Ethanol Withdrawal-Induced Impaired Recognition Is Reversed by Chronic Exposure to Stress and the Acute Administration of Corticosterone in Mice

Hideaki Kato,* Minoru Tsuji, Kazuya Miyagawa, Kotaro Takeda, and Hiroshi Takeda

Department of Pharmacology, School of Pharmacy, International University of Health and Welfare; 2600–1 Kitakanemaru, Ohtawa, Tochigi 324–8501, Japan.

Received March 24, 2016; accepted July 10, 2016

The present study was designed to ascertain the effects of repeated exposure to stress and the acute administration of corticosterone (1, 3, 10 mg/kg, intraperitoneally (i.p.)) on the ethanol withdrawal-induced impairment of novel object recognition in mice. Mice were chronically treated with 3% ethanol for 7 d, with or without exposure to restraint stress for 1 h/d. A significant decrease in cognitive function was observed in the ethanol plus no stress group at 48 h after the discontinuation of ethanol treatment. This impaired recognition was recovered in the ethanol plus stress group. Moreover, we investigated the effects of acute pretreatment with corticosterone, which is a corticosteroid-type hormone produced in the cortex of the adrenal glands, on the impaired recognition after the discontinuation of ethanol treatment in mice. The impaired recognition in the 3% ethanol alone-treated group at 48 h after the discontinuation of ethanol treatment was recovered by treatment with the middle dose (3 mg/kg) of corticosterone, but not with the low or high doses (1, 10 mg/kg). These results suggest that chronic stress during the development of ethanol dependence may reduce the impaired recognition after the discontinuation of ethanol treatment. Moreover, acute pretreatment with the middle dose of corticosterone also recovered the impaired recognition after the discontinuation of ethanol treatment in mice. Adequate regulation of the hypothalamic–pituitary–adrenal (HPA) axis by corticosterone may improve the impaired recognition after the discontinuation of ethanol treatment.

Key words ethanol withdrawal; stress; corticosterone; hypothalamic–pituitary–adrenal axis; novel object recognition test; mouse

Alcohol is one of the most commonly abused substances, and chronic excessive intake leads to the development of ethanol dependence in both humans and laboratory animals. The development of ethanol dependence is associated with a withdrawal syndrome when ethanol consumption is discontinued or substantially reduced. Many studies have reported that stressful conditions are a significant risk factor for future excessive alcohol (ethanol) consumption, and thereby increase the risk for dependence and alcoholism. Several animal models have been used to better understand the relationship between stress and ethanol intake. Ethanol dependence and its withdrawal syndrome are thought to result from adaptive changes in the neuronal network. On the other hand, chronic stress is the response of the brain to unpleasant events for a prolonged period, and is thought to lead to changes in several neurotransmission systems, including cognitive function. It is well known that alcohol withdrawal impairs learning, memory and recognition in humans, such as abstinent alcoholic patients. Our previous results indicated that the hypothalamic–pituitary–adrenal (HPA) axis plays a critical role in the response to stress, and its function may regulate the development of ethanol dependence and expression of the withdrawal syndrome. Corticosterone, the major glucocorticoid in rodents, also plays a central role in the regulation of the HPA axis and modulates learning and memory processes. Interestingly, the plasma concentration of corticosterone is increased by acute ethanol challenge, but this response is attenuated by chronic exposure. It has been reported that corticosterone promotes learning and memory in non-stressed rodents, while the chronic administration of corticosterone has opposite effects. Although there is increasing evidence that the stress hormone corticosterone may modulate learning and memory, the effects of chronic exposure to stress and acute administration of corticosterone on the impaired recognition after ethanol withdrawal are not sufficiently clear.

Therefore, the present study was designed to ascertain the influence of chronic stress on cognitive function using the novel object recognition test after the discontinuation of ethanol treatment in mice. We also investigated the effects of acute pretreatment with corticosterone on the impaired recognition after the discontinuation of ethanol treatment in mice.

