Heterogeneity of Hippocampal $GABA_A$ Receptors: Regulation by Corticosterone

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Chronic stressors produce changes in hippocampal neurochemistry, neuronal morphology, and hippocampal-dependent learning and memory processes. In rats, stress-induced changes in CA3 apical dendritic structure are mediated by corticosterone (CORT) acting, in part, on excitatory amino acid neurotransmission. CORT also alters GABA-mediated inhibitory neurotransmission, so the $GABA_A$ receptor system may also contribute to dendritic remodeling and other stress-related changes in hippocampal function. A previous study indicated that chronic CORT treatment produces complex changes in $GABA_A$ receptor subunit mRNA levels, so we hypothesized that CORT alters the pharmacological properties of hippocampal $GABA_A$ receptors. To test this, adult male rats were treated with CORT or vehicle pellets for 10 d, after which we quantified [^{35}S]t-butylbicyclophosphorothionate ([^{35}S]TBPS) and [^{3}H]flunitrazepam binding to $GABA_A$ receptors using *in vitro* receptor autoradiography. Pharmacological properties of receptors were assessed by examining the allosteric regulation of binding at both sites by GABA and 5α-pregnane-3α,21-diol-20-one (THDOC), an endogenous anxiolytic steroid. We found striking regional differences in the modulation of [^{35}S]TBPS binding, particularly between strata radiatum and strata oriens, suggesting a functional heterogeneity among hippocampal $GABA_A$ receptors even within the apical versus basal dendrites of pyramidal neurons. Furthermore, we found that CORT treatment decreased the negative modulation of hippocampal [^{35}S]TBPS binding by both GABA and THDOC and increased the enhancement of [^{3}H]flunitrazepam binding by GABA and THDOC in the dentate gyrus. Together, these data suggest that prolonged exposure to stress levels of corticosteroids may alter hippocampal inhibitory tone by regulating the pharmacological properties of $GABA_A$ receptors in discrete dendritic subfields.

Key words: chronic stress; corticosterone; hippocampus; $GABA_A$ receptors; neurosteroid; 5α-pregnane-3α,21-diol-20-one; testosterone

Repeated exposure to stressors produces neurochemical, synaptic, and morphological changes in the hippocampus, including selective regression of the apical dendrites in CA3 pyramidal neurons (Woolley et al., 1990; Magariños and McEwen, 1995; Magariños et al., 1996, 1998) and impaired performance in some spatial memory tasks (Luine et al., 1994; Conrad et al., 1996; Endo et al., 1996). These stress effects are mediated, in part, by corticosteroids acting on excitatory amino acid neurotransmission (McEwen, 1999). However, corticosterone (CORT) also modifies the actions of GABA, and GABA regulates hippocampal excitability (Freund and Buzsaki, 1996) and neurite extension (Mattson and Kater, 1989). This suggests that chronic stressors may alter the balance between excitation and inhibition leading to dendritic remodeling and other changes in hippocampal function. Previous studies have shown that CORT alters GABA release and uptake (Miller et al., 1978; Ravindran et al., 1994), radioligand binding to $GABA_A$ receptors (Goeders et al., 1986; Miller et al., 1988; Drugan et al., 1989; Weizman et al., 1990; Wilson and Biscardi, 1994), and expression of $GABA_A$ receptor subunits (Kang et al., 1991; Orchinik et al., 1995). In addition, benzodiazepines, positive allosteric modulators of $GABA_A$ receptors, can block stress-induced dendritic regression (Magariños et al., 1999) and the neurodegeneration produced by diminished expression of $GABA_A$ receptors (Karle et al., 1997). However, these studies have not provided enough resolution to reveal how CORT and GABA interact to produce region-specific effects within the hippocampus.

The $GABA_A$ receptor is a pentamer, assembled from a diversity of subunits, that gates a chloride conductance (Nayeem et al., 1994; Hevers and Luddens, 1998; Mehta and Ticku, 1999). The receptor complex contains multiple, allosterically interacting binding sites, usually including sites for GABA, barbiturates, benzodiazepines ([^{3}H]flunitrazepam), chloride channel antagonists [t-butylbicyclophosphorothionate ([^{35}S]TBPS)], and endogenous anxiolytic steroids such as 5α-pregnane-3α,21-diol-20-one (THDOC) (Majewska et al., 1986; Turner et al., 1989). Native and recombinant $GABA_A$ receptors composed of different subunit combinations differ in pharmacological properties such as channel kinetics and allosteric modulation (Pritchett et al., 1989; Vicini, 1999). Recent evidence is consistent with the idea that specific subunits are targeted to specific intracellular sites, producing functionally distinct receptor isoforms within the same neurons (Buhl et al., 1994; Koulen et al., 1996; Nusser et al., 1996; Ruano et al., 1997; Fritschy et al., 1998; Brickley et al., 1999; Banks and Pearce, 2000).

A previous study found that chronic exposure to CORT produced site- and subunit-specific changes in the mRNA levels of

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$GABA_A$ receptor subunits (Orchinik et al., 1995). The complexity of these transcriptional changes led us to hypothesize that CORT alters the pharmacological properties of hippocampal $GABA_A$ receptors, rather than receptor number per se. We tested this hypothesis by analyzing the allosteric regulation of radioligand binding to $GABA_A$ receptors using *in vitro* receptor autoradiography. The study revealed that the pharmacological properties of $GABA_A$ receptors were strikingly different between dendritic subfields and that CORT treatment did appear to alter the pharmacological properties of hippocampal $GABA_A$ receptors.

MATERIALS AND METHODS

Animal treatments. Young adult male Sprague Dawley rats (~300 gm) were housed two to three in a cage and maintained on a 14:10 hr light/dark cycle (lights on 5 A.M.). Animals were treated in accordance with the principles and procedures of the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*. The animals were divided into six treatment groups ($n = 9$), and surgery was performed under Metofane anesthesia (Pitman-Moore, Washington Crossing, NJ) using standard aseptic surgical techniques. All animals were adrenally intact. Half of the animals received subcutaneous implants of four 100 mg CORT pellets, and half received vehicle (VEH) pellets (Innovative Research, Toledo, OH) through an incision in the dorsolateral abdominal region. The CORT pellets produced circulating corticosterone levels similar to those seen in stressed animals (Orchinik et al., 1995). Two-thirds of the CORT- and VEH-treated animals were castrated, whereas one-third received sham gonadectomy. Castrates received either a 30 mm SILASTIC capsule filled with crystalline testosterone (T) or a blank capsule, creating six treatment groups. In Experiment 1, all six groups were used, but preliminary analysis indicated that T levels were not correlated with any response variables. Therefore, in subsequent experiments gonadally intact or castrated, testosterone-treated animals (Experiment 3) were used.

Ten days after surgery the animals were killed between 10 and 11 A.M. Animals were killed by decapitation, and the brains rapidly removed, frozen on dry ice, and stored at −70°C until sectioning. Coronal sections (12 μm) were collected from dorsal hippocampus between the approximate coordinates 2.5–4.0 mm posterior to bregma (Paxinos and Watson, 1986) on gelatin-coated slides using a cryostat microtome and stored at −70°C until use. These sections were used for *in vitro* receptor autoradiography.

[^{35}S]TBPS receptor autoradiography. Sections were preincubated in assay buffer (50 mM Tris-HCl, 200 mM NaCl, pH 7.4) for 30 min at 23°C and then incubated with 2–3 nM [^{35}S]TBPS (New England Nuclear) (108–137 Ci/mmol) in assay buffer for 2 hr at 23°C. For steroid modulation of [^{35}S]TBPS binding (Experiment 1), sections were incubated with 3 nM [^{35}S]TBPS in buffer containing 1.0 μM GABA in the presence or absence of 100 nM THDOC (Sigma, St. Louis, MO). GABA was included in the assay buffer because the sensitivity of [^{35}S]TBPS binding to inhibition by steroids is enhanced by GABA (Gee et al., 1988; Orchinik et al., 1994). For GABA modulation of [^{35}S]TBPS binding (Experiments 2 and 3), paired sections were incubated with 2 nM [^{35}S]TBPS in the presence or absence of 1.5 μM GABA (unless indicated otherwise). After incubation with [^{35}S]TBPS, slides were rinsed 2 ×r 2.5 min in ice-cold assay buffer and dipped in ice-cold ddH_2O. Excess H_2O was rapidly removed from slides and the sections were dried under a stream of cool air. The sections, as well as [^{14}C] microscales (Amersham, Arlington Heights, IL), were exposed to scientific imaging film (Kodak SB5, Rochester, NY) for 2 d before development. Nonspecific binding was determined in alternate sections in the presence of 10 μM picrotoxin and was negligible.

