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Effects of nefazodone on voluntary ethanol consumption induced by isolation stress in young and aged rats

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Abstract

Late-onset ethanol (EtOH) consumption is related to life and social stressors of aging. The stress system (hypothalamic–pituitary–adrenal, HPA, axis) coordinates the adaptive response of the organism to stressors, but age-related deficits in HPA function seem to be associated with disorders such as late-onset EtOH consumption, anxiety and depression. In the present study, we examined whether HPA dysfunction is associated with stress-related EtOH consumption in aged rats and whether the treatment with nefazodone hydrochloride, a phenylpiperazine antidepressant, partially reverses the adverse effects of isolation (ISOL) stress. The animals were offered two-bottle choice consumption of 0.2% saccharin and 10% EtOH/0.2% saccharin, and then exposed to 4 days of ISOL stress on an irregular, unpredictable schedule. ISOL stress-induced increases in corticosterone secretion and EtOH consumption both during and following the stress (recovery period) in aged rats. Nevertheless, this effect at the recovery period was not evident in young stressed rats. Nefazodone caused a significant decrease in plasma corticosterone levels and EtOH consumption. The attenuation of stress-induced corticosterone by nefazodone was correlated with reduced EtOH consumption. These findings link the effect of ISOL stress to the induction of voluntary EtOH consumption following the end of the stressor and the limitation of aged HPA to down-regulated corticosterone.

Keywords: Social stress; Isolation; Nefazodone; Ethanol; Hippocampus; Corticosterone; Aging

1. Introduction

Demographic trends reveal the elderly to be the fastest growing segment of the population. Findings from epidemiological studies suggest that late-onset EtOH consumption, defined as onset of the first ethanol (EtOH) problem at or after age 60 (Atckinson et al., 1990), is a growing public health problem that is related to life and social stressors of aging. Moreover, chronic stress is positively related to EtOH dependence and problems (Welte, 1998) and there is a relationship between voluntary EtOH intake and escape from the consequences of stress (Mills et al., 1977a,b). In terms of the latter, exposure to stress can increase EtOH consumption and EtOH can attenuate the behavioral and biochemical effects of stress (Bowers et al., 1997). The hypothalamic–pituitary–adrenal (HPA) axis, mediated by corticosterone, appears to play an important role in the processes of stress- and EtOH-induced sensitization (Phillips et al., 1997). Furthermore, some data demonstrates cross-sensitization between stress and EtOH (Roberts et al., 1995). Nevertheless, experimental approaches using animal models of alcohol self-administration have had widely varying outcomes depending on drinking paradigm used, the subject's basal EtOH intake prior to testing, short or continuous stressor, as well as on the animal strain used (including genetically preferring animal models) (van Erp and Miczek, 2001).

On the other hand, stress has a predictive value for the occurrence of depression (Kivela et al., 1996) or is a frequent trigger for this pathology in old age (Vogel, 2000). Also, among people older than 65, those with alcoholism are approximately three times more likely to exhibit a major depressive disorder than are those without alcoholism (Grant and Harford, 1995). Depression has been

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associated with impaired mineralocorticoid receptor function, restrained glucocorticoid receptor feedback at the level of the HPA axis, raised cortisol level and increased corticotropin-releasing factor activity, which may act in concert to induce the signs and symptoms of the disorder (Steckler et al., 1999). Such an HPA disturbance in feedback control can be acquired as a result of stressful life experiences and be computed by age (Holsboer et al., 1995).

HPA dysfunction and glucocorticoid hypersecretion have been implicated in the degenerative changes in the region of the brain that normally inhibits glucocorticoid release—the hippocampus. Studies have shown that stress-related glucocorticoid secretion causes cell atrophy and death in certain areas of the hippocampus, and the hippocampus of depressed patients was, on average, 12–15% smaller than those of controls of the same age, weight, level of education and handedness. It is possible that the death of new neurons triggered by a stress-induced spike in glucocorticoids leads to depression (Vogel, 2000). In addition, chronic EtOH exposure, which reduces hippocampus neuron number, is associated with hyperactivity of the HPA (Walker et al., 1980).

