

Original Research Article

Susceptibility of drug-seeking and taking behaviors increases through dysregulation of copper and zinc and impaired prefrontal function in addiction period in male rats

Abstract

Introduction: Drug addiction is a condition that in some occasions occurs with relapsing episodes. Reducing these relapsing episodes slows progression of recreational abuse with least adverse effects to everyday abuse with the most debilitating adverse effects.

Method and material: In this study 16 male Sprague-Dawley rats weighing 200 to 250 gram were divided into 2 groups: control and morphine-received. At the end of experiment sucrose consumption, salt appetite, novelty-seeking behavior, zinc in serum, copper in serum and prefrontal cortex stress-oxidative status for MDA (Malondialdehyde) were assessed.

Result: Sucrose and salt consumption were increased in morphine-receiving rats compared to control rats. Also, Novelty-seeking behavior was increased in morphine-receiving rats compared to control rats. Copper in serum was increased in morphine-receiving rats compared to control rats. Paradoxically zinc in serum was reduced in morphine-receiving rats compared to control rats. Oxidative-stress status as assessed for MDA in prefrontal cortex was increased in morphine-receiving rats compared to control rats.

Conclusion: involvement of prefrontal cortex in morphine-receiving period can be responsible for occurrence of drug-seeking and taking behavior. In this sense, copper and zinc can play a pivotal role.

Key words: morphine, sucrose, salt, MDA, prefrontal, glutathione, copper and zinc

22 **Introduction**

23 There is a wide range of variability for the outcome of addiction in different people. The adverse
 24 effects vary from mild cognitive and emotional disturbances to compulsive drug seeking and
 25 taking behavior (1). One of the main problem with these adverse effects is that addicted person
 26 seeks for more abusing despite its adverse effects (2). Applying effective treatments for avoiding
 27 entrance of addicted person to compulsive drug taking during abusing drugs such as morphine
 28 can be an effective strategy.

29 Addiction is a multistage process that finally develops to compulsive-drug taking behavior in a
 30 proportion of abusers (3). The main problem is why some abusers develop such devastating
 31 behavior. In this study for answering this question rats received morphine every day for
 32 inducing addiction with normal dose of morphine. According to previous studies for inducing
 33 addiction constant dose can induce addiction and high dosage application makes no difference
 34 (4). This protocol induces every day abuse (4).

35 Prefrontal cortex is the brain region responsible for personality and performing planable
 36 executive function (5, 6). Also memory function is dependent on proper function of prefrontal
 37 cortex (7). In recent studies prefrontal cortex dysfunction has been associated with relapse and
 38 compulsive drug seeking behavior (8). However, the role of oxidative-stress in this regard has
 39 not been studied.

40 In animal studies the experiments that is applied for assessing for relapse and drug seeking and
 41 taking behavior is increase in salt appetite (9), sucrose preference test (10) and novelty seeking
 42 behavior (11).

Therefore this study was designed for investigating the devastating effects of morphine on normal Prefrontal function, and assessing the resultant increase to more abuse with the above behavioral test. Also, the importance of copper and zinc were investigated in this regard.

Method and Material

Animal care: the experimental protocols followed in this study were conformed to the guidelines for the care and use of laboratory animals published by national institution of health (NIH Publication No. 85-23, revised 1996) and was further approved by the institutional ethical committee at Tehran University of medical science (Tehran, Iran).

Animals: In this study male sprague-dawley rats weighting 200 grams were used. In each group we use 8 rats.

Addiction: For induction of addiction rats were received 0.75 mg/rat/interaperitoneally/day morphine (4). Based on previous studies there was no difference between applying high dose of morphine and medium dose of morphine for addiction induction (4). During behavioral experiments morphine injection was done as described.

Sucrose preference test

The rats were submitted to a 48 h period drinking both sucrose and water for adaptation to sucrose solution taste. For 3 days water was the only available fluid. In the day of experiment rats were placed individually in the test cage, first with no access to fluid for 3 h, and then they had access to the two bottles, one containing tap water and the other a 1% sucrose solution. Fluid intake was measured by percentage sucrose consumption to total fluid intake (10).

Evaluation of NaCl appetite

64 In this experiment we removed food for 24h. After that we offered NaCl 3% for one hour. Total
65 consumption was calculated (9).

