trusible tongue is mounted on the hyoid, and hyoid movement everts it or positions it beyond the mouth for further propulsion. *Bufo* and all known anurans lack such an intrinsic lingual skeleton (*2, 5*). Instead, there are sets of muscle fibers locked into a connective-tissue framework that limits their shortening. Stimulation transforms these sets into rigid rods that transmit forces at right angles to the lines of action of their component muscle fibers. Thus, it is the swelling of the muscle as much as its shortening that rotates the lingual frame and produces the ballista. While lateral transmission of muscular force appears unusual, it does occur in a number of other situations, an obvious example being the protrusible human tongue (*12*).

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9. High-speed cinematography (140, 200, and 400 frames per second) and synchronized electromyograms (EMG) of the lingual and hyoid muscles were recorded from 17 unanesthetized and unrestrained toads (*Bufo marinus*) fed on mealworms. Analysis included 36 feeding sequences with good simultaneous cine and EMG, 76 sequences with EMG only, and 100 films of control sequences. High-speed films were obtained with a Hycam 16-mm movie camera from which a signal from the shutter mechanism provided a frame marker that was stored on tape and permitted correlation of tongue position with muscular activity. After an intraperitoneal injection of tricaine methanesulfonate (0.04 mg per gram of body weight), bipolar fine-wire electrodes (0.076-mm Teflon-coated stainless steel; 1-mm bared tips; Medwire Inc.), inserted into 22-gauge hypodermic needles, were passed subcutaneously from the parotid region toward the buccal cavity and submucosally into the appropriate muscles. The wire was kinked several times during removal of the hypodermic needle. Reverse stimulation of the electrode was used to check electrode placement. Generally, four electrodes were implanted for each experiment and their external ends were soldered to a harness of earphone wire which, in turn, was sutured to the animal's back. One to two days after the electrodes were implanted, the animals were offered food in a photo cage with a 45° mirror that provided simultaneous frontal and lateral views of the head. Electromyogram signals were amplified through 26A2 Tektronix preamplifiers, Honeywell 117 DC amplifiers, and stored on a Honeywell 5600 1-inch tape recorder. Films projected from a Lafayette analytical projector were analyzed frame by frame, and the position of the tongue relative to the head was traced; EMG signals were processed and plotted with a minicomputer (J. Beach, G. C. Gorniak, C. Gans, *J. Biomech.*, in press).
10. The EMG magnitudes were keyed to the film frames, reflecting 5- or 7-msec intervals (150 or 200 frames per second). The delay between mechanical muscle activity and electrical stimulation in frogs at 20°C is also 5 msec [B. C. Abbott and A. J Brady, in *Physiology of the Amphibia*. J. A. Moore, Ed. (Academic Press, New York, 1964), vol. 1, p. 329].
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## Tritiated Imipramine Binding Sites Are Decreased in the Frontal Cortex of Suicides

Abstract. *Binding characteristics of tritiated imipramine were determined in the frontal cortex of suicides and well-matched controls. Maximal binding was significantly lower in brains from the suicides. This finding is consistent with reports of decreased tritiated imipramine binding in the platelets of patients diagnosed as having a major affective disorder.*

Briley *et al.* (*1*) reported decreased binding of [$^3$H]imipramine in the platelets of patients suffering from clinically significant depression. The change in this binding characteristic appears to be related more to diagnostic category (affective disorder) than to clinical status or mood, since binding properties are not correlated with measures of depression and remain unaltered as a function of treatment outcome (*2*). A recent report (*3*) indicated that [$^3$H]imipramine binding is increased in platelets from patients with unipolar or bipolar disorders. However, the lack of specific information regarding the diagnostic criteria as well as the absence of binding data make evaluation of the report difficult. Binding sites for [$^3$H]imipramine have also been described in various regions of the brains of animals (*4*) and humans (*5*). Both receptor sites—brain and platelet—possess virtually identical binding characteristics, and it has been postulated that they may serve as useful biological substrates for advancing our understanding of affective disorders.