Since we have previously observed that benzodiazepines, buspirone and fluoxetine partially reverse many effects of stress in rodents (Freire-Garabal et al., 1992, 1993, 1995, 1997, 2000; Núñez et al., 1999) and because there is a combination of alcoholism, anxiety and/or depression in humans, we initiated studies to examine psychopharmacological interventions in order to avoid their negative consequences on the appearance and evolution of late-onset drinking. In this respect, our first study showed that alprazolam, a benzodiazepine agonist drug, partially reversed the stress-induced voluntary choice EtOH consumption in aged rats (Núñez et al., 1999). The goal of this paper is to determine whether nefazodone, a recently released antidepressant drug that seems to be more anxiolytic than other antidepressants (Davis et al., 1997), could modify voluntary EtOH consumption in aged stressed rats, and focus on the potential effect of this drug on the stress-induced increase in corticosterone secretion and hippocampus damage. This drug was selected because it has the potential for therapeutic activity in the treatment of anxiety and depression or both (Graeff et al., 2001; Garfield et al., 2001) and possesses potent 5-HT_{2A} receptor antagonism properties (Olausson et al., 1998), and this receptor is an important component of the neural substrates underlying EtOH intake and behaviours related to anxiety and stress (Blakley et al., 2001).

2. Methods

2.1. Animals

Female aged (19–22 months old) and young (4–6 months old) rats of the Sprague–Dawley strain (Interfauna Ibérica, Barcelona, Spain) were used. They were housed 7 days before experiments, four per cage, in a temperature (22–24 °C)- and humidity-controlled animal room, with an alternating 12-h light–dark cycle (lights on at 0600 h, lights off at 1800 h), with food (Ultrasorb; PANLAB, Barcelona, Spain) and drink available ad libitum.

2.2. Experimental procedure

After 1 week of habituation to the colony room and handling, animals received 2 weeks of 24-h access to EtOH and saccharin in a two-bottle choice paradigm. Screening was then conducted for 2 weeks prior to the initiation of the experimental procedure. Those animals consuming a minimum of 1.5 g/kg body weight/day EtOH were selected for subsequent experimental procedure. Then, of the 120 rats that were originally screened for EtOH consumption, 50 that drank to criterion levels were randomly divided into five groups of 10 animals according to the treatment they will be submitted to: Group A, controls; Group B, unstressed rats injected with saline; Group C, unstressed rats injected with nefazodone; Group D, stressed rats injected with saline; Group E, stressed rats injected with nefazodone. The EtOH administration routine was kept identical to the screening procedure and lasted for 18 days (baseline, stress and recovery periods). We considered the possibility that a subject's experience in some of the earlier screening procedure might have influenced its behavior in subsequent experiment. Great care was taken to minimize these effects across groups; schedules and procedures were held identical for all groups and all subjects received identical time courses and procedures through 18 days of the experimental period. At this point, rats were sacrificed and the brain was removed and frozen at −70 °C until analysis. The University of Santiago de Compostela Review Committee for the use of Animal Subjects approved this experimental protocol.

2.3. Drinking solutions

Two drinking solutions were used. One consisted of a 0.2% saccharin solution and the other consisted of the same solution containing 10% (vol/vol) EtOH. The solutions were prepared on alternate days and stored in airtight containers until used.

2.4. Induction of stress

Isolation (ISOL) stress was conducted by placing the animals alone in a novel environment. In this experiment, we used unpredictable stress in which the timing of stress exposures varied from 1 to 4 h on a random, irregular schedule (Sprague and Maickel, 1994) since predictable stress has been previously reported (Haile et al., 2001) inefficient on animal drug addiction. Unstressed rats were exposed only to the normal activity of the animal room. Groups D and E were submitted to the same ISOL exposure. Following a 10-day baseline period, the animals

were given 4 days of ISOL, followed by 4-day recovery period (Sprague and Maickel, 1994). Cages (Cage FI, Iffa-Credo; Criffa, Barcelona, Spain) of 305 (depth) × 180 (width) × 184 mm (height) internal dimensions were used both for control housing conditions and ISOL. They were always placed in the same animal room (Núñez et al., 1999).

2.5. Quantification of EtOH consumption

Saccharin was presented in one tube and EtOH–saccharin in the other tube for the duration of the study (from basal to recovery periods) (Sprague and Maickel, 1994). The volumes remaining in each tube were recorded daily during the period 1100–1300 h, the tubes were refilled and their positions (left–right) alternated to prevent the development of positional preference (Sprague and Maickel, 1994). Daily fluid consumption (both EtOH and saccharin) and body weight were monitored. EtOH intake, expressed as grams per kilograms per day, was also daily calculated (Spigelman et al., 1991). Low, medium and high EtOH consumptions were defined by means of daily EtOH consumption (1.5–2.5, 2.5–4.5, 4.5–6.0 g/kg/day, respectively) (Rockman and Glavin, 1986).

