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Influences of Housing Conditions and Ethanol Intake on Binding Characteristics of D_2, 5-HT_{1A}, and Benzodiazepine Receptors of Rats

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RILKE, O., T. MAY, J. OEHLER AND J. WOLFFGRAMM. *Influences of housing conditions and ethanol intake on binding characteristics of D_2, 5-HT_{1A}, and benzodiazepine receptors of rats.* PHARMACOL BIOCHEM BEHAV **52**(1) 23–28, 1995.—The effects of different housing conditions and ethanol treatment (6 vol % in the drinking water) on the in vitro binding characteristics of striatal dopaminergic D_2 ([^{3}H]spiperone), hippocampal serotonergic 5-HT_{1A} ([^{3}H]8-OH-DPAT), and cortical benzodiazepine ([^{3}H]flunitrazepam) receptors have been examined. Social deprivation due to contact caging, short- (1 day) and long-term isolation (5 weeks) yielded a significant decrease of striatal D_2 receptor density with the greatest decrease after long-term isolation (-21% B_{max}) without changes of K_d in comparison to group animals. The effect of ethanol on striatal D_2 receptor density depended on the housing conditions. Whereas ethanol treatment reduced receptor density of group animals (down to 88%), chronic exposure to ethanol under long-term isolation elicited no significant alteration of D_2 receptor density compared with group animals. Different housing and ethanol treatment had no effect on 5-HT_{1A} receptor affinity and density. Alterations of benzodiazepine receptor density were not found, but social deprivation as well as ethanol treatment of group animals caused an increased affinity of [^{3}H]flunitrazepam (reduced K_d value). These results indicate that different housing conditions of adult rats evoked significant alterations in D_2 and benzodiazepine receptor binding assays, which were modified by ethanol treatment in the case of striatal D_2 receptor density.

Social isolation Ethanol D_2 receptor 5-HT_{1A} receptor Benzodiazepine receptor Rat

SOCIAL isolation is known to affect synaptic systems in the brain of rodents. Among others, dopaminergic, serotonergic, and GABAergic signal transmission are concerned. Most studies have examined the effect of rearing in isolation, i.e., the influence of early social deprivation on the developing central nervous system. For example, dopaminergic activity has been shown to be increased in the nucleus accumbens and the striatum of isolated rats (5). The turnover of central 5-HT has been postulated to be increased (11) or decreased (24) in isolated animals. Changes in receptor sensitivity have been suggested from observations of the effects of the dopamine receptor agonist apomorphine (39) and the 5-HT_{1A} agonist 8-hydroxy-2-[di-n-propylamino]tetraline (8-OH-DPAT) (12) as well as by receptor binding studies of dopamine D_2 (2,15) and benzodiazepine receptors (10).

There are, however, limited data available on adaptive changes of receptors in response to different housing conditions of adult rats. There is growing evidence that social factors can modify even behavioral and neurochemical parameters at later stages of life, e.g., isolated rats display an increased alcohol consumption (40) and differ in response to diazepam compared to group-housed animals (17).

Neurochemical alterations produced by treatment with ethanol remain controversial and incompletely understood. In view of different ethanol intake of isolated and group-housed animals (40), we suggest a housing condition-dependent action

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of ethanol. Direct and indirect evidence have suggested that some of the central actions of ethanol may be mediated via the dopaminergic system. In vivo, ethanol increases the extracellular dopamine concentration in the striatum and the nucleus accumbens (9). Chronic exposure to ethanol reduces both dopamine D_1 and D_2 receptor densities (20). Studies have shown that chronic ethanol administration results in alterations in the structure and function of $GABA_A$ receptors.

After chronic ethanol treatment $GABA_A$ receptors α_1 subunit mRNA and polypeptide levels are reduced in the rat cortex (23). The involvement of the CNS serotonin system in regulating alcohol drinking behavior and mediation of ethanol effects is indicated by neurochemical studies: a) rats selectively bred for ethanol preference have higher densities of 5-HT_{1A} receptors in the cerebral cortex and hippocampus (22); b) the density of 5-HT_{1A} receptors is decreased in the hippocampus during chronic ethanol intoxication and withdrawal (37).