MATERIALS AND METHODS

Animals Male ICR mice (25–30 g) were obtained from Japan SLC, Inc. (Shizuoka, Japan). Each mouse was kept individually in a polycarbonate cage (W22×L32×H13.5 cm) with sterilized PaperClean Bedding (Japan SLC Inc.) at a room temperature of 23±1°C and humidity of 50±5% with a 12 h light–dark cycle (light on 7:00 a.m.–7:00 p.m.). Food and water were available ad libitum until the experiment. All experiments were performed in accordance with the guidelines for the Care and Use of Laboratory Animals of International University of Health and Welfare and the Japanese Pharmacological Society.

Repeated Restraint Stress Procedure Mice were individually placed in a 50-mL plastic syringe pump (Terumo Co., Tokyo, Japan) once a day for 1 h from day 0 to 6. Restraint stress was applied at the same time each day (between 9:00 a.m., 10:00 a.m.) for 7 consecutive days, according to the
procedure we described previously. Liquid Diet Method
To prepare the liquid diet, 99.5% ethanol (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and sucrose (Wako Pure Chemical Industries, Ltd.) were mixed with skimmed milk according to the method described by Iso,13,14) Narita et al.15,16) and Kato et al.6) with minor modification. For chronic ethanol treatment, mice were individually housed and given access to a measured amount of liquid diet containing 3% (w/w) ethanol as their sole nutrient source for 7 d (days 0–7). Control mice were pair-fed a liquid diet in which ethanol was replaced by an isocaloric amount of sucrose. Every 24 h, the liquid diet was replaced by fresh normal or ethanol-containing liquid diet.

Ethanol Withdrawal
Withdrawal was induced by replacing the ethanol-containing liquid diet with a normal liquid diet on day 7 of ethanol treatment.

Drugs
Corticosterone (Sigma-Aldrich, St. Louis, MO, U.S.A.) was suspended in 0.2% carboxymethylcellulose (CMC; Wako Pure Chemical Industries, Ltd.) solution containing 0.2% Tween20 (Wako Pure Chemical Industries, Ltd.) dissolved in saline. The drug solution was prepared immediately before injection.

Novel Object Recognition Test
The novel object recognition test was carried out as described previously.17–19) Briefly, the test was conducted in an open field arena consisting of a polycarbonate box (W27×L44×H19 cm) with two different kinds of objects. A video camera connected to a video recorder and monitor was positioned above the box. The two objects had the same height and volume, but differed in shape and appearance. During habituation for 10 min on day 8, the animals were allowed to explore the empty arena. Twenty-four hours after habituation, the animals were exposed to the familiar arena with two identical objects placed at an equal distance (acquisition session for 10 min, day 9). One hour after this acquisition session, the mice were allowed to explore the open field in the presence of the familiar object and a novel object to test recognition memory (test session for 10 min, day 9). Throughout the experiments, the objects were used in a counterbalanced manner in terms of their emotional neutrality. The access counts, the time spent exploring each object and the exploratory preference percentage of counts and time spent were recorded. The exploratory preference percentage in the test session, which is the ratio of the access counts or time spent exploring the novel object to the total access counts or time spent exploring both objects, was used to measure cognitive function. In the acquisition session, the exploratory preference percentage was calculated as a ratio of the access counts or time spent exploring the object that was replaced by the novel object in the test session to the total access counts or time spent exploring both objects. Corticosterone (1, 3, 10 mg/kg, intraperitoneally (i.p.)) or vehicle (10 mL/kg) was administered to each mouse 1 h before the acquisition session.

Measurement of Serum Corticosterone Concentrations
Mice were decapitated 48 h after the discontinuation of chronic ethanol treatment, and their blood was collected. In the preliminary study, mice were decapitated 1 h after the acute administration of corticosterone (1, 3, 10 mg/kg, i.p.), and their blood was collected. Blood samples were centrifuged at 1500×g for 15 min, and serum was stored at −20°C for future analysis. The corticosterone concentrations were determined by immunoassay (AssayPro, St. Charles, MO, U.S.A.).

Statistical Analysis
All data are presented as the mean±standard error of the mean (S.E.M.). The Wilcoxon matched pair or Bonferroni multiple comparison test was used for the statistical evaluation of behavioral data (p<0.05, 0.01).