66 Social interaction test

67 The social interaction test was first introduced by File and Hyde (1978). This test involves
68 placing a pair of animals in an arena and measuring the amount of time engaged in such
69 behaviors such behavior as grooming, sniffing, and boxing. Social interaction has been validated
70 repeatedly as an index of anxiety related disorder (12). A modification of the standard social
71 interaction test was used to reduce the number of animals needed for experiment. According to
72 file (1993), the most sensitive procedure is to match up pairs of rats that have the same treatment
73 on the basis of their body weights and then treat the number of interactions by pairs as the unit of
74 measure. During the 5 minute session time spent in social interaction (grooming, sniffing,
75 following) were scored individually for each rats (13).

76 Copper and zinc assessment

77 For obtaining plasma, after thoracotomy before paraformaldehyde perfusion five milliliter blood
78 was taken from left heart. After processing the final solution was examined with atomic
79 spectroscopy (Varian-220-FS-aa). After obtaining absorbed wavelength it was adjusted with
80 calibration curve and expressed as p.p.m (14).

81 MDA (Malondialdehyde) assessment

82 Total MDA was measured according to thobarbitic acid (TBA) reaction. Simply for performing
83 this analysis TBA 1% in HCl 5N and trichloroacetic acid (TCA) 20% in NaOH 1N were mixed
84 together. Then 100 microliter of homogenized sample was added to the above mixture. After 90

85 minutes of boiling, the final solution developed brown color, and maximum absorbance was
86 measured at 532 nm with the spectrometer (15).

87 Glutathione (Malondialdehyde) assessment

88 DTNB was used as the reaction substrate for estimating the amount of reduced glutathione. For
89 performing this experiment Tris buffer, DTNB and methanol were used. 100 microliter of
90 homogenized sample was added to the above mixture. The final solution developed yellow color,
91 and maximum absorbance was measured at 412 nm with the spectrometer (16).

92

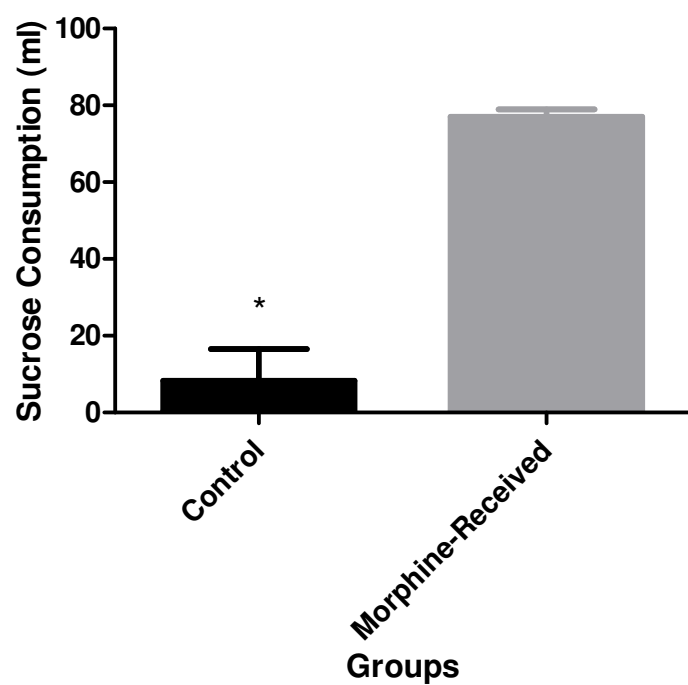
93 Statistic

94 Data were analyzed using SPSS version 21 and Graphpad Prism 5. Independent samples two-
95 tailed t-test was performed for all experiments. Data are as represented as mean $\pm$ SEM and
96 $P < 0.05$ considered significant.