2.6. Corticosterone assay

Blood was collected daily, from basal to recovery periods, in polypropylene beakers containing disodium EDTA, centrifuged and plasma was frozen at − 70 °C until assayed as described Zenker and Berstein (1958) for corticosterone. A duplicate sample was used for each animal. The standard curve is plotted and the amount, A, in sample is read from that graph. The concentration, C, was obtained by the formula: $C = A \times 100/V$, where V is the number of milliliters of plasma used. The results were expressed as micrograms per 100 ml (mean ± S.E.M.).

2.7. Hippocampus cell counting

Four animals from each group were randomly selected for the analysis of hippocampus measures. Cell counting was performed on 20-μm cresyl violet-stained sections of the dorsal hippocampus as previously described (Meaney et al., 1988). Three to five sections were analyzed for each animal. Cell counts of the pyramidal cell fields were transformed into measures of neuron density per 0.1 mm^2. We also estimated the rostro-to-caudal extent of the dorsal hippocampus in each animal by counting the number of 20-μm sections between plates 19 and 23 of the atlas of Paxinos and Watson (1997) (Issa et al., 1990).

2.8. Drug treatments

Nefazodone hydrochloride (10 mg/kg; Bristol-Myers-Squibb, Madrid, Spain) was subcutaneously injected in a volume of 1 ml/kg 0.9% saline solution (Freire-Garabal et al., 2000). Control stressed mice were subcutaneously injected with 1 ml/kg 0.9% saline solution as placebo. As the goal of this paper is to determine whether nefazodone could modify voluntary EtOH consumption, drugs were administered daily throughout baseline, stress and recovery periods.

2.9. Statistical analysis

Mean 24-h fluid consumption for each group of animals was computed in grams per kilograms body weight ± S.E.M. Statistical analysis was performed using the three-way repeated-measures ANOVA, with two between-group factors [Group (placebo, nefazodone, stress + placebo, stress + nefazodone) and Age (old, young)] and one within-group factor [Time period (Basal, ISOL, Recovery)]. A posteriori tests were carried out by means of Student–Newman–Keuls multiple range tests with significance set at $P < .05$ (Sprague and Maickel, 1994). Correlation analysis (Pearson's r) was conducted on values from rats to evaluate the relationship between EtOH consumption and corticosterone plasmatic levels.

3. Results

Given the groups of animals and experimental procedures conducted, results are organized according to the stress, age and nefazodone effects on EtOH intake, corticosterone plasmatic levels and hippocampus parameters. The ANOVA test showed that main effects of Time, Age and Group were significant (all of them with $P < .001$) both for EtOH consumption and corticosterone plasmatic levels. Two-way interactions Time × Age ($P < .001$), Group × Age ($P < .001$) and Time × Group ($P < 0.001$) were significant. Three-way interaction Time × Age × Group ($P < .001$) was observed. Neuron density in hippocampus pyramidal cell fields was correlated with age (Pearson's coefficient $r = .9253$, $P < .01$).

3.1. Effect of stress

Measurement of EtOH consumption and corticosterone plasmatic levels are shown in Figs. 1 and 2. Since Time × Age interaction was highly significant, we analyzed these data with more detail. ISOL stress resulted in EtOH consumption and corticosterone plasmatic levels that were increased in young and old stressed rats during the period of stress compared to prestress period ($P < .001$). Using the Newman–Keuls multiple range tests, pairwise comparisons between groups were significant in relation to EtOH consumption. Corticosterone plasmatic levels showed similar results except in the case of placebo and nefazodone groups. With regard to the recovery period, for young rats, EtOH consumption and corticosterone samples taken daily showed a trend to recover