[^{3}H]flunitrazepam receptor autoradiography. Sections were preincubated in assay buffer (100 mM Tris-HCl, pH 7.4) for 30 min at 4°C and then incubated with 0.5 nM [^{3}H]flunitrazepam (New England Nuclear) (85.8 Ci/mmol) for 1 hr at 4°C. Alternate sections were incubated in assay buffer containing either 100 nM THDOC or 3 μM GABA or no modulator. Slides were rinsed 2 × 1 min in ice-cold assay buffer and dipped in ice cold ddH_20. Excess H_2O was rapidly removed, and the sections were dried under a stream of cool air. Autoradiograms were produced by exposing labeled sections, as well as brain-mash tritium standards, to Hyperfilm-^{3}H (Amersham) for 1 week before development. Nonspecific binding was determined in the presence of 3 μM diazepam and was negligible.

Data collection. Receptor autoradiograms were analyzed by computer-assisted densitometry (MCID and AIS/C systems from Imaging Research, St. Catherines, Ontario, Canada). Optical density values in hippocampal regions of interest were quantified using an automated "paintbrush" function and converted to picomoles per gram of dry tissue using [^{14}C] microscales ([^{35}S]TBPS binding) or to femtomoles per milligram of protein using [^{3}H] brain-mash standards (flunitrazepam binding). Mean values for each region were derived from sampling the left and right side of each section, two sections per slide, and 2 slides per animal. We used the following nomenclature conventions. We distinguished between the ectal (external) and endal (internal) limbs of the dentate gyrus and refer to them as the superior and inferior blades, respectively. We analyzed binding in the superior and inferior dendritic fields of the layer of polymorph cells that extends from the CA3 pyramidal cell layer between the blades of the dentate gyrus; these regions are referred to as CA4, strata radiatum, and strata oriens. Data from CA4 are reported in the Tables but were not included in statistical analyses because binding in this region was often quite diffuse. In Ammon's horn, the area referred to as stratum radiatum may include some of the stratum lucidum.

Statistical analysis. We used a three-way nested, repeated-measures ANOVA model to test hypotheses about the equivalence of the treatment means (CORT-treated vs vehicle-treated animals) and about the equivalence of the means at different dendritic subfields for each response variable (basal [^{35}S]TBPS or [^{3}H]flunitrazepam binding, and the percentage modulation of [^{35}S]TBPS or [^{3}H]flunitrazepam binding by THDOC or GABA). In the ANOVAs, the between group factor was the treatment (CORT-treated vs vehicle-treated animals). The repeated-measure, hippocampal dendritic subfield was nested within a third factor, called hippocampal region, that had two levels: Ammon's horn and dentate gyrus. There were six dendritic subfields within Ammon's horn and two dendritic subfields within the dentate gyrus (molecular layers of inferior and superior blades). The normality assumption was investigated for all of the analyses by inspecting the normal plots of the residuals. In all cases, this inspection indicated that the errors were approximately normally distributed.

Rather than assuming a spherical covariance structure for the repeated measure (hippocampal dendritic subfield), which implies that the variances of the differences of the observations for all pairs of treatment levels are equivalent, we performed a maximum likelihood test for each variable to determine whether a more general covariance structure provided a superior fit. In each case, the maximum likelihood test indicated that the sphericity condition was too restrictive. Therefore, we modeled the relationships among the repeated measures (dendritic subfields) using an unstructured covariance matrix in all of the analyses.

When the interaction between treatment and dendritic subfield was significant (Experiments 1 and 2), we performed hypothesis tests using contrasts (Set A) to determine whether CORT treatment produced unique changes in CA3 stratum radiatum (where dendritic remodeling occurs) or the dentate gyrus (which provides excitatory input to CA3). The Set A contrasts tested the following: (1) the mean level of binding in CA3 stratum radiatum of CORT-treated animals equals the mean level of binding in CA3 stratum radiatum of vehicle-treated animals, (2) the mean level of binding in the dentate gyrus of CORT-treated animals equals the mean level of binding in the dentate gyrus of vehicle-treated animals, and (3) the difference between the mean level of binding in CA3 stratum radiatum of CORT-treated animals minus the level in CA3 stratum radiatum of vehicle-treated animals equals the mean level of binding in CA1 stratum oriens of CORT-treated animals minus the mean level in CA1 oriens of vehicle-treated animals. (4) This latter contrast was repeated for CA3 stratum radiatum versus CA1 stratum radiatum, (5) CA3 stratum radiatum versus CA2 stratum oriens, (6) CA3 stratum radiatum versus CA2 stratum radiatum, and (7) CA3 stratum radiatum versus CA3 stratum oriens. A familywise $\alpha = 0.05$ significance level was used for all tests, and the Bonferroni method was used to control for simultaneous testing.

When the interaction between treatment and hippocampal region was significant (Experiment 4), we performed two different hypothesis tests (Set B contrasts) that tested the following: (1) the mean level of binding in Ammon's horn of CORT-treated animals equals the mean level of binding in Ammon's horn of vehicle-treated animals and (2) the mean level of binding in the dentate gyrus of CORT-treated animals equals the mean level of binding in the dentate gyrus of vehicle-treated animals. In Experiment 3, we performed an additional ANOVA to determine

whether the means of the percentage modulation of CA3 strata radiatum, oriens, and pyramidale were equivalent.

The unexpected differences in the modulation of radioligand binding by THDOC and GABA within Ammon's horn led us to develop exploratory hypothesis tests and construct additional contrasts (Set C) to examine binding in CA3 stratum radiatum relative to other dendritic subfields. When the main effect of dendritic subfield on the mean percentage modulation was significant, we performed Set C contrasts, which tested for the equivalence of (1) the mean percentage modulation in CA3 stratum radiatum and the mean percentage modulation in CA3 stratum oriens, (2) the mean percentage modulation in CA3 stratum radiatum and the mean percentage modulation in CA1 stratum radiatum, and (3) the mean percentage modulation CA3 stratum radiatum and the mean percentage modulation of CA1 oriens, CA1 radiatum, CA2 oriens, and CA2 radiatum. Again, a familywise $\alpha = 0.05$ significance level and the Bonferroni method were applied. All statistical tests are summarized in Table 3.

RESULTS

Experiment 1: CORT effects on the modulation of [^{35}S]TBPS binding by THDOC

Preliminarily, we examined the possibility that changes in $GABA_A$ receptors induced by CORT treatment are mediated by the suppression of circulating testosterone levels by examining [^{35}S]TBPS binding in animals of mixed gonadal status. Pearson's correlations were computed for plasma testosterone levels versus [^{35}S]TBPS binding for each hippocampal region for basal binding of [^{35}S]TBPS, binding in the presence of THDOC, and the degree of modulation of [^{35}S]TBPS binding by THDOC. All correlations with testosterone were nonsignificant at the 0.05 level. Therefore, for subsequent statistical analyses we ignored gonadal status.

We tested the hypothesis that chronic exposure to stress levels of CORT alters the pharmacological properties of $GABA_A$ receptors. More specifically, we examined the modulation of [^{35}S]TBPS binding by THDOC, an endogenous steroid with agonist-like activity at $GABA_A$ receptors. CORT treatment appeared to decrease the sensitivity of [^{35}S]TBPS binding to allosteric modulation by THDOC (Fig. 1). In addition, $GABA_A$ receptors in CA3 stratum radiatum (apical dendritic region) appeared to differ pharmacologically from those in the stratum oriens (basal dendritic region). These results, detailed below, are consistent with our hypothesis.

There was no main effect of CORT treatment on the mean levels of basal [^{35}S]TBPS binding, but there was a significant interaction between dendritic subfield and CORT treatment ($F_{(6,52)} = 5.39$; $p = 0.0002$) (Table 1), indicating that the effect of CORT- versus vehicle-treatment on basal [^{35}S]TBPS binding differed with dendritic subfield. However, none of the Set A contrasts were significant, indicating that CORT versus vehicle effects were not localized in CA3 stratum radiatum or dentate gyrus. There were differences between the mean levels of basal [^{35}S]TBPS binding in Ammon's horn versus dentate gyrus ($F_{(1,52)} = 1323$; $p \leq 0.0001$) and between dendritic subfields within either Ammon's horn or the dentate gyrus, or both ($F_{(6,52)} = 1014$; $p \leq 0.0001$).

