

Published in final edited form as:

Brain Res. 2006 December 13; 1125(1): 138–146.

Normal [³H]Flunitrazepam Binding to GABA_A Receptors in the Locus Coeruleus in Major Depression and Suicide

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Abstract

Major depression and suicide are associated with altered concentrations of specific noradrenergic proteins in the human locus coeruleus (LC). Based on experimental studies that can reproduce these LC abnormalities in laboratory animals, we hypothesized that noradrenergic pathobiology in depression is a result of over-activity of the LC. LC activity is under the control of both excitatory and inhibitory inputs. A major inhibitory input to the LC is GABAergic, arising from the nucleus prepositus hypoglossi. Numerous studies demonstrating low levels of GABA in the CSF and plasma of subjects with major depressive disorder (MDD) raise the possibility that LC over-activity in depression may be secondary to reduced GABAergic input to the LC. Here, GABAergic input to the LC in depression was evaluated by studying the binding of [³H]flunitrazepam to GABA_A receptors at three anatomically-defined levels of the human postmortem LC. LC tissues were collected from subjects with MDD, subjects with depressive disorders including MDD that died as a result of suicide, and psychiatrically normal control subjects. A modest rostral-caudal gradient of GABA_A receptor binding density was observed amongst all subjects. No significant differences in the amount of binding to GABA_A receptors were observed between control subjects (n=21) and MDD subjects (n=9) or depressed suicide victims (n=17). These results demonstrate that GABA_A receptor binding in the LC measured with [³H]flunitrazepam is not altered in subjects with depressive illnesses.

Keywords

postmortem brain; major depressive disorder; suicide, norepinephrine; GABA_A receptor; flunitrazepam; locus coeruleus

1. Introduction

The locus coeruleus (LC) is the largest noradrenergic nucleus in the brain projecting extensively to a number of cortical and subcortical regions [57]. Norepinephrine (NE) released from LC neurons plays a crucial role in the regulation of emotional activation, vigilance, sleep-

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wake cycle, and mood. Disruptions of noradrenergic transmission have long been believed to underlie certain psychiatric disorders, particularly depression. Converging evidence from studies of the LC from postmortem depressed subjects and from catecholamine-depletion studies in living depressed subjects has provided compelling evidence that depression is associated with a deficit in noradrenergic transmission [12,36,39]

Previous observations from postmortem studies have revealed that major depressive disorder (MDD) and suicide are associated with altered concentrations of several noradrenergic proteins in the LC. For example, elevated levels of tyrosine hydroxylase [42,69], increased agonist binding to α_2 -adrenergic receptors [40,43] and reduced levels of norepinephrine transporters [20] have been reported in the LC from MDD subjects and/or from suicide victims. Studies utilizing laboratory animals provide interesting insights into the possible basis for these postmortem findings. For example, depletion of NE or repeated stress in rats increases tyrosine hydroxylase expression, increases binding to α_2 -adrenergic receptors, and decreases binding to the norepinephrine transporter [10,23,30,61,63,64,66]. Together, these findings are highly suggestive of dysfunctional noradrenergic neurotransmission in depression, possibly through sustained stress-induced LC activation and the ultimate depletion of NE.

The possibility that there is an elevated LC activity in depression brings attention to the possibility of depression-associated deficits in inputs to the LC from neurons that regulate LC activity. Recently, abnormalities in excitatory inputs (corticotropin releasing factor, glutamate) to the LC in depression and/or suicide have been reported [2,7,17,32]. It is reasonable to speculate that elevated LC activity in depression may be simultaneously associated with diminished inhibitory input to the LC. GABAergic fibers, originating in the nucleus prepositus hypoglossi, project to the LC and inhibit LC firing [1]. Receptors responding to GABA in the LC include GABA_A receptors [45,67], which are ligand-gated chloride channels [for review, see 3,35]. Interestingly, there is a wealth of information suggesting that there is a deficit of inhibitory neurotransmitter, GABA, in depression [8,26,31,54,55,56,62].

Given the putative role of GABA in depressive disorders and GABA-modulation of the noradrenergic LC, the present study investigated the distribution of the GABA_A receptors along the rostral-caudal axis of the human LC and potential abnormalities in the concentrations of GABA_A binding sites in the LC that might occur in MDD and suicide. MDD and suicide subjects were matched with control subjects for age, sex, cigarette smoking history and postmortem interval as closely as possible. Brain tissue was collected from carefully screened subjects (post-mortem) who were diagnosed retrospectively with depressive disorders at the time of death, and from control subjects who lacked major (Axis I) psychiatric disorders, except as indicated for nicotine dependence.