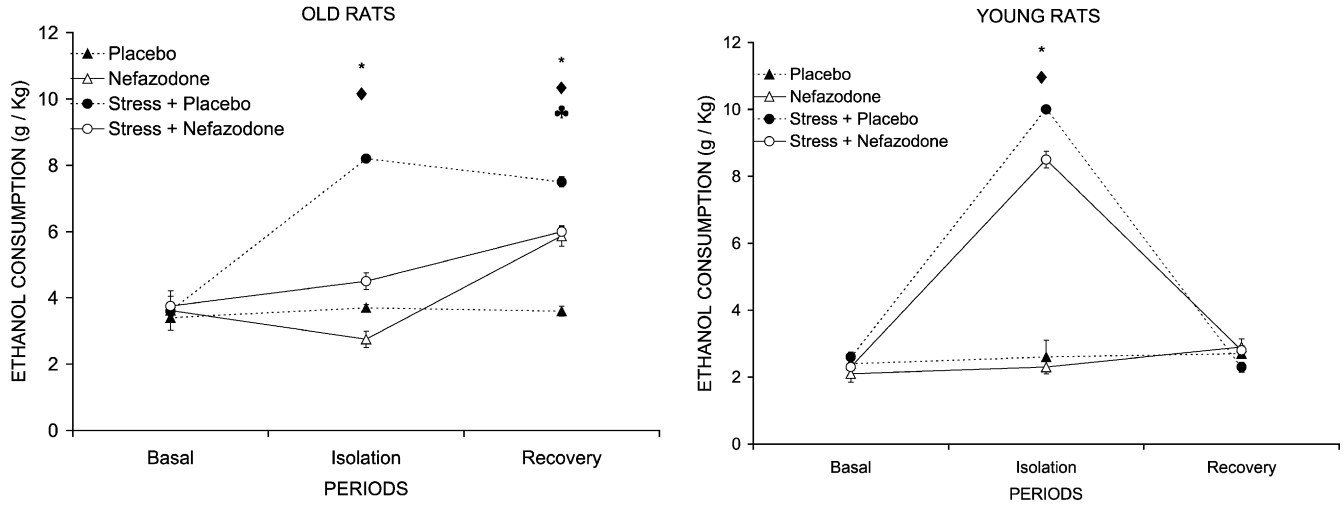

Fig. 1. Effects of nefazodone on EtOH consumption in old and young stressed rats. Mean EtOH consumption of basal (prestress) and stress periods were equivalent for old and aged rats, but over the course of the recovery period (after stress), aged rats were reliably impaired relative to young ones in recovery at the basal values. Nefazodone reduced stress-induced EtOH consumption in the old and young rats. Each value represents the mean 24-h consumption value (g/kg ± S.D.) of 10 rats over 4-day periods. Statistical analysis was performed using the ANOVA with grouping of means by Student–Newman–Keuls multiple range tests with significance set at $P < .05$. * Differences between stressed and unstressed rats injected with placebo significant at $P < .001$. (♦) Differences between saline and nefazodone stressed rats significant at $P < .001$. (♣) Differences between saline and nefazodone unstressed rats significant at $P < .001$. Differences between old and young rats at the same point significant at $P < .001$ except for the unstressed + nefazodone group on the isolation period.

basal levels compared to the stress period ($P < .001$), whilst the aged rats values showed also a significant trend to decreasing compared to the stress period ($P < .001$), but without return to the basal levels. Saccharin consumption was not influenced by ISOL stress. Total fluid consumption was significantly increased in stressed rats because of the significant increase in EtOH consumption in stressed rats as compared to control and unstressed groups (Table 1).

There were no significant group differences neither in the length of any of the four pyramidal cell fields for each of the four cell fields of Ammon's horn nor neuron density in