Addition of 100 nM THDOC to the assay buffer reduced [^{35}S]TBPS specific binding to 30–50% of basal binding. We analyzed the modulation of [^{35}S]TBPS binding by THDOC as the percentage of basal binding, [^{35}S]TBPS binding with THDOC/[^{35}S]TBPS binding without THDOC × 100, normalized for each animal (Fig. 1). There was a significant treatment effect on the mean percentage modulation of [^{35}S]TBPS binding by THDOC ($F_{(1,52)} = 7.28$; $p = 0.0094$): THDOC inhibited a smaller fraction of [^{35}S]TBPS binding in CORT-treated animals than in vehicle-treated animals. There were also differences between the mean levels of modulation by THDOC in Ammon's horn versus dentate gyrus ($F_{(1,52)} = 1213$; $p \leq 0.0001$) and between dendritic subfields within either Ammon's horn or the dentate gyrus, or both ($F_{(6,52)} = 295.4$; $p \leq 0.0001$).

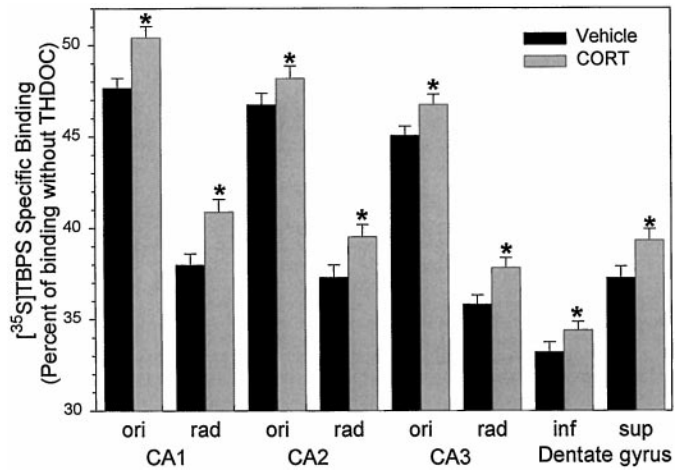

Figure 1. Effect of CORT on the modulation of [^{35}S]TBPS binding by 100 nM THDOC in dorsal hippocampus. Male rats of mixed gonadal status received CORT pellets or vehicle pellets subcutaneously for 10 d. Data are expressed as the specific binding of [^{35}S]TBPS in the presence of THDOC as percentage of binding in the absence of THDOC, normalized for each animal. Shown are means and error bars representing SEM; $n = 27$ for CORT- and vehicle-treated animals. There was an overall significant main effect of CORT treatment on the mean percentage modulation by THDOC in hippocampus ($F_{(1,52)} = 7.28$; $p = 0.0094$), indicated by an *asterisk*. There were also significant regional differences between mean levels of modulation by THDOC, averaged across treatments, between CA3 stratum radiatum and CA3 stratum oriens ($t = -34.65$; $p \leq 0.0001$), and between CA3 stratum radiatum and CA1 stratum radiatum ($t = -9.95$; $p \leq 0.0001$). See Table 3 for summary of statistical analysis.

The sensitivity of [^{35}S]TBPS binding to modulation by THDOC appeared to differ between the strata oriens and radiatum (Fig. 1), so we performed three exploratory hypothesis tests (Set C) on the data averaged over both treatment levels (see Table 3). These tests indicated that there were significant differences in the mean levels of percentage modulation of [^{35}S]TBPS binding by THDOC between CA3 stratum radiatum and CA3 stratum oriens ($t = -34.65$; $p \leq 0.0001$; THDOC inhibited a greater fraction of [^{35}S]TBPS binding in CA3 stratum radiatum than stratum oriens), between CA3 stratum radiatum and CA1 stratum radiatum ($t = -9.95$; $p \leq 0.0001$), and between CA3 stratum radiatum and the average of CA1 and CA2, stratum oriens and stratum radiatum ($t = 29.99$; $p \leq 0.0001$). These data suggest that the pharmacological properties of $GABA_A$ receptors in CA3 radiatum differ from those in the stratum oriens of CA3 and from other regions of Ammon's horn.

Experiment 2: CORT effects on modulation of [^{35}S]TBPS binding by GABA

We further tested the hypothesis that CORT treatment alters the pharmacological properties of $GABA_A$ receptors, reflected in the allosteric modulation of [^{35}S]TBPS binding by GABA. Preliminary studies indicated that 2.0 mM GABA had variable, region-specific effects on [^{35}S]TBPS binding, whereas 5 μM GABA uniformly inhibited [^{35}S]TBPS binding (Fig. 2). We used 1.5 μM GABA to modulate [^{35}S]TBPS binding (Experiments 2 and 3) and 3 μM GABA to modulate [^{3}H]flunitrazepam binding (Experiment 4) because these concentrations were in a physiologi-

Table 1. Effect of CORT on [^{35}S]TBPS binding in the absence and presence of 100 nM THDOC

	CA1 oriens	CA1 radiatum	CA2 oriens	CA2 radiatum	CA3 oriens	CA3 radiatum	Inferior dentate	Superior dentate	CA4 oriens	CA4 radiatum
Basal										
VEH + T	11.74 ± 0.20	8.54 ± 0.18	8.93 ± 0.19	7.47 ± 0.17	10.02 ± 0.16	9.72 ± 0.22	11.85 ± 0.42	14.37 ± 0.46	9.99 ± 0.37	7.94 ± 0.21
VEH − T	11.58 ± 0.15	8.49 ± 0.16	8.95 ± 0.12	7.34 ± 0.20	10.19 ± 0.22	9.70 ± 0.26	11.56 ± 0.34	14.23 ± 0.34	9.94 ± 0.28	7.60 ± 0.24
VEH/INT	11.82 ± 0.20	8.79 ± 0.26	9.17 ± 0.18	7.53 ± 0.17	10.43 ± 0.17	9.98 ± 0.21	12.06 ± 0.26	14.52 ± 0.27	10.26 ± 0.25	8.17 ± 0.27
CORT + T	10.79 ± 0.19	7.76 ± 0.19	8.98 ± 0.21	7.41 ± 0.15	9.70 ± 0.17	9.19 ± 0.20	11.18 ± 0.33	13.46 ± 0.28	9.86 ± 0.25	7.37 ± 0.17
CORT − T	11.12 ± 0.14	8.25 ± 0.18	9.09 ± 0.15	7.33 ± 0.11	10.04 ± 0.21	9.59 ± 0.18	11.87 ± 0.37	14.45 ± 0.34	10.24 ± 0.41	7.60 ± 0.20
CORT/INT	11.36 ± 0.16	8.40 ± 0.18	9.26 ± 0.14	7.43 ± 0.12	10.29 ± 0.10	9.85 ± 0.16	12.00 ± 0.26	14.50 ± 0.21	10.05 ± 0.34	7.57 ± 0.27
+ THDOC										
VEH + T	5.45 ± 0.12	3.17 ± 0.09	4.11 ± 0.14	2.64 ± 0.08	4.46 ± 0.10	3.40 ± 0.07	3.90 ± 0.13	5.18 ± 0.19	3.26 ± 0.18	2.21 ± 0.08
VEH − T	5.46 ± 0.11	3.23 ± 0.10	4.16 ± 0.06	2.77 ± 0.10	4.58 ± 0.10	3.48 ± 0.11	3.72 ± 0.12	5.16 ± 0.12	3.15 ± 0.16	2.28 ± 0.10
VEH/INT	5.79 ± 0.12	3.40 ± 0.06	4.38 ± 0.11	2.92 ± 0.08	4.83 ± 0.11	3.67 ± 0.09	4.23 ± 0.12	5.68 ± 0.16	3.45 ± 0.15	2.44 ± 0.06
CORT + T	5.36 ± 0.15	3.19 ± 0.10	4.27 ± 0.12	2.90 ± 0.09	4.49 ± 0.12	3.45 ± 0.13	3.79 ± 0.15	5.26 ± 0.18	3.27 ± 0.20	2.19 ± 0.09
CORT − T	5.64 ± 0.09	3.26 ± 0.08	4.45 ± 0.10	2.82 ± 0.09	4.69 ± 0.06	3.53 ± 0.08	4.02 ± 0.13	5.58 ± 0.09	3.39 ± 0.11	2.31 ± 0.07
CORT/INT	5.85 ± 0.14	3.52 ± 0.13	4.43 ± 0.10	3.05 ± 0.09	4.84 ± 0.09	3.85 ± 0.09	4.24 ± 0.12	5.81 ± 0.14	3.52 ± 0.13	2.49 ± 0.12

Data are expressed as picomoles [^{35}S]TBPS specifically bound per gram dry tissue (means ± SEM; $n = 9$). Buffer included 3 nM [^{35}S]TBPS and 1 μM GABA.