The aim of this study was to determine the effects of different housing conditions (individual caging, partial social deprivation, and group caging) as well as the combined influence of ethanol treatment and social deprivation on dopaminergic D_2, serotonergic 5-HT_{1A}, and benzodiazepine receptors.

METHOD

Animals and Housing

The experiments were performed with male Wistar rats (n = 72, breeder: Lippische Versuchstierzucht, Extertal, Germany); body weight at the beginning of the experiments ranged from 250 to 330 g. To study the effects of gradual social deprivation and ethanol consumption in a period of 5 weeks, the animals were divided randomly into six experimental groups of each 12 animals.

Group I. The rats were housed in groups of four rats (group animals, G).

Group II. Under the same housing condition the rats were forced to drink 6% (v/v) ethanol solution in tap water (group animals + ethanol treatment, GE).

Group III. Four animals, each, were kept in contact cages (contact animals, C).

Group IV. The animals were housed individually for 1 day (short-term isolation, S).

Group V. The animals were housed individually for 5 weeks (long-term isolation, L).

Group VI. The rats were housed individually like group V with 6% ethanol as the drinking fluid (long-term isolation + ethanol treatment, LE).

Individually housed animals were kept in single Makrolon cages (43 × 26 × 15 cm). Contact housing used Makrolon cages of the same dimension for each individual, but there was a contact area in the middle of the four cages in which vertical bars positioned at a distance of 1 cm to each other allowed the rats to communicate with the other three members of the group (41). Group-housed rats were kept together (four individuals) in a large Makrolon cage (60 × 38 × 20 cm). The animals were kept in a controlled environment at 21 ± 2°C and 40–60% air humidity with an 12L : 12D cycle, with lights on between 0500 and 1700 h. Standard diet (Altromin 1324) was available ad lib. The drinking fluid consisted of tap water or 6% ethanol solution depending on the experimental conditions. Body weight, food consumption, and fluid intake were measured two times a week.

In Vitro Binding Studies

The animals were killed 7 h after fluid withdrawal by decapitation, and brains were rapidly removed on ice. Cerebral cortex, striatum, and hippocampus were dissected, frozen in liquid nitrogen, and stored at −80°C until use. The thawed tissues were homogenized (10 strokes with a glass-teflon homogenizer, 1000 turns per min) in 100 volumes (w/v) of 50 mM Tris-HCl, pH 7.4, and centrifuged for 10 min at 40,000 × g. The resulting pellets were rehomogenized (5 strokes) in the same buffer, hippocampal membranes were incubated at 37°C for 10 min to remove endogenous serotonin (26), and recentrifuged. Striatal membranes were rehomogenized in 50 mM Tris-HCl, 120 mM NaCl, pH 7.4, with a final tissue concentration of 15 mg orginal wet weight per ml, cortical membranes in 50 mM Tris-HCl, pH 7.4, (final tissue concentration of 20 mg per ml), and hippocampal membranes in 50 mM Tris-HCl, pH 7.4, 2 mM $CaCl_2$ with a final tissue concentration of 15 mg per ml.

Receptor-binding assays were performed using [^{3}H]spiperone, [^{3}H]flunitrazepam, and [^{3}H]8-OH-DPAT as binding ligands for dopaminergic D_2, benzodiazepine, and serotonergic 5-HT_{1A} receptors, respectively.

[^{3}H]Spiperone (specific activity: 23.5 Ci/mmol; Amersham Buchler, Braunschweig, Germany) was used as the radioligand for labeling dopamine D_2 receptors in rat striatal preparations (32). The membrane homogenate (100 µl) was incubated in 3.0 ml Tris-HCl, 120 mM NaCl, pH 7.4, including six concentrations (0.01–0.3 nM) of [^{3}H]spiperone at 23°C for 90 min. Nonspecific binding was defined in the presence of 10 µM S(−)sulpiride.