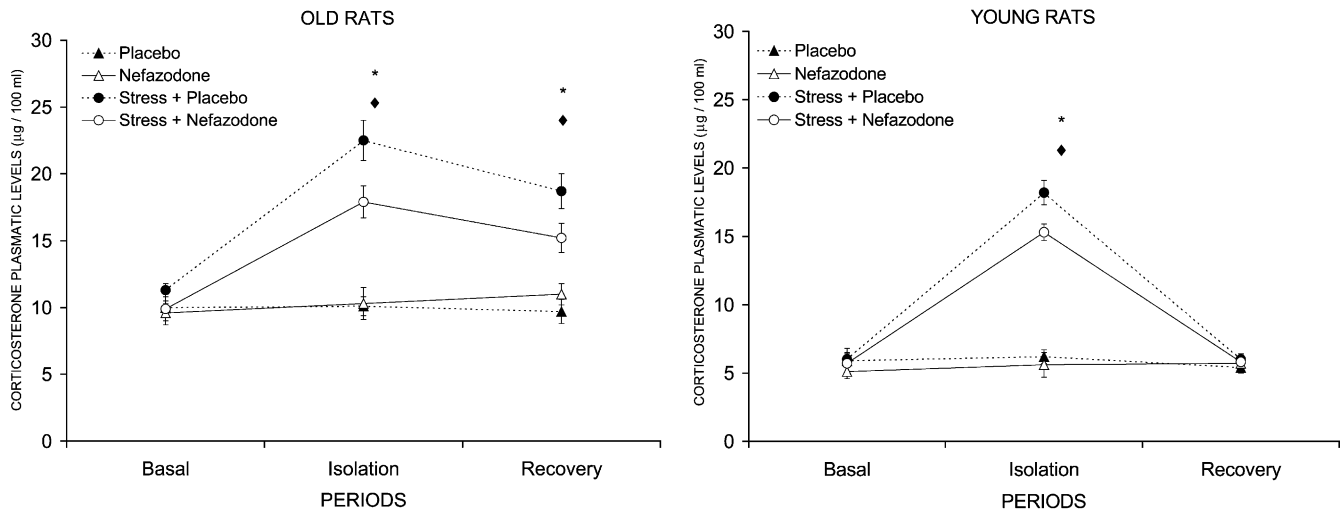

Fig. 2. Effect of nefazodone on corticosterone plasmatic levels in old and young rats. Plasma corticosterone measured from aged and young rats prior to stress (basal), during stress and after isolation stress (recovery). Each value represents the mean value (µg/100 ml ± S.D.) of 10 rats. Statistical analysis was performed using the ANOVA with grouping of means by Student–Newman–Keuls multiple range tests with significance set at $P < .05$. (♦)Differences between saline and nefazodone stressed rats significant at $P < .001$. (♣) Differences between saline and nefazodone unstressed rats significant at $P < .001$. Differences between old and young rats at the same point significant at $P < .001$.

Table 1
Fluid consumption

		Baseline		Isolation		Recovery	
		Old rats	Young rats	Old rats	Young rats	Old rats	Young rats
Control	Saccharine	11.8 ± 0.6	9.3 ± 0.6	–	–	–	–
	Total	15.4 ± 0.8	10.7 ± 1.1	–	–	–	–
Placebo	Saccharine	11.5 ± 0.5	9.1 ± 1.2	12.0 ± 0.3	9.8 ± 0.3	11.1 ± 0.5	10.1 ± 2.0 **
	Total	14.9 ± 0.7	11.5 ± 1.5	15.7 ± 0.5	12.4 ± 0.4	14.7 ± 0.8	12.8 ± 2.2 **
Nefazodone	Saccharine	15.6 ± 1.6	10.1 ± 0.2	14.7 ± 0.6	10.5 ± 0.3	14.1 ± 1.6	9.9 ± 0.5
	Total	19.2 ± 1.7	12.2 ± 0.7	17.4 ± 1.1	12.8 ± 0.7	19.9 ± 2.2	12.7 ± 1.0
Stress + placebo	Saccharine	12.8 ± 0.1	10.0 ± 0.8	12.6 ± 0.7	10.2 ± 0.3	13.0 ± 0.4	9.9 ± 0.5
	Total	16.4 ± 0.2	12.6 ± 1.1	20.8 ± 2.6 *,***	20.2 ± 0.5 *,***	20.0 ± 1.6 *,***	12.2 ± 0.8 *,***
Stress + nefazodone	Saccharine	14.0 ± 0.4	9.7 ± 0.1	13.8 ± 0.4	9.9 ± 0.4	11.6 ± 0.8	9.8 ± 0.3
	Total	15.9 ± 0.1	12.0 ± 0.7	18.3 ± 0.2 *,***	18.4 ± 2.9 *,**,***	19.1 ± 1.5 *,***	12.6 ± 0.6 *,***

Total fluid consumption was significantly increased in stressed rats because of the significant increase in EtOH consumption as compared to control and unstressed groups. Each value represents the mean 24-h consumption value (g/kg ± S.D.) of 10 rats over 4-day periods. Statistical analysis was performed using the ANOVA with grouping of means by Student–Newman–Keuls multiple range tests with significance set at $P < .05$.

* Differences between stressed and unstressed rats injected with saline significant at $P < .001$.

** Differences between old and young rats at the same point significant at $P < .001$.

*** Differences between saline and nefazodone stressed rats significant at $P < .001$.

hippocampus pyramidal cell fields between stressed or unstressed rats (Table 2).

3.2. Effect of age

Prior to the stress period, all rats, irrespective of age, had uniformly medium EtOH consumption (2.5–4.5 g/kg/day), but the aged rats showed higher corticosterone levels relative to young ones ($P < .001$). Analysis of the daily assessments revealed that stress induced an increase in EtOH consumption and plasma corticosterone concentrations in old versus young animals ($P < .001$). Taking differences in basal corticosterone levels into consideration, the stress-induced change in levels was similar between the young and old rats, or may be even greater in the young. Subsequent comparisons that were made at each day after stress indicated that the stressed groups differed significantly from the 2- to 4-day time points ($P < .001$). For young stressed group, EtOH consumption and corticosterone levels showed a relatively faster rate of decline over time that approached basal values by 4 days after the cessation of stress. Aged stressed rats demonstrated higher EtOH consumption and corticosterone concentrations for a longer period of time after ISOL stress than young ones. That revealed a significant effect of age during the recovery period. As we already mentioned, we found significant differences between groups in neuron density of hippocampus pyramidal cell fields in aged rats compared to young adult controls ($P < .01$).

