# Neurotransmitter Receptors in Frontal Cortex of Schizophrenics

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• **Frontal cerebral cortex brain samples from schizophrenics and controls have been assayed for binding associated with muscarinic cholinergic, serotonin (5HT), γ-aminobutyric acid (GABA), and β adrenergic receptors as well as for the activity of the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD). Binding levels of tritium-LSD, presumably associated with postsynaptic 5HT receptors, were reduced 40% to 50% in samples from schizophrenics in three independent studies, whereas no other consistent alteration was observed in levels of binding associated with other receptors or in the activity of GAD. This change in receptor binding levels does not seem to be attributable to postmortem changes, to influences of drugs received by the patients, or to demographic features of the patient populations.**

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Evidence of an important genetic component in schizophrenia implies that there are associated biochemical defects.[1-4] Effects of drugs on the behavior of schizophrenics, such as the therapeutic actions of neuroleptic phenothiazines and butyrophenones and the exacerbation of acute schizophrenic symptoms by amphetamines, suggest possible pathophysiological roles for neurotransmitters, including the biogenic amines,[5-8] although the results of studies of neurotransmitter concentrations and of associated enzymes in the brains of schizophrenics have been equivocal.[9]

Recently developed binding techniques permit direct labeling of synaptic neurotransmitter receptors in animal and human brains.[10] We compared receptor binding associated with muscarinic cholinergic, serotonin (5HT), γ-aminobutyric acid (GABA), and β-adrenergic receptors, as well as the activity of the GABA-forming enzyme glutamic acid decarboxylase (GAD), in frontal cerebral cortex brain samples from schizophrenics and nonschizophrenics.

## MATERIALS AND METHODS

Human brains were obtained from persons who had died and were autopsied at either St Elizabeth's Hospital or the office of the District of Columbia medical examiner. For each subject, the time interval from death to arrival at the morgue was either documented by witnesses or estimated by the pathologist. At the morgue, the bodies were stored at 4 °C until autopsied. At the time of autopsy, the brain was removed and samples from the right frontal cortex were immediately frozen in dry ice. In each brain, a single sample was dissected from the same region corresponding approximately to Brodmann areas 6, 8-11, and 44-47. These samples were then stored at −80 °C until the time of assay.

The psychiatric diagnoses had been made by attending psychiatrists prior to the death of the subject and were verified by review of the records. The diagnoses corresponded to DSM-II categories.

Circumstances surrounding each death were noted by interviewing relevant physicians, policemen, and relatives. The drugs the subjects were taking had been prescribed for prolonged periods and the subjects were presumably taking them in the dosages prescribed until the time of death. In most cases, verification of this was not possible, nor was it possible to ascertain the exact duration of drug-free periods for subjects not receiving drugs at the time of death. In most cases, the drug-free interval was reported to be at least two months.

For analysis, the frozen tissue was homogenized with an automatic instrument in 10 v/v of distilled water. Portions of this homogenate were analyzed for muscarinic receptor binding[11] and GAD activity.[12] The remainder was centrifuged at 48,000 g for ten minutes, the supernatant fluid was separated, and the pellet resuspended in 20 v/v of distilled water. This homogenate was separated into several portions, one for each of the remaining receptor assays, and centrifuged again as before.

The individual receptors were assayed as previously described for human brain.[10] Briefly, muscarinic cholinergic receptors were assayed by measuring the membrane binding of tritium-quinuclidinyl benzilate (QNB) (1.7 to 16 Ci/mmole), an extremely potent muscarinic cholinergic antagonist,[13] and the β-adrenergic receptor was assayed by measuring binding of tritium-dihydroalprenolol[14,15] (DHA) (30 to 48 Ci/mmole) or $^{125}$I-hydroxybenzylpindolol ($^{125}$I-HYP),[16] both potent β-adrenergic antagonists. Sodium-independent binding of GABA (36.7 Ci/mmole) was measured to

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Table 1.—Neurotransmitter Receptor Binding and Glutamic Acid Decarboxylase,