The benzodiazepine binding assay was performed with cerebral cortex membranes according to the method described by Rägo et al. (28), with slight modifications. Binding of [^{3}H]flunitrazepam (0.2–5.0 nM; specific activity 84.3 Ci/mmol; Amersham Buchler, Braunschweig, Germany) was carried out in a total incubation volume of 500 µl of 50 mM Tris-HCl buffer, pH 7.4, in the presence (defining nonspecific binding) and the absence of 1 µM flumazenil (gift from Hoffmann-LaRoche, Basel, Switzerland). The binding assay was performed for 60 min at 0°C.

[^{3}H]8-OH-DPAT was used to label 5-HT_{1A} receptors in hippocampal membranes according to the modified method of Hall et al. (16) including $CaCl_2$ in the assay buffer to enhance the specific binding of [^{3}H]8-OH-DPAT. Membranes were incubated at 23°C for 30 min in a final volume of 0.3 ml of 50 mM Tris-HCl, pH 7.4, containing 2 mM $CaCl_2$ and [^{3}H]8-OH-DPAT (0.05–1.5 nM; specific activity: 162.9 Ci/mmol; Amersham Buchler, Braunschweig, Germany). Nonspecific binding was determined by incubation of samples with unlabeled serotonin (10 µM).

All incubations were stopped by rapid filtration with a Brandel cell harvester through GF/B filters (presoaked in 0.1% polyethyleneimine) under reduced pressure. The filters were washed twice with 4 ml of ice-cold 50 mM Tris-HCl, pH 7.4, and the radioactivity was determined by a liquid scintillation counter (counting efficiency about 60%). Analysis of binding data was performed according to Scatchard (31).

Protein content was measured by the BioRad® assay according to Bradford (7), with bovine serum albumin (Sigma) as the standard.

Statistical Analysis

All parametric data were analysed using an analysis of variance (CoStat 4.2., CoHort Software, Berkeley, CA). Significant F ratios were further analysed by a Duncan's multiple range test. Interrelationships between different parameters were studied by linear regression analysis.

TABLE 1

EFFECTS OF DIFFERENT HOUSING AND CHRONIC ETHANOL TREATMENT ON [^{3}H]SPIPERONE BINDING AFFINITY (STRIATUM), [^{3}H]8-OH-DPAT BINDING PARAMETERS (HIPPOCAMPUS), AND [^{3}H]FLUNITRAZEPAM BINDING DENSITY (CORTEX)

Group	G	GE	C	S	L	LE
[^{3}H]Spiperone binding affinity:						
K_d (pM)	26.6 ± 0.6	27.0 ± 0.5	27.3 ± 0.8	27.2 ± 0.6	26.9 ± 0.7	26.8 ± 0.8
[^{3}H]8-OH-DPAT binding parameters:						
B_{max} (fmol/mg protein)	363 ± 19	340 ± 13	348 ± 18	370 ± 11	350 ± 14	340 ± 12
K_d (pM)	323 ± 13	344 ± 24	324 ± 17	345 ± 21	336 ± 18	318 ± 19
[^{3}H]Flunitrazepam binding density:						
B_{max} (pmol/mg protein)	2.50 ± 0.06	2.48 ± 0.07	2.53 ± 0.08	2.54 ± 0.07	2.54 ± 0.09	2.56 ± 0.06

Means ± SEM (n = 12).

G: group animals; GE: group animals + ethanol treatment; C: contact animals; S: short-term isolation; L: long-term isolation; LE: long-term isolation + ethanol treatment.

RESULTS

Housing Data

The body weight of the rats was 250–330 g at the beginning of the treatment. During the observation period of 5 weeks, the increases of body weights remained constant in all groups. Animals treated with ethanol (GE + LE) had reduced food intake (down to 81%) due to the caloric properties of ethanol. The ethanol intake of these groups did not differ significantly (GE: 6.2 ± 0.3 g/kg per day, LE: 6.0 ± 0.2 g/kg per day).

