the effects of stress-induced drug addiction in the brain. However, it is often impractical to extract such information from humans.

In order to study the impact of early life impoverishment on the brain, the rodent isolation model may be used. Conversely, rodent enriched conditions may be used to produce the opposite effect, stimulating a nurturing atmosphere for adolescents. Previous studies have shown that rodents raised in isolated conditions self-administer greater levels of stimulant drugs, such as cocaine, amphetamine, and methamphetamine, compared to their enriched counterparts (Bardo *et al*, 2001; Burke *et al*, 2015; Green *et al*, 2010; Lewis *et al*, 2015).

However, only a few studies have examined the effects of enriched and isolated conditions on opioid reward (Bozarth *et al*, 1989; el Rawas *et al*, 2009). When rats raised in impoverished conditions self-administer opioids, they may be at a greater risk for experiencing changes in the brain. Unfortunately, little is known regarding what opioid-induced changes occur in the brain in differentially reared rats. The present study used the short-acting μ opioid remifentanyl to measure changes in cell activity in the nucleus accumbens of impoverished and enriched rats. The nucleus accumbens is associated with motivation and addiction in the body and is a critical part of the reward circuit of the brain (Quinteros *et al*, 2013). Cells in this region

contain c-fos, a transcription factor involved in regulating the transcription of genetic material. C-fos is an immediate early gene (IEG), becoming activated to a variety of cellular stimuli faster than other transcription factors. The study focused on c-fos because its expression can be used as a marker for cellular activation (Hoffman *et al*, 1993). Cells that express c-fos change the genes that are expressed and are more likely to release neurotransmitters. By understanding how opioids affect cell signaling and gene expression, researchers can create new opioid drugs to treat pain, while at the same time reducing their addictive liability.

The aim of the current study was to quantify the number of c-fos positive cells in the nucleus accumbens after administration of remifentanyl in differentially housed rats. It was hypothesized that regardless of the drug received, rats raised in isolated conditions would have more c-fos positive cells compared to rats raised in enriched conditions. Furthermore, regardless of the living condition, remifentanyl was predicted to increase c-fos expression. Finally, since isolated rats self-administer more remifentanyl than enriched rats (results not shown), it was hypothesized that isolated rats would show a greater remifentanyl-induced change in c-fos expression than enriched rats.

Materials and Methodology

Animals/Housing

Thirty-two male Sprague-Dawley rats arrived at the laboratory 21 days after birth. Upon arrival, rats were randomly placed in 1 of 2 housing conditions, enriched or isolated. The enriched condition (EC) consisted of 8 rats in a metal cage (122 x 61 x 45.5 cm), which included bedding and 14 objects that were switched daily. EC rats were handled daily for approximately 5 minutes. The isolated condition (IC) consisted of 1 rat per metal cage (17 x 24 x 20 cm) with grid floors, no objects, and no bedding. All rats were kept on a 12h light-dark cycle, with lights on at

7:00AM. Rats were given access to food and water *ad libitum*. All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Kentucky and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Surgical Procedures

Between 55-58 days after birth, rats underwent surgery to implant a catheter into the jugular vein. Rats were anesthetized with Ketamine/Xylazine/Acepromazine (75/7.5/0.75 mg/kg). A catheter was inserted into the right jugular vein, threaded under the skin, and attached to a cannula. The cannula was secured to the skull with dental acrylic and four screws. Two rats were lost during the surgeries. All surgical procedures were performed by skilled post-baccalaureate trainees in the laboratory of Dr. Bardo [as detailed in (Hofford *et al*, 2015)].

Test Day/Tissue Collection

Six or seven days after surgery, rats were placed inside standard operant conditioning boxes (28 × 24 × 21 cm; ENV-008CT; MED Associates, St. Albans, VT, USA) with syringe pumps on the outside that provided remifentanil or saline (PHM-100; MED Associates). The chambers consisted of an active lever that gave an infusion of remifentanil or saline and an inactive lever that gave no infusion. Each lever had a cue light located above it. Rats were connected to the syringe pump via tubing strung through a leash, where they received 10 i.v. infusions of 3 µg/kg remifentanil (Sigma-Aldrich, St. Louis MO; experimental group) or saline (control group) through the jugular vein over 15 minutes (one infusion every 90 seconds, 3.4 second infusion time).

Eighty-five minutes after the last infusion occurred, rats were anesthetized with Ketamine/Xylazine/Acepromazine (75/7.5/0.75 mg/kg). Prior to brain removal, rats underwent

perfusions in order to preserve protein configuration and tissue structure. Rats were perfused with 1xPBS followed by 4% paraformaldehyde. After removal, brains were placed in 4% paraformaldehyde overnight at 4 degrees C.