2. Materials and Methods

Tissue collection and psychiatric autopsy

Human brain tissue was obtained at autopsy at the Cuyahoga County Coroner's Office, Ohio, in accordance with an approved Institutional Review Board protocol. The description of the tissue collection process has been described previously [20]. Briefly, causes of death were determined by the coroner and information on lifetime and latest psychiatric status, psychotropic medication, and possible illicit drug use of all subjects was obtained in structured clinical interviews by a trained interviewer with the next of kin. The interviewer used the Schedule for Affective Disorders and Schizophrenia-Lifetime version (SADS-L) [14] supplemented by questions from the Diagnostic Interview Schedule (DIS-III-R) [28]. Responses from the depressed subjects evaluated with the SADS-L were also recorded with the Structured Clinical Interview for DSM-IV Psychiatric Disorders (SCID) [15] and these subjects met criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth

edition (DSM-IV; American Psychiatric Association, 1994) for major depressive disorder, dysthymia, adjustment disorder with depressed mood or alcohol induced mood disorder. A total of 17 subjects who had a depressive disorder at the time of death were studied (Table 1), 9 of which had a diagnosis of MDD. Evaluations of drug and alcohol abuse and dependency were assessed using the DIS-III-R. Axis I diagnoses of MDD were made by experienced psychiatrists and a clinical psychologist, based on the data gathered from the structured interviews and, when available, hospital and physician's records. Control subjects were sudden death victims, free of any psychiatric diagnoses at the time of death (Table 2), except nicotine dependence where noted. A toxicological screen of blood, bile, and urine from all subjects was performed by the Cuyahoga County Coroner's Office as described previously. No antidepressant drugs were detected in any of the subjects selected for the study. Control subjects were matched as closely as possible to MDD and suicide subjects based on age, brain pH value, gender, postmortem interval and cigarette smoking. Past histories of cigarette smoking were not considered in the comparison between smokers and non-smokers.

Dissection

Details of the dissection of tissue blocks of human dorsal pons containing the LC have been described previously [20]. Briefly, blocks containing the LC were bisected at the midline and only right side LC nuclei were studied. Left side nuclei were used for other experiments. We have previously found no differences in right and left side biochemical or cellular data (37,41,42). Tissue blocks were then sectioned sequentially using a cryostat microtome (-16°C) in a transverse plane perpendicular to the floor of the fourth ventricle throughout the entire length of the LC beginning near its rostral end, collecting all tissue sections. The LC had its rostral border defined as a point where at least 25 ± 5 neuromelanin-containing cells were identified (at the frenulum). The caudal border was defined near the caudal end at a point where 25 ± 5 or less neuromelanin-containing cells were present. Equivalent anatomical levels of the LC were identified in Nissl-stained sections by computing 3 levels that represented 25% (rostral), 50% (middle), and 75% (caudal) of the total rostrocaudal length of the LC (as define above). After confirming anatomically equivalent levels in each subject, four adjacent sections ($20\ \mu\text{m}$ each) from each level (totally 12 sections from each subject) were chosen for these experiments. After dissection, sections were dried overnight at 4°C and placed into the freezer (-80°C) until assayed.

Immunohistochemical staining

Nissl staining and tyrosine hydroxylase (TH) immunostaining of sections were used to create anatomical templates for measuring the densities of radioligand binding by quantitative autoradiography. For Nissl staining, the same sections used for the radioligand autoradiography were stained after film exposure with cresyl violet for the identification of LC cell bodies. For tyrosine hydroxylase immunostaining, sections were fixed in 4% paraformaldehyde in 0.05 M phosphate-buffered saline (PBS) for 1 h at 21°C . Sections were then pre-incubated in 5% normal horse serum in 0.05 M PBS (containing 0.1 % Triton X-100 and 0.005 % sodium azide) for 30 min followed by incubating for 24 h at 4°C in the same solution containing mouse anti-tyrosine hydroxylase monoclonal antibody (1:500; Chemicon, Temecula, CA, USA). Sections were subsequently washed in 0.05 M PBS, and incubated for 4 h at 21°C in biotinylated horse anti-mouse IgG (1:200) in 0.05 M PBS (Vector, Burlingame, CA, USA). The sections were then processed using the Vectastain ABC immunoperoxidase kit (Vector, Burlingame, CA, USA) for 24 h at 4°C and the antibody distribution was detected using 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO, USA). *Quantitative autoradiography.* The autoradiographic method used for [^3H]flunitrazepam binding was a modification of that reported by Benes et al. [5]. At the time of assay, tissue sections were first thawed at 4°C for 1 h and then incubated with 1 nM [^3H]flunitrazepam (84.5 Ci/mmol; PerkinElmer Life Sciences, Inc. Boston, MA) in 0.17 M Tris-HCl, pH 7.4, at 21°C for 40 min

in the presence (non-specific binding, in duplicate) or absence (total binding, in duplicate) of 1 μM diazepam (Sigma Chemical Co., St. Louis, MO). The slide-mounted sections were washed in two changes of ice-cold Tris-HCl for 1 min each, and then dipped rapidly in ice-cold distilled water. Slides were dried at 21°C overnight and then apposed, along with [^3H] standards calibrated in μCi of ^3H (American Radiolabeled Chemicals, Inc., St. Louis, MO), to ^3H -hyperfilm (Amersham, Arlington Hts. IL) for one month. Densitometric measurements of autoradiograms were made using a computer-aided imaging system (MCID Elite, Imaging Research, St. Catherine, Ontario, Canada). LC autoradiograms were analyzed by simultaneously overlaying the image of the autoradiogram with the template created by Nissl staining of the same section and with a template created by the tyrosine hydroxylase immunostaining of an adjacent section. The cell-dense region of the LC was outlined and binding density was computed. The reported binding density is the average of radioactivity, determined from the standard, measured across hundreds of pixels located within a defined area of the film (representing the LC). The specific binding of [^3H]flunitrazepam to GABA_A receptors was determined by subtracting non-specific binding (computed in adjacent sections) from the total binding.