[^{3}H]Spiperone Binding

No significant differences of the K_d values were found between the different six groups (Table 1). However, analysis of variance (ANOVA) revealed a significant effect of housing conditions on striatal dopamine D_2 receptor density, $F(5, 66) = 7.32$, $p < 0.001$. Long-term isolated rats (L) displayed a 21% reduced B_{max} value compared with group animals (G). Ethanol treatment of long-term isolated animals (LE group) reversed the isolation dependent downregulation of the D_2 receptor density, because no differences were found between group animals (G) and long-term isolated animals with ethanol treatment (LE). However, ethanol treatment of group housed animals resulted in a downregulation to 88%. Contact caging and short-term isolation elicited also a decrease of D_2 receptor density (to 85% and 86%). Figure 1 presents these results.

[^{3}H]8-OH-DPAT Binding

Analyses of displacement data as well as linear Scatchard plots ($r > 0.97$) revealed a single high affinity binding site. In the present study, a single-site model of [^{3}H]8-OH-DPAT binding was most consistent with the observed data, and no evidence was obtained for labeling of a second, low affinity binding site as detected by Gozlan et al. (14) in cortical membranes.

We have found that receptor binding affinity and density of the 5-HT_{1A} receptor measured in the hippocampus were neither affected by different housing conditions nor by alcohol treatment (Table 1).

[^{3}H]Flunitrazepam Binding

Different housing conditions and ethanol treatment did not influence the benzodiazepine receptor density in the cortex (Table 1). However, there was a significant difference in the affinity (K_d) of [^{3}H]flunitrazepam to benzodiazepine receptors as a result of housing conditions, $F(5, 66) = 4.3$, $p < 0.002$. Post hoc comparisons revealed that long-term and short-term isolation, contact caging, and ethanol treatment of group-housed animals produced a significant decrease of the K_d value (reduction of K_d down to 85%). Ethanol treatment of long-term isolated rats (LE) did not further decrease the K_d value compared to isolated animals without ethanol treatment (Fig. 2).

Interrelationships

Interrelationships between all individually measured receptor binding data as well as housing data were analyzed. Figure

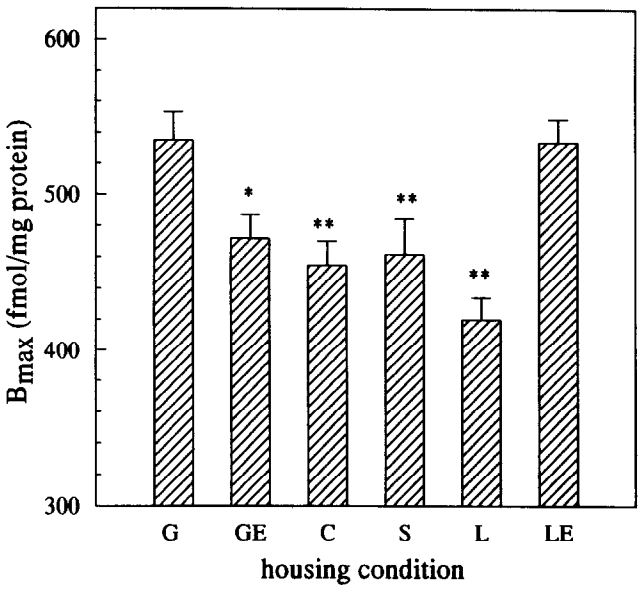

FIG. 1. Effect of different housing and chronic ethanol treatment on [^{3}H]spiperone binding (B_{max}) in the striatum. Error bars indicate SEM (n = 12). *$p < 0.05$, **$p < 0.01$, compared to group housing -G- (Duncan's multiple range test). G: group animals; GE: group animals + ethanol treatment; C: contact animals; S: short-term isolation; L: long-term isolation; LE: long-term isolation + ethanol treatment.