97 Result

98 Sucrose consumption

99 Sucrose consumption was increased in morphine-receiving rats compared to control rats (figure
100 1).



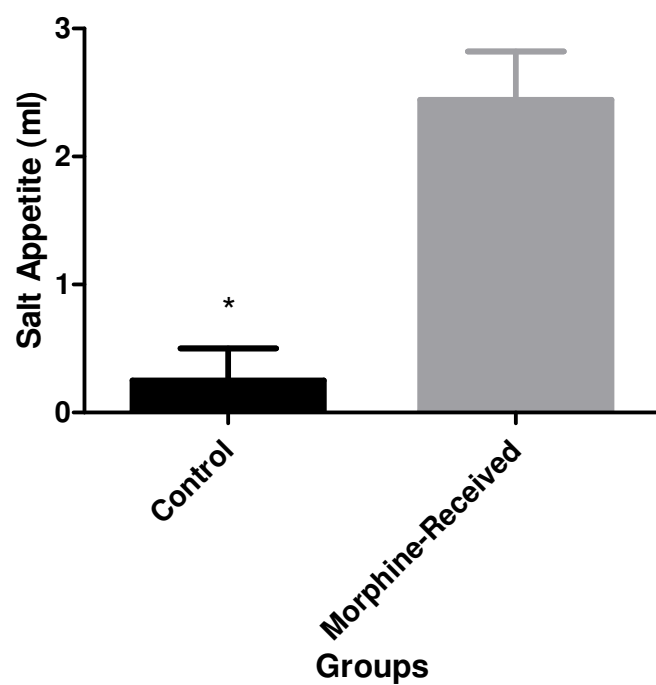
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102 Figure 1) Sucrose consumption was assessed in sucrose preference test. Data is represented as mean $\pm$
 103 SEM (n=8).

104 Salt consumption

105 Salt consumption was increased in morphine-receiving rats compared to control rats (figure 2).

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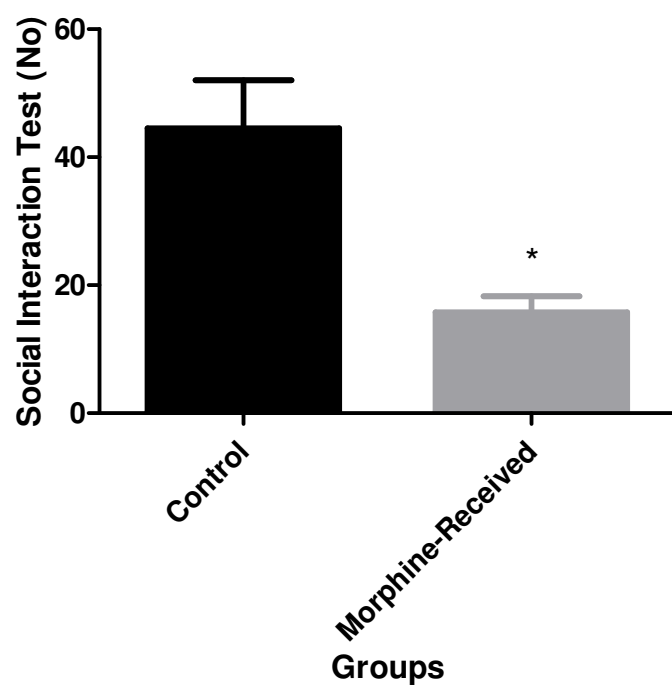
108 Figure 2) Salt consumption was assessed in salt appetite test. Data is represented as mean $\pm$ SEM (n=8).

109 Social interaction test

110 Novelty seeking behavior was increased in morphine-receiving rats compared to control rats.

111 Also this experiment can be used as an indicator of anxiety (figure 3).

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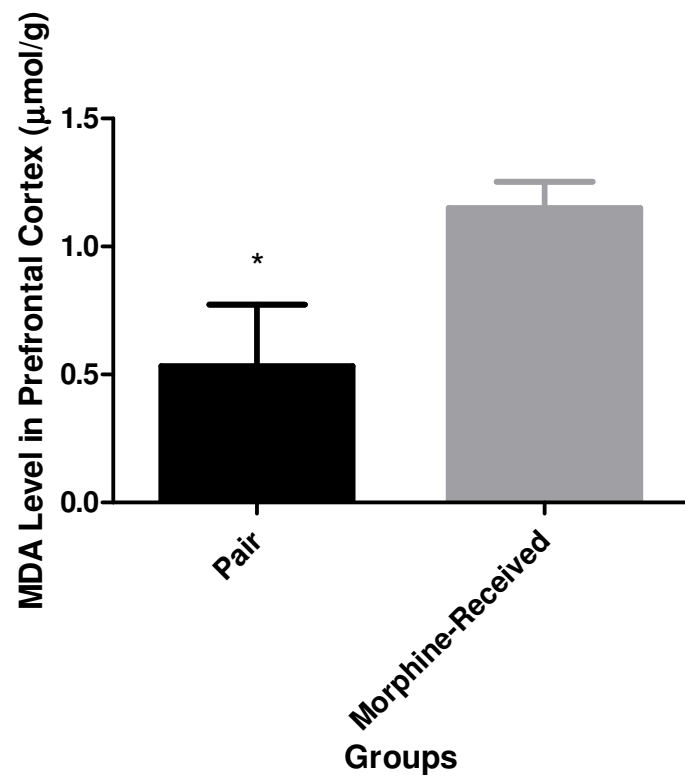
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114 Figure 3) Novelty seeking behavior was assessed in social interaction test. Data is represented as mean $\pm$
 115 SEM (n=8).

