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Regional Densities of Benzodiazepine Sites in the CNS of Alcohol-Naive P and NP Rats

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THIELEN, R. J., W. J. MCBRIDE, E. CHERNET, L. LUMENG AND T.-K. LI. *Regional densities of benzodiazepine sites in the CNS of alcohol-naive P and NP rats.* PHARMACOL BIOCHEM BEHAV **57**(4) 875–882, 1997.—The regional densities of benzodiazepine (BDZ) recognition sites coupled to $GABA_A$ receptors were studied in ethanol-naive alcohol-preferring (P) and -nonpreferring (NP) lines of rats by using quantitative autoradiography to measure the amount of 2 nM [^{3}H]flunitrazepam (FNZ) binding in the absence and presence of 100 μM GABA. Lower values ($p < 0.025$) for [^{3}H]FNZ binding (in the absence of GABA) were observed in the prefrontal cortex, layer 4 of the parietal cortex, and the nucleus accumbens shell of the P relative to the NP line. GABA significantly ($p < 0.025$) stimulated [^{3}H]FNZ binding in all 50 central nervous system regions examined in both the P and the NP rats. The largest percent increases (190–220%) were observed in the prefrontal, cingulate, frontal, and parietal cortices; shell and core nucleus accumbens; caudate putamen; dorsal lateral, intermediate lateral, ventral lateral, and medial septal nuclei; and lateral hypothalamus. In several layers of the frontal and parietal cortices, a 25–30% greater net or percent increase ($p < 0.025$) in GABA-enhanced [^{3}H]FNZ binding was observed in the P rats compared with the NP rats. In contrast, lower net or percent increases ($p < 0.025$) in GABA-enhanced [^{3}H]FNZ binding were found in the entorhinal cortex, the mediodorsal thalamus, and the dorsal CA3 area and middle dentate gyrus of the posterior hippocampus of the P line relative to the NP line. The present findings suggest that there are innate regional differences between P and NP rats in the densities and/or affinities of BDZ recognition sites and in the coupling between the $GABA_A$ and BDZ binding sites. © 1997 Elsevier Science Inc.

Alcohol-preferring rats Flunitrazepam binding Benzodiazepine recognition sites $GABA_A$ receptors Autoradiography

ETHANOL, at physiologically relevant concentrations, can enhance muscimol- or GABA-stimulated $^{36}Cl^-$ uptake into membrane preparations from the cerebral cortex and cerebellum of mice (2,10) and rats (27,34). However, ethanol potentiation of GABA-activated $^{36}Cl^-$ influx into membrane preparations has been shown to be highly dependent on assay conditions (24). Several electrophysiological studies with cultured dorsal root ganglion neurons (28) and with cultured mouse cortical/hippocampal neurons (1,41) also support ethanol potentiation of GABA-mediated chloride currents. These data suggest that the $GABA_A$–benzodiazepine–chloride receptor complex ($GABA_A$/BDZ/Cl^-) may be involved in mediating some of the actions of ethanol on the central nervous system (CNS).

The long-sleep (LS) and short-sleep (SS) mice were selectively bred for differential sensitivity to the hypnotic effects of ethanol (22). In addition to being more sensitive to the acute intoxicating effects of ethanol, LS mice also show a longer duration of sleeptime in response to a number of CNS depressants, including benzodiazepines (18,19), suggesting that there might be differences in the $GABA_A$/BDZ/Cl^- complex between the LS and SS mice. GABA has been shown to enhance [^{3}H]flunitrazepam (FNZ) binding to a greater extent in the cerebral cortex of SS compared with LS mice (4,23). Similar findings have been reported for the alcohol-tolerant (AT) and alcohol-nontolerant (ANT) rats, which were selectively bred for differences in alcohol-induced ataxia (17).

The alcohol-preferring (P) and -nonpreferring (NP) lines of rats were selectively bred for their disparate alcohol drinking behaviors (14,15). However, in addition to differences in alcohol drinking behavior, the P rats show greater locomotor stimulation at low ethanol doses (40) and less motor impairing

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effects at high ethanol doses (16,39) than do the NP rats. If these dissimilar behavioral effects of ethanol between the lines are due in part to innate regional CNS diversities in the $GABA_A$/BDZ/Cl^- receptors, then it might be possible to detect receptor differences between the P and NP rats with quantitative autoradiography. Therefore, the present study was undertaken to determine the CNS regional densities of [^{3}H]FNZ binding sites in alcohol-naive P and NP rats and to assess the functional coupling between the $GABA_A$ site and the BDZ site by examining GABA-stimulated [^{3}H]FNZ binding.