VEH + T, Vehicle-treated castrates with T replacement (30 mm SILASTIC capsule filled with crystalline testosterone); VEH − T, vehicle-treated castrates with blank capsule; VEH/INT, vehicle-treated, gonadally intact; CORT + T, CORT-treated castrates with T replacement; CORT − T, CORT-treated castrates with blank capsule; CORT/INT, CORT-treated, gonadally intact. There was no significant main effect of CORT treatment on mean levels of basal [^{35}S]TBPS binding, but there was a significant interaction between dendritic subfield and treatment ($F_{(6,52)} = 5.39$; $p = 0.0002$).

cally relevant range, based on GABA EC_{50} values (Costa, 1998), and provided good modulatory contrasts. The results, detailed below, indicate that CORT treatment altered (probably decreased) the sensitivity of [^{35}S]TBPS binding to modulation by GABA (Fig. 3) and, again, that $GABA_A$ receptors in CA3 stratum radiatum appeared to differ from those in other regions of Ammon's horn.

As in Experiment 1, there was a significant interaction between dendritic subfield and CORT treatment on basal levels of [^{35}S]TBPS binding ($F_{(6,16)} = 4.30$; $p = 0.0091$). However, as in Experiment 1, all of the Set A contrasts were nonsignificant. There were differences between the mean levels of basal binding in Ammon's horn and the dentate gyrus ($F_{(1,16)} = 976.0$; $p \leq 0.0001$) and between the mean levels of basal binding within the dendritic subfields in Ammon's horn, within the dentate gyrus, or within both ($F_{(6,16)} = 212.4$; $p \leq 0.0001$).

At 1.5 μM, GABA had dendritic region-specific effects on [^{35}S]TBPS binding. GABA enhanced [^{35}S]TBPS binding in the stratum oriens of CA1, CA2, and CA3 but inhibited binding or had no effect on binding in the stratum radiatum (Fig. 3). Analysis of normalized data indicated that the mean percentage modulation of [^{35}S]TBPS binding by GABA differed between CORT- and vehicle-treated animals (Fig. 3) ($F_{(1,16)} = 4.80$; $p = 0.044$); the percentage of basal binding was greater in CORT-treated animals. There were also significant differences between the mean level of modulation by GABA in Ammon's horn versus the mean level of modulation in dentate gyrus ($F_{(1,16)} = 267.1$; $p \leq 0.0001$), and between the means of the various dendritic subfields ($F_{(6,16)} = 420.3$; $p \leq 0.0001$).

The Set C contrasts (see Table 3) on normalized data averaged over both treatment levels indicated that there were significant differences in mean percentage modulation of [^{35}S]TBPS binding by GABA between CA3 stratum radiatum and CA3 stratum oriens ($t = -13.56$; $p \leq 0.0001$; GABA enhanced [^{35}S]TBPS binding in CA3 stratum oriens but not in stratum radiatum), between CA3 stratum radiatum and CA1 stratum radiatum ($t = 5.15$; $p \leq 0.0001$), and between CA3 stratum radiatum and the average of CA1 and CA2, strata oriens, and radiatum ($t = 7.13$; $p \leq 0.0001$). As with the modulation of [^{35}S]TBPS binding by THDOC, these regional differences in the sensitivity to GABA indicate that the functional properties of $GABA_A$ receptors in CA3 stratum radiatum are likely to differ from those in the stratum oriens.

Experiment 3: Region-specific modulation of [^{35}S]TBPS binding by GABA

Experiment 2 revealed striking differences between the modulatory effect of GABA in the stratum radiatum versus stratum oriens (apical vs basal dendritic fields of pyramidal cells, respectively). Given this unexpected polarity, and the selective susceptibility of apical dendrites to stress-induced remodeling, we performed Experiment 3 to confirm that [^{35}S]TBPS binding is modulated in opposite directions by GABA in apical versus basal dendritic subfields. We further investigated the apparent heterogeneity of $GABA_A$ receptors in CA3 by examining binding in the pyramidal cell layer. The results, detailed below, confirmed that 1.5 μM GABA enhanced [^{35}S]TBPS binding in the stratum oriens of CA1, CA2, and CA3 but inhibited binding or had no effect on [^{35}S]TBPS binding in the stratum radiatum. These data are consistent with the idea that hippocampal pyramidal neurons selectively sort $GABA_A$ receptor subtypes between the apical and basal dendrites.

As in Experiments 1 and 2, there was a significant interaction ($F_{(6,16)} = 10.33$; $p \leq 0.0001$) between hormone treatment and dendritic subfield on the basal levels of [^{35}S]TBPS binding, and the Set A contrasts were again nonsignificant. Unlike Experiment 2, which used gonadally intact animals, there was no significant effect of CORT versus vehicle treatment on the mean percentage modulation by GABA; Experiment 3 used castrated animals. The focus of Experiment 3, however, was on regional differences in modulation of [^{35}S]TBPS binding by GABA (Fig. 4). There were again differences between the mean percentage modulation by GABA in Ammon's horn versus the dentate gyrus ($F_{(1,16)} = 127.6$; $p \leq 0.0001$) and between the means of the individual dendritic subfields within either Ammon's horn or the dentate gyrus, or both ($F_{(6,16)} = 278.0$; $p \leq 0.0001$). As in Experiment 2,

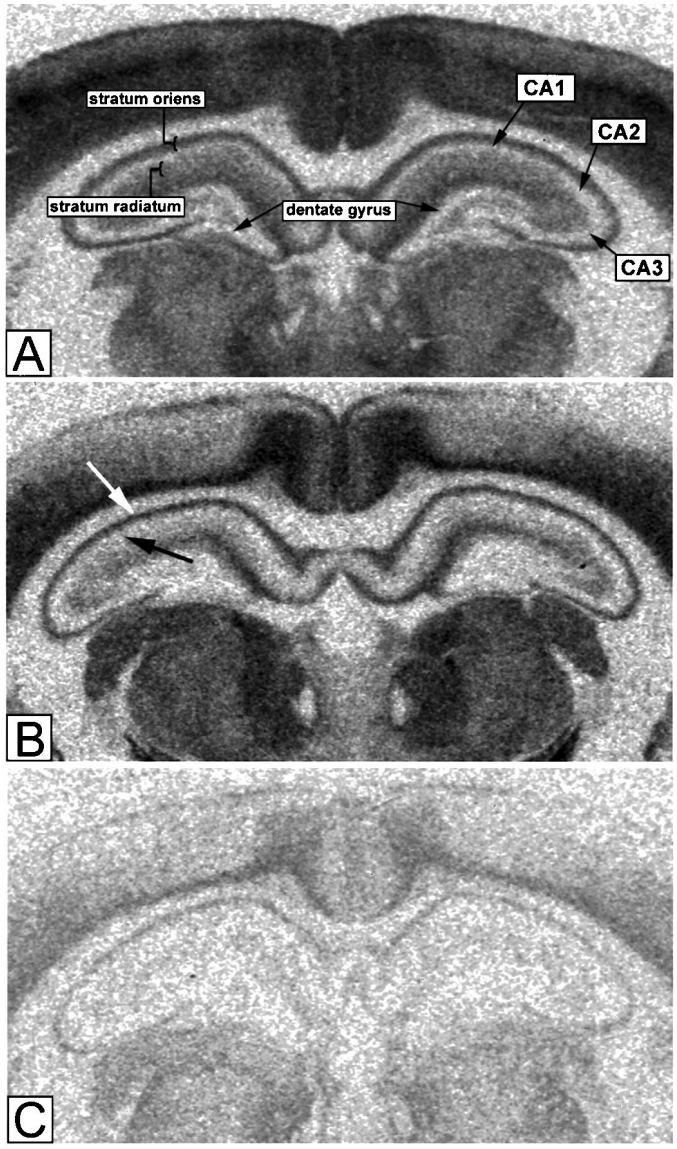

Figure 2. Representative autoradiograms showing the modulation of 2 nM [^{35}S]TBPS binding by GABA in the dorsal hippocampus. In *A*, incubation buffer included no exogenous GABA; in *B*, 2.0 μM GABA; in *C*, 5 μM GABA. In *A*, CA1–CA3 in Ammon's horn and dentate gyrus are indicated with *arrows* pointing to the pyramidal or granule cell layers. In *B*, note the effect of GABA on [^{35}S]TBPS binding: an increase in (CA1) stratum oriens (*white arrow*) and a decrease in stratum radiatum (*black arrow*), relative to control binding in *A*. [^{35}S]TBPS binding was inhibited in all regions by 5 μM GABA.