116

117 MDA in prefrontal cortex

118 MDA was increased in prefrontal cortex of rats exposed to morphine compared to rats that have
 119 received morphine (figure 4).



120

121 Figure 4) MDA was assessed in prefrontal cortex. Data is represented as mean $\pm$ SEM (n=8).

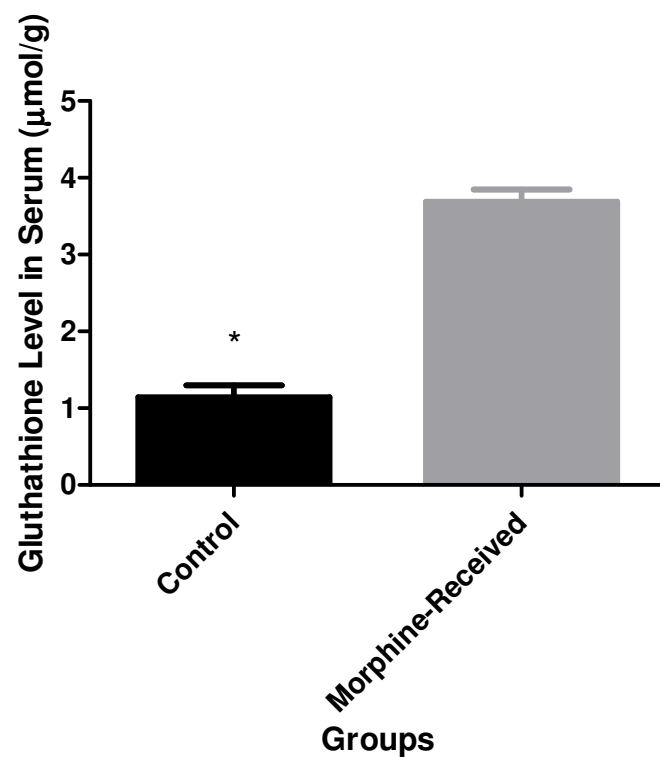
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123 Glutathione in serum

124 Glutathione was increased in serum of rats exposed to morphine compared to rats that have

125 received morphine (figure 5).

126



127

128 Figure 5) Glutathione was assessed in serum. Data is represented as mean $\pm$ SEM (n=8).

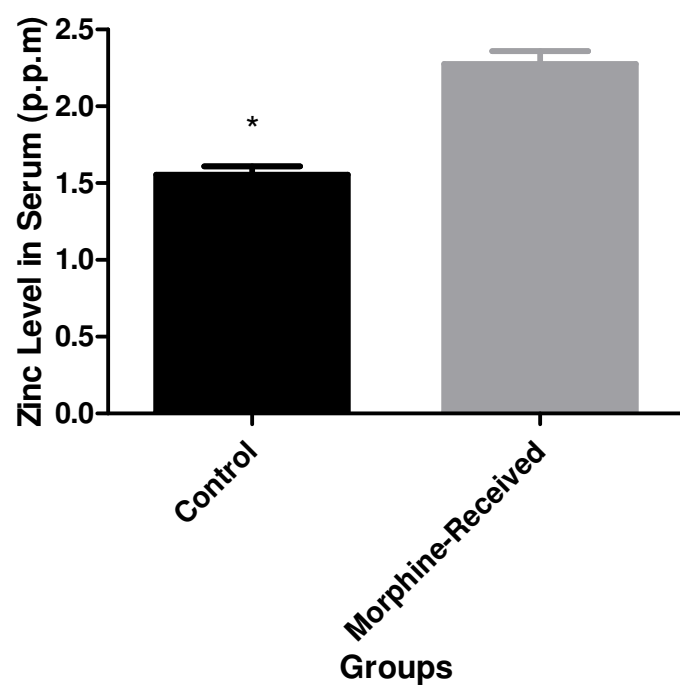
129

130 Zinc in serum

131 Zinc in serum was reduced in serum of rats exposed to morphine compared to rats that have

132 received morphine (figure 6).