Statistical analysis

Mean values of the specific binding of [^3H]flunitrazepam to GABA_A receptors in the LC for groups of MDD, suicide, and control subjects were compared statistically using a one-way ANOVA followed by Bonferroni post tests. Comparisons between smokers and non-smokers were made using the Student's t-test. A p value < 0.05 was considered statistically significant. All data are presented as means \pm S.E.M. Pearson correlation analysis was used to compute potential relationships between binding densities and age, postmortem interval, and brain pH.

3. Results

A representative autoradiogram of the binding of [^3H]flunitrazepam to GABA_A receptors in the middle LC from a control subject is shown in Figure 1. The amount of specific binding of [^3H]flunitrazepam to GABA_A receptors was measured in the immediate region of the compact neuromelanin-containing cell body region of the LC, shown by Nissl stains of same section. The specific binding of [^3H]flunitrazepam to GABA_A receptors in the entire region of the LC was low relative to the density of binding in the cerebellum, as observed in the upper left corner of Figure 1. Both the compact cellular region of the LC and the area of the dendritic expanse of the LC demonstrated relatively low [^3H]flunitrazepam binding levels. There was an uneven distribution of [^3H]flunitrazepam binding along the rostrocaudal axis of the LC ($F=10.74_{2,20}$, $p<0.0001$), with the density of binding in caudal portion of the LC being significantly lower than that in the rostral and medial portions of the LC ($p<0.001$). The rostrocaudal gradient of binding density did not correlate with the uneven distribution of noradrenergic cell number along the rostrocaudal axis of the LC (data not shown, see ref. 20). No significant differences in the amount of [^3H]flunitrazepam binding to GABA_A receptors were observed between MDD and control subjects at any anatomical level of the LC (Figure 2). Likewise, no differences in binding were observed comparing suicide victims to control subjects (Figure 3).

The possibility was considered that [^3H]flunitrazepam binding over the cell dense region, as described above, might be different in density from [^3H]flunitrazepam binding to the dendritic field of the LC, and might be differentially affected in depression. Tyrosine hydroxylase immunostains could not be used as a guide to analyze binding on autoradiograms in the dendritic region because of the confounding issue of increased tyrosine hydroxylase immunoreactivity in the LC of MDD subjects [42,69]. Therefore, the binding of [^3H]flunitrazepam in the dendritic field of the LC was estimated using two concentric circles centered over the cellular region of the LC as observed in stained tissue sections (overlaid onto the binding autoradiogram). The inner concentric circle (area of 5 mm^2) encompassed the

compact cellular region of the LC while the outer concentric circle (total area of 10 mm²) defined an area (5 mm²) that laid immediately outside the compact cellular region of the LC, i.e the immediate dendritic region of the LC. There was significantly lower binding in the dendritic region of the LC as compared to the compact cellular region in all three study groups (e.g. for controls at the middle level: cellular region was 8.1 ± 0.7 μCi/g; dendritic region was 6.4 ± 0.6 μCi/g; p < 0.0001, paired Student's t-test). However, there were no significant differences in the amount of [³H]flunitrazepam binding to GABA_A receptors in the dendritic region of the LC comparing the different study groups (data not shown).

Table 3 summarizes the demographic information regarding all study subjects. There were no significant differences in ages or brain pH between control and MDD subjects or between control and suicide subjects. Postmortem intervals of the control group were significantly lower than the MDD group (p = 0.04), but not the suicide group. Given that there were no significant differences in binding between control and MDD and control and suicide groups, data from all subjects were merged to examine possible correlations between binding and demographic variables. No significant correlations were found between PMI values and binding at any individual level (rostral, middle and caudal, respectively). In addition, there were no significant correlations between brain pH and [³H]flunitrazepam binding in the LC. There was a very modest correlation between age and [³H]flunitrazepam binding in the caudal portion of the LC (r² = 0.10, p = 0.04), but other levels of the LC did not demonstrate this correlation. Smokers and nonsmokers were matched across groups as closely as possible, primarily because we have previously observed differences in the biochemistry of the LC between these groups [21]. Given a lack of effect of depression or suicide, all smokers were compared to all nonsmokers (regardless of other diagnoses) and no significant difference in [³H]flunitrazepam binding to GABA_A receptors was observed between these two groups at any level of the LC (Figure 4).

4. Discussion

The present study is the first examination of [³H]flunitrazepam binding to GABA_A receptors in the human locus coeruleus of depressed subjects. This report demonstrates that the amount of [³H]flunitrazepam binding is not different in major depressive subjects as compared to psychiatrically normal controls. Additionally, suicide is not associated with altered benzodiazepine binding to GABA_A receptors in the LC. These findings do not provide support for the hypothesis that altered biochemistry in the LC associated with depression and suicide [20,40,42,43,69] results from disrupted GABAergic input to the LC.