3.3. Effect of nefazodone

Under stress conditions, treatment with nefazodone (10 mg/kg) partially reversed that effect of ISOL stress. Thus, in old stressed rats, nefazodone reduced around 50% the EtOH consumption compared to old stressed rats injected

Table 2
Hippocampus measures

Measure	Control		Placebo		Nefazodone		Placebo + stress		Nefazodone + stress	
	Old	Young	Old	Young	Old	Young	Old	Young	Old	Young
CA_1 length	9.1 ± 0.2	9.5 ± 0.4	9.6 ± 0.1	9.8 ± 0.4	9.7 ± 0.4	9.6 ± 0.4	9.3 ± 0.2	9.7 ± 0.7	9.3 ± 0.8	9.7 ± 0.6
CA_2 length	2.7 ± 0.2	2.7 ± 0.2	2.3 ± 0.5	2.3 ± 0.5	2.0 ± 0.4	2.3 ± 0.1	1.9 ± 0.1	2.2 ± 0.4	2.3 ± 0.2	2.2 ± 0.1
C_3 length	5.1 ± 0.2	5.1 ± 0.2	5.3 ± 0.1	5.3 ± 0.1	5.5 ± 0.4	5.3 ± 0.4	5.0 ± 0.8	5.1 ± 0.4	5.6 ± 0.7	5.3 ± 0.8
CA_4 length	5.6 ± 0.3	5.6 ± 0.3	5.0 ± 0.2	5.0 ± 0.2	5.6 ± 0.6	5.4 ± 0.2	5.5 ± 0.9	5.6 ± 0.1	5.6 ± 0.2	5.6 ± 0.3
Rostral–caudal extent	225 ± 9	225 ± 9	223 ± 15	223 ± 15	222 ± 10	236 ± 5.7	223 ± 14	218 ± 9.1	225 ± 17	223 ± 18
Neuron density										
CA_1	22.5 ± 3.8 *	87.6 ± 4.5	25.3 ± 3.3 *	83.0 ± 2.9	29.1 ± 0.7 *	89.9 ± 2.7	27.3 ± 1.7 *	89.1 ± 1.8	24.2 ± 2.7 *	88.5 ± 1.7
CA_2	36.8 ± 4.1 *	49.3 ± 7.1	34.2 ± 1.9 *	49.5 ± 0.1	39.1 ± 1.2 *	49.0 ± 2.2	37.8 ± 2.3 *	49.1 ± 0.5	37.5 ± 3.3 *	48.7 ± 6.9
CA_3	25.5 ± 3.2 *	51.6 ± 3.8	29.2 ± 1.1 *	54.5 ± 6.9	29.3 ± 1.4 *	54.1 ± 2.0	29.5 ± 0.8 *	55.2 ± 2.3	27.3 ± 1.7 *	55.2 ± 0.8
CA_4	24.9 ± 1.5 *	42.4 ± 2.1	27.2 ± 3.1 *	40.6 ± 1.9	26.3 ± 2.5 *	42.6 ± 0.9	29.0 ± 1.9 *	42.5 ± 1.3	27 ± 3.9 *	43.2 ± 1.7

There were no significant group differences neither in the length of any of the four pyramidal cell fields for each of the four cell fields of Ammon's horn nor neuron density in hippocampus pyramidal cell fields between stressed or unstressed rats.

Values of CA lengths are expressed as mm ± S.E.M.; values for the rostral–caudal extent are expressed as the number of 20-μm sections. Mean (± S.E.M.) neuron density (per 0.1 mm^2) in hippocampus pyramidal cell fields in three to four animals per group.

* Significant difference from young rats ($P < .01$).

with saline. Also, treatment with nefazodone (10 mg/kg) reduced significantly ($P<.001$) corticosterone plasmatic levels during the stress period and thereafter, following the termination of the stressor in old ($P<.001$) and young ($P<.001$) rats. Corticosterone plasmatic levels were correlated with EtOH consumption and the attenuation of stress-induced corticosterone by nefazodone was correlated with reduced EtOH consumption (Pearson's coefficient $r=.9406$, $P<.01$).