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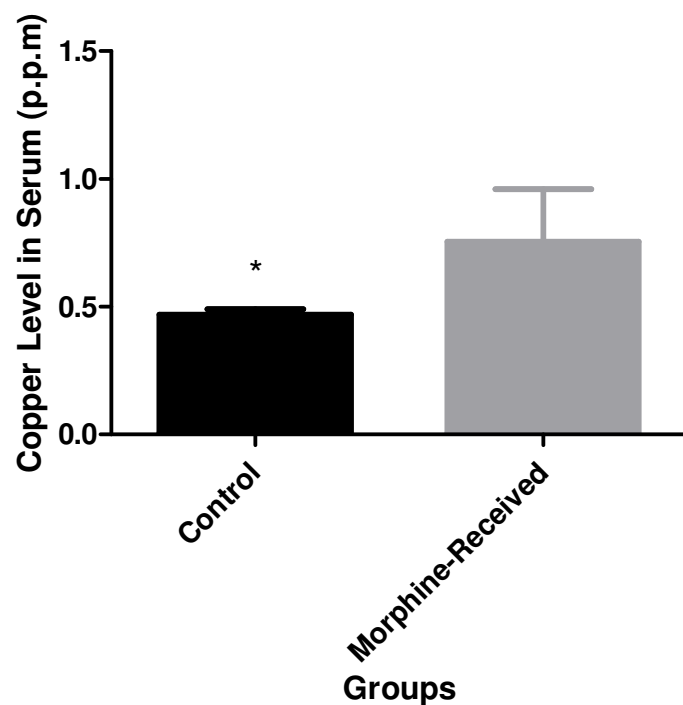
135 Figure 6) Zinc was assessed in serum. Data is represented as mean $\pm$ SEM (n=8).

136

137 Copper in serum

138 Copper in serum was increased in serum of rats exposed to morphine compared to rats that have
 139 received morphine (figure 7).

140



141

142 Figure 7) Copper was assessed in serum. Data is represented as mean $\pm$ SEM (n=8).

143

144

145 Discussion

146 The conventional research on relapse to drugs and development of drug-seeking behavior in
 147 abstinence period has focused on dopaminergic system. The new approach to such behaviors in
 148 recent research has been focused on prefrontal cortex.

149 The underlying mechanism and behaviors that dysfunction of prefrontal cortex causes relapse in
 150 recent studies says some brain dysfunctions is necessary. Brain capabilities necessary for
 151 stopping relapse are self-control, proper emotional regulation, motivation, interoception,
 152 cognitive flexibility, learning and memory and salient attributions.

153 In this study some novel behaviors investigated to find out new mechanism for prefrontal cortex
154 for regulation of addictive behaviors. During abstinence period three maladaptive behaviors
155 develop that causes relapse: excessive salient to drugs and drug-related cues, decreased
156 sensitivity to non-drug reinforces and decreased ability to inhibit maladaptive behaviors.

157 Sucrose preference test in previous studies has been used for two reasons. First for assessing
158 craving for drugs and the second for assessing behaviors that is respond to non-drugs reinforces.
159 Prefrontal cortex regulation of these behaviors has been established in previous studies, but the
160 underlying mechanism has not been fully understood.

161 Salt appetite in previous studies has been used for assessing the probability of relapse in
162 abstinence period. However in this study this experiment was used also for assessing if addiction
163 itself causes relapse or something unrelated to direct effects of abuse drugs to brain causes such
164 behaviors.

165 Novelty seeking behavior in this experiment has been assessed with social interaction test. This
166 test also can be used for assessing anxiety and mood balance that is important as co-morbid
167 psychiatric disorder important for abstinence period for controlling relapse to drug abuse.

168 Prefrontal cortex involves in learning and memory (17), categorization (18), inhibitory control
169 (19) and cognitive flexibility (20). There should be many interconnections to other brain areas
170 for accomplishing these tasks. Also prefrontal cortex receives inputs from other parts of brain
171 that are part of rewarding center including cingulate, prelimbic and infralimbic cortices (5). In
172 previous studies it was well demonstrated that prefrontal cortex gives afferents to hippocampus.
173 Hippocampus is of known areas that regulates addictive behaviors. For investigating other
174 mechanisms that prefrontal cortex may regulate addictive behavior, recent studies suggest it can

175 regulate addictive behavior directly (8). In this sense learning ability deficiency causes relapse to
 176 drug abuse. Also prefrontal area modulates VTA, one of the main areas that its disturbance
 177 causes relapse to drug abuse (21). Of known behaviors that cause relapse to drug abuse is
 178 impulse control that as said prefrontal actually play as an inhibitor to impulsive behaviors (19).
 179 The most region of prefrontal cortex responsible for drug-seeking behavior is prelimbic
 180 prefrontal cortex area (22).