METHODS

Animals

Alcohol-naive adult (90–105 days) male P and NP rats from the S33–S35 generations (13,16) were used in this study. Animals were housed in a temperature-controlled room, maintained on a 12 L:12 D cycle (lights on at 0600 h), with food and water available ad lib. Animals were housed in pairs (P with P, NP with NP) in standard plastic animal containers.

Chemicals

Tris base, concentrated HCl, GABA, and clonazepam were purchased from Sigma Chemical Co. (St. Louis, MO, USA). [^{3}H]Flunitrazepam (85 Ci/mmol) was purchased from Amersham (Arlington Heights, IL, USA). Cresyl violet was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA).

[^{3}H]FNZ Binding in the Presence and Absence of 100 μM GABA

Animals were killed by decapitation and the brains were rapidly removed and placed in liquid nitrogen for 17 s. Frozen

TABLE 1

DENSITIES OF BENZODIAZEPINE RECOGNITION SITES LABELLED WITH [^{3}H]FLUNITRAZEPAM (FNZ) IN THE ABSENCE AND PRESENCE OF 100 μM GABA IN CEREBRAL CORTICAL REGIONS OF ALCOHOL-NAIVE P AND NP RATS

		fmol/mg Protein (mean ± SEM, n = 4)			
Region		FNZ	FNZ + GABA	Net Increase	% Increase
PF	P	509 ± 22*	969 ± 36	460 ± 36	191 ± 9
	NP	606 ± 49	963 ± 78	357 ± 30	159 ± 2
Cg	P	631 ± 36	1209 ± 24	599 ± 49	193 ± 12
	NP	666 ± 52	1149 ± 60	482 ± 24	173 ± 6
Fr 1–3	P	722 ± 4	1353 ± 31	631 ± 32	187 ± 5
	NP	773 ± 30	1345 ± 43	572 ± 19	174 ± 3
Fr 4	P	733 ± 40	1442 ± 26	709 ± 60*	198 ± 14
	NP	813 ± 43	1381 ± 34	569 ± 12	170 ± 5
Fr 5, 6	P	456 ± 18	934 ± 13	478 ± 20*	206 ± 9
	NP	458 ± 38	844 ± 44	386 ± 10	185 ± 6
Par 1–3	P	615 ± 8	1129 ± 5	514 ± 4*	184 ± 2*
	NP	690 ± 25	1059 ± 55	369 ± 54	154 ± 9
Par 4	P	624 ± 12*	1137 ± 9	513 ± 17	182 ± 4
	NP	707 ± 31	1173 ± 40	466 ± 31	166 ± 5
Par 5, 6	P	361 ± 8	727 ± 17	365 ± 24	202 ± 9*
	NP	409 ± 18	701 ± 50	292 ± 39	171 ± 9
Oc 1–3	P	993 ± 41	1317 ± 93	317 ± 82	132 ± 9
	NP	1018 ± 32	1403 ± 66	384 ± 92	138 ± 10
Oc 4	P	1082 ± 40	1392 ± 104	287 ± 120	126 ± 12
	NP	1097 ± 38	1437 ± 44	339 ± 80	131 ± 8
Oc 5, 6	P	748 ± 14	1088 ± 67	339 ± 62	145 ± 8
	NP	757 ± 36	1089 ± 35	331 ± 53	144 ± 9
Te 1–3	P	910 ± 96	1212 ± 67	321 ± 130	140 ± 22
	NP	906 ± 31	1263 ± 46	354 ± 60	139 ± 8
Te 4	P	992 ± 54	1295 ± 38	296 ± 71	130 ± 9
	NP	978 ± 33	1325 ± 45	347 ± 58	135 ± 7
Te 5, 6	P	640 ± 25	874 ± 42	241 ± 50	139 ± 10
	NP	595 ± 30	923 ± 38	327 ± 50	155 ± 11
Ent	P	896 ± 29	1023 ± 38	126 ± 13*	114 ± 1*
	NP	854 ± 41	1052 ± 38	199 ± 29	123 ± 4
Ant Pir	P	543 ± 13	877 ± 20	334 ± 24	162 ± 5
	NP	581 ± 24	916 ± 35	335 ± 16	158 ± 3
Post Pir	P	1002 ± 51	1299 ± 88	297 ± 88	130 ± 9
	NP	908 ± 70	1225 ± 56	317 ± 65	136 ± 9

Net and % increases are reported for FNZ + 100 μM GABA binding with respect to FNZ binding alone. *$p < 0.025$ for P vs. NP values. Cortical regions: PF, prefrontal; Cg, cingulate; Fr, frontal, layers 1–6; Par, parietal, layers 1–6; Oc, occipital, layers 1–6; Te, temporal, layers 1–6; Ent, entorhinal; Ant Pir, anterior piriform; Post Pir, posterior piriform.