It is worth pointing out that previously unstressed nefazodone-treated aged rats, at the recovery period, showed a significantly increase ($P<.001$) in the EtOH consumption compared to baseline and ISOL periods (Fig. 1). Also, according to the data showed above, at the recovery period, nefazodone-treated rats, whether stressed or not, exhibited a significantly greater total fluid consumption compared to baseline period ($P<.001$). As shown in Figs. 1 and 2, nefazodone was not associated with any change in EtOH consumption or corticosterone levels in nonstressed nefazodone-treated young rats.

There were no significant differences ($P>.05$) in neither EtOH consumption nor corticosterone levels at each time point between unstressed animals injected with saline or nefazodone and control animals. Nevertheless, there was a significant difference in unstressed nefazodone-treated old rats EtOH consumption compared to placebo-treated old rats ($P<.01$).

4. Discussion

Depressive disorders are more common among the elderly than among younger people and tend to co-occur with EtOH misuse (Welte, 1998). Both stress and depression are associated with an increase in cortisol and corticosterone plasmatic levels (Kim and Yoon, 1998; McEwen, 1992).

One biological parameter that came under scrutiny in the past few years is the HPA axis, an endocrine closed-loop system controlling the secretion of stress hormones (glucocorticoids). A prominent feature of aging is the deficiency in HPA response to stress as well as decline in negative feedback sensitivity to glucocorticoids and the development of glucocorticoid hyper secretion in later life (McEwen, 1992). Perhaps it could explain alcoholism and depression in later life—two disorders characterized by increased glucocorticoid secretion in a significant proportion of patients.

In our study, we observed that aged rats exposed to ISOL stressful stimuli increased their EtOH intake during the stress period and maintained this increase throughout the entire poststress period as compared to nonstressed animals. Whereas young rats exhibit complete tolerance to the stimulatory effects of EtOH on corticosterone levels, impaired adaptation of the HPA axis to EtOH in aged rats was observed. Thus, the loss in the ability to down-regulate both stress and EtOH responses, and the elevated glucocorticoids might induce increased EtOH consumption during post-stress period noted in our study.

The hippocampus, a brain region involved in learning, memory and a number of neurological disease states (depression, etc.), is a target of adrenal steroids (McEwen, 1992; Vogel, 2000) and is through to play a role in the function of the adrenal–hypothalamic–pituitary axis (Issa et al., 1990). The hippocampus is enriched with receptors for corticosterone (a glucocorticoid hormone released in response to stress) and plays a role in glucocorticoid negative feedback and, therefore, some hippocampus functioning might be particularly susceptible to stress (Kim and Yoon, 1998; Vogel, 2000). The capacity of the hippocampus to show such down-regulation declines with age and results in a progressive destruction of hippocampus neurons, leading to progressively rising titers of adrenal steroids (McEwen, 1992). Our old animals showed marked decreases in neuron density in all areas examined and higher levels of corticosterone. These could be an evidence for a age-related deficit in HPA function during and following stress, which is associated with higher and prolonged EtOH consumption compared to younger rats.

The 5-HT_{2A} receptor is an important component of the neural substrates underlying EtOH intake and behaviours related to anxiety and stress (Blakley et al., 2001). Also, 5-HT_{2A} receptors have a partial role in controlling affective states. Then their modulation by corticosteroids provides a potential mechanism by which these hormones may regulate mood (Haile et al., 2001). These receptors are found with the highest density in the prefrontal cortex, the piriform cortex, the claustrum and in several structures associated with reward and reinforcement pathways, as well as with some structures associated with stress and anxiety, such as prefrontal cortex, amygdala, dorsal raphe nucleus and hippocampus. CNS-wide reduction of 5-HT_{2A} receptors decreased EtOH consumption, demonstrating that compounds that down-regulate 5-HT_{2A} receptors also reduce EtOH consumption. 5-HT_{2A} receptor knockdowns also produced changes in anxiety and stress and modulated plasma corticosterone (Blakley et al., 2001).

Nefazodone hydrochloride is a phenylpiperazine antidepressant with a mechanism of action that is distinct from those of other currently available drugs. It potently and selectively blocks postsynaptic 5-HT_{2A} receptors and moderately inhibits serotonin and noradrenaline reuptake (Davis et al., 1997). The influence of nefazodone on the neuroendocrine response to stress should be also mainly involved in the regulation of voluntary EtOH intake. Nefazodone attenuates stress-induced (Brotto et al., 2001) or chronic corticosterone administration-induced (Hanson et al., 1998) increases in 5-HT_{2A} receptor-mediated behaviours in rats. Also, this compound partially reverses the stress-induced increase in ACTH secretion and it has a stimulatory effect of nefazodone on ACTH and corticosterone secretion in unstressed rats (Matheson et al., 1997). In our study, we observed that nefazodone reduced the EtOH consumption