181 Co morbid psychiatric disorders such as depression and anxiety causes relapse to drug abuse
 182 with some known and unknown mechanisms (23). In this study in social interaction test rats in
 183 morphine-received group showed dysregulations in mood balance and this can cause increase in
 184 drug-taking and seeking behavior.

185 Oxidative stress (OS) disturbance has been known in previous studies to cause mood
 186 dysregulations. In this study OS occurred both in prefrontal cortex. In previous studies it well
 187 approved that OS disturbance can disturb neuronal function through different mechanisms (24-
 188 26). The exact neuron type that is responsible for development of such maladaptive behaviors is
 189 not well demonstrated. In one study pyramidal neurons has been shown to exert most effects
 190 (27). These neuronal damages have been well proved the occurrence of addictive behaviors
 191 through different mechanisms. However in this study novel mechanisms with different behaviors
 192 were experimented.

193 OS also acts through different neurotransmitter. In one study GABA and in another GLU
 194 neurotransmitters has been implicated (28, 29). Hormones in this regard in some studies have
 195 been proved to have positive effects. Melatonin and estrogen have been proved to enhance brain
 196 cognitive abilities through improvement of inflammation and acetyl cholinesterase activity (30,

31). Improper prostaglandin production also is a factor that works in concert to MDA elevation (32). Disturbances of immune system function through improper cytokines production such as TNF- α and IL-10 is another contributing factor that that may cause elevation of MDA (33). Adverse effects of psychoactive drugs such as haloperidol also act through MDA dysregulation (34).

MDA elevation in previous studies has been associated with many disturbances. In brain research MDA and glutathione elevation have been associated with Alzheimer and some psychiatric diseases such as depression and autism (35, 36). In this study the have been associated with habits that are representatives of addictive behaviors.

Metals are necessary for many brain functions. For reasons that are not completely understood brain neurons needs metals to sustain enough spontaneous activity. There are two types of metals necessary for brain function 1) alkali and alkaline for proper signaling 2) transition metals for tolerating OS injury. In one study copper has been shown to be essential for normal spontaneous activity (37).

Copper is essential element necessary for many cellular functions. In brain copper is necessary for the function of a verity of enzymes. Copper is of known elements that is known to works in parallel with OS status for maintaining neuronal proper function (38).

Zinc is an essential part of many different protein involve in different cellular function. Zinc also is necessary for the function of zinc-finger proteins that is located in genome. Zinc containing neurons are glutamatergic neurons. In these neurons zinc is responsible for storage, release, re-uptake and modulation of glutamate receptors. The most prominent parts of brain that contain zinc are cortex and amygdales (39).