There is considerable preclinical and clinical evidence that depression is associated with reduced GABA function [26,47]. For example, plasma GABA levels have been reported to be low in patients with major depression [48,49,50]. Using magnetic resonance spectroscopy, Sanacora et al. [54,56] demonstrated low GABA levels in the occipital cortex of depressed patients. A significant decrease in 3α, 5α-tetrahydroprogesterone(3α, 5α-THP) and 3α, 5β-THP concentrations, both of which are neuroactive steroids and positive modulators of the GABA_A receptor, was observed in MDD patients and this abnormality could be corrected by treatment with antidepressant fluoxetine [53]. Interestingly, GABA agonists demonstrate activity in animal models useful for identifying antidepressant compounds [4,24,25,70]. There is also evidence that facilitation of GABAergic transmission produces antidepressant effects in humans [34]. Together, these findings suggest that depression is associated with a GABA deficiency and that correcting this deficiency may be beneficial to depressed patients.

If GABA neurotransmission is deficient in depression, then a consequence of this could be an adaptive change in the density of GABA receptors. The regulation of the GABA_A receptor is complex, typical of receptor-operated channels [51,65,68]. Nevertheless, chronic GABA agonist exposure to rats induces down-regulation and allosteric uncoupling of the GABA_A

receptor [51], demonstrating that GABA_A receptors can adapt to changes in activation. However, studies of the GABA_A receptor binding in postmortem tissues from suicide and depressed subjects have not consistently demonstrated GABA_A receptor alterations. An increase in the percentage of type I benzodiazepine binding sites was observed in the hippocampus of suicide subjects [29]. A modest decrease in the affinity of [³H]flunitrazepam for GABA_A receptors in several hippocampal layers of violent suicide victims compared with matched controls has been reported [52]. An elevated density of GABA_A/benzodiazepine receptors has been reported in the cerebral cortex (Brodmann's area 10) [46] and frontal cortex [9] of depressed suicide victims. However, Stocks and coworkers [60] reported that the density and affinity of benzodiazepine binding sites did not differ significantly in the hippocampus and amygdala between depressed suicide victims and controls. Similarly, Kugaya et al. [22] also reported no differences between patients with major depression and healthy control subjects in GABA_A receptor binding measured *in vivo* with a benzodiazepine radioligand. In addition, Merali and coworkers [31] reported low levels of mRNAs encoding several alpha subunits of the GABA_A receptor in the frontal cortex of suicide victims. Overall, postmortem studies of radioligand binding to GABA_A receptors, including the present study, have not provided strong support for GABA dysregulation as a pathological marker of depression. There are many possible reasons why postmortem studies have failed to consistently identify GABA receptor abnormalities in depression and/or suicide and several reviews have addressed in detail common shortcomings of postmortem research [38,59].

In the present study, 5 of the depressed subjects (1 MDD and 4 depressed suicide) had an alcohol use disorder (see Table 1). Since GABA_A receptors are targets of ethanol action in the brain [11], the potential effect of ethanol on [³H]flunitrazepam binding to GABA_A was carefully considered. The average binding levels of these 5 subjects was not significantly different from the depressed suicide and MDD non-drinkers, or from the control group (data not shown). Hence, it is unlikely that ethanol exposure influenced the findings of this study.

GABA neurons projecting to the LC originate largely in the nucleus prepositus hypoglossi [13]. There are apparently no GABA cell bodies intrinsic to the LC, but glutamic acid decarboxylase immunoreactive nerve terminals are present, closely juxtaposed to noradrenergic cell bodies and dendrites [6]. GABA inhibits the firing of LC neurons primarily by activation of GABA_A receptors [44], but compared to other brain regions, the human LC has a low density of GABA_A receptors. Benzodiazepine binding to the GABA_A receptor in the LC is displaced by the triazolopyridazine Cl 218872, indicating type I benzodiazepine binding sites [18,67]. The findings of the present study confirmed relatively low binding density of GABA_A receptors in the human LC and further demonstrated an uneven distribution of the binding of [³H]flunitrazepam to GABA_A receptors along the rostral-caudal axis of the human LC. Although there is a modest density gradient of GABA_A receptor binding in the LC, this gradient did not correlate with the number of neuromelanin-containing (noradrenergic) neurons along the LC axis. These findings indicate that the majority of [³H]flunitrazepam binding to GABA_A receptors within the region of the LC does not likely occur on the cell bodies of noradrenergic neurons in the LC, since other proteins (e.g. monoamine oxidase A, norepinephrine transporter, α_{2A} adrenoceptors) known to reside on the noradrenergic cell bodies demonstrate high correlations of binding density relative to cell density along the axis of the LC [19,37,41]. Since LC neurons do express GABA_A subunit mRNAs [16,27,33], it is likely that they do indeed have receptors on their cell bodies or dendrites, though the level of expression may be low, and that other cells (e.g. glia) or terminals of neurons projecting to the LC (serotonin, glutamate etc) likely also express GABA_A receptors. Despite the low levels of benzodiazepine binding to the GABA_A receptor, infusion of benzodiazepines into the rat LC reduces spontaneous firing of LC neurons, demonstrating functional benzodiazepine binding sites in this region [58].