| No. | Age | Sex | Subtype | Psychoactive Medication, mg/Day | Cause of Death | Death to Morgue, hr | Morgue to Autopsy, hr | Freezer to Storage, mo |
|---|---|---|---|---|---|---|---|---|
| 1 | 48 | M | WMD | None | Fall | 4 | 17 | 36 |
| 2 | 22 | M | WMD | None | Drowning | 1 | 8 | 36 |
| 3 | 30 | M | WMD | None | Gunshot | 1 | 9 | 36 |
| 4 | 22 | M | WMD | None | Gunshot | 1 | 18 | 12 |
| 5 | 51 | M | WMD | None | Myocardial infarction | 1 | 7 | 36 |
| 6 | 33 | M | WMD | None | Stroke | 1 | 1 | 36 |
| 7 | 25 | M | WMD | None | Gunshot | 2 | 14 | 36 |
| 8 | 62 | M | WMD | None | Fall | 1 | 14 | 36 |
| 9 | 73 | F | WMD | None | Pulmonary edema | 2 | 36 | 39 |
| 10 | 65 | M | WMD | None | Pulmonary embolism | 1 | 2 | 36 |
| 11 | 63 | M | WMD | None | Gunshot | 1 | 33 | 24 |
| 12 | 12 | M | WMD | None | Gunshot | 1 | 17 | 36 |
| **Mean 42.2±8.0** | | | | | | | | **32±3** |
| 13 | 84 | F | Ch Undiff | None | Sepsis | 1 | 34 | 36 |
| 14 | 25 | M | Paranoid | CPZ, 450 | Fall | 7 | 2 | 24 |
| 15 | 77 | F | Paranoid | None | Pneumonia | 1 | 27 | 36 |
| 16 | 32 | M | Ch Undiff | THDZ, 400; FLU, 6 | Fall | 1 | 68 | 36 |
| 17 | 33 | M | Paranoid | None | Gunshot | 1 | 43 | 36 |
| 18 | 65 | M | Ch Undiff | CPZ, 800 | Myocardial infarction | 1 | 51 | 36 |
| 19 | 66 | F | Ch Undiff | CPZ, 600; PRO, 20 | Diarrhea; emesis | 1 | 46 | 36 |
| 20 | 28 | M | Paranoid | None | Fall | 1 | 22 | 36 |
| 21 | 24 | M | Ch Undiff | THDZ, 600; HAL, 10; CPZ, 300; DZP, 10; IMI, 25; PRO, 15 | Suffocation | 2 | 12 | 37 |
| 22 | 81 | M | Paranoid | None | Malignant melanoma | 1 | 36 | 37 |
| 23 | 86 | F | Paranoid | 3§ | Cardiovascular collapse | 1 | 4 | 38 |
| 24 | 84 | F | Paranoid | None | Diabetic acidosis; pulmonary edema | 5 | 38 | 38 |
| **Mean 57.1±7.6** | | | | | | | | **31±4** |

*All values are given as mean±SEM. Tritium-ligand concentrations in the incubation media were tritium-3 quinuclidinyl benzilate, 1 nM; tritium-serotonin, 8nM; tritium-LSD, 4 nM; tritium-γ-aminobutyric acid, 8nM. Tritium-3 quinuclidinyl benzilate was used at a concentration exceeding its $K_D$[9] because of the relatively low specific activity of the available supply. The lower levels of tritium-quinuclidinyl benzilate binding compared to earlier findings[11] might reflect the longer storage of the present brain samples.

†QNB indicates 3-quinuclidinyl benzilate; 5HT, serotonin; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase activity (nmole carbon dioxide/mg protein/h); CPZ, chlorpromazine hydrochloride; THDZ, thioridazine hydrochloride; HAL, haloperidol; FLU, fluphenazine hydrochloride; DZP, diazepam; IMI, imipramine hydrochloride; PRO, procyclidine hydrochloride.

‡Significantly lower than control ($P<.05$).

§These received a combination of dihydroergocornine mesylate, dihydroergocristine mesylate, and dihydroergokryptine mesylate (Hydergine).

Table 2.—Neurotransmitter Receptor Binding, Study 2*

| No. | Age | Sex | Subtype | Psychoactive Medication, mg/Day | Cause of Death | Death to Morgue, hr | Morgue to Autopsy, hr | Freezer Storage, mo |
|---|---|---|---|---|---|---|---|---|
| 4 D | 40 | M | WMD | ETOH | Gunshot | 1 | 9 | 24 |
| 10 G | 66 | M | WMD | ETOH | Heart failure | 2.5 | .5 | 24 |
| 10 F | 49 | M | WMD | None | Acute gastrointestinal bleeding | 2.5 | 8 | 20 |
| 37/2 | 22 | M | WMD | ETOH | Drowning | 1 | 8 | 6 |
| 10 A | 20 | M | WMD | Narcotics, ETOH | Gunshot | .5 | 15.5 | 33 |
| 36/2 | 22 | M | WMD | None | Gunshot to face | 1.5 | 14 | 36 |
| 26/1 | 51 | M | WMD | None | Heart failure | .5 | 7 | 36 |
| 19/1 | 33 | M | WMD | None | Heart failure | 1 | 1 | 36 |
| 33/2 | 22 | M | WMD | None | Gunshot | 1 | 18 | 36 |
| **Mean 36.1±1.8** | | | | | | | | **24±3** |
| 4 F | 71 | F | Paranoid | None | Pneumonia | 1 | 4.5 | 19 |
| 11 E | 54 | M | Paranoid | THDZ, ETOH | Uremia | 24-36 | 16 | 22 |
| 10 C | 104 | F | Paranoid | Digoxin, Keflex | Pneumonia | 1 | 17.5 | 21 |
| 11 L | 78 | M | Hebephrenic | None | Heart failure | .5 | 89 | 24 |
| 25/1 | 84 | M | Undiff | THDZ, 100 | Pulmonary edema | 1 | 30 | 38 |
| **Mean 78.2±8.2** | | | | | | | | **27±3** |

*All values are given as mean±SEM. Ligand concentrations in the incubation media were $^{125}$I-hydroxybenzylpindolol, 20 pM; tritium-dihydroalprendolol, 1nM; tritium-LSD, 2nM; tritium-serotonin, 5nM.

†HYP indicates hydroxybenzylpindolol; DHA, dihydroalprendolol; 5HT, serotonin; WMD, without mental disease; ETOH, ethyl alcohol; THDZ, thioridazine hydrochloride.

‡Significantly different from control ($P<.5$).