TABLE 2

DENSITIES OF BENZODIAZEPINE RECOGNITION SITES LABELLED WITH [^{3}H]FLUNITRAZEPAM (FNZ) IN THE ABSENCE AND PRESENCE OF 100 μM GABA IN BASAL GANGLIA AND SELECTED LIMBIC AREAS OF ALCOHOL-NAIVE P AND NP RATS

		fmol/mg Protein (mean ± SEM, n = 4)			
Region		FNZ	FNZ + GABA	Net Increase	% Increase
Acb					
Shell	P	341 ± 19*	691 ± 21	350 ± 17	203 ± 9
	NP	456 ± 32	676 ± 35	220 ± 51	150 ± 15
Core	P	321 ± 23	651 ± 7	324 ± 13	203 ± 10
	NP	415 ± 47	645 ± 18	236 ± 52	162 ± 20
OTu	P	471 ± 10	829 ± 10	358 ± 18	176 ± 6
	NP	512 ± 11	866 ± 19	353 ± 16	169 ± 4
VP	P	493 ± 16	855 ± 44	362 ± 33	173 ± 6
	NP	504 ± 14	854 ± 38	350 ± 29	169 ± 5
VTA	P	209 ± 17	368 ± 29	165 ± 53	186 ± 28
	NP	245 ± 9	387 ± 10	143 ± 14	159 ± 7
CPu	P	227 ± 13	504 ± 9	276 ± 5	223 ± 9
	NP	277 ± 20	494 ± 28	217 ± 21	180 ± 10
SN	P	491 ± 42	653 ± 25*	181 ± 19	139 ± 8
	NP	552 ± 27	778 ± 50	226 ± 50	141 ± 9

Net and % increases are reported for FNZ + 100 μM GABA binding with respect to FNZ binding alone. *$p < 0.025$ for P vs. NP values. Acb, nucleus accumbens, lateral and medial; OTu, olfactory tubercle; VP, ventral pallidum; VTA, ventral tegmental area; CPu, caudate putamen; SN, substantia nigra.

brains were stored in sealed bags at −70°C until sectioned. The frozen brains were placed in the cryostat (Reichert-Jung HistoSTAT Cryostat Microtome; Cambridge Instruments, Buffalo, NY, USA) and maintained at −17°C for approximately 15–30 min prior to sectioning. Coronal brain sections (20 μm) were prepared and thaw mounted onto subbed slides. Subbed slides were prepared by immersing slides twice in a subbing solution (0.5 g gelatin and 50 mg chromium potassium phosphate dissolved in 100 ml distilled–deionized H_2O) and allowing them to dry overnight. For each rat, a total of 180 sections from selected brains regions were cut. A total of 36 sections was used for total [^{3}H]FNZ binding, and 36 adjacent sections were used to define nonspecific binding. Another 72 sections, adjacent to the previous sections, were used for [^{3}H]FNZ binding in the presence of 100 μM GABA: 36 sections were used for total binding and 36 sections for nonspecific binding. Sections were stored at −20°C for 2–8 days before incubation with [^{3}H]FNZ. Finally, another 36 sections, adjacent to the previous sections, were prepared for histological staining with cresyl violet.