Numerous pathological alterations in the LC from depressed suicide victims have been described [20,40,42,43,69], and many of these are consistent with the hypothesis that depression is associated with a stress-related over-activity of the LC, and a concomitant deficiency of norepinephrine [36]. One possible mediator of an overactive LC could be reduced GABAergic input to this cell group, based on considerable evidence of deficient GABAergic transmission in depression as described above. The present data demonstrate that benzodiazepine binding to the GABA_A receptor is normal in depression and do not provide support for altered GABAergic input to the LC in depression. However, given the subunit complexity of the GABA_A receptor, the present study is insufficient to conclude that there is no abnormality of this receptor in the LC in depression, or that GABA input to the LC is not disrupted in depression. Several other tools can be used to evaluate GABAergic input to the LC in postmortem tissues from depressed subjects, including but not limited to receptor subunit immunoreactivities, GABA transporter immunoreactivities, GABA levels, and synthetic enzyme immunoreactivities.

Acknowledgements

We gratefully acknowledge the work of Drs. James C. Overholser, Herbert Y. Meltzer, Bryan L. Roth, George Jurjus, and Ginny Dilley, Lisa Konick, and Lesa Dieter in the retrospective psychiatric diagnoses. The excellent assistance of the Cuyahoga County Coroner's Office, Cleveland, OH is greatly appreciated. This project was supported by grants from the National Institute of Mental Health (MH63187, MH46692, MH 67996, and MH02031) and the National Center for Research Resources (RR17701).

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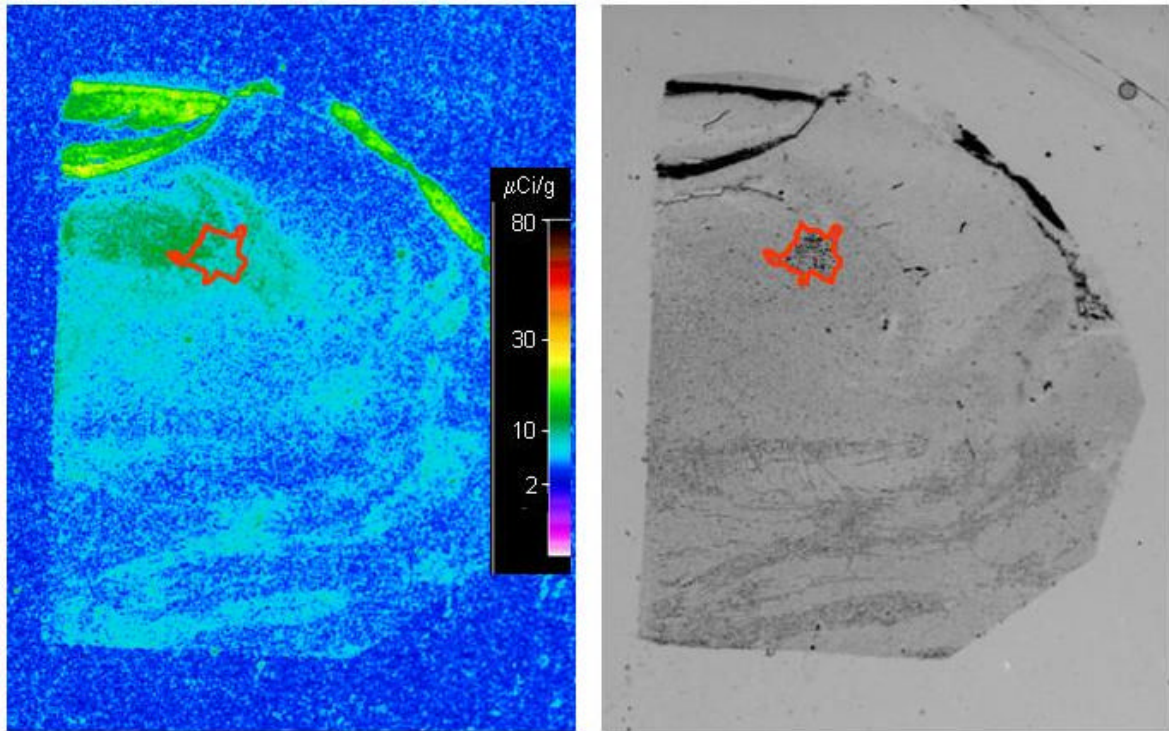


Figure 1.

Digital images of an autoradiogram depicting the specific binding of [³H]flunitrazepam to GABA_A receptors (left panel) and the Nissl staining of same section (right panel) at the middle level of a representative control subject. The image of specific binding was generated by digitally subtracting the image of non-specific binding from that of total binding using autoradiograms generated from adjacent tissue sections. The drawing on image of the Nissl stained section, overlaid onto the autoradiogram, depicts the region of the compact LC cell group that was measured.