219 References

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- 221 1. Ramo DE, Prince MA, Roesch SC, Brown SA. Variation in substance use relapse episodes among
222 adolescents: a longitudinal investigation. *Journal of substance abuse treatment*. 2012;43(1):44-52.
- 223 2. Bornovalova MA, Cashman-Rolls A, O'Donnell JM, Ettinger K, Richards JB, deWit H, et al. Risk
224 taking differences on a behavioral task as a function of potential reward/loss magnitude and individual
225 differences in impulsivity and sensation seeking. *Pharmacology, biochemistry, and behavior*.
226 2009;93(3):258-62.
- 227 3. Coppens CM, de Boer SF, Koolhaas JM. Coping styles and behavioural flexibility: towards
228 underlying mechanisms. *Philosophical transactions of the Royal Society of London Series B, Biological*
229 *sciences*. 2010;365(1560):4021-8.
- 230 4. Di Chiara G, Bassareo V, Fenu S, De Luca MA, Spina L, Cadoni C, et al. Dopamine and drug
231 addiction: the nucleus accumbens shell connection. *Neuropharmacology*. 2004;47 Suppl 1:227-41.
- 232 5. Groenewegen HJ, Uylings HB. The prefrontal cortex and the integration of sensory, limbic and
233 autonomic information. *Progress in brain research*. 2000;126:3-28.
- 234 6. Mars RB, Grol MJ. Dorsolateral prefrontal cortex, working memory, and prospective coding for
235 action. *J Neurosci*. 2007;27(8):1801-2.
- 236 7. Kane MJ, Engle RW. The role of prefrontal cortex in working-memory capacity, executive
237 attention, and general fluid intelligence: an individual-differences perspective. *Psychonomic bulletin &*
238 *review*. 2002;9(4):637-71.
- 239 8. Goldstein RZ, Volkow ND. Dysfunction of the prefrontal cortex in addiction: neuroimaging
240 findings and clinical implications. *Nature reviews*. 2011;12(11):652-69.
- 241 9. Roitman MF, Na E, Anderson G, Jones TA, Bernstein IL. Induction of a salt appetite alters
242 dendritic morphology in nucleus accumbens and sensitizes rats to amphetamine. *J Neurosci*.
243 2002;22(11):RC225.
- 244 10. Romieu P, Host L, Gobaille S, Sandner G, Aunis D, Zwiller J. Histone deacetylase inhibitors
245 decrease cocaine but not sucrose self-administration in rats. *J Neurosci*. 2008;28(38):9342-8.
- 246 11. Parkitna JR, Sikora M, Golda S, Golembiowska K, Bystrowska B, Engblom D, et al. Novelty-
247 seeking behaviors and the escalation of alcohol drinking after abstinence in mice are controlled by
248 metabotropic glutamate receptor 5 on neurons expressing dopamine d1 receptors. *Biological psychiatry*.
249 2013;73(3):263-70.
- 250 12. Overstreet DH, Knapp DJ, Breese GR. Modulation of multiple ethanol withdrawal-induced
251 anxiety-like behavior by CRF and CRF1 receptors. *Pharmacology, biochemistry, and behavior*.
252 2004;77(2):405-13.
- 253 13. Kampov-Polevoy AB, Garbutt JC, Janowsky DS. Association between preference for sweets and
254 excessive alcohol intake: a review of animal and human studies. *Alcohol and alcoholism*. 1999;34(3):386-
255 95.
- 256 14. Gümüş M, Yüksel H, Evliyaoğlu O, Kapan M, Büyük A, Önder A, et al. Effects of ellagic acid on
257 copper, zinc, and biochemical values in serum and liver of experimental cholestatic rats. *Biological trace*
258 *element research*. 2011;143(1):386-93.
- 259 15. Zhang H, Wang ZY, Yang L, Yang X, Wang X, Zhang Z. In vitro antioxidant activities of sulfated
260 derivatives of polysaccharides extracted from *Auricularia auricular*. *International journal of molecular*
261 *sciences*. 2011;12(5):3288-302.
- 262 16. Khan RA, Khan MR, Sahreen S. Brain antioxidant markers, cognitive performance and
263 acetylcholinesterase activity of rats: efficiency of *Sonchus asper*. *Behavioral and brain functions : BBF*.
264 2012;8:21.