Labeling of BDZ recognition sites was performed as previously described (25). Sections were preincubated in buffer (0.17 M Tris-HCl, pH 7.7) at 0–4°C for 40 min. Sections were then incubated at room temperature for 60 min in buffer containing 2 nM [^{3}H]FNZ, a nonselective BZD receptor agonist, with or without 100 μM GABA, for total binding. Previous studies have demonstrated this concentration of FNZ to be approximately the K_d for binding to both BZD_1 and BZD_2 receptor subtypes (6,30). Nonspecific binding was determined in the presence of 10 μM clonazepam under the same conditions as those for total binding. The sections were then washed three times in ice-cold buffer followed by ice-cold deionized–glass distilled H_2O. The sections were dried by passing a stream of cold dry air over them and allowing them to stand at room temperature overnight. Brain sections from P and NP rats, along with tritium-labeled brain paste standards (20-μm sections thaw mounted on subbed slides as described above), were placed into standard X-ray cassettes (10 × 12 inches). Cassettes had one set of standards and sections, for total and nonspecific binding in the presence and absence of 100 μM GABA, from the same brain areas of P and NP rats. Tritium-sensitive Ultrofilm (LKB, Mager Scientific, Dexter, MI, USA) was apposed to the sections, and the cassettes were stored at 4°C. After 10 days, the Ultrofilm was developed and fixed.

Quantitative microdensity measurements of the autoradiograms were made using an AIC MicroImage (v. 2.30) image analysis system (Analytical Imaging Concept, Inc., Atlanta, GA, USA). Brain paste standards (dpm/mg protein) were corrected for tritium decay and converted to fmol [^{3}H]FNZ/mg protein.

Brain areas were identified using the rat brain atlas of Paxinos and Watson (29). Total and nonspecific binding in the presence and absence of 100 μM GABA in most areas was usually determined by taking three to six bilateral readings per animal by outlining the areas of interest. Specific binding was determined by subtracting nonspecific binding from total binding for adjacent sections. Specific binding for each area in each animal was determined by taking the average of the three to six values. The mean values for each area in different animals were grouped by line and treatment (±100 μM GABA) for statistical analysis.

Net GABA-stimulated [^{3}H]FNZ binding (fmol/mg protein) was defined as [^{3}H]FNZ binding density in the presence of 100μM GABA minus [^{3}H]FNZ binding density in the absence of GABA. Percent enhancement was determined by dividing the total amount of 100 μM GABA-stimulated [^{3}H]FNZ binding by the amount of [^{3}H]FNZ binding in the absence of GABA and multiplying by 100%.

TABLE 3

DENSITIES OF BENZODIAZEPINE RECOGNITION SITES LABELLED WITH [^{3}H]FLUNITRAZEPAM (FNZ) IN THE ABSENCE AND PRESENCE OF 100 μM GABA IN AMYGDALA, HYPOTHALAMIC, AND CENTRAL GRAY REGIONS OF ALCOHOL-NAIVE P AND NP RATS

		fmol/mg Protein (mean ± SEM, $n = 4$)			
Region		FNZ	FNZ + GABA	Net Increase	% Increase
Amygdala					
B1/Lat	P	845 ± 33	1193 ± 54	306 ± 16	141 ± 8
	NP	837 ± 77	1102 ± 33	264 ± 56	133 ± 9
Med	P	718 ± 48	1059 ± 53	341 ± 32	147 ± 6
	NP	764 ± 52	1067 ± 71	303 ± 33	140 ± 4
Cort	P	981 ± 75	1323 ± 129	342 ± 99	135 ± 10
	NP	965 ± 82	1300 ± 81	334 ± 78	135 ±9
Hypothalamus					
LH	P	213 ± 17	402 ± 27	189 ± 27	190 ± 16
	NP	239 ± 17	428 ± 21	189 ± 16	180 ± 5
VMH	P	588 ± 18	880 ± 66	291 ± 49	149 ± 7
	NP	600 ± 51	980 ± 67	379 ± 33	164 ± 6
DMH	P	475 ± 36	713 ± 24	237 ± 28	151 ± 10
	NP	479 ± 44	751 ± 61	271 ± 86	159 ± 22
Central gray					
CGD	P	506 ± 38	685 ± 7	179 ± 36	136 ± 9
	NP	500 ± 55	728 ± 29	229 ± 69	149 ± 18
CG	P	386 ± 28	502 ± 22	116 ± 36	131 ± 11
	NP	416 ± 48	561 ± 14	145 ± 56	138 ± 18

Net and % increases are reported for FNZ + 100 μM GABA binding with respect to FNZ binding alone. There are no significant differences ($p < 0.025$) between P vs. NP values. Bl/Lat, basolateral/lateral amygdala; Med, medial amygdala; Cort, cortical amygdala; LH, lateral hypothalamus; VMH, ventromedial hypothalamus; DMH, dorsomedial hypothalamus; CGD, dorsal central gray; CG, central gray.