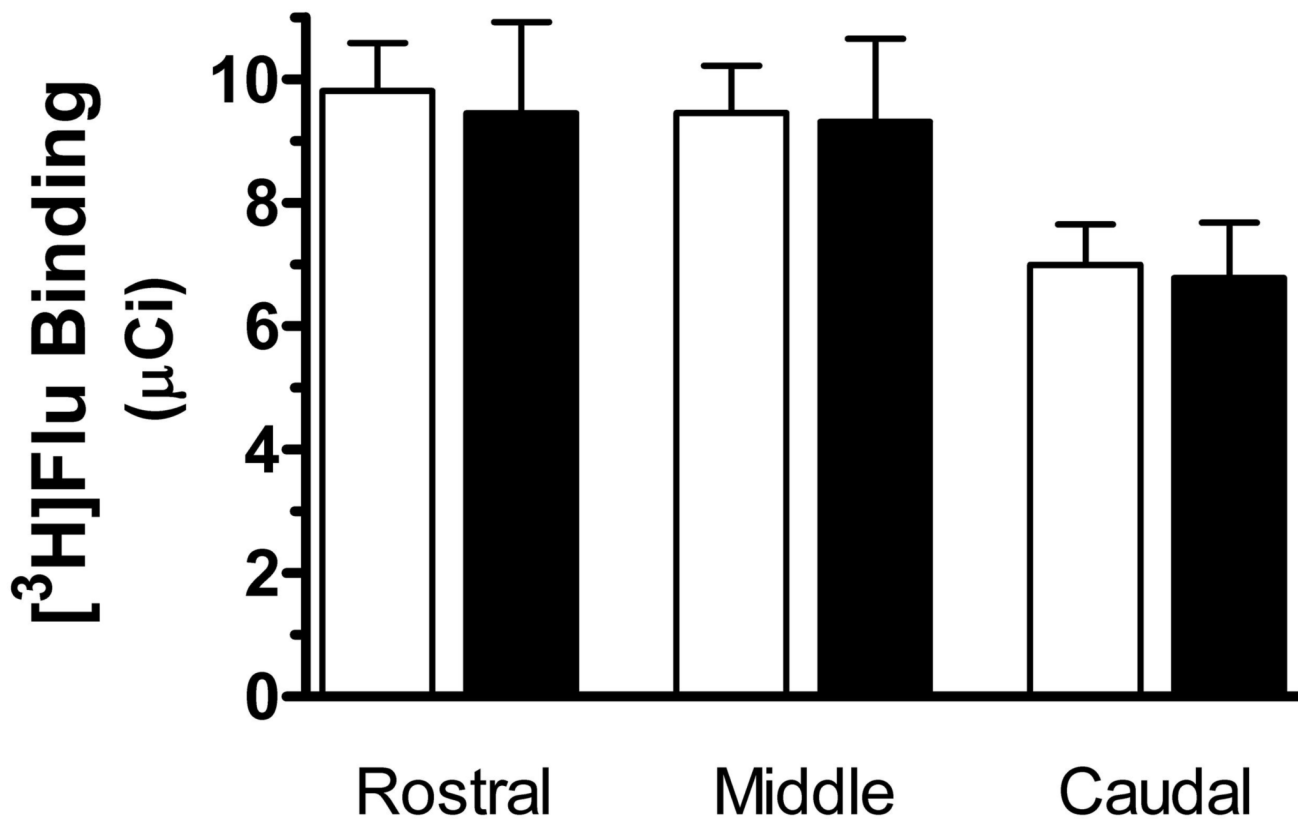


Figure 2. The specific binding of [³H]flunitrazepam ([³H]Flu) to GABA_A receptors at rostral (R), medial (M), and caudal (C) levels of the LC from normal control subjects (open bars, n = 21) and subjects with MDD (filled bars, n = 9). Data shown were generated by measuring the cell-dense region of the LC. No significant differences between the two study groups were observed.