the Set C contrasts indicated that there were significant differences (see Table 3) in mean percentage modulation of [^{35}S]TBPS binding by GABA between CA3 stratum radiatum and CA3 stratum oriens ($t = -17.82$; $p \leq 0.0001$), between CA3 radiatum and CA1 stratum radiatum ($t = 8.40$; $p \leq 0.0001$), and between CA3 stratum radiatum and the average of CA1 and CA2, stratum oriens, and stratum radiatum ($t = 9.46$; $p \leq 0.0001$). Furthermore, we found that the mean percentage modulation of [^{35}S]TBPS binding by GABA differed greatly between the strata oriens, radiatum, and pyramidale of CA3 ($F_{(2,17)} = 229.0$; $p \leq 0.0001$), ranging from 30% enhancement in stratum oriens to 40% inhibition in the pyramidal cell layer.

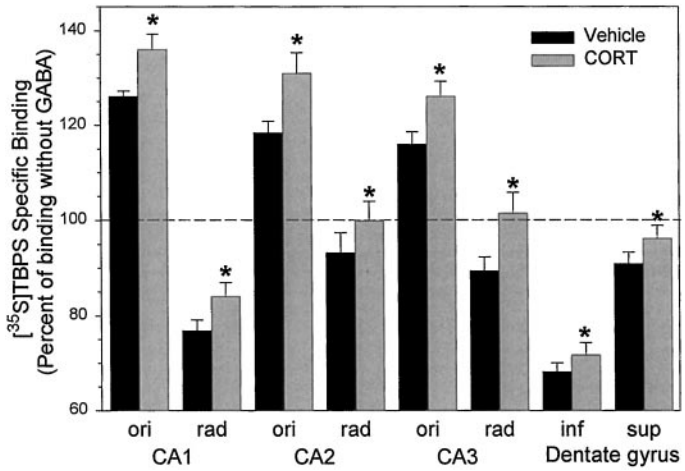

Figure 3. Effect of CORT on the modulation of [^{35}S]TBPS binding by GABA in the dorsal hippocampus. Experiment 2 used brain sections from gonadally intact CORT- or vehicle-treated animals incubated with 2.3 [^{35}S]TBPS, with or without 1.5 μM GABA. Data are expressed as the specific binding of [^{35}S]TBPS, percentage of binding in the presence of GABA relative to basal binding in the absence of GABA. Shown are means and error bars representing SEM; $n = 9$. There was an overall significant main effect of CORT versus vehicle treatment in hippocampus ($F_{(1,16)} = 4.80$; $p = 0.044$), indicated by an *asterisk*. Set C contrasts indicated significant differences in the mean levels of modulation of [^{35}S]TBPS binding by GABA, averaged across treatments, between CA3 radiatum and CA3 oriens ($t = -13.56$; $p \leq 0.0001$), and between CA3 radiatum and CA1 radiatum ($t = 5.15$; $p \leq 0.0001$).

Experiment 4: CORT effects on modulation of [^{3}H]flunitrazepam binding by GABA and THDOC

We further tested the hypothesis that chronic CORT treatment alters the pharmacological properties of $GABA_A$ receptors by examining the allosteric regulation of [^{3}H]flunitrazepam binding by GABA and THDOC. Benzodiazepines bind to a site on $GABA_A$ receptors distinct from the [^{35}S]TBPS binding site (Braestrup and Squires, 1977), so CORT treatment may alter [^{3}H]flunitrazepam binding independently of changes in [^{35}S]TBPS binding. Brain sections were incubated with 0.5 nM [^{3}H]flunitrazepam in buffer alone or buffer containing either 100 nM THDOC or 3 μM GABA. We used 100 nM THDOC, although this concentration had only minimal effects on [^{3}H]flunitrazepam binding in preliminary studies, because THDOC levels in the plasma of stressed rats tend to be in the lower nanomolar range (Purdy et al., 1991). In contrast to the [^{35}S]TBPS binding studies, modulation of benzodiazepine binding by GABA and THDOC was similar throughout all regions of the hippocampus. Also in contrast to the [^{35}S]TBPS binding studies, CORT treatment apparently increased the sensitivity of [^{3}H]flunitrazepam binding to modulation by both GABA and THDOC, but only in the dentate gyrus. The results, detailed below, further support the hypothesis that CORT alters the pharmacological properties of $GABA_A$ receptors.

There was a significant interaction between hippocampal region (Ammon's horn vs dentate gyrus) and CORT treatment on the mean levels of basal [^{3}H]flunitrazepam binding ($F_{(1,16)} = 8.50$; $p = 0.0101$). Set B contrasts, however, indicated that basal binding was not significantly different between CORT- and vehicle-treated animals in either Ammon's horn or dentate gyrus. There were regional differences (Table 2) in mean basal [^{3}H]flunitrazepam binding between dentate gyrus and Ammon's horn ($F_{(1,16)} = 3385$; $p \leq 0.0001$) and between dendritic subfields

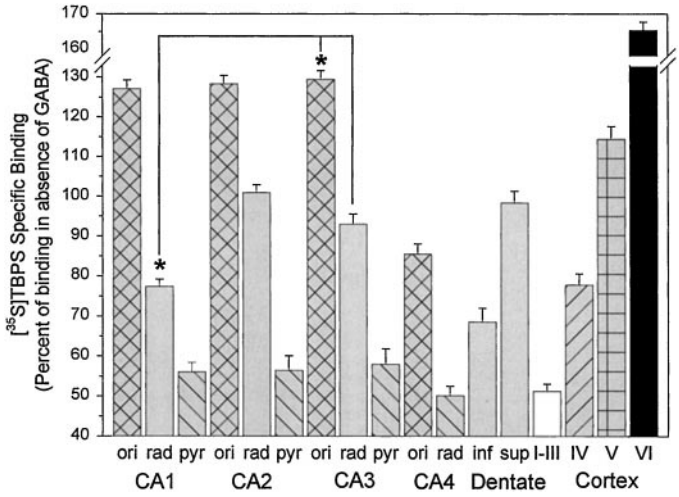

Figure 4. Modulation of [^{35}S]TBPS binding by GABA in the dorsal hippocampus and cortex. In Experiment 3, brain sections were incubated with 2 nM [^{35}S]TBPS with or without 1.5 μM GABA. Data are expressed as [^{35}S]TBPS specific binding in the presence of 1.5 μM GABA as the percentage of binding in the absence of GABA. Shown are means and error bars representing SEM averaged across CORT- and vehicle-treated groups; $n = 18$. There were significant regional differences between the modulation of [^{35}S]TBPS by GABA in Ammon's horn and dentate gyrus ($F_{(1,16)} = 127.6$; $p \leq 0.0001$) and between the means of the individual dendritic subfields ($F_{(6,16)} = 278.0$; $p \leq 0.0001$) in Ammon's horn or dentate gyrus, or both. Set C contrasts indicated significant differences, indicated by an *asterisk*, between the mean modulation of [^{35}S]TBPS binding by GABA in CA3 stratum radiatum versus CA3 stratum oriens ($t = -17.82$; $p \leq 0.0001$) and between CA3 stratum radiatum and CA1 stratum radiatum ($t = 8.40$; $p \leq 0.0001$). Also note the striking differences in mean percentage modulation by GABA between layers I–III, IV, V, and VI of the parietal cortex.

within either Ammon's horn or the dentate gyrus, or both ($F_{(6,16)} = 202.7$; $p \leq 0.0001$).