Study 1

| Receptor Ligand Binding, fmole/mg Protein* | | | | |
|---|---|---|---|---|
| Tritium-QNB† | Tritium-5HT | Tritium-LSD | Tritium-GABA | GAD |
| 388 | 109 | 160 | 126 | 9 |
| 877 | 96 | 261 | 82 | ... |
| 454 | 39 | 130 | 88 | 9 |
| 677 | 58 | 165 | 62 | ... |
| 671 | 40 | 75 | 167 | 7 |
| 475 | 120 | 141 | 56 | 13 |
| 572 | 153 | 157 | 77 | 18 |
| 546 | 70 | 85 | 139 | 12 |
| 582 | 94 | 97 | 187 | 6 |
| 481 | 98 | 103 | 36 | 22 |
| 489 | 98 | 101 | 116 | 14 |
| 626 | 91 | 113 | 100 | 18 |
| 569±38 | 88.9±9.5 | 132±15 | 103±13 | 12.8±2 |
| ... | 98 | ... | ... | ... |
| 657 | 76 | 108 | 115 | 6 |
| 347 | 12 | 48 | 153 | ... |
| 180 | 36 | 24 | 134 | 5 |
| 444 | 59 | 110 | 66 | 16 |
| 515 | 136 | 110 | 75 | 8 |
| 280 | 65 | 43 | 101 | 4 |
| 579 | 136 | 113 | 57 | 16 |
| 296 | 98 | 76 | 105 | 13 |
| 470 | 97 | 101 | 95 | 10 |
| 570 | 89 | 75 | 87 | 25 |
| 600 | 123 | 115 | 55 | 18 |
| 449±46 | 85±11 | 84±10‡ | 95±9 | 12±2 |

| Receptor Ligand Binding, fmole/mg Protein | | | |
|---|---|---|---|
| $I^{125}$-HYP† | Tritium-DHA | Tritium-LSD | Tritium-5HT |
| 12.1 | 96 | 111.4 | 113.0 |
| 14.4 | 74 | 75.5 | 86.6 |
| 12.4 | 64 | 78.8 | 153.0 |
| 10.9 | 88 | 69.2 | ... |
| 8.0 | 43 | 57.5 | 42.1 |
| 7.9 | 48 | 64.2 | 50.1 |
| 6.4 | 42 | 57.3 | 19.3 |
| 8.7 | 47 | 28.8 | 66.5 |
| 8.0 | 35 | 71.8 | 20.7 |
| 9.8±0.9 | 59.7±7.3 | 68.2±7.3 | 68.9±16.5 |
| 9.1 | 66 | 14.5 | 18.3 |
| 10.8 | 60 | 18.9 | 63.3 |
| 15.0 | 77 | 46.3 | 75.3 |
| 9.7 | 58 | 52.9 | 53.6 |
| 14.6 | 69 | 35.4 | 58.0 |
| 11.8±1.2 | 66.0±3.4 | 33.6±7.9‡ | 63.7±9.6 |

determine GABA receptors[17] and the binding of tritium-LSD (16 to 26 Ci/mmole)[16] and tritium-5HT[17] (13 Ci/mmole) were measured to monitor 5HT receptors. The ratio of total to nonspecific binding levels in brains from controls was 8:12 for tritium-QNB, 5:8 for tritium-DHA, 7:10 for $^{125}$I-HYP, 1.5:3.0 for tritium-GABA, 4:7 for tritium-LSD, and 2:3 for tritium-5HT. Previous studies have shown that these binding agents selectively label the appropriate receptor in human brain membranes.[11] Samples of each suspension were also assayed for protein.[18]

Each sample was analyzed in triplicate and the significance of the difference between groups was analyzed using the nonparametric Mann-Whitney *U* test. Rank correlations were calculated using the Spearman correlation analysis.

## RESULTS

Receptor binding levels observed here in normal frontal cerebral cortex (Tables 1, 2, 3) are essentially the same as previously detected in monkey and rat brain tissue as well as in other human postmortem brains,[3,9,11-13,17] suggesting the absence of major postmortem changes. Moreover, no correlation could be detected between the interval between death and freezing of the brain or between freezing of the brain and assay of receptor binding for any of the receptors studied, with Spearman correlation coefficients yielding $P > .4$ for all comparisons.

Three separate groups of patients and controls were studied sequentially. The second and third studies were conducted to replicate results obtained with the first group.

### Study 1

Using the Mann-Whitney test, no significant differences between brains from schizophrenics and brains from controls were apparent for the activity of GAD or levels of GABA, muscarinic cholinergic, or tritium-5HT receptor binding (Table 1). Tritium-LSD binding levels, however, were significantly reduced in brains from schizophrenics by 36% ($P < .05$).

Some of the schizophrenic patients had received substantial doses of neuroleptic drugs. These, in vitro, can inhibit tritium-LSD binding associated with 5HT receptor[16,17] (Table 1). If the reduction in levels of tritium-LSD binding in brain from schizophrenics was attributable to such drugs, extensive washing should tend to remove the drugs and elevate the lowered binding levels. Accordingly, three cerebral cortex samples from schizophrenics with the lowest LSD binding levels and three samples of brain from schizophrenics with LSD binding levels in the normal range were preincubated and washed to remove membrane-bound drugs. However, no augmentation in LSD binding levels were detectable after the samples were subjected to an additional ten-minute preincubation at 37 °C and subjected to two additional washes over and above the standard procedure.