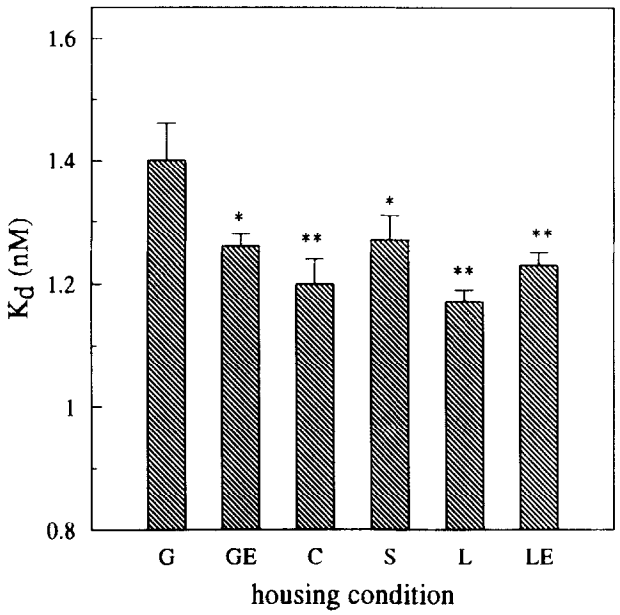

FIG. 2. Effect of different housing and chronic ethanol treatment on [^{3}H]flunitrazepam binding affinity (K_d) in the cortex. Error bars indicate SEM (n = 12). *p < 0.05, **p < 0.01, compared to group housing -G- (Duncan's multiple range test). G: group animals; GE: group animals + ethanol treatment; C: contact animals; S: short-term isolation; L: long-term isolation; LE: long-term isolation + ethanol treatment.

3 shows a significant correlation of D_2 receptor density and ethanol consumption observed in the LE group (n = 12, r = 0.764, p < 0.01).

An apparent negative correlation was established in the two ethanol groups (GE and LE: n = 24, r = −0.705, p < 0.001) between receptor density of 5-HT_{1A} and benzodiazepine receptors (Fig. 4). This relation was not found in the animals receiving tap water.

DISCUSSION

Binding studies of dopamine D_2 receptors in the striatum revealed a significant decrease of the density under social deprivation (contact caging, short- and long-term isolation). The reduction in striatal dopamine D_2 receptor density after 5 weeks of isolation is consistent with a recent study indicating that the binding density is decreased in the striatum of rats after 40 days of isolation (2). However, we have found that even a short isolation period of 24 h produced a significant decrease in dopamine D_2 receptor density. Dopaminergic activity has been shown to be increased in the nucleus accumbens and the striatum of isolated (8 weeks) Sprague-Dawley rats (5). Corresponding increases of tyrosine hydroxylase mRNA concentration (1) as well as activity (33) have been found in isolated animals. The reduced dopamine D_2 receptor density can, therefore, be interpreted as a consequence of higher dopamine concentration at the synaptic receptor site. A parallel to our results is provided by studies on stress-induced changes in the dopaminergic activity. Seven weeks exposure to the chronic mild stress procedure increased the concentration of dopamine and yielded a downregulation of dopamine D_2 receptors in the nucleus accumbens (38). Conversely, haloperidol treatment decreased dopaminergic activities (18) and resulted in an upregulation of dopamine D_2 receptor density in rat striatum (2,42). The effect of ethanol treatment on striatal dopamine D_2 receptor density was dependent on the housing conditions. Whereas ethanol treatment reduced the B_{max} value of group animals, ethanol treatment in long-term isolation reversed the isolation mediated downregulation of dopamine D_2 receptor density and resulted in no alterations of the B_{max}