RESULTS
Influence of Repeated Exposure to Stress on Ethanol Withdrawal-Induced Impaired Recognition
We examined whether ethanol withdrawal-induced impaired recognition was reversed by repeated exposure to stress. In the novel

![Fig. 1. Influence of Chronic Stress on Cognitive Function after the Discontinuation of Ethanol Treatment in Mice, with Respect to the Mean Exploratory Preference in Terms of Counts](image-url)
Object recognition test, there was no significant difference among groups with respect to access counts or time spent exploring the two identical objects during the acquisition session (Figs. 1A, 2A). All of the groups, except the 3% ethanol plus no stress group, showed a significant preference for the novel object during the test session (Wilcoxon matched pair test, \( p < 0.01 \) vs. the exploratory preference percentage for the familiar object). A significant decrease in cognitive function was observed in the ethanol plus no stress group (\( p < 0.01 \) vs. control milk plus no stress group, \( p < 0.01 \) vs. control milk plus stress group). This impaired recognition was significantly recovered in the ethanol plus stress group with respect to time spent (\( p < 0.05 \) vs. 3% ethanol plus no stress group), but not with respect to access counts (Figs. 1B, 2B).

**Serum Corticosterone Concentrations after Ethanol Withdrawal**
A significant increase in the serum corticosterone concentration was observed in the ethanol plus stress group compared with all other groups (\( p < 0.01 \)) at 48h after the discontinuation of ethanol treatment (Fig. 3). The serum corticosterone concentration (ng/mL) in each of the groups was 67.7±10.2 (control milk plus no stress group), 139.5±22.3 (control milk plus stress group), 155.6±21.9 (3% ethanol plus no stress group) and 335.0±50.1 (3% ethanol plus stress group), respectively. On the other hand, the serum corticosterone concentration (ng/mL) at 1h after acute administration in each corticosterone (1, 3, 10 mg/kg, i.p.)-treated group (\( n = 5 \)) was 170.5±30.8, 385.3±105.3 and 1075.9±136.6, respectively (data not shown).

**Effects of Acute Administration of Corticosterone on Ethanol Withdrawal-Induced Impaired Recognition**
We examined whether ethanol withdrawal-induced impaired recognition was reversed by acute pretreatment with corticosterone (1, 3, 10 mg/kg, i.p.). In the novel object recognition test, there was no significant difference among groups with respect to access counts or time spent exploring the two identical objects during the acquisition session (Figs. 4A, 5A). In terms of the access counts, all of the groups except the 3% ethanol plus vehicle-treated group showed a significant preference for the novel object during the test session (Wilcoxon matched pair test, \( p < 0.05 \) or \( p < 0.01 \) vs. the exploratory preference percentage for the familiar object). In terms of the time spent, both the control milk plus vehicle-treated and 3% ethanol...
plus corticosterone (3 mg/kg, i.p.)-treated groups showed a significant preference for the novel object during the test session (Wilcoxon matched pair test, \( p < 0.01 \) vs. the exploratory preference percentage for the familiar object). A significant decrease in cognitive function was observed in the 3% ethanol plus vehicle-treated group (\( p < 0.05 \) or \( p < 0.01 \) vs. control milk plus vehicle-treated group). This impaired recognition in the 3% ethanol plus vehicle-treated group was only recovered by treatment with the middle dose (3 mg/kg, \( p < 0.05 \) vs. 3% ethanol plus vehicle-treated group) of corticosterone, but not with the low or high doses (1, 10 mg/kg) (Figs. 4B, 5B).