THDOC

Addition of 100 nM THDOC to the assay buffer (without exogenous GABA) produced a modest inhibition of [^{3}H]flunitrazepam binding in vehicle-treated animals (Table 2). Analysis of normalized data (Fig. 5) indicated that there was a significant effect of CORT versus vehicle treatment on the mean percentage modulation of [^{3}H]flunitrazepam binding by THDOC ($F_{(1,16)} = 9.81$; $p = 0.0064$) but also a significant interaction ($F_{(1,16)} = 6.23$; $p = 0.0239$) between CORT treatment and hippocampal region (Ammon's horn vs dentate gyrus). Subsequent hypothesis tests (set B) indicated that there was a significant effect of CORT treatment on the mean percentage modulation of [^{3}H]flunitrazepam binding by THDOC in the dentate gyrus ($t = -3.98$; $p = 0.0011$) but not Ammon's horn (Table 3); [^{3}H]flunitrazepam binding was inhibited by THDOC in CORT-treated animals but not vehicle-treated animals.

GABA

Addition of 3 μM GABA to the buffer enhanced [^{3}H]flunitrazepam binding in all regions of the hippocampus (Table 2). Analysis of normalized data (Fig. 6) indicated that there was a significant effect of CORT versus vehicle treatment on the mean percentage modulation of [^{3}H]flunitrazepam binding by GABA ($F_{(1,16)} = 4.79$; $p = 0.0437$) but also a significant interaction ($F_{(1,16)} = 5.89$; $p = 0.0274$) between CORT treatment and hippocampal region. Set B contrasts indicated that the mean percentage modulation of [^{3}H]flunitrazepam binding by GABA differed between CORT- and vehicle-treated animals in the dentate gyrus ($t = -2.65$; $p = 0.0173$), but not Ammon's horn (Table 3); there was a greater enhancement of [^{3}H]flunitrazepam binding by GABA in the dentate gyrus of CORT-treated animals relative to vehicle-treated controls. There were also significant regional differences in mean levels of modulation by GABA between Ammon's horn versus dentate gyrus ($F_{(1,16)} = 28.57$; $p \leq 0.0001$) and between dendritic subfields within either Ammon's horn or the dentate gyrus, or both ($F_{(6,16)} = 2.79$; $p = 0.0474$). Exploratory hypothesis tests (Set C) indicated that there were no significant differences in the mean level of modulation of [^{3}H]flunitrazepam binding by GABA between the apical and basal dendritic subfields of Ammon's horn (Table 3).

Ratio of [^{35}S]TBPS to [^{3}H]flunitrazepam binding

We compared the ratio of chloride channel to benzodiazepine sites as another indicator of hippocampal $GABA_A$ receptor heterogeneity. Ratios were calculated from the total binding data in Experiments 2 and 4 in both CORT- and vehicle-treated animals. These experiments used nonsaturating concentrations of [^{35}S]TBPS and [^{3}H]flunitrazepam in buffer with no exogenous GABA. There was a significant interaction between CORT treatment and dendritic subfields within either Ammon's horn or the dentate gyrus, or both ($F_{(6,16)} = 7.06$; $p = 0.0008$) on the ratio means. However, Set A contrasts failed to detect a significant difference in ratio means between CORT- and vehicle-treated animals in CA3 stratum radiatum or dentate gyrus specifically. There were regional differences in the mean ratio of [^{35}S]TBPS to [^{3}H]flunitrazepam binding as follows. (1) The difference in ratios between Ammon's horn and dentate gyrus was significant ($F_{(1,16)} = 10.33$; $p = 0.0054$), with a greater [^{35}S]TBPS to [^{3}H]flunitrazepam ratio in the dentate gyrus than Ammon's horn, particularly compared with the stratum radiatum; (2) there were differences in ratio means in individual dendritic subfields within either Ammon's horn or the dentate gyrus, or both ($F_{(6,16)} = 165.1$; $p \leq 0.0001$). Additional hypothesis tests, performed for each level of treatment, indicated that the mean ratio of [^{35}S]TBPS to [^{3}H]flunitrazepam binding was different (higher) in CA3 stratum oriens from CA3 stratum radiatum in both vehicle-treated ($t = -7.54$; $p \leq 0.0001$) and CORT-treated ($t = -8.74$; $p \leq 0.0001$) animals; different (higher) in CA3 stratum radiatum from CA1 radiatum in both vehicle-treated ($t = 15.47$; $p \leq 0.0001$) and CORT-treated ($t = 14.47$; $p \leq 0.0001$) animals; different in CA3 stratum radiatum from the average of CA1 and CA2, stratam oriens, and stratum radiatum means in both vehicle-treated ($t = -9.86$; $p \leq 0.0001$) and CORT-treated ($t = -7.11$; $p \leq 0.0001$) animals.

DISCUSSION

There were two major findings in this study. First, prolonged CORT treatment altered the sensitivity of $GABA_A$ receptors to modulation by GABA and THDOC. The altered sensitivity to endogenous modulators is consistent with a CORT-induced shift in the balance between excitatory and inhibitory neurotransmission. Second, there were marked intrahippocampal differences in the pharmacological properties of $GABA_A$. These differences in radioligand binding are likely to represent differences in the receptor subtypes found in Ammon's horn relative to dentate gyrus and in the stratum radiatum relative to the stratum oriens within Ammon's horn. The latter suggests that $GABA_A$ receptor subtypes are selectively expressed in the apical versus basal dendrites within pyramidal neurons.

Table 2. Effect of CORT on the modulation of 0.5 nM [^{3}H]flunitrazepam by 100 nM THDOC or 3 μM GABA

	CA1 oriens	CA1 radiatum	CA2 oriens	CA2 radiatum	CA3 oriens	CA3 radiatum	Inferior dentate	Superior dentate	CA4 oriens	CA4 radiatum
Basal										
Vehicle	182.9 ± 3.4	220.3 ± 3.8	153.0 ± 4.3	193.1 ± 4.9	154.5 ± 2.7	191.6 ± 4.3	271.6 ± 5.5	294.8 ± 5.6	249.3 ± 5.0	265.2 ± 4.1
CORT	176.6 ± 3.1	221.7 ± 5.1	149.8 ± 3.0	194.2 ± 2.6	154.6 ± 3.0	193.8 ± 3.1	252.1 ± 2.9	293.6 ± 3.7	241.6 ± 5.5	261.8 ± 3.7
+ THDOC										
Vehicle	173.9 ± 2.4	206.2 ± 3.1	149.3 ± 3.1	185.9 ± 3.6	152.2 ± 4.0	189.4 ± 5.8	241.2 ± 7.3	273.8 ± 5.6	234.0 ± 7.2	246.7 ± 5.6
CORT	179.2 ± 3.5	221.4 ± 5.1	153.7 ± 2.5	196.8 ± 3.7	153.6 ± 3.6	197.7 ± 4.2	259.3 ± 4.8	298.0 ± 7.6	250.5 ± 4.4	266.2 ± 6.1
+ GABA										
Vehicle	207.8 ± 4.4	250.6 ± 4.1	171.6 ± 3.6	212.2 ± 3.9	170.9 ± 4.6	212.6 ± 4.1	298.3 ± 6.8	348.6 ± 5.2	282.0 ± 6.8	299.9 ± 4.0
CORT	210.2 ± 4.3	260.8 ± 5.3	183.8 ± 4.1	227.1 ± 4.5	183.7 ± 4.8	228.3 ± 4.2	313.5 ± 7.3	368.1 ± 9.4	298.6 ± 8.1	317.1 ± 8.3

Data are expressed as femtomoles [^{3}H]flunitrazepam specifically bound per milligram protein (means ± SEM; $n = 9$).

There was a significant interaction between CORT treatment and hippocampal region (Ammon's horn vs dentate gyrus) on basal levels of [^{3}H]flunitrazepam binding ($F_{(1,16)} = 8.50$; $p = 0.0101$).