Most of the brain's drug content should be released into the supernatant fluid during homogenization and membrane preparation. If drugs in subjects' brains accounted for reduced levels of tritium-LSD binding, supernatant preparations from such brains should inhibit tritium-LSD binding to animal brain membranes.

Supernatant fractions from brains of schizophrenics and brains of controls were added to incubations containing rat

forebrain membranes, which were then assayed for tritium-LSD binding associated with 5HT receptors (Table 4). The supernatant from only one schizophrenic patient (19) reduced level of tritium-LSD binding to rat brain membranes. This finding, as well as the studies of additional membrane washings, suggests that the reduced levels of tritium-LSD binding in brain from schizophrenics cannot be attributed solely to drugs ingested and still present in the subjects. Moreover, although LSD binding in drug-treated schizophrenics is somewhat lower than in schizophrenics not receiving drugs, the reduction is not statistically significant (Table 5).

Tritium-QNB binding levels associated with muscarinic cholinergic receptors were reduced somewhat in brains from schizophrenics, though the decrement was not statistically significant (Table 1). Two of the patients had received the potent anticholinergic agent procyclidine, whereas another two received thioridazine hydrochloride, a phenothiazine with substantial central anticholinergic activity. To ascertain whether reduced levels of QNB binding might be associated with drug treatment, the influence of supernatant preparations from all brains on tritium-QNB binding to muscarinic receptors of rat forebrain was determined (Table 4). Substantial reduction of levels of binding was observed for brain from three schizophrenic subjects, two of whom had received procyclidine whereas the third had received treatment with thioridazine. Rat brain QNB binding values for brain supernatants from these three subjects (16, 19, 21) were, respectively, 78%, 71%, and 79% of control rat brain muscarinic receptor binding (Table 4). Since these are the three lowest QNB binding values of the schizophrenic group, the tendency for reduced levels of QNB binding in schizophrenic brain is probably attributable to the presence in these subjects of anticholinergic medication.

| No. | Age | Sex | Subtype | Psychoactive Medication mg/Day | Cause of Death |
|---|---|---|---|---|---|
| 1 | 78 | F | WMD | None | Cerebral Hemorrhage |
| 2 | 65 | M | WMD | None | Pulmonary embolism |
| 3 | 63 | M | WMD | None | Gunshot to neck |
| 4 | 30 | M | WMD | None | Gunshot to chest |
| 5 | 50 | M | WMD | None | Internal hemorrhage |
| 6 | 33 | M | WMD | None | Pneumonia |
| 7 | 42 | M | WMD | ETOH | Pneumonia |
| 8 | 69 | F | WMD | None | Overdose |
| 9 | 17 | M | WMD | None | Gunshot to chest |
| 10 | 42 | M | WMD | ETOH | Pneumonia |
| Mean 46.9±6.2 | | | | | |
| 11 | 32 | M | Ch Undiff | THDZ, 100; FLU, 80; Insulin | Multiple fractures from jump |
| 12 | 66 | F | Ch Undiff | CPZ, 200; Kemadrin, 5 | Cardiac arrest |
| 13 | 24 | M | Ch Undiff | CPX, 100; THDZ, 200; HAL, 5; DZP, 5 | Suffocation |
| 14 | 35 | F | Ch Undiff | None | Undetermined |
| Mean 36.8±9.9 | | | | | |

## Study 2

To determine whether the findings in the initial experiments were replicable, we obtained a second group of brains from five schizophrenic and ten control subjects (Table 2). In frontal cerebral cortex specimens, levels of LSD binding were reduced by 50% ($P < .05$), confirming findings in the initial group of brains. Tritium-LSD binding levels in brain from controls in this study were lower than in study 1, conducted several months earlier. We are unaware of what factors may have accounted for this discrepancy. The duration of brain storage and characteristics of the tritium-LSD preparation seemed the same in the two studies. Again, no changes were demonstrable in binding of tritium-5HT. In this group, $\beta$-noradrenergic receptors were assayed by measuring the levels of binding of the $\beta$-antagonists $^{125}$I-HYP and tritium-DHA. No significant difference was detected between the levels of binding of these $\beta$-adrenergic receptor ligands in specimens from schizophrenics and from controls.

## Study 3

To further replicate the results of the first two studies, a third group of brains from four schizophrenics and ten controls were assayed for tritium-LSD, tritium-QNB, and tritium-DHA binding (Table 3). Confirming results of the first two studies, levels of tritium-LSD binding in cerebral cortex from schizophrenics was only 50% of that of samples from controls ($P < .05$). Also, as observed before, levels of tritium-DHA binding did not differ between brain from schizophrenics and from controls. Tritium-QNB binding levels were lower in brain from schizophrenics than in brain from controls, a difference that was statistically significant ($P < .05$).

As in study 1, we attempted to ascertain whether drugs or other substances in supernatant fractions of the brains from schizophrenics could account for the reduced tritium-LSD and tritium-QNB binding. Unlike findings in study 1, supernatant fractions from cerebral cortex of schizophrenics in study 3 did not inhibit rat brain tritium-QNB binding. Only one of the four supernatant fractions for brain from schizophrenics inhibited tritium-LSD binding and the reduction was about 25%.