**DISCUSSION**

Stress can be a key factor that underlies both alcohol addiction and dependence.\(^{20}\) We have previously demonstrated that repeated exposure to restraint stress during ethanol consumption changes withdrawal-induced emotional abnormality without changing locomotor activity in ICR mice.\(^{6}\) These observations suggest that repeated exposure to stress may affect the development of ethanol dependence and change the expression of withdrawal-induced emotional abnormality.
Several previous sociocultural studies have shown that stress may increase the risk of alcoholism. Exposure to stress is an environmental factor that has long been thought to increase alcohol consumption and predispose people to the development of alcoholism.21–25) Numerous studies have reported that 50–75% of detoxified alcoholics have some type of cognitive or memory disturbance.4,5) However, the influence of repeated stress on ethanol withdrawal-induced impaired recognition is not sufficiently clear. In our previous study, withdrawal signs, such as piloerection, tremor and seizure almost were disappeared at 48h after the discontinuation of ethanol treatment.6) Therefore, we examined the influence of chronic exposure to stress on ethanol withdrawal-induced impaired recognition according to our previous experimental schedule. In the present study, ethanol-dependence-and-withdrawal mice were prepared by using the liquid diet method.13,14,26) In the liquid diet procedure, animals consume significant quantities of ethanol to develop physical dependence, and withdrawal signs emerge when ethanol is omitted from the diet. On the other hand, previous studies have shown that stress adaptation developed under repeated restraint stress for 1 h/d, but not 4 h/d in mice.27,28) These results suggest that the repeated restraint stress for 1 h/d, which was applied in the present study, may be appropriate for producing a mild to moderate level of stress in mice. A significant increase in cognitive function was observed in both the control milk plus no stress and control milk plus stress groups at 48 h after the discontinuation of ethanol treatment. This result suggests that repeated stress is hardly any influence on cognitive function under our experimental conditions. On the other hand, a significant decrease in the cognitive function was observed in the ethanol plus no stress group. A growing body of evidence from animal studies has indicated that memory impairment is induced by chronic ethanol treatment.28–30) These results are in good agreement with our present findings. Interestingly, this impairment in novel object recognition induced by ethanol withdrawal was reversed by chronic exposure to stress. These results suggest that chronic exposure to stress during ethanol consumption may improve ethanol withdrawal-induced impaired recognition in mice. Moreover, these findings indicate that the time spent exploring a novel object may be more sensitive than the access count for a novel object for detecting an improvement in impaired recognition. It is still not fully clear how chronic exposure to stress reversed ethanol withdrawal-induced the impaired recognition.

The HPA axis has been hypothesized to play a role in alcoholism and dependence.21,22) Ethanol acutely activates the HPA axis, which leads to glucocorticoid release from the adrenal glands.30) Glucocorticoid hormones are the final step in the activation of the HPA axis and are known to function in the biological response to stress.31) In alcohol dependence, the HPA axis is dysregulated in both humans35) and rodents.36–37) but the effects of this dysregulation are still unclear. Here, we speculated that regulation of the HPA axis, such as by changes in the blood corticosterone level, may play an important role in the impaired recognition after the discontinuation of ethanol treatment. Therefore, we also measured the serum corticosterone concentration in ethanol-withdrawal mice. A significant increase in the serum corticosterone concentration was observed in the ethanol plus stress group compared with all other groups at 48 h after the discontinuation of ethanol treatment. The improvement of ethanol withdrawal-induced impaired recognition, which was reversed by chronic exposure to stress, may be associated with an increase in the serum corticosterone level. Corticosterone, the major endogenous glucocorticoid in rodents, plays a critical role in the regulation of the HPA axis, which is the major neuroendocrine system that regulates stress responses and modulates learning and memory processes.7) Corticosterone also promotes learning and memory in non-stressed rodents and under certain stressful test conditions.9,10,38,39) It has been reported that chronic co-treatment with corticosterone protects against memory impairments induced by chronic treatment with a low dose of ethanol.30) We next investigated the effects of acute pretreatment with corticosterone, instead of chronic treatment with corticosterone, on the impaired recognition after the discontinuation of ethanol treatment in mice.