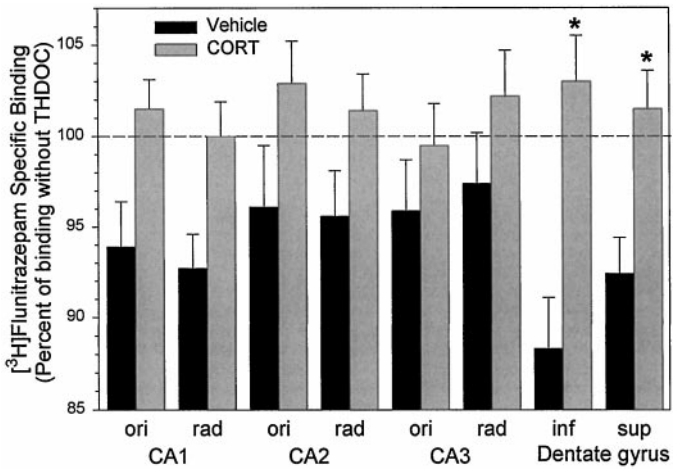

Figure 5. Effect of CORT on the modulation of [^{3}H]flunitrazepam binding by THDOC in the dorsal hippocampus. Brain sections from CORT- or vehicle-treated, gonadally intact animals were incubated with 0.5 nM [^{3}H]flunitrazepam in buffer alone or buffer containing 100 nM THDOC. Data presented are [^{3}H]flunitrazepam-specific binding in the presence of THDOC expressed as the percentage of basal binding in the absence of THDOC. Shown are means and error bars representing SEM; $n = 9$. The CORT- versus vehicle-treatment factor was significant ($F_{(1,16)} = 4.79$; $p = 0.0437$) but so was the interaction between treatment and hippocampal region (Ammon's horn vs dentate gyrus; $F_{(1,16)} = 5.89$; $p = 0.0274$). Set B contrasts indicated that the percentage modulation of [^{3}H]flunitrazepam binding by THDOC differed between CORT- and vehicle-treated animals in the dentate gyrus ($t = -2.65$; $p = 0.0173$), indicated by an *asterisk*.

To test our hypothesis that chronic exposure to stress levels of CORT alters pharmacological properties of hippocampal $GABA_A$ receptors, we examined the allosteric regulation of [^{35}S]TBPS and [^{3}H]flunitrazepam binding by GABA and THDOC. CORT treatment produced the following results supporting our hypothesis. (1) It decreased the fraction of [^{35}S]TBPS binding inhibited by THDOC (Fig. 1), suggesting a decrease in sensitivity to THDOC, and (2) it enhanced the potentiation of [^{35}S]TBPS binding by GABA in stratum oriens and attenuated the inhibition of [^{35}S]TBPS binding by GABA in stratum radiatum (Fig. 3). Because higher doses of GABA uniformly inhibited [^{35}S]TBPS binding, CORT apparently decreased sensitivity of [^{35}S]TBPS binding to modulation by GABA. (3) It enhanced the potentiation of [^{3}H]flunitrazepam binding by a low dose of THDOC in dentate gyrus (Fig. 5). Because higher doses of THDOC uniformly potentiate benzodiazepine binding, CORT apparently increased sensitivity of [^{3}H]flunitrazepam to modulation by THDOC in the dentate gyrus. (4) It enhanced the potentiation of [^{3}H]flunitrazepam binding by GABA in dentate gyrus (Fig. 6), apparently increasing sensitivity of [^{3}H]flunitrazepam binding to modulation by GABA.

These changes in allosteric regulation of binding probably reflect changes in receptor function, because the ability of agonist-like steroids, such as THDOC, to regulate GABA-gated Cl^- conductance is highly correlated with their ability to modulate [^{35}S]TBPS and [^{3}H]flunitrazepam binding (Hawkinson et al., 1994). A decreased sensitivity of [^{35}S]TBPS binding to modulation by THDOC and GABA would be consistent with a general increase in hippocampal excitability, whereas the increased sensitivity of [^{3}H]flunitrazepam binding to THDOC and GABA in the dentate gyrus suggests a localized increase in inhibitory tone. One hypothesis is that chronic CORT alters information flow through the hippocampus such that the massive excitatory input to CA3 from mossy fibers originating in the dentate gyrus is inhibited, whereas pyramidal cell responsiveness to excitatory input from entorhinal cortex, septum, or commissural fibers is enhanced. Stress-induced dendritic regression occurs in distal apical dendrites of CA3 that receive innervation from the entorhinal cortex, and, interestingly, lesions of the entorhinal cortex block this dendritic remodeling (Sunanda et al., 1997).

CORT treatment had little effect on basal levels of [^{35}S]TBPS or [^{3}H]flunitrazepam binding, so CORT effects on hippocampal circuits may be most apparent when THDOC levels are elevated, such as during stress (Paul and Purdy, 1992). THDOC also has anxiolytic and anticonvulsant activity (Gasior et al., 1999), in part mediated by the dorsal hippocampus (Bitran et al., 1999). CORT treatment may decrease the efficacy or potency of THDOC as an anxiolytic in the hippocampus, and this may contribute to the anxiogenic actions of corticosteroids seen in some experimental paradigms (File et al., 1979; Smythe et al., 1997; Bitran et al., 1998; Conrad et al., 1999; Tronche et al., 1999) or contribute to the anxiety associated with major depression (Wong et al., 2000). Consistent with this idea, fluoxetine, commonly used to treat mood disorders, increases the brain levels of 5α-pregnan-3α-ol-20-one (Uzunov et al., 1996), a neurosteroid that modulates $GABA_A$ receptors in a manner very similar to THDOC. A similar mechanism may occur in prenatal stress, a condition that decreases the potency of 5α-pregnan-3α-ol-20-one as an anticonvulsant in adulthood (Frye and Bayon, 1999).

Table 3. Summary of statistical tests

	CORT effects on basal [^{35}S]TBPS binding	Modulation of [^{35}S]TBPS binding by THDOC	Modulation of [^{35}S]TBPS binding by GABA	Modulation of [^{35}S]TBPS binding by GABA	CORT effects on basal [^{3}H]flunitrazepam binding	Modulation of [^{3}H]flunitrazepam binding by THDOC	Modulation of [^{3}H]flunitrazepam binding by GABA
	Experiments 1 and 2	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 4	Experiment 4
Significant ANOVA results	CORT X dendritic subfield Main effect of dendritic subfield Main effect of dendritic subfield	Main effect of CORT Main effect of dendritic subfield	Main effect of CORT	Main effect of dendritic subfield	CORT X hippocampal region	Main effect of CORT CORT X hippocampal region	Main effect of CORT CORT X hippocampal region
Hypothesis tests							
CORT vs VEH							
Set A contrasts	All NS[a]	N/A	N/A	N/A	N/A	N/A	N/A
Set B contrasts	N/A	N/A	N/A	N/A			
(1)					NS[a]	NS[a]	NS[a]
(2)					NS[a]	Significant[a]	Significant[a]
Regional differences							
Set C contrasts	N/A				N/A	N/A	
(1)		Significant[a]	Significant[a]	Significant[a]			NS[a]
(2)		Significant[a]	Significant[a]	Significant[a]			NS[a]
(3)		Significant[a]	Significant[a]	Significant[a]			NS[a]
ANOVA[b]				Main effect of region			

Set A contrasts performed when the interaction between CORT versus VEH treatment and dendritic subfield was significant tested the equivalence of (1) the mean level of binding in CA3 stratum radiatum of CORT-treated animals and CA3 stratum radiatum of vehicle-treated animals, (2) the mean level of binding in the dentate gyrus of CORT-treated animals and dentate gyrus of vehicle-treated animals, (3) the difference between the mean level of binding in CA3 stratum radiatum of CORT-treated animals minus the level in CA3 stratum radiatum of vehicle-treated animals and the mean level of binding in CA1 stratum oriens of CORT-treated animals minus the mean level in CA1 oriens of vehicle-treated animals. (4) This latter contrast was repeated for CA3 stratum radiatum versus CA1 stratum radiatum, (5) CA3 stratum radiatum versus CA2 stratum oriens, (6) CA3 stratum radiatum versus CA2 stratum radiatum, (7) CA3 stratum radiatum versus CA3 stratum oriens.

Set B contrasts performed when the interaction between CORT versus VEH treatment and hippocampal region was significant tested the equivalence of (1) the mean level of binding in Ammon's horn of CORT-treated animals and in Ammon's horn of vehicle-treated animals, (2) the mean level of binding in the dentate gyrus of CORT-treated animals and in dentate gyrus of vehicle-treated animals.

Set C contrasts performed when there was a significant main effect of dendritic subfield on the mean percentage modulation tested the equivalence of (1) the mean percentage modulation in CA3 stratum radiatum and in CA3 stratum oriens, (2) the mean percentage modulation in CA3 stratum radiatum and in CA1 stratum radiatum, (3) the mean percentage modulation CA3 stratum radiatum and in CA1 oriens, CA1 radiatum, CA2 oriens, and CA2 radiatum.