Studies in rat and monkey brain indicated that 5HT and LSD both bind to postsynaptic 5HT receptors.[16,17] However, differences in drug specificity indicate that they label distinct populations of 5HT receptors or separate states of one interconverting receptor[16,17] (S. J. Peroutka, MD, and S. H. Snyder, MD, unpublished data, October 1978). Such differences could explain the lack of reduction of levels of 5HT binding in the brains from schizophrenics in spite of the significant lowering of levels of LSD binding in these subjects. To ascertain whether binding of 5HT and LSD as well as other neurotransmitter receptor ligands might be mutually related, Spearman correlation coefficients were computed for the various binding and enzymatic activities. The only correlations that attain statistical significance for

Table 3.—Neurotransmitter Receptor Binding, Study 3*

| Death to Morgue, hr | Morgue to Autopsy, hr | Freezer Storage, mo | Receptor Ligand Binding, fmole/mg Protein | | | Effect of Brain Supernatant on Tritium Ligand Binding, % Control | |
|---|---|---|---|---|---|---|---|
| | | | Tritium-LSD | Tritium-QNB† | Tritium-DHA | Tritium-QNB | Tritium-LSD |
| 2.75 | 1.75 | 6 | 11.7 | ... | 13.1 | >90 | >90 |
| 1 | 2 | 36 | 38.9 | 478 | 23.1 | >90 | >90 |
| 1 | 9.5 | 36 | 28.0 | 490 | 19.0 | >90 | >90 |
| 1 | 19 | 24 | 57.0 | 447 | 22.2 | >90 | >90 |
| .25 | 1 | 12 | 43.8 | 483 | 19.0 | >90 | >90 |
| 2 | 19 | 18 | 66.3 | 364 | 21.2 | >90 | 86 |
| .5 | 18 | 1 | 55.4 | 313 | 23.0 | >90 | >90 |
| 2 | 11 | 1 | 85.0 | 485 | 20.8 | >90 | >90 |
| 1 | 5.5 | 1 | 125.0 | 659 | 32.9 | >90 | >90 |
| 0.5 | 18 | ... | 75.8 | 527 | 30.0 | >90 | >90 |
| | | 15.0±1.8 | 58.7±9.1 | 471±32 | 22.4±1.8 | | |
| 1 | 68 | 24 | 24.0 | 294 | 14.7 | >90 | 85 |
| .7 | 48 | 24 | 24.0 | 259 | 14.8 | >90 | >90 |
| 2 | 12 | 36 | 45.5 | 362 | 19.7 | >90 | >90 |
| 5 | 2 | 6 | 24.9 | 210 | 19.3 | >90 | 75 |
| | | 23±6 | 29.6±5.3§ | 281±32§ | 17.1±1.4 | | |

*Values are given as mean±SEM. Ligand concentrations in the incubation media were tritium-LSD, 2nM; tritium-3 quinuclidinyl benzilate, 1 nM; tritium-dihydroalprenolol, 0.4 nM.

†QNB indicates 3-quinuclidinyl benzilate; DHA, dihydroalprenolol, WMD, without mental disease; ETOH, ethyl alcohol; THDZ, thioridazine hydrochloride; FLU, fluphenazine hydrochloride; CPZ, chlorpromazine hydrochloride; CPX, chlorprothixene hydrochloride; HAL, haloperidol; DZP, diazepam; Ch Undiff, chronic undifferentiated.

‡The methodology is the same as in Table 4.

§Significantly lower than control ($P<.5$).

both schizophrenics and controls are between the binding of tritium-5HT and tritium-LSD (Fig 1) ($P < .01$) and $^{125}$I-HYP and tritium-DHA (Fig 2) ($P < .01$). This finding suggests that LSD and 5HT may to a certain extent label the same or similar sites, confirming similar correlations observed previously in brains from patients with Huntington's chorea and from other control human brains.[11] However, the fact that reductions were observed in levels of LSD but not of 5HT binding shows that the two ligands must, in part, label different sites. These observations also confirm the conclusion that both $^{125}$I-HYP and tritium-DHA label $\beta$-adrenergic receptors.

### Relationships Between Biochemical and Demographic Features

Many biochemical aspects of brain function change with age. In this investigation, the mean age of the schizophrenics was significantly higher than the mean age of controls in studies 1 and 2, whereas in study 3, the mean age of controls was higher.

We examined the relationships of age to all the biochemical measurements assayed in brains from both schizophrenics and controls. No significant correlation was found between subject age and levels of tritium-QNB, tritium-5HT, tritium-DHA, or $^{125}$I-HYP binding, or GAD activity. In studies 1 and 3, tritium-LSD binding levels correlated negatively with age for the control groups but not for the schizophrenic groups (Fig 3). The correlation for study 1 was −0.85 ($P< .01$) and for study 3 the correlation was −0.90 ($P < .01$).