The impaired recognition in the 3% ethanol plus no stress group at 48 h after the discontinuation of ethanol treatment was recovered by treatment with the middle dose (3 mg/kg) of corticosterone, but not with the low or high doses (1, 10 mg/kg). This U-shaped dose–response curve for improvement of the impaired recognition induced by corticosterone is in agreement with the results of many other previous studies.5,30) The basis for this bell-shaped effect is unknown, but, as proposed by Munck et al.,40) such a pattern could reflect a dissociation between the physiological and pharmacological actions of glucocorticoids. It has been previously reported that there are two distinct types of receptor systems for corticosterone, i.e., mineralocorticoid receptors (MRs; type I adrenal steroid receptor) and glucocorticoid receptors (GRs; type II adrenal steroid receptor).41) Circulating corticosterone readily crosses the blood–brain barrier where it binds directly to both receptors. MRs bind corticosterone with much higher affinity than GRs.41–45) Therefore, while MRs are predominantly occupied under low levels of circulating corticosterone levels predominantly occupy MRs, whereas GRs become extensively occupied only under high levels of circulating corticosterone.44) Such an inverted U-shaped dose–response effect of corticosterone on learning, memory and cognition consolidation has often been explained in terms of the dose-dependent activation of high-affinity MRs and low-affinity GRs.46) The adequate activation of MRs and GRs by corticosterone during ethanol withdrawal may improve the impaired recognition after the discontinuation of ethanol treatment. Therefore, regulation of the HPA axis, which includes corticosterone and its receptors, may be closely related to cognitive function under the ethanol-withdrawal state. The present results suggest that regulation of the HPA axis, which includes corticosterone and its receptors, may play an important role in recognition memory in the ethanol-withdrawal state in mice. Therefore, it is necessary to examine the change in levels of MRs and GRs with time course in the several brain regions, such as hippocampus and hypothalamus, using the same animal model to clarify a role of these receptors. In our present study, for the first time, we showed that acute treatment with corticosterone significantly improved the impaired recognition in ethanol-withdrawal mice. These results suggest that glucocorticoids may be effective for the treatment of cognitive deficits in chronic or abstinent alcoholics. However, additional experiments beyond the scope of this study will be necessary to fully test this hypothesis.
CONCLUSION

The present study demonstrated that repeated exposure to a mild to moderate level of stress, during ethanol consumption may improve the impaired recognition in ethanol-withdrawal mice. Furthermore, acute pretreatment with a medium dose of corticosterone also recovered the impaired recognition after the discontinuation of ethanol treatment in mice. Adequate regulation of the HPA axis, such as the regulation of corticosterone-mediated MR and GR function, during ethanol withdrawal may improve the impaired recognition after the discontinuation of ethanol treatment. Additional neurochemical and/or molecular biological experiments based on the present behavioural findings should help to explain the processes that underlie the improvement of ethanol withdrawal-induced impaired recognition via the HPA axis.

Conflict of Interest  The authors declare no conflict of interest.