On the day following each perfusion, the brains obtained from the rats were transferred to 30% sucrose in 1 x Phosphate-buffered saline (PBS). Three days after the brains were transferred, they were frozen. Brains were stored at -80 degrees C. Brains were sliced at a thickness of 40 µm on a cryostat and transferred immediately into a 24 well cell culture plate filled with a PBS-thimerosal solution (0.1% w/v thimerosal in 1 x PBS) for storage at 4 degrees C. Three consecutive slices were placed in each well.

Immunohistochemistry

The process of immunohistochemistry was started. Immunohistochemistry was performed on all 30 brains simultaneously. Slices between 2.2 mm and 0.70 mm rostral to bregma were chosen for immunohistochemistry. Tissue was washed 2 times using 1 x PBS for 5 minutes each. Quenching was then performed for 12 minutes using a solution of 1% hydrogen peroxide in methanol. The tissue was then washed 3 times in 1 x PBS for 10 minutes each. Blocking was then done to reduce nonspecific binding. A solution of PBS-thimerosal, 3% donkey serum, and 0.3% Tween20 was prepared, placed on tissue, and incubated for 2 hours. After blocking, c-fos antibody (c-fos H-125, sc-7202, Santa Cruz Biotechnology, Santa Cruz CA) was added to PBS-thimerosal, 3% donkey serum, and 0.3% Tween20 at a concentration of 1:250. The tissue was shaken for 24 hours at room temperature and then for another 24 hours at 4 degrees C.

The next day involved 2 washes of 3 x PBS for 15 minutes each. Two additional washes of 1 x PBS were performed for 15 minutes each. The secondary antibody solution was prepared

in 20 mL PBS-T (0.1% Tween20 in 1xPBS), per assay kit instructions (goat biotinylated anti-rabbit antibody, Vector Laboratories, Burlingame CA). The solution was applied to the tissue, which shook for 1.5 hours.

Three washes of PBS-T were performed for 15 minutes each. The avidin-biotin complex (ABC) was prepared 30 minutes prior to being added to the tissue in 20 mL of PBS-T, per assay kit instructions (Vector Laboratories, Burlingame CA). ABC ran for 1 hour on the tissue.

After the ABC step was completed, the tissue was washed 3 times with PBS-T for 15 minutes each. After the PBS-T washes, two more washes were performed in 1 x PBS for 10 minutes each. 3, 3'-diaminobenzidine (DAB) was prepared in distilled water to stain the tissue and react with the avidin-biotin complex to visualize the c-fos. The DAB was prepared using modified assay instructions provided in the kit (Vector Laboratories, Burlingame CA). Specifically, in 40 mL double distilled water, 6 drops of "buffer," 10 drops of DAB, 6 drops of "H₂O₂," and 4 drops of "nickel solution" were added. Each well was developed with DAB for 40 seconds. DAB was then removed and 1 x PBS was added to stop the reaction. After 5 minutes, the 1 x PBS was removed and a second wash of 1 x PBS was performed for 5 minutes.

Tissue was mounted on slides and cover-slipped using Cytoseal. After drying 24 hours, pictures of the nucleus accumbens were taken using bright field imaging at a magnification of 40x, performed using a microscope with a mounted camera.

Analysis

Average c-fos positive cell number per slice in the nucleus accumbens shell and core were obtained by two people blind to the treatment groups using the cell counter plugin in ImageJ. A two-way ANOVA with living condition and drug treatment as factors was conducted to determine if there was an association between living condition and c-fos expression or

between drug received and c-fos expression. Additionally, an interaction between living condition and drug received and its effect on c-fos expression may be determined. P values less than 0.05 were deemed statistically significant.

Results

Using a 2x2 between subjects ANOVA, with drug (saline, remifentanyl) and environment (enriched, isolated) as factors, this research demonstrated a main effect of environment on c-fos expression in the shell ($F(1, 26) = 15.18, p < 0.05$) and core ($F(1, 26) = 17.40, p < 0.05$) of the nucleus accumbens. IC rats had more c-fos positive cells in the shell and core of the nucleus accumbens compared to EC rats (Figures 1 and 2). However, there was no significant difference found between groups receiving remifentanyl and saline, both in the shell ($F(1, 26) = 1.18, p > 0.05$) and core ($F(1, 26) = 0.53, p > 0.05$) of the nucleus accumbens. Furthermore, there was not a statistically significant interaction found between drug and environment in the shell ($F(1, 26) = 1.81, p > 0.05$) or core ($F(1, 26) = 0.34, p > 0.05$) of the nucleus accumbens.