Could the decrease with age in tritium-LSD binding levels explain the reduced levels in brain from schizophrenics? This seems unlikely for several reasons. Whereas schizophrenics were older than controls in studies 1 and 2, they were younger in study 3. Yet, levels of tritium-LSD binding in brain from schizophrenics were reduced compared to control values in study 3 to the same or greater extent as in studies 1 and 2. Moreover, whereas the negative correlation of levels of tritium-LSD binding and age was detected in control subjects, no such correlation was apparent among schizophrenics. To analyze statistically whether the age-related change in levels of LSD binding might account at all for the decreased binding observed in schizophrenics, we computed the age-predicted levels of tritium-LSD binding levels in controls and schizophrenics for study 1, since only in study 1 were there both an age-related decline in LSD binding and a higher mean age for the schizophrenic population compared to the control population. Tritium-LSD binding levels for controls and schizophrenics still differ significantly after correcting for the predicted influence of age on binding (Fig 3). This further supports the conclusion that age-related changes are not responsible for the reduced tritium-LSD binding levels observed in schizophrenics. The only other age-

Table 4 —Effect of Brain Supernatant on Rat Brain Tritium-QNB† and Tritium-LSD Binding, Study 1*

| No. | Binding, % of Control† | |
|---|---|---|
| | Tritium-QNB | Tritium-LSD |
| 1 | 100 | 96 |
| 2 | 94 | 98 |
| 3 | 101 | 93 |
| 4 | 98 | 99 |
| 5 | 98 | 98 |
| 6 | 102 | 100 |
| 7 | 92 | 92 |
| 8 | 93 | 100 |
| 9 | 93 | 100 |
| 10 | 95 | 100 |
| 11 | 94 | 104 |
| 12 | ... | ... |
| 13 | 95 | 97 |
| 14 | 104 | 98 |
| 15 | 101 | 98 |
| 16 | 76‡ | 94 |
| 17 | 100 | 100 |
| 18 | 95 | 106 |
| 19 | 71‡ | 67‡ |
| 20 | 88 | 98 |
| 21 | 79‡ | 96 |
| 22 | 97 | 94 |
| 23 | 93 | 94 |
| 24 | 95 | 98 |

*3-quinuclidinyl benzilate.
†Supernatants from human brain samples (equivalent to 100 mg tissue) were added to standard rat brain tritium-LSD and tritium-QNB binding assays. Results are expressed as percent of control (no added supernatant) rat brain ligand binding. Patient numbers are same as in Table 1.
‡Significantly different from control ($P<.01$) in $t$ test for samples assayed in quadruplicate.

related change observed was a positive correlation between tritium-GABA binding levels and age (Fig 4) ($r = .86$, $P < .01$).

## COMMENT

In the present study, ligand binding associated with a number of neurotransmitter synaptic receptors was assayed in autopsied brains of schizophrenics and controls in three independent populations. Only levels of LSD binding were consistently altered and were reduced 40% to 7% in samples from schizophrenics. The failure of additional membrane washing to elevate these values and the lack of influence of supernatant fractions from schizophrenics on tritium-LSD binding to rat brain membranes suggest that the reduced levels of LSD binding in brains from schizophrenics is not primarily related to the presence of drugs in the samples. However, the fact that in study 1,

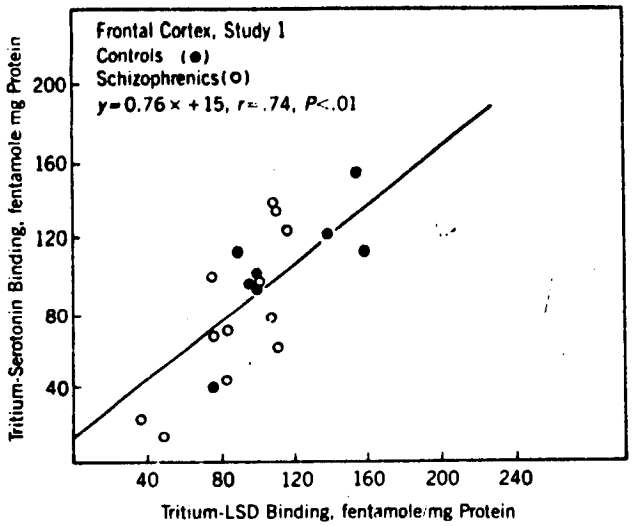


Fig 1.—Correlation between tritium-LSD and tritium-5HT binding to frontal cortex samples from study 1.

tritium-LSD binding levels were somewhat lower in the drug-treated than in drug-free patients, suggests that drug treatment may have played some role in the reduced binding. Chronic neuroleptic pretreatment of animals might provide contributory information.

Whereas in the corpus striatum of calf brain tritium-LSD can bind to dopamine as well as to 5HT receptors[11] in the cerebral cortex of calf and rat brain, LSD binding seems to be associated exclusively with 5HT receptors. Moreover, LSD binding to cerebral cortex membranes of brains from both schizophrenics and controls was not influenced by dopamine except at very high concentrations. Thus, tritium-LSD binding in human cerebral cortex seems to label sites with characteristics of 5HT receptors.

It seems unlikely that reduced tritium-LSD binding levels are simply related to postmortem changes. There were no significant correlations between values for any neurotransmitter receptor binding and the interval between death and arrival at the morgue (4 °C), arrival at the morgue and autopsy, or between freezing and the time of assay. Age differences between groups also do not seem to fully explain the tritium-LSD binding differences between brain from controls and from schizophrenics. To examine this more fully, the following statistical manipulations were performed: A two-way analysis of variance for independent groups showed that there was a large difference in tritium-LSD binding levels between controls and

Table 5.—Receptor Binding in Drug Treated and Nontreated Individuals, Study 1

| Type | Receptor Binding* (fmole/mg Protein) | | | |
|---|---|---|---|---|
| | Tritium-QNB† | Tritium-5HT | Tritium-LSD | Tritium-GABA |
| Schizophrenic, drug treated | 386±87(5) | 87±16(5) | 73±17(5) | 106±10(5) |
| Schizophrenic, no drug treatment | 502±40(6) | 85±16(7) | 93±11(6) | 86±15(6) |
| Total schizophrenic population | 449±46(11) | 85±11(12) | 84±10‡(11) | 95±9(11) |
| Control | 569±38(12) | 89±10(12) | 132±15(12) | 103±13(12) |

*Values are the mean ±SEM of the number of determinations in parenthesis.
†QNB indicates 3 quinuclidinyl benzilate; 5HT, serotonin; GABA, γ-aminobutyric acid.
‡Significantly different from control values by the Mann Whitney test, $P<.5$.