and the corticosterone plasmatic levels induced by ISOL stress, both in young and aged rats. Therefore, nefazodone seems to be useful both in young and aged stressed rats, but the age-related shut-off of glucocorticoid secretion could be one reason for the lower effect of nefazodone on the ISOL stress effects at the recovery period compared to young ones. Nevertheless, nefazodone enhances EtOH consumption in nonstressed old rats during the recovery period but not in their younger counterparts. These contrasting findings may be attributed to recent data demonstrating nefazodone-decreased EtOH intake in high—but not low—consuming rats (Olausson et al., 1998). This finding would be in agreement with our data since old rats consume more EtOH than young ones in the present study. Additionally, a recent paper (Blakley et al., 2001) showed that the 5-HT_{2A} receptor can positively or negatively regulate EtOH intake and plasma glucocorticoid levels, depending upon the central nervous system structure targeted (prefrontal cortex, central amygdaloidal nucleus, medial and lateral division, dorsal raphe nucleus or hippocampus). Down-regulation of 5-HT_{2A} receptors in the prefrontal cortex resulted in an EtOH consumption increase, suggesting that the 5-HT_{2A} receptors in this central nervous system structure have a tonic inhibitory effect on EtOH, whereas down-regulation of 5-HT_{2A} receptors in the central amygdaloidal nucleus decreased EtOH consumption (Blakley et al., 2001). They suggested that those data, combined with the moderate success seen using serotoninergic agents to decrease alcohol consumption and treat stress-related disorders, imply that the down-regulation of 5-HT_{2A} receptor produced by chronic use of these compounds may be one mechanism through which they affect such behaviours. Age- and EtOH-related changes in 5-HT_{2A} receptors and 5-HT reuptake sites (Druse et al., 1997) may also contribute to EtOH consumption in our nonstressed nefazodone-treated old rats but not in younger ones. Both aging and chronic EtOH intake were associated with a decline in the concentration of 5-HT_{2A} receptors in the nucleus accumbens and in the frontal cortex. In contrast, 5-HT reuptake sites were increased in older rats in the frontal cortex, amygdala and CA3 region of the hippocampus (Druse et al., 1997).

Since EtOH intake increased in nonstressed nefazodone-treated old rats, in the absence of a similar enhancement of corticosterone levels, it is possible that factors other than or in addition to alterations in HPA function can modify EtOH intake in old rats. First, serotonin mediates EtOH intake as a part of its larger role in behavior modulation, such that increases in serotoninergic functioning decrease EtOH intake, and decreased serotoninergic functioning increases EtOH intake. EtOH produces transient increases in serotoninergic functioning that activate the mesolimbic dopaminergic reward system (LeMarquand et al., 1994). Aging and EtOH-decreased serotonin level could be due to the decreased transport of precursor amino acid (tryptophan) across the blood–brain barrier, the diminished activity of the anabolic enzymes and/or enhanced activity of catabolic enzymes, which could lower the neurotransmitters level in aging brain, and the decline of the transport of tryptophan (Hussain and Mitra, 2000). Second, EtOH-increased levels of endorphins have important implications on EtOH self-administration as well, as opioids antagonist may exert an inhibitory effect on EtOH self-administration (Kranzler, 2000). Third, melatonin could have effects on EtOH intake, a serotoninergic Type 2A (5-HT_{2A}) receptor-mediated behaviour, because it is a putative 5-HT_{2A} antagonist (Brotto et al., 1999), but melatonin declines with aging (Trentini et al., 1992) and EtOH reduces melatonin synthesis in rat pinealocytes (Chick and Ho, 1992). Fourth, chronic EtOH consumption results in significant abnormalities in both the dopaminergic and the serotoninergic systems of aged rats, which may contribute to the problems in EtOH abuse found in the aged (Woods and Druse, 1996).

Steckerler et al. (1999) suggest that normalization of HPA function is critical for relief of the clinical symptoms of affective disorders such as depression. In our study, there is evidence suggesting that corticosterone hypersecretion contributes to the enhanced EtOH consumption and that reduction of stress-induced corticosterone levels is necessary to lower EtOH consumption. Work by several neuroscientists has shown that there is a cross-sensitization between stress and EtOH consumption mediated by corticosterone plasmatic levels (Roberts et al., 1995). Those insights put together, the normalization of this hormone might be the first step to reduce stress-induced EtOH consumption and EtOH-related problems.

In summary, the present results suggest that exposure to ISOL stress produces enhancement of EtOH consumption at the stress and poststress periods in aged rats, which are partially reversed by nefazodone.

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