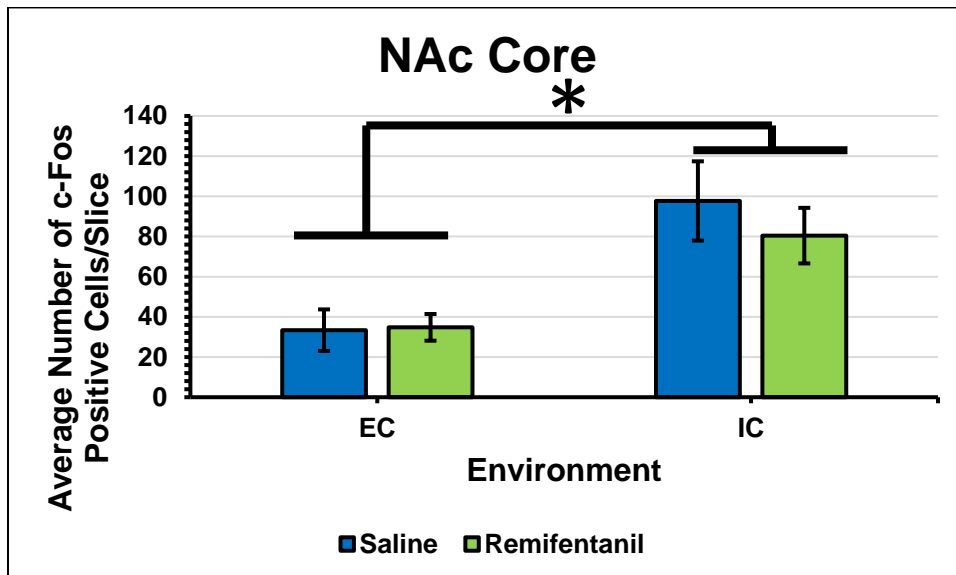


Figure 1: Effect of environment and drug on average number of c-fos positive cells per slice of the nucleus accumbens core.

Rats raised in isolated conditions had significantly more c-fos positive cells in the core of the nucleus accumbens compared to rats raised in enriched conditions. Remifentanyl did not have any statistically significant effect on the number of c-fos positive cells.

* Indicates a significant difference ($p < 0.05$) between rats raised in isolated and enriched conditions.

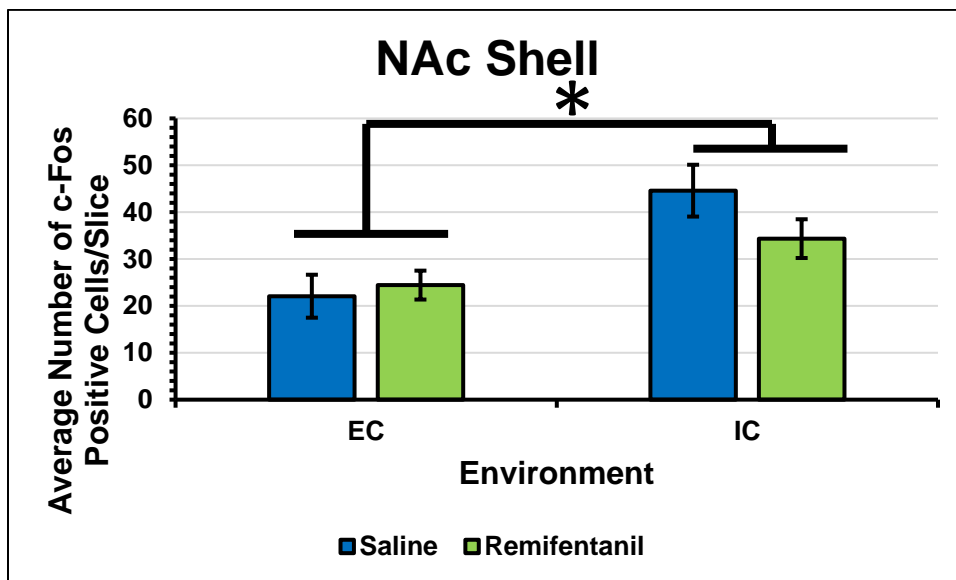


Figure 2: Effect of environment and drug on average number of c-fos positive cells per slice of the nucleus accumbens shell.

Rats raised in isolated conditions had significantly more c-fos positive cells in the shell of the nucleus accumbens compared to rats raised in enriched conditions. Remifentanyl did not have any statistically significant effect on the number of c-fos positive cells.