Statistical Analysis

All data are expressed as the mean ± SEM. The data for each area were analyzed by two-way analysis of variance (line × ±GABA treatment) with repeated measures (±GABA treatment) followed by post hoc Newman–Keuls tests, when multiple comparison were carried out, using CSS: Statistica (v. 3.1). The Mann–Whitney *U*-test was used when individual comparisons between pairs of means for net and percent change data were carried out. Significance was set at $p < 0.025$.

RESULTS

The densities of [^{3}H]FNZ binding sites, in the absence of GABA, were highest in several regions of the posterior cerebral cortex (layers 1–3 and 4 of the occipital and temporal cortices, posterior piriform cortex, and entorhinal cortex; Table 1), parts of the amygdaloid complex (basolateral/lateral and cortical amygdala; Table 3), the superficial gray layer of the superior colliculus (Table 4), and regions of the anterior dorsal hippocampus (CA4 and dentate gyrus; Table 5). The densities of [^{3}H]FNZ binding sites, in the absence of GABA, were 15–25% lower ($p < 0.025$) in the prefrontal cortex, layer 4 of the parietal cortex, and shell nucleus accumbens (Tables 1, 2). There was also a tendency ($p < 0.05$) for lower densities of FNZ sites in layers 5 and 6 of the parietal cortex (Table 1) and the substantia nigra (Table 2).

GABA significantly ($p < 0.025$) stimulated [^{3}H]FNZ binding in all 50 CNS regions examined for both the P and the NP rats. In the presence of 100 μM GABA, the densities of [^{3}H]FNZ-labeled sites were highest in layers 1–3 and 4 of the frontal, parietal, occipital, and temporal cortices, as well as in the posterior piriform and cingulate cortices (Table 1), the amygdaloid complex (Table 3), the superficial gray layer of the superior colliculus (Table 4), the dorsal dentate gyrus (both anterior and posterior), and the anterior dorsal CA4 region of the hippocampus (Table 5). The lowest densities of [^{3}H]FNZ recognition sites, both in the absence and in the presence of GABA, were found in the caudate putamen, ventral tegmental area (Table 2), lateral hypothalamus (Table 3), intermediate lateral septum (Table 4), and mediodorsal and posterior ventral thalamic nuclei (Table 4). The densities of [^{3}H]FNZ binding in the presence of GABA were significantly lower ($p < 0.025$) in the substantia nigra, mediodorsal thalamus, and middle dentate gyrus (Tables 2, 4, and 5) and higher in the CA2 region of the anterior dorsal hippocampus (Table 5) of the P compared with the NP line.

The largest percent increases (190–220%) were observed in several cortical regions (prefrontal, cingulate, frontal, and parietal; Table 1), in the shell and core nucleus accumbens and caudate putamen (Table 2), in the lateral hypothalamus (Table 3), and in the dorsal lateral, intermediate lateral, ventral lateral, and medial septal nuclei (Table 4). The highest net increases (>500 fmol/mg protein) in GABA-stimulated FNZ binding occurred in the cingulate cortex and in layers 1–3 and 4 of the frontal and parietal cortices (Table 1).

Among the 17 cerebral cortical regions examined (Table 1), the net or percent increase in GABA-stimulated FNZ binding was different ($p < 0.025$) between the P and NP rats in 5 areas. In four of the five areas, the values for the P rats were 25–30% higher than values for the NP rats. These cere-

TABLE 4

DENSITIES OF BENZODIAZEPINE RECOGNITION SITES LABELLED WITH [^{3}H]FLUNITRAZEPAM (FNZ) IN THE ABSENCE AND PRESENCE OF 100 μM GABA IN SEPTAL NUCLEI, THALAMIC REGIONS, AND SUPERFICIAL GRAY LAYER OF THE SUPERIOR COLLICULUS