REFERENCES

1) Enoch MA, Genetic and environmental influences on the development of alcoholism: resilience vs. risk. Ann. N. Y. Acad. Sci., 1094, 193–203 (2006).
2) Uhart M, Wood G, Stress, alcohol and drug interaction: an update of human research. Addict. Biol., 14, 43–64 (2009).
3) Sillaber I, Henniger MS, Stress and alcohol drinking. Ann. Med., 36, 596–605 (2004).
4) Parsons OA, Nixon SJ, Neurobehavioral sequelae of alcoholism. Neurol. Clin., 11, 205–218 (1993).
5) Smith DM, Atkinson RM, Alcoholism and dementia. Int. J. Addict., 30, 1843–1869 (1995).
6) Kato H, Tsuji M, Miyagawa K, Takeda K, Takeda H. Repeated exposure to stress stimuli during ethanol consumption prolongs withdrawal-induced emotional abnormality in mice. Eur. J. Pharmacol., 721, 29–34 (2013).
7) Blundell J, Blaiss CA, Lajace DC, Eisch AJ, Powell CM. Block of glucocorticoid synthesis during re-activation inhibits extinction of an established fear memory. Neurobiol. Learn. Mem., 95, 453–460 (2011).
8) Richardson HN, Lee SY, O’Dell LE, Koob GF, Rivier CL. Alcohol self-administration acutely stimulates the hypothalamic–pituitary–adrenal axis, but alcohol dependence leads to a dampened neuroendocrine state. Eur. J. Neurosci., 28, 1641–1653 (2008).
9) McCormick CM, McNamara M, Mukhopadhyay S, Kelsey JE. Acute corticosterone replacement reinstates performance on spatial and nonspatial memory tasks 3 months after adrenalectomy despite degeneration in the dentate gyrus. Behav. Neurosci., 111, 518–537 (1997).
10) Roorenstraal B, Okuda S, Van der Zee EA, McGaugh JL. Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. Proc. Natl. Acad. Sci. U.S.A., 103, 6741–6746 (2006).
11) Coburn-Litvak PS, Potthakes K, Tata DA, McCloskey DP, Anderson BJ. Chronic administration of corticosterone impairs spatial reference memory before spatial working memory in rats. Neurobiol. Learn. Mem., 80, 11–23 (2003).
12) Tsuji M, Takeuchi T, Miyagawa K, Ishit D, Imai T, Takeda K, Kitajima M, Takeda H. Yokukansen, a traditional Japanese herbal medicine, alleviates the emotional abnormality induced by maladaptation to stress in mice. Phytotherapy, 21, 363–371 (2014).
13) Iso H. Temporal changes in avoidance learning in physically alcohol-dependent rats following withdrawal. Yakubutsu Seishin Kodo, 4, 243–248 (1984).
14) Iso H. The development of theories of avoidance learning and its application to behavioral pharmacology. Yakubutsu Seishin Kodo, 10, 381–392 (1990).
15) Narita M, Soma M, Narita M, Mizoguchi H, Tseng LF, Suzuki T. Implications of the NR2B subunit-containing NMDA receptor localized in mouse limbic forebrain in ethanol dependence. Eur. J. Pharmacol., 401, 191–195 (2000).
16) Narita M, Soma M, Tamaki H, Narita M, Suzuki T. Intensification of the development of ethanol dependence in mice lacking dopamine (D3) receptor. Neurosci. Lett., 324, 129–132 (2002).
17) Tang Y-P, Shimizu E, Dube GR, Rampon C, Kerchner GA, Zhuo M, Liu G, Tsien JZ. Genetic enhancement of learning and memory in mice. Nature, 401, 63–69 (1999).
18) Nagai T, Yamada K, Kim HC, Kim YS, Noda Y, Imura A, Nabeshima Y, Nabeshima T. Cognition impairment in the genetic model of aging klotho gene mutant mice: a role of oxidative stress. FASEB J., 17, 50–52 (2003).
19) Carlini VP, Martini AC, Schiöth HB, Ruiz RD, Fiol de Cunéo M, de Barrogiolo SC. Decreased memory for novel object recognition in chronically food-restricted mice is reversed by acute ghlrtin administration. Neuroscience, 153, 928–934 (2008).
20) Zebenhek JE, Clark BW, Smith BD, Traumatic experiences and substance abuse: mapping the territory. J. Psychoactive Drugs, 26, 327–344 (1994).
21) Horton DJ. The function of alcohol in primitive societies. Q. J. Stud. Alcohol, 4, 199–320 (1943).
22) Conger JJ, Alcoholism: theory and problem and challenge. II. Reinforcement theory and the dynamics of alcoholism. Q. J. Stud. Alcohol, 17, 296–305 (1956).
23) Pohorecky LA. The interaction of alcohol and stress. Neurosci. Biobehav. Rev., 5, 209–229 (1981).
24) Pohorecky LA. Stress and alcohol interaction: an update of human research. Alcohol. Clin. Exp. Res., 15, 438–459 (1991).
25) Volpicelli J, Balaranam G, Hahn J, Wallace H, Bux D. The role of uncontrollable trauma in the development of PTSD and alcohol addiction. Alcohol Res. Health, 23, 256–262 (1999).
26) Lieber CS, DeCarli LM. The feeding of alcohol in liquid diets: two decades of applications and 1982 update. Alcohol. Clin. Exp. Res., 6, 525–531 (1982).
27) Tsuji M, Takeda H, Matsumiya T, Protective effects of 5-HT1A receptor agonists against emotional changes produced by stress stimuli are related to their neuroendocrine effects. Br. J. Pharmacol., 134, 585–595 (2001).
28) Melis F, Stancampiano R, Imperato A, Carta G, Fadda F, Chronic ethanol consumption in rats: correlation between memory performance and hippocampal acetylcholine release in vivo. Neuroscience, 74, 155–159 (1996).
29) White AM, Simson PE, Best PJ, Comparison between the effects of ethanol and diazepam spatial working memory in the rat. Psychopharmacology, 133, 256–261 (1997).
30) Eskada ME, Latif LM, Kendall IA, Pardon MC, Corticosterone protects against memory impairments and reduced hippocampal BDNF levels induced by a chronic low dose of ethanol in C57BL/6J mice. Rom. J. Morphol. Embryol., 55, 1303–1316 (2014).
31) Wand GS, Dobs AS, Alterations in the hypothalamic–pituitary–adrenal axis in actively drinking alcoholics. J. Clin. Endocrinol. Metab., 72, 1290–1295 (1991).
32) Froehlich J, O’Malley S, Hyttia P, Davidson D, Farren C, Preclinical and clinical studies on naltrexone: what have they taught each other? Alcohol. Clin. Exp. Res., 27, 533–539 (2003).
33) Ellis FW, Effect of ethanol on plasma corticosterone levels. J. Pharmacol. Exp. Ther., 153, 121–127 (1966).
34) Selby H, The evolution of the stress concept. Am. Sci., 61, 692–699 (1973).
35) Costa A, Bono G, Martignoni E, Merlo P, Sances G, Nappi G, An...
assessment of hypothalamic–pituitary–adrenal axis functioning in non-depressed, early abstinent alcoholics. Psychoneuroendocrinology, 21, 263–275 (1996).