* Indicates a significant difference ($p < 0.05$) between rats raised in isolated and enriched conditions.

Discussion

The results of this study demonstrate that environment alters c-fos expression in the reward-relevant nucleus accumbens. IC rats had more c-fos positive cells in the nucleus accumbens compared to EC rats. Thus, the hypothesis of this study was partially confirmed. The previous literature on c-fos expression after IC and EC housing differs from the results of the present study. One study demonstrates that IC rats have more Fos+ cells in the nucleus accumbens compared to animals raised in group housing. (Schiavone *et al*, 2009). In contrast, another study shows that Fos levels in the nucleus accumbens do not differ between EC and IC rats (Gill *et al*, 2014). Neither of these studies exposed the rats to a novel environment (operant chambers) with sensory stimulation, which was performed in the present study. Novel environments can increase c-fos expression in the nucleus accumbens (Stone *et al*, 2006), and IC rats are behaviorally more sensitive to novel environments (Levine *et al*, 2007). Thus, it was not surprising that IC rats expressed more c-fos+ cells than EC rats in the nucleus accumbens, despite previous literature.

The hypothesis of the present study also suggested that there would be an interaction between drug and environment, and that remifentanyl would lead to an increase in c-fos positive cells. However, these conclusions were not supported by the results of the study. This study demonstrated that remifentanyl does not have an effect on c-fos expression in the nucleus accumbens, at least not under the conditions used in the current study. However, previous studies show that opioids, such as heroin, increase c-fos expression in the nucleus accumbens (Shalev *et al*, 2003). Additionally, exposure to morphine produces an increase in c-fos positive cells in the

shell and core of the nucleus accumbens (Enoksson *et al*, 2012). Thus, most studies have found that most opioids produce an increase in c-fos in the nucleus accumbens.

There may be several possible reasons why remifentanil did not lead to an increase in c-fos. The c-fos antibody used in the current study was not the same as those reported elsewhere. It is possible this antibody was not specific enough to c-fos to observe any small changes in c-fos expression. Additionally, the 3 µg/kg dose of remifentanil may have been too low to produce any significant changes in c-fos expression. The 90-minute time point after which brains were extracted may have been too soon, as the c-fos may not have been fully expressed. Furthermore, due to the limited number of previous studies, it is possible that remifentanil does not have an effect on c-fos expression. Thus, future studies must be conducted before any definite conclusions are drawn.

In conclusion, this study helped elucidate the cellular consequences of being raised in different living conditions. Individuals raised under high levels of stress may be at risk of altered cell signaling and gene expression in the reward system of the brain.

References

- Bali, A., Randhawa, P., & Jaggi, A. (2015). Stress and opioids: Role of opioids in modulating stress-related behavior and effect of stress on morphine conditioned place preference. *Neuroscience & Biobehavioral Reviews*, *51*(138-50), 138-150. Retrieved December 13, 2015, from <http://www.ncbi.nlm.nih.gov/pubmed/25636946>
- Bardo, M., Klebaur, J., Valone, J., & Deaton, C. (2001). Environmental enrichment decreases intravenous self-administration of amphetamine in female and male rats. *Psychopharmacology*, *155*(3), 278-284. Retrieved December 13, 2015, from <http://www.ncbi.nlm.nih.gov/pubmed/11432690>
- Bozarth, M., Murray, A., & Wise, R. (1989). Influence of housing conditions on the acquisition of intravenous heroin and cocaine self-administration in rats. *Pharmacology Biochemistry and Behavior*, *33*(4), 903-907. Retrieved December 13, 2015, from <http://www.ncbi.nlm.nih.gov/pubmed/2616610>
- Burke, A., & Miczek, K. (2015). Escalation of cocaine self-administration in adulthood after social defeat of adolescent rats: Role of social experience and adaptive coping behavior. *Psychopharmacology*, *232*(16), 3067-3079. Retrieved December 13, 2015, from <http://www.ncbi.nlm.nih.gov/pubmed/25943168>
- Chaby, L., Cavigelli, S., Hirrlinger, A., Caruso, M., & Braithwaite, V. (2014). Chronic unpredictable stress during adolescence causes long-term anxiety. *Behavioural Brain Research*, *278*(492-5), 492-495. Retrieved December 13, 2015, from <http://www.ncbi.nlm.nih.gov/pubmed/25448433>
- Enoksson, T., Bertran-Gonzalez, J., & Christie, M. (2012). Nucleus accumbens D2- and D1-receptor expressing medium spiny neurons are selectively activated by morphine