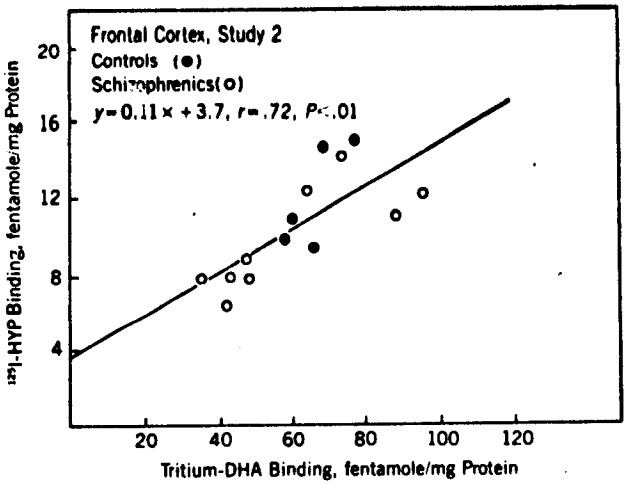


Fig 2.—Correlation between levels of tritium-dihydroalprenolol (tritium-DHA) and ($^{125}$I-HYP) binding to frontal cortex samples from study 2.

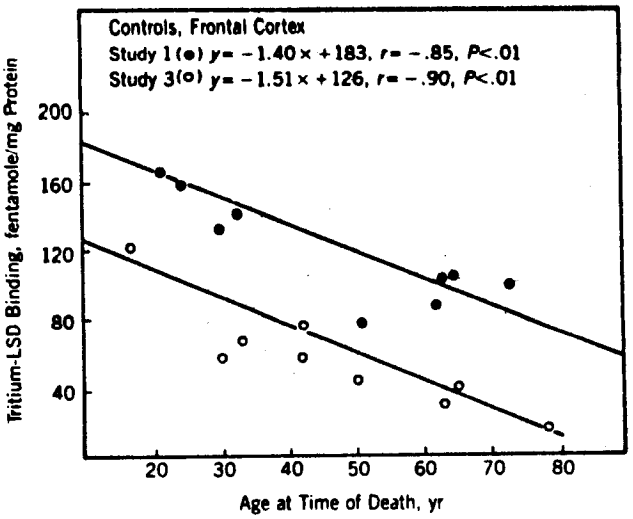


Fig 3.—Correlation between tritium-LSD binding level and age of patients for control frontal cortex samples, studies 1 and 3. In study 2, there was no correlation of tritium-LSD binding and age in control population. In all studies, there was no correlation between subject age and tritium-LSD binding for schizophrenic populations. From regression line for study 1, predicted mean control tritium-LSD binding is 124 ± 29 (SD) (observed is 132 ± 51 [SD]). The age-predicted mean schizophrenic tritium-LSD binding is 103 ± 37 (SD) (observed is 84 ± 33 [SD]). The age-predicted schizophrenic binding is still significantly different from observed schizophrenic binding ($t$ = 4.22, $df$ = 20, $P < .001$). Thus, age-related decline in tritium-LSD binding found for controls in study 1 does not account for lower tritium-LSD binding found in older schizophrenic population.

schizophrenics ($F$ = 13.15, $df$ = 1,45, $P < .001$) and across the three studies ($F$ = 14.85, $df$ = 2,45, $P < .0001$), indicating that there was variation in the assay itself over time.

To control for variation between assays, the data was adjusted by means of Z transform. Using an independent group $t$-test, the difference between controls and schizophrenics remained highly significant ($t$ [49] – 4.40; $P < .0002$).

For all subjects, regression of age ($X_1$) and time from morgue to autopsy ($X_2$) on levels of tritium-LSD binding Z transform ($Y$) allowed us to derive the following equation using a multiple linear regression: $Y = 0.9736 - 0.016 (X_1) - 0.0101 (X_2)$. The data adjusted with this formula using a two-tailed test remained highly significant ($t$ = 2.65; $P < .02$) (Fig 5). The combined variance accounted for by these two factors was 16% of the total variance. The reduced levels of tritium-LSD binding do not seem to be fortuitous because they were replicated in three independent populations of controls and schizophrenics over a two-year interval.

Although the drug specificity of both LSD and 5HT binding to brain membranes suggests an association with postsynaptic 5HT receptors, the two sites differ in absolute affinities for various drugs. In general, antagonists are more potent at LSD sites and 5HT at sites labeled by tritium-5HT.[17-20] These findings were consistent with a labeling of two states of one interconvertible 5HT receptor[17] as proposed for opiate and dopamine receptors.[8] Recent findings favor a different interpretation. Tritium-spiroperidol labels 5HT receptors in the cerebral cortex.[21,22] Although both tritium-5HT and tritium-spiroperidol label sites with the characteristics of 5HT receptors, recent studies show that the two ligands bind to distinct and noninterconverting receptors (S. J. Peroutka, MD, and S. H. Snyder, MD, unpublished data, October 1978). These sites are distinct in the same sense that muscarinic and nicotinic cholinergic receptors or $\alpha$- and $\beta$-noradrenergic receptors are distinct. Spiroperidol has about 1,000-fold greater affinity for the sites labeled by tritium-spiroperidol than by tritium-5HT. Concentrations of unlabeled spiroperidol that occupy all the tritium-spiroperidol sites fail to reduce the binding of tritium-5HT.