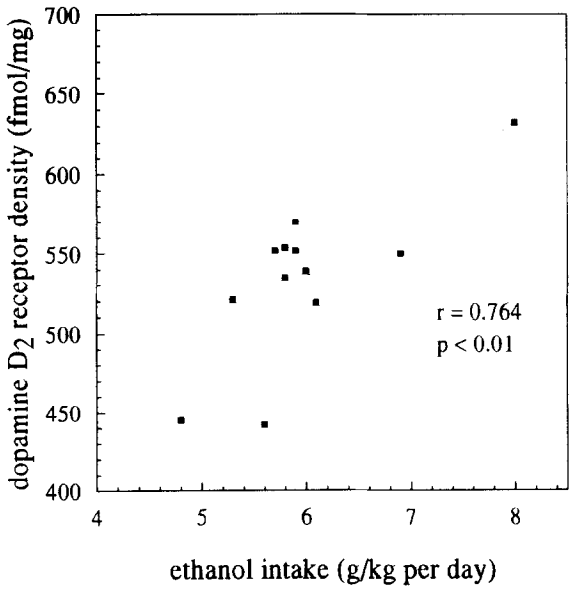

FIG. 3. Correlation between D_2 receptor density and individual ethanol intake (means) of long-term isolated animals with chronic ethanol treatment (LE).

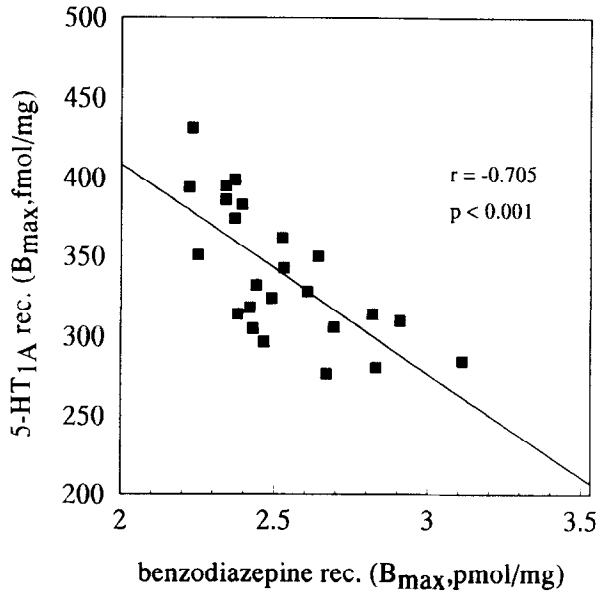

FIG. 4. Correlation between the receptor densities of 5-HT_{1A} (hippocampus) and benzodiazepine receptor (cortex) of the two alcohol groups: group animals and long-term isolated animals with chronic ethanol treatment (GE and LE).

values of long-term isolated animals with ethanol treatment vs. the control group animals. Such interferences of receptor regulation by ethanol have also been reported in the reduced upregulation of dopamine D_2 receptors after subchronic haloperidol treatment (42) and in the prevented downregulation of β-adrenoceptors after chronic treatment with desipramine (30). The mechanisms by which such regulations are influenced are still unclear, but they could consist in a specific interaction with the process of receptor adaptation.

Controversy exists in the literature regarding the effect of ethanol treatment on dopamine content and its metabolites as well as on dopamine receptor densities. Authors reported an increase (29) or decrease (34) of the dopamine D_2 receptor density after chronic administration of ethanol. No changes (13) and enhancement (8) of striatal dopaminergic activity after ethanol treatment have been found. Thus, our present results support the view that housing conditions should be considered in discussions of ethanol effects on dopaminergic systems.