Region		fmol/mg Protein (mean ± SEM, $n = 4$)			
		FNZ	FNZ + GABA	Net Increase	% Increase
Septum					
LSD	P	356 ± 24	733 ± 13	377 ± 36	210 ± 19
	NP	390 ± 17	640 ± 36	249 ± 27	164 ± 7
LSI	P	226 ± 16	486 ± 14	260 ± 23	219 ± 17
	NP	265 ± 11	480 ± 28	215 ± 18	181 ± 5
LSV	P	362 ± 17	709 ± 43	347 ± 39	196 ± 12
	NP	418 ± 28	714 ± 60	296 ± 62	173 ± 17
MS	P	443 ± 15	829 ± 24	388 ± 32	188 ± 10
	NP	483 ± 35	821 ± 33	338 ± 20	171 ± 7
Thalamus					
PVP	P	588 ± 33	863 ± 61	275 ± 38	147 ± 6
	NP	575 ± 33	900 ± 18	325 ± 43	157 ± 11
MD	P	223 ± 7	340 ± 8*	117 ± 9*	152 ± 5
	NP	222 ± 13	383 ± 9	161 ± 14	173 ± 11
PV	P	208 ± 8	330 ± 8	121 ± 10	159 ± 7
	NP	197 ± 8	338 ± 15	140 ± 16	171 ± 9
Superior colliculus					
SuG	P	865 ± 36	1115 ± 61	251 ± 48	129 ± 5
	NP	872 ± 49	1155 ± 37	283 ± 58	133 ± 8

Net and % increases are reported for FNZ + 100 μM GABA binding with respect to FNZ binding alone. *$p < 0.025$ for P vs. NP values. LSD, dorsal lateral septum; LSI, intermediate lateral septum; LSV, ventral lateral septum; MS, medial septum; PVP, posterior paraventricular thalamic nucleus; MD, mediodorsal thalamic nucleus; PV, posterior ventral thalamic nucleus; SuG, superficial gray layer of the superior colliculus.

bral cortical regions included layers 1–3 of the parietal cortex, layer 4 of the frontal cortex, and layers 5 plus 6 of the frontal and parietal areas. There was a trend ($p < 0.05$) for a higher net increase in GABA-stimulated FNZ binding in the prefrontal and cingulate cortical areas in P rats compared with NP rats (Table 1). All six of these differences between the P and NP lines were observed in the anterior portion of the cerebral cortex. The only cerebral cortical area where the value for net or percent increase was lower for the P than that for the NP line was the entorhinal cortex. Other than in the entorhinal cortex, there were few differences between the lines in GABA-enhanced FNZ binding in any of the posterior cerebral cortical regions (Table 1).

In addition to the entorhinal cortex, three other regions had significantly ($p < 0.025$) lower net or percent increases in GABA-enhanced FNZ binding in the P relative to the NP line. These regions include the mediodorsal thalamic nucleus (Table 4) and the dorsal CA3 and middle dentate gyrus of the posterior hippocampus (Table 5). Three regions showed a trend ($p < 0.05$) toward a greater net or percent increase in GABA-stimulated FNZ binding in the P compared with the NP line. These regions include the shell nucleus accumbens, caudate putamen, and dorsal lateral septum (Tables 2, 4).

DISCUSSION

The most robust differences observed between the P and NP lines were in the net or percent increase in GABA-enhanced FNZ binding observed in various CNS regions. A greater net or percent increase occurred in layers 1–3 of the parietal cortex, layer 4 of the frontal cortex, and layers 5 plus 6 of the frontal and parietal cortices (Table 1) of the P rats relative to the NP rats. A trend ($p < 0.05$) towards a greater net or percent increase was observed in the prefrontal cortex, cingulate cortex, parietal cortex layer 4 (Table 1), shell nucleus accumbens, caudate putamen (Table 2), and dorsal lateral septum (Table 4) of P compared with NP rats. The greater percent increase suggests that the coupling between the GABA and BDZ sites is more effective in the P line than in the NP line in these CNS regions. On the other hand, there were four regions (entorhinal cortex, mediodorsal thalamus, and dorsal CA3 and middle dentate gyrus of the posterior hippocampus) that showed a smaller net or percent increase in GABA-enhanced FNZ binding in the P relative to the NP line (Tables 1, 4, 5). These data suggest less effective coupling between the GABA and BDZ binding sites in these CNS regions of the P rats. Studies using in vitro expression systems demonstrate that changing the subunit composition of the $GABA_A$/BDZ receptor alters the coupling between the $GABA_A$ and BDZ sites (31,32,36,38). Therefore, the dissimilar amounts of percent increases in GABA-enhanced [^{3}H]FNZ binding between the lines could indicate differences in the expression of receptor subunits within these CNS regions.