[a]Significance level set at a familywise $\alpha = 0.05$; Bonferroni method was used to control for simultaneous testing; NS, nonsignificant; N/A, nonapplicable.

[b]Additional repeated-measures ANOVA tested equivalence between the mean percentage modulation in CA3 strata radiatum, oriens, and pyramidale.

The conclusion that $GABA_A$ receptors in the stratum radiatum displayed different pharmacological properties relative to those in the stratum oriens is supported by the following. (1) [^{35}S]TBPS binding was more sensitive to inhibition by THDOC in the radiatum than in the oriens. (2) GABA enhanced [^{35}S]TBPS binding in stratum oriens but inhibited or had no effect on binding in the stratum radiatum. Within CA3, 1.5 μM GABA enhanced [^{35}S]TBPS binding in stratum oriens by 30%, inhibited binding in the stratum pyramidale by 40%, and had little effect in CA3 stratum radiatum. (3) The ratio of [^{35}S]TBPS to [^{3}H]flunitrazepam binding was higher in CA3 oriens than in CA3 radiatum. These data suggest that receptor subtypes are differentially targeted within hippocampal pyramidal neurons. Further supporting this hypothesis, different subcellular regions of pyramidal neurons are innervated by different types of GABAergic interneuron (Buhl et al., 1994), display unique physiological responses to GABA (Brickley et al., 1999; Banks and Pearce, 2000), and stain for distinct $GABA_A$ receptor subunits (Nusser et al., 1996; Fritschy et al., 1998).

There may be other explanations for regional differences in radioligand binding. First, modulation of [^{35}S]TBPS binding by THDOC is enhanced by GABA, so subcellular differences in sensitivity to THDOC may reflect regional differences in the abundance of GABA rather than receptor subtypes. However, we preincubated sections of tissues for 30 min before binding assays to minimize the contribution of endogenous GABA (Sapp et al., 1992). Second, radioligand binding in the stratum oriens and stratum radiatum probably does not reflect binding to pyramidal cell receptors exclusively. GABAergic interneurons in Ammon's horn have extensive dendritic and axonal arbors in the stratum oriens and stratum radiatum (Buhl et al., 1994; Freund and Buzsaki, 1996). These interneurons may be innervated by other GABAergic interneurons (Freund and Buzsaki, 1996), and they express $GABA_A$ receptors (Gao and Fritschy, 1994; Sperk et al., 1997). Therefore, some fraction of radioligand binding in the stratum oriens and stratum radiatum probably reflects binding to $GABA_A$ receptors in interneurons. Clearly, the functional outcomes would be quite different whether CORT altered the pharmacological properties of $GABA_A$ receptors in GABAergic interneurons rather than pyramidal cells, so it is important to determine the cellular targets for CORT action.

In addition to altering radioligand binding, chronic CORT treatment produced complex changes in $GABA_A$ receptor subunit mRNA levels (Orchinik et al., 1995). The relative abundance of subunits may alter many properties of $GABA_A$ receptors and regulate the assembly, trafficking, or insertion of specific receptor

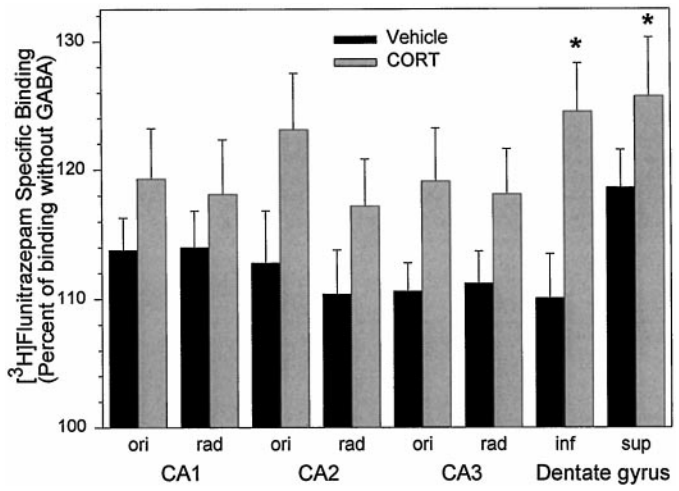

Figure 6. Effect of CORT on the modulation of [^{3}H]flunitrazepam binding by GABA in the dorsal hippocampus. Brain sections from CORT- or vehicle-treated, gonadally intact animals were incubated with 0.5 nM [^{3}H]flunitrazepam in buffer alone or buffer containing 3 μM GABA. Data presented are [^{3}H]flunitrazepam-specific binding in the presence of GABA, expressed as the percentage of binding in the absence of GABA. Shown are means and error bars representing SEM; $n = 9$. There was a significant CORT- versus vehicle-treatment effect ($F_{(1,16)} = 9.81$; $p = 0.0064$), and a significant interaction between treatment and hippocampal region ($F_{(1,16)} = 6.23$; $p = 0.0239$). Set B contrasts indicated that mean percentage modulation of [^{3}H]flunitrazepam binding by GABA differed between CORT- and vehicle-treated animals in the dentate gyrus ($t = -3.98$; $p = 0.0011$), indicated by an *asterisk*.

subtypes in neuronal membranes (Mehta and Ticku, 1999; Vicini, 1999). CORT treatment increased $\gamma 2$ subunit mRNA levels in Ammon's horn. This subunit, required for benzodiazepine binding to $GABA_A$ receptors (Pritchett et al., 1989), also alters the dose–response curve for steroid inhibition of [^{35}S]TBPS binding (Srinivasan et al., 1999) and decreases the sensitivity of $GABA_A$ receptors to GABA (Zhu et al., 1996b). Therefore, the CORT-induced increase in $\gamma 2$ mRNA levels may underlie the decreased sensitivity of [^{35}S]TBPS binding to modulation by GABA or THDOC. The $\gamma 2$ subunit is also required for clustering of $GABA_A$ receptors at the cell surface (Essrich et al., 1998), and decreased receptor clustering in $\gamma 2$ deficient mice is associated with increased anxiety (Crestani et al., 1999). CORT treatment also increased $\beta 2$ mRNA levels throughout the hippocampus and altered mRNA levels of $\alpha 1$, $\alpha 2$, $\beta 1$, and $\beta 3$ mRNA levels in dentate gyrus (Orchinik et al., 1995). Therefore, the increased sensitivity of [^{3}H]flunitrazepam binding to modulation by GABA and THDOC seen in the dentate gyrus may be related to a decreased ratio of $\alpha 1$ or $\alpha 2$ to $\gamma 2$ or $\beta 2$ subunits. Given that the δ subunit is abundant in hippocampus (Sperk et al., 1997) and inhibits the sensitivity of GABA-gated currents to enhancement by THDOC (Zhu et al., 1996a), CORT treatment may also increase expression of the δ subunit.

In conclusion, differences in the allosteric modulation of radioligand binding by THDOC and GABA revealed that $GABA_A$ receptors are pharmacologically heterogeneous within the hippocampus, probably within individual pyramidal neurons of Ammon's horn. Chronic treatment with stress levels of CORT selectively altered pharmacological properties such that the sensitivity to GABA and THDOC was changed. The differential sensitivity to THDOC or GABA between dendritic subfields, in conjunction with CORT-induced changes in $GABA_A$ receptor properties, may contribute to dendritic remodeling or other changes in hippocampal function. It is likely that hormonally driven swapping of $GABA_A$ receptor subunits underlies these changes in $GABA_A$ receptors, and it may be a more general mechanism for regulating neural plasticity (Smith et al., 1998; Brussaard and Herbison, 2000). The persistently elevated levels of corticosterone in this study may not be ideal for understanding the dynamics of stress, but prolonged elevations of corticosteroids also occur in conditions such as Cushing's disease and major depression (Gold et al., 1996; Mitchell and Dening, 1996; De Kloet et al., 1997; O'Toole et al., 1997; Wong et al., 2000). Similar to chronic stress, these conditions are also associated with anxiety and deficits in hippocampus-dependent memory and hippocampal atrophy (Lupien et al., 1994, 1998). The close association between stress, corticosteroids, anxiety, depressed mood, and cognitive disorders suggests that CORT-induced changes in hippocampal $GABA_A$ receptor function may play a role in the etiology of major depression.

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