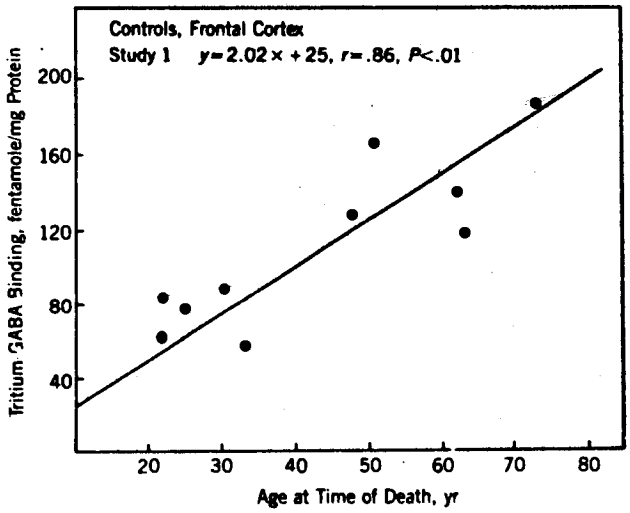


Fig 4.—Correlation between levels of γ-aminobutyric acid tritium-GABA binding and age of patients for control frontal cortex samples, study 1.

Several lines of evidence indicate that tritium-LSD labels both the tritium-spiroperidol and the tritium-5HT sites. The number of tritium-LSD sites equals the sum of tritium-spiroperidol and tritium-5HT sites in rat cerebral cortex (S. J. Peroutka, MD, and S. H. Snyder, MD, unpub-

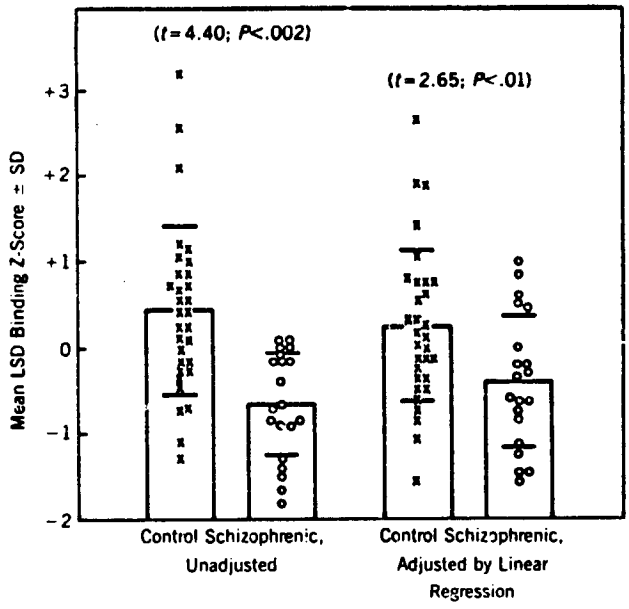


Fig 5.—Tritium-LSD binding levels in brains from all controls and s ophrenics. Unadjusted values are Z transforms derived as described in text. For each of three studies, mean and standard deviations were calculated for all values. Individual values were then expressed as their position in normal distribution. First set of numbers is not adjusted for age and time of arrival at morgue to autopsy; second set is adjusted for these variables.

lished data, October 1978). Moreover, for an extensive series of drugs, potencies in inhibiting tritium-LSD binding are the logarithmic mean between potencies of these agents in reducing tritium-spiroperidol and tritium-5HT binding levels.

Thus, whereas tritium-LSD and tritium-5HT in part label the same sites, they also label distinct 5HT receptors. Since levels of tritium-5HT binding were not reduced in brain from schizophrenics, the reduction of tritium-LSD binding levels presumably reflected that portion of binding associated with the sites labeled preferentially by tritium-spiroperidol. Accordingly, one would predict that tritium-spiroperidol binding levels would be reduced to even a greater extent in the frontal cortex of schizophrenics.

is unclear what factors account for the postulated change in 5HT receptors. First, it should be emphasized that the change in binding might result from an alteration in some associated tissue required for tritium-LSD binding and not necessarily in the receptor site itself. Whether the changes observed in the part of the brain studied (frontal cortex) occur in other parts of the brain is not known. Whether the reductions in tritium-LSD binding levels are related to the enhancement of dopamine receptor binding in the corpus striatum and nucleus accumbens of schizophrenics[23,24] or the elevated levels of 5HT in blood in autistic children[25] is unclear. Moreover, the present findings do not permit judgment of whether the alterations in tritium-LSD binding relate to the schizophrenic process or are merely effects of chronic drug treatment or institutionalization. Attempts to control for the role of institutional hospitalization and to evaluate other brain areas will require further investigation.

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## Nonproprietary Name and Trademark of Drug

Thioridazine hydrochloride—*Mellaril Hydrochloride.*

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