- 265 17. Warden MR, Miller EK. Task-dependent changes in short-term memory in the prefrontal cortex.
266 J Neurosci. 2010;30(47):15801-10.
- 267 18. Freedman DJ, Riesenhuber M, Poggio T, Miller EK. Categorical representation of visual stimuli in
268 the primate prefrontal cortex. Science (New York, NY. 2001;291(5502):312-6.
- 269 19. Dalley JW, Everitt BJ, Robbins TW. Impulsivity, compulsivity, and top-down cognitive control.
270 Neuron. 2011;69(4):680-94.
- 271 20. Gruber AJ, Calhoon GG, Shusterman I, Schoenbaum G, Roesch MR, O'Donnell P. More is less: a
272 disinhibited prefrontal cortex impairs cognitive flexibility. J Neurosci. 2010;30(50):17102-10.
- 273 21. Lewis BL, O'Donnell P. Ventral tegmental area afferents to the prefrontal cortex maintain
274 membrane potential 'up' states in pyramidal neurons via D(1) dopamine receptors. Cereb Cortex.
275 2000;10(12):1168-75.
- 276 22. Mihindou C, Guillem K, Navailles S, Vouillac C, Ahmed SH. Discriminative inhibitory control of
277 cocaine seeking involves the prelimbic prefrontal cortex. Biological psychiatry. 2013;73(3):271-9.
- 278 23. Chambers RA, Krystal JH, Self DW. A neurobiological basis for substance abuse comorbidity in
279 schizophrenia. Biological psychiatry. 2001;50(2):71-83.
- 280 24. Jacintho JD, Kovacic P. Neurotransmission and neurotoxicity by nitric oxide, catecholamines, and
281 glutamate: unifying themes of reactive oxygen species and electron transfer. Current medicinal
282 chemistry. 2003;10(24):2693-703.
- 283 25. Ramalingam M, Kim SJ. Reactive oxygen/nitrogen species and their functional correlations in
284 neurodegenerative diseases. Journal of neural transmission. 2012;119(8):891-910.
- 285 26. Choi DH, Cristovao AC, Guhathakurta S, Lee J, Joh TH, Beal MF, et al. NADPH oxidase 1-mediated
286 oxidative stress leads to dopamine neuron death in Parkinson's disease. Antioxidants & redox signaling.
287 2012;16(10):1033-45.
- 288 27. Chen BT, Yau HJ, Hatch C, Kusumoto-Yoshida I, Cho SL, Hopf FW, et al. Rescuing cocaine-induced
289 prefrontal cortex hypoactivity prevents compulsive cocaine seeking. Nature. 2013;496(7445):359-62.
- 290 28. Michels L, Schulte-Vels T, Schick M, O'Gorman RL, Zeffiro T, Hasler G, et al. Prefrontal GABA and
291 glutathione imbalance in posttraumatic stress disorder: preliminary findings. Psychiatry research.
292 2014;224(3):288-95.
- 293 29. Ozyurt B, Ozyurt H, Akpolat N, Erdogan H, Sarsilmaz M. Oxidative stress in prefrontal cortex of
294 rat exposed to MK-801 and protective effects of CAPE. Progress in neuro-psychopharmacology &
295 biological psychiatry. 2007;31(4):832-8.
- 296 30. Pourganji M, Hosseini M, Soukhtanloo M, Zabihi H, Hadjzadeh MA. Protective role of
297 endogenous ovarian hormones against learning and memory impairments and brain tissues oxidative
298 damage induced by lipopolysaccharide. Iranian Red Crescent medical journal. 2014;16(3):e13954.
- 299 31. Buyukokuroglu ME, Cemek M, Yurumez Y, Yavuz Y, Aslan A. Antioxidative role of melatonin in
300 organophosphate toxicity in rats. Cell biology and toxicology. 2008;24(2):151-8.
- 301 32. Jeng W, Ramkissoon A, Parman T, Wells PG. Prostaglandin H synthase-catalyzed bioactivation of
302 amphetamines to free radical intermediates that cause CNS regional DNA oxidation and nerve terminal
303 degeneration. FASEB journal : official publication of the Federation of American Societies for
304 Experimental Biology. 2006;20(6):638-50.
- 305 33. Ahmed MA, El Morsy EM, Ahmed AA. Pomegranate extract protects against cerebral
306 ischemia/reperfusion injury and preserves brain DNA integrity in rats. Life sciences. 2014;110(2):61-9.
- 307 34. Abdel-Sattar EA, Mouneir SM, Asaad GF, Abdallah HM. Protective effect of Calligonum comosum
308 on haloperidol-induced oxidative stress in rat. Toxicology and industrial health. 2014;30(2):147-53.
- 309 35. Dringen R, Hirrlinger J. Glutathione pathways in the brain. Biological chemistry.
310 2003;384(4):505-16.

- 311 36. Talarowska M, Galecki P, Maes M, Gardner A, Chamielec M, Orzechowska A, et al.
312 Malondialdehyde plasma concentration correlates with declarative and working memory in patients
313 with recurrent depressive disorder. *Molecular biology reports*. 2012;39(5):5359-66.
- 314 37. Dodani SC, Firl A, Chan J, Nam CI, Aron AT, Onak CS, et al. Copper is an endogenous modulator
315 of neural circuit spontaneous activity. *Proceedings of the National Academy of Sciences of the United*
316 *States of America*. 2014;111(46):16280-5.
- 317 38. Chen X, Guo C, Kong J. Oxidative stress in neurodegenerative diseases. *Neural regeneration*
318 *research*. 2012;7(5):376-85.
- 319 39. Frederickson CJ, Suh SW, Silva D, Frederickson CJ, Thompson RB. Importance of zinc in the
320 central nervous system: the zinc-containing neuron. *The Journal of nutrition*. 2000;130(5S Suppl):1471S-
321 83S.