Biochemical (2,10,27,34) and electrophysiological (1,28,41) evidences support a role for the $GABA_A$/BDZ receptor in mediating some of the acute actions of ethanol. Furthermore, the effect of ethanol at the $GABA_A$/BDZ channel complex appears to be dependent on the expression of different subunit combinations (7,37). Therefore, the difference in behavioral sensitivity to the acute sedative/hypnotic effects of etha-

TABLE 5

DENSITIES OF BENZODIAZEPINE RECOGNITION SITES LABELLED WITH [^{3}H]FLUNITRAZEPAM (FNZ) IN THE ABSENCE AND PRESENCE OF 100 μM GABA IN HIPPOCAMPAL REGIONS OF ALCOHOL-NAIVE P AND NP RATS

Region		fmol/mg Protein (mean ± SEM, $n = 4$)			
		FNZ	FNZ + GABA	Net Increase	% Increase
Anterior dorsal					
DG	P	875 ± 37	1223 ± 63	348 ± 26	140 ± 1
	NP	849 ± 58	1165 ± 39	316 ± 61	138 ± 10
CA1	P	762 ± 29	1022 ± 41	259 ± 30	134 ± 4
	NP	707 ± 48	998 ± 44	291 ± 51	142 ± 10
CA2	P	573 ± 35	842 ± 12*	268 ± 37	148 ± 9
	NP	526 ± 40	753 ± 31	227 ± 39	144 ± 11
CA3	P	724 ± 32	1042 ± 43	317 ± 21	144 ± 3
	NP	683 ± 37	980 ± 29	297 ± 54	144 ± 11
CA4	P	922 ± 49	1249 ± 62	327 ± 57	135 ± 7
	NP	843 ± 49	1214 ± 33	370 ± 63	145 ± 11
Posterior dorsal					
DG	P	733 ± 13	1096 ± 48	338 ± 36	146 ± 5
	NP	818 ± 21	1177 ± 51	359 ± 66	144 ± 8
CA1	P	731 ± 48	984 ± 21	253 ± 39	135 ± 8
	NP	700 ± 21	977 ± 41	277 ± 47	140 ± 7
CA3	P	687 ± 57	945 ± 38	257 ± 25*	138 ± 6*
	NP	657 ± 23	1006 ± 18	359 ± 30	156 ± 6
Posterior ventral					
AVA	P	544 ± 43	780 ± 62	237 ± 20	143 ± 2
	NP	494 ± 11	726 ± 11	232 ± 7	147 ± 2
VDG	P	599 ± 22	842 ± 44	222 ± 35	137 ± 6
	NP	582 ± 13	873 ± 48	291 ± 55	150 ± 10
MDG	P	657 ± 33	834 ± 11*	176 ± 23*	127 ± 5*
	NP	644 ± 13	916 ± 25	273 ± 25	142 ± 4

Net and % increases are reported for FNZ + 100 μM GABA binding with respect to FNZ binding alone. *$p < 0.025$ for P vs. NP values. DG, dentate gyrus; AVA, anterior ventral area; VDG, ventral dentate gyrus; MDG, middle dentate gyrus.

nol observed between the P and NP lines (16,39) might be partially due to regional differences in the density of the $GABA_A$/BZD complex or the response of the $GABA_A$/BDZ receptor complex to ethanol as a result of alterations in the expression of different subunit combinations between the lines. GABA has been shown to enhance [^{3}H]FNZ binding to a greater extent in cerebral cortical membranes from SS than from LS mice (4,23). Similar findings have been reported with cerebral cortical membranes from AT compared with ANT rats (17). The greater enhancement of [^{3}H]FNZ binding by GABA in the frontal and parietal cortices of the P line relative to the NP line is in agreement with the membrane binding studies with SS and LS mice and with AT and ANT rats. Therefore, the greater net or percent increase in [^{3}H]FNZ binding produced by GABA in the frontal and parietal cortices (Table 1) may indicate fundamental differences in the coupling between the GABA and BDZ binding sites in these areas, which may be a factor contributing to the differences in ethanol sensitivity between the P and NP lines.