36) Roberts AJ, Lessov CN, Phillips TJ. Critical role for glucocorticoid receptors in stress- and ethanol-induced locomotor sensitization. J. Pharmacol. Exp. Ther., 275, 790–797 (1995).

37) Rasmussen DD, Boldt BM, Bryant CA, Mitton DR, Larsen SA, Wilkinson CW. Chronic daily ethanol and withdrawal: I. Long-term changes in the hypothalamic–pituitary–adrenal axis. Alcohol. Clin. Exp. Res., 24, 1836–1849 (2000).

38) Akirav I, Kozenicky M, Tal D, Sandi C, Venero C, Richter-Levin G. A facilitative role for corticosterone in the acquisition of a spatial task under moderate stress. Learn. Mem., 11, 188–195 (2004).

39) Conboy L, Sandi C. Stress at learning facilitates memory formation by regulating AMPA receptor trafficking through a glucocorticoid action. Neuropsychopharmacology, 35, 674–685 (2010).

40) Deroche V, Marinelli M, Le Moal M, Piazza PV. Glucocorticoids and behavioral effects of psychostimulants. II: Cocaine intravenous self-administration and reinstatement depend on glucocorticoid levels. J. Pharmacol. Exp. Ther., 281, 1401–1407 (1997).

41) Munck A, Guyre PM, Holbrook NJ. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev., 5, 25–44 (1984).

42) de Kloet ER, Vreugdenhil E, Oitzl MS, Joëls M. Brain corticosteroid receptor balance in health and disease. Endocr. Rev., 19, 269–301 (1998).

43) Real JMHM, de Kloet ER. Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology, 117, 2505–2511 (1985).

44) Real JMHM, van den Bosch FR, de Kloet ER. Differential response of type I and type II corticosteroid receptors to changes in plasma steroid level and circadian rhythmicity. Neuroendocrinology, 45, 407–412 (1987).

45) Sutanto W, De Kloet ER. Species-specificity of corticosteroid receptors in hamster and rat brains. Endocrinology, 121, 1405–1411 (1987).

46) Lupien SJ, McEwen BS. The acute effects of corticosteroids on cognition: integration of animal and human model studies. Brain Res. Brain Res. Rev., 24, 1–27 (1997).