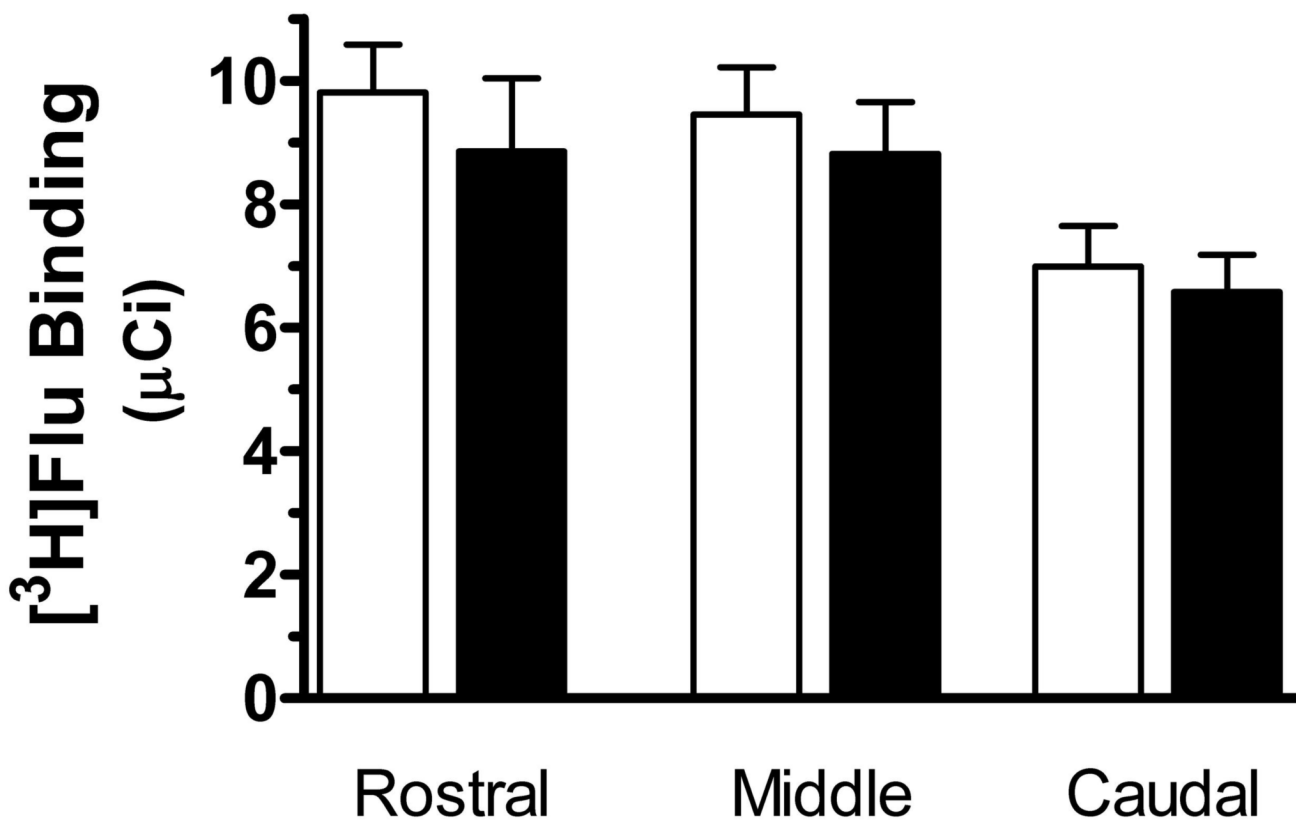


Figure 3. The specific binding of [³H]flunitrazepam ([³H]Flu) to GABA_A receptors at rostral (R), medial (M), and caudal (C) levels of the LC from normal control subjects (open bars, n = 21) and suicide subjects (filled bars, n = 17). Data shown were generated by measuring the cell-dense region of the LC. No significant differences between the two study groups were observed.

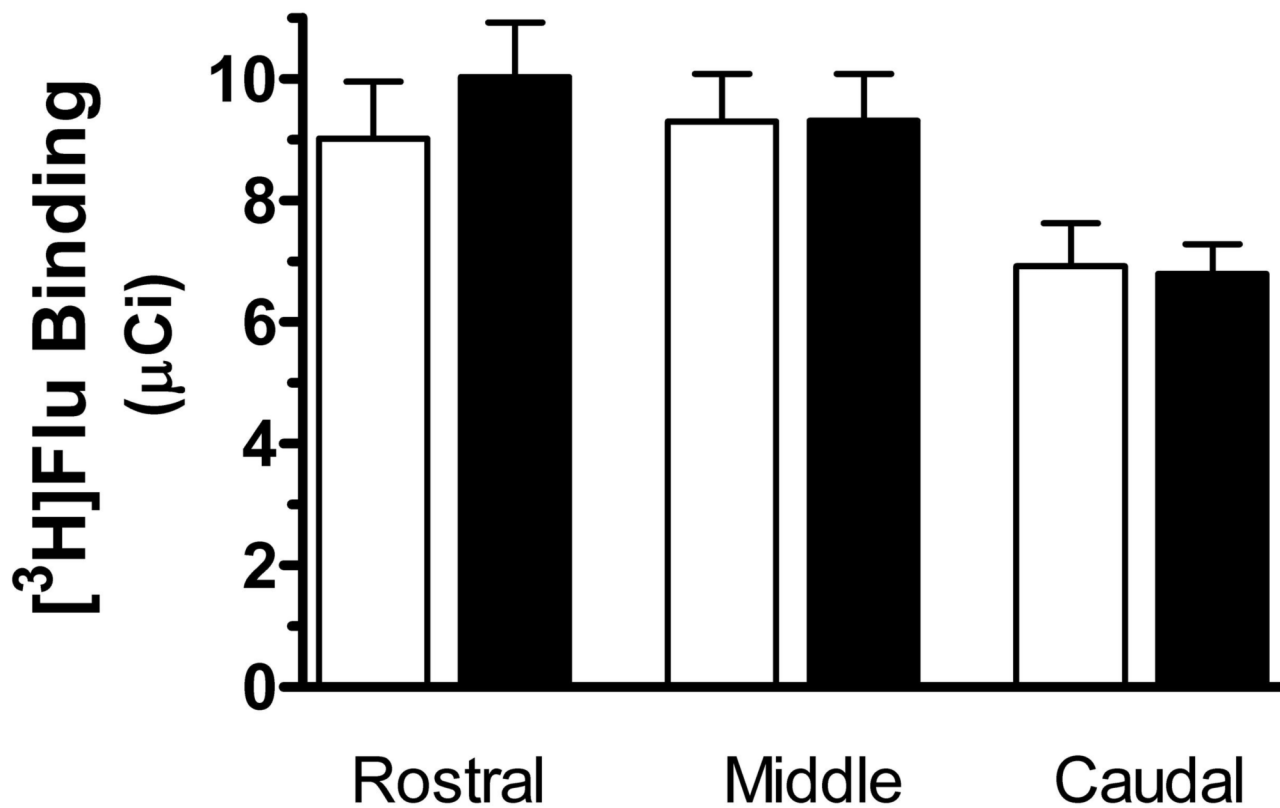


Figure 4. The specific binding of [³H]flunitrazepam ([³H]Flu) to GABA_A receptors at rostral (R), medial (M), and caudal (C) levels of the LC from non-smokers (open bars, n = 21) and smokers (filled bars, n = 19). Data shown were generated by measuring the cell-dense region of the LC. No significant differences between the two study groups were observed.