The more generalized differences in GABA enhancement of [^{3}H]FNZ binding to cortical $GABA_A$/BZD receptors between SS and LS mice compared with the more localized differences in the frontal and parietal cortices between P and NP rats may result from the different selection pressures on the two lines. The SS and LS mice are selectively bred for differences in the duration of the loss of the righting reflex ("sleep-time") after an acute intoxicating dose of ethanol (22). As mentioned above, P and NP rats, selected for differences in ethanol-seeking behavior, also differ in their ability to perform a complex motor task while intoxicated (16,39). Coordination and planning of motor function with a moving visual target (i.e., the descending platform in the jump test) are required to perform this task. The difference in P relative to NP rats in GABA enhancement of [^{3}H]FNZ binding density in the parietal cortex (an area involved in spatial coordination of motor function) and frontal cortex (an area involved in planning of motor action), along with the reduced density of FNZ binding in the presence of GABA in the substantia nigra (an area involved in planning and execution of motor function), may be related to the improved performance while intoxicated of P rats compared with NP rats in the electrical shock-motivated descending platform jump task (16,39).

Previous studies indicated that FNZ enhancement of GABA-stimulated $^{36}Cl^-$ influx into cortical microsacs was greater in individually housed P rats than in NP rats, but there was no difference between the lines when rats were housed in pairs (35). The differences observed in the enhancement of [^{3}H]FNZ in the frontal and parietal cortices (Table 1) are not due to isolation-induced stress, because the rats used in the present study were housed in pairs (P with P and NP with

NP). Furthermore, the lack of any differences observed between the pair-housed P and NP rats (35) could indicate that measuring $^{36}Cl^-$ influx with the microsac preparation is not as sensitive as the present procedure for detecting differences in the $GABA_A/BDZ/Cl^-$ complex between P and NP rats.

The dissimilar response of [^{3}H]FNZ binding in the presence of GABA might also be associated with certain behavioral differences between the P and NP lines. The results of a recent study (33) indicated that P rats exhibited more anxiety in certain behavioral tests than did NP rats. The trends and significant differences in response to GABA of [^{3}H]FNZ binding could indicate differences between the lines in the functioning of the GABA system in CNS regions mediating emotionality, e.g., limbic cortex, nucleus accumbens shell, mediodorsal thalamus, and hippocampus. Kang et al. (12) and Montpied et al. (26) have shown that stress produces changes in the expression of subunits of the $GABA_A$/BDZ receptor complex. In addition, an increase in GABA-enhanced [^{3}H]FNZ binding was observed in the cerebral cortex of SS but not LS mice when confined to the open arms of a plus maze to induce stress (4,5). Differences have previously been reported in both the density of 5-HT_{1A} receptors (21) and immunostained 5-HT fibers (42,43) between the lines. Both the serotonergic system, particularly the 5-HT_{1A} receptor (9), and the GABAergic system have been implicated in the etiology of anxiety disorders. Therefore, differences in coupling of the GABA and BZD sites or the number of GABA-enhanced BZD sites, along with the differences in the 5-HT system, in certain CNS regions may be factors related to the higher anxiety state of the P rat.

Previous neurochemical/neuroanatomical studies indicated that there are innate differences in the GABA and serotonin systems between P and NP rats. Hwang et al. (11) reported greater numbers of GABAergic terminals in the nucleus accumbens of the P line compared with the NP line, whereas no differences were found between the lines in the striatum or septum. In the present study, innate lower densities of FNZ, in the absence of GABA, were found in the nucleus accumbens. Whether this lower amount of FNZ binding is a compensatory response to the higher numbers of GABA nerve terminals in the nucleus accumbens is unclear. Higher densities of 5-HT_{1A} receptors have been found throughout most layers of the cerebral cortex (21), whereas lower densities of 5-HT_{2A} sites were reported in layer 4 of the cerebral cortex (20) of the P than the NP rat. Therefore, it appears that multiple genetic factors are influencing the development of 5-HT and $GABA_A$ receptors within the anterior cerebral cortex. However, whereas similar differences in the densities of the two 5-HT receptor subtypes were observed between the P and the NP rats in both the anterior (frontal, parietal, and cingulate cortices) and posterior (occipital and temporal cortices) cerebral cortex, the differences between the lines in the coupling of the GABA and benzodiazepine sites were observed in the frontal and parietal cortices and not in the occipital and temporal areas.

Although this study has revealed some differences in the density of BZD recognition sites between the P and NP rats, it does not address whether differences in the density of specific BZD receptor subtypes exist between the lines. Previous studies have suggested that the actions of ethanol may be mediated through the BZD_1 receptor subtype (7,8). Labeled selective agonists (e.g., [^{3}H]zolpidem) exist for this BZD receptor subtype (3). It would be of interest, therefore, to compare the density of [^{3}H]zolpidem binding sites in CNS regions of the P and the NP rats.

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