- withdrawal and acute morphine, respectively. *Neuropharmacology*, 62(8), 2463-2471.
Retrieved December 13, 2015, from <http://www.ncbi.nlm.nih.gov/pubmed/22410393>
- Gill, M., Weiss, M., & Cain, M. (2014). Effects of differential rearing on amphetamine-induced c-fos expression in rats. *Drug and Alcohol Dependence*, 145(231-4), 231-234. Retrieved December 13, 2015, from <http://www.ncbi.nlm.nih.gov/pubmed/25454411>
- Green, T., Alibhai, I., Roybal, C., Winstanley, C., Theobald, D., Birnbaum, S., . . . Nestler, E. (2010). Environmental Enrichment Produces a Behavioral Phenotype Mediated by Low Cyclic Adenosine Monophosphate Response Element Binding (CREB) Activity in the Nucleus Accumbens. *Biological Psychiatry*, 67(1), 28-35. Retrieved December 13, 2015, from <http://www.ncbi.nlm.nih.gov/pubmed/19709647>
- Hoffman, G., Smith, M., & Verbalis, J. (1993). C-Fos and Related Immediate Early Gene Products as Markers of Activity in Neuroendocrine Systems. *Frontiers in Neuroendocrinology*, 14(3), 173-213. Retrieved December 13, 2015, from <http://www.ncbi.nlm.nih.gov/pubmed/8349003>
- Levine, J., Youngs, R., Macdonald, M., Chu, M., Leeder, A., Berthiaume, F., & Konradi, C. (2007). Isolation rearing and hyperlocomotion are associated with reduced immediate early gene expression levels in the medial prefrontal cortex. *Neuroscience*, 145(1), 42-55. Retrieved February 16, 2016.
- Lewis, C., Staudinger, K., Tomek, S., Hernandez, R., Manning, T., & Olive, M. (2015). Early life stress and chronic variable stress in adulthood interact to influence methamphetamine self-administration in male rats. *Behavioural Pharmacology*. Retrieved December 13, 2015, from <http://www.ncbi.nlm.nih.gov/pubmed/26176409>

- Quinteros, G. (2013). Role of nucleus accumbens glutamatergic plasticity in drug addiction. *NDT Neuropsychiatric Disease and Treatment*, 9(1499-512), 1499-1499. Retrieved December 13, 2015, from <http://www.ncbi.nlm.nih.gov/pubmed/24109187>
- Rawas, R., Thiriet, N., Lardeux, V., Jaber, M., & Solinas, M. (2008). Environmental enrichment decreases the rewarding but not the activating effects of heroin. *Psychopharmacology*, 203(3), 561-570. Retrieved December 13, 2015, from <http://www.ncbi.nlm.nih.gov/pubmed/19005643>
- Schiavone, S., Sorce, S., Dubois-Dauphin, M., Jaquet, V., Colaianna, M., Zotti, M., . . . Krause, K. (2009). Involvement of NOX2 in the Development of Behavioral and Pathologic Alterations in Isolated Rats. *Biological Psychiatry*, 66(4), 384-392. Retrieved December 13, 2015, from <http://www.ncbi.nlm.nih.gov/pubmed/?term=Schiavone 2009 fos>
- Shalev, U., Robarts, P., Shaham, Y., & Morales, M. (2003). Selective induction of c-Fos immunoreactivity in the prelimbic cortex during reinstatement of heroin seeking induced by acute food deprivation in rats. *Behavioural Brain Research*, 145(1-2), 79-88. Retrieved December 13, 2015, from <http://www.ncbi.nlm.nih.gov/pubmed/14529807>
- Sinha, R. (2008). Chronic Stress, Drug Use, and Vulnerability to Addiction. *Annals of the New York Academy of Sciences*, 1141, 105-130. Retrieved December 13, 2015, from <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2732004/>
- Stone, E., Yan, L., Ahsan, M., Lehmann, M., Yeretsian, J., & Quartermain, D. (2006). Role of CNS α 1-adrenoceptor activity in central fos responses to novelty. *Synapse*, 59(5), 299-307. Retrieved December 13, 2015, from <http://www.ncbi.nlm.nih.gov/pubmed/?term=Stone 2006 novel fos>

Young, C., & Dietrich, M. (2015). Stressful Life Events, Worry, and Rumination Predict Depressive and Anxiety Symptoms in Young Adolescents. *J Child Adolesc Psychiatr Nurs Journal of Child and Adolescent Psychiatric Nursing*, 28(1), 35-42. Retrieved December 13, 2015, from <http://www.ncbi.nlm.nih.gov/pubmed/25808272>