Table 1

Demographic data of major depressive and suicide subjects, including disorders evident at the time of death.

Subject	Brain pH	PMI ^d (h)	Toxicology	Depressive Disorder Diagnosis	Other Diagnoses
TT	6.52	24	NDD ^b	MDD (S ^{cd})	None
DD	6.48	17	CO ^e	MDD (S) dysthymic disorder	None
KS8	6.68	44	NDD	MDD (S)	None
JJ	6.24	23	CO, phenytoin, phenobarbital ethanol	MDD (S)	None
GG	6.91	18	chlorthalimide	MDD (S)	nicotine dependence
KS 12	6.24	19		MDD (N ^f)	nicotine dependence alcohol abuse,
KS 17	6.84	27	NDD	MDD (N ^g)	nicotine dependence
KS 24	6.85	26	ethanol, phenyl- propranolamine	MDD (S)	nicotine dependence
KS 28	6.78	33	NDD	MDD (S)	nicotine dependence
KS 2	6.52	24	NDD	adjustment disorder with depressed mood (S)	None
KS 5	6.31	20	ethanol, propoxyphene acetaminophen	dysthymic disorder (S)	None
KS 16	6.60	5	ethanol, CO	adjustment disorder with depressed mood (S)	None
KS 1	6.74	22	NDD	dysthymia (S)	alcohol abuse
KS 25	6.15	27	NDD	adjustment disorder with depressed mood (S)	None
KS 29	6.87	16	propoxyphene, lidocaine	adjustment disorder w/ depressed mood (S)	None
KS 3	6.89	12	ethanol	dysthymic disorder (S)	None
KS 4	7.07	22	ethanol	alcohol induced mood disorder (depressed) (S)	alcohol dependence, nicotine dependence
KS 13	6.47	18	ethanol	adjustment disorder with depressed mood (S)	nicotine dependence alcohol dependence, pathological gambling,
KS 9	6.945	6	NDD	Schizoaffective (S)	nicotine dependence

^aPMI, postmortem interval^bNDD, no drugs detected^cMDD, major depressive disorder^dS, suicide^eCO, carbon monoxide^fN, natural death – hypertrophic cardiomyopathy^gN, natural death – pulmonary thromboemboli

Table 2

Demographic data of control subjects.

Subject	Brain pH	PMI ^d (h)	Toxicology	Cause of death	Diagnosis
RR	6.47	17	NDD ^b	acute hemorrhagic pancreatitis	no diagnosis
BB	6.28	17	NDD	heart	no diagnosis
KS6	6.42	22	NDD	crushing impact	no diagnosis
KS15	5.99	17	CO	asphyxiation due to CO	no diagnosis
UU	6.71	16	NDD	Heart	no diagnosis
EE	7.09	29	NDD	Heart	no diagnosis
KS11	6.58	26	meperidine, promethazine, morphine	acute gastric ulcer	no diagnosis
KS22	6.9	20	ethanol, quinine	cardiomyopathy	no diagnosis
KS20	6.32	24	NDD	homicide	no diagnosis
HH	6.87	17	NDD	heart disease	no diagnosis
KS26	6.7	26	brompheniramine	heart disease	no diagnosis
FF	6.88	17	NDD	homicide	nicotine dependence
KS21	6.98	9	NDD	heart disease	nicotine dependence
KS23	6.78	21	ethanol	heart disease	nicotine dependence
KS27	6.62	21	NDD	abdominal aortic aneurysm	nicotine dependence
SS	6.46	11	NDD	hypertensive cardiomyopathy	nicotine dependence
II	6.71	23	NDD	aortic aneurism	nicotine dependence
CC	6.85	26	NDD	myocardial infarction	nicotine dependence
KS7	6.65	28	NDD	atherosclerosis/ myocardial infarction	nicotine dependence
KS14	6.71	10	CO	asphyxiation due to CO	nicotine dependence
KS19	6.86	9	NDD	heart disease	nicotine dependence

^dPMI, postmortem interval^bNDD, no drugs detected^cCO, carbon monoxide

Table 3

Summary of vital statistics of study subjects.

Group	n	M/F	Age	pH	Postmortem interval	No. of smokers
Control	21	17/4	49 ± 3	6.66 ± 0.06	19.3 ± 1.3	10
Major depressive disorder	9	9/2	50 ± 5	6.61 ± 0.09	25.5 ± 2.8*	5
Suicide [†]	17	15/2	49 ± 4	6.65 ± 0.07	21.2 ± 2.5	7

[†]This group includes a number of different affective disorders including 7 of the 9 major depressive disorder subjects (see Table 1).

* p < 0.05 compared to control subjects (t-test)