The amount of ethanol intake of long-term isolated animals with ethanol treatment correlates positively with dopamine D_2 receptor density. This finding suggests that the amount of ethanol intake can determine the increase (compared with long-term isolated animals without ethanol treatment) of dopamine D_2 receptor density in long-term isolation. On the other hand, Bisaga and Kostowski (4) have suggested that individual differences in the acquisition of high ethanol intake may be influenced, at least partially, by the differences in the sensitivity of brain dopaminergic neurotransmission. In view of the enhanced ethanol consumption by isolated rats (40), different effects of ethanol and housing condition on dopamine D_2 receptors could modify drug taking behavior in animals: we found that social deprivation reduced striatal dopamine D_2 sensitivity (downregulation). Socially deprived rats tend to drink more ethanol (40). In a similar way, the downregulation induced by subchronic administration of lisuride (27) also causes a significant increase of voluntary ethanol consumption (21). This is not a proof, but an indication for a correlation between free choice intake of ethanol and dopaminergic sensitivity. Because the interaction between ethanol ingestion and housing condition is nonadditive, the D_2 receptor density would be normalized in socially deprived rats (but not in group-housed ones) by means of ethanol drinking.

It has been found that the selective serotonergic 5-HT_{1A} receptor agonist 8-OH-DPAT produces greater responding in isolated rats, suggesting supersensitivity of the postsynaptic 5-HT_{1A} receptor (43).

However, our data show unchanged hippocampal 5-HT_{1A} receptor density and affinity in the tested housing conditions, not disproving that other 5-HT_{1A}-rich areas such as raphe dorsal and median nuclei could be involved in such suggested receptor regulations. Thus, Beer et al. (3) found that 8-OH-DPAT administration is accompanied by a 25% downregulation of the number of 5-HT_{1A} binding sites in the raphe but not in the hippocampus. We could not confirm the results of Ulrichsen (37), who detected a decrease in [^{3}H]8-OH-DPAT binding in the hippocampus of Wistar rats during chronic ethanol intoxication and withdrawal, respectively. In accordance with the present study, on the other hand, hippocampal [^{3}H]8-OH-DPAT binding has been reported to be unaffected by chronic ethanol administration in Sprague-Dawley rats (19), suggesting that the ethanol feeding technique represents an important factor in view of alterations in the [^{3}H]8-OH-DPAT binding characteristics produced by chronic ethanol treatment.

Behavioral data of differently housed rats in a shock-probe procedure (unpublished results) and different responses to diazepam (17) indicate modifications in the GABA/benzodiazepine receptor system. Although alteration of benzodiazepine binding site density, affinity, and coupling were not measured in isolated Sprague-Dawley rats (24), in our experiments isolated (short- and long-term) and contact caged Wistar rats displayed an increased in vitro affinity of [^{3}H]flunitrazepam. Thielen et al. (36) have shown an increase in the efficacy of flunitrazepam to enhance GABA-stimulated $^{36}Cl^-$ influx in microsacs prepared from cerebral cortex of isolated rats. These results are consistent with the present findings of reduced K_d values (increased affinity) of [^{3}H]flunitrazepam in cortical membranes of isolated animals. The authors suggested that altered response to GABA and flunitrazepam might result from alterations in $GABA_A$/benzodiazepine receptor subunit expression as well as receptor phosphorylation.

However, it is possible that the differences in [^{3}H]flunitrazepam affinity may result from different concentrations of allosteric modulators of the $GABA_A$/benzodiazepine receptor complex remained in the membrane homogenates, such as neuroactive steroids and GABA. Because GABA is a known positive allosteric effector that potentiates the binding affinity of benzodiazepine agonists (35), a higher level of GABA could produce the reduced K_d value in isolated animals. On the contrary, isolation was shown to significantly reduce levels of GABA in several parts of the limbic system of male Long Evans rats (6).

Although different housing conditions did not influence receptor densities of cortical benzodiazepine receptors and hippocampal 5-HT_{1A} receptors, we have found a negative correlation between these receptors in the alcohol treated groups. This relation was not found in groups with water treatment, but our results suggest that ethanol directly or indirectly affects both 5-HT_{1A} and benzodiazepine receptor systems.

In summary, our results provide evidence that different housing conditions of adult rats can produce significant alterations in brain neurochemistry. The observed neurochemical alterations may represent brain mechanisms of adaptation to social deprivation leading to an ethanol sensitive dopamine D_2 receptor density regulation and a regulation of affinity of benzodiazepine receptors.

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