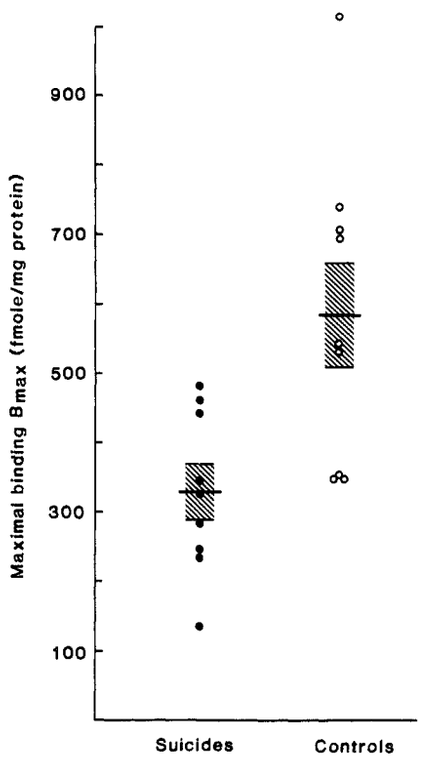


Fig. 1. Comparison of maximal binding of [$^3$H]imipramine in frontal cortex samples from suicide and control subjects. Each point represents an individual subject. Binding values were calculated by Scatchard analysis with six concentrations of [$^3$H]imipramine. Solid lines and hatched areas represent the means and standard errors, respectively, for each group. The difference in $B_{max}$ values is statistically significant ($P < .01$).

Evidence that [$^3$H]imipramine binding sites in both platelets and brain tissue are functionally related is provided by preliminary animal studies. Arbilla *et al.* (*6*) reported parallel decreases in [$^3$H]imipramine binding in cat platelets and brain tissue after long-term imipramine treatment. However, to determine whether the decrease in [$^3$H]imipramine binding observed in platelets from patients with affective disorders is an indicator of similar changes in the brain, it is necessary to examine binding in samples from deceased patients with a similar diagnosis. Because of the prevalence of affective disorders in people who commit suicide (*7*), we decided to determine the binding characteristics of [$^3$H]imipramine in the frontal cortex of suicide victims and matched controls.

Brain samples from nine suicide victims and nine controls were obtained at autopsy at the New York City Medical Examiner's Office (*8*). There were no significant differences between the two groups with respect to age, sex, and elapsed time between death and autopsy (Table 1). Frontal cortex samples corre-


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Table 1. Binding characteristics of [$^3$H]imipramine in frontal cortex from suicides and controls.*

| Cause of death | Age | Time between death and autopsy (minutes) | $B_{max}$ | $K_d$ |
|---|---|---|---|---|
| Suicides | | | | |
| Hanging | 46 | 1920 | 464 | 10.0 |
| Gunshot wound | 13 | 1140 | 249 | 6.8 |
| Hanging | 15 | 555 | 345 | 7.5 |
| Gunshot wound | 25 | 1560 | 328 | 5.3 |
| Gunshot wound | 33 | 1020 | 285 | 9.1 |
| Hanging | 41 | 990 | 237 | 7.6 |
| Hanging | 58 | 2250 | 443 | 12.9 |
| Jumping from height | 55 | 1140 | 136 | 2.2 |
| Hanging | 30 | 1320 | 483 | 10.9 |
| Mean ± standard error | 35.11 ± 5.40 | 1322 ± 172 | 330 ± 39† | 8.04 ± 1.06 |
| Controls | | | | |
| Gunshot wound (homicide) | 45 | 1650 | 740 | 4.4 |
| Gunshot wound (homicide) | 21 | 1190 | 707 | 13.9 |
| Myocardial infarction (apparent) | 22 | 750 | 353 | 6.8 |
| Gunshot wound (homicide) | 20 | 1570 | 350 | 5.1 |
| Myocardial infarction (apparent) | 31 | 880 | 350 | 4.8 |
| Auto accident (hit and run) | 40 | 550 | 531 | 7.7 |
| Gunshot wound (homicide) | 42 | 1440 | 1017 | 4.0 |
| Myocardial infarction (apparent) | 47 | 1200 | 695 | 1.8 |
| Myocardial infarction (apparent) | 39 | 1455 | 544 | 8.8 |
| Mean ± standard error | 34.15 ± 3.60 | 1187 ± 128 | 587.44 ± 75.13 | 6.36 ± 1.17 |

*All subjects were male. †Significantly different from corresponding control value ($P < .01$).

sponding to Brodmann's areas 8 and 9 were dissected out, immediately frozen on dry ice, and stored at −80°C until assayed.

Binding of [$^3$H]imipramine was determined in accordance with the methods described by Rehavi *et al.* and Langer *et al.* (*5*). A Brinkmann Polytron was used to homogenize the brain samples in 50 volumes of cold 0.05*M* tris-HCl buffer (*p*H 7.4) containing 0.12*M* NaCl and 0.005*M* KCl. The homogenate was centrifuged at 30,000*g* for 10 minutes and the resulting pellet was washed and centrifuged twice. After the last centrifugation the pellet was resuspended to yield a tissue concentration of approximately 30 mg (wet weight) per milliliter of buffer.

Samples (180 μl) of the homogenate were incubated in triplicate at 0°C with 35 μl of [$^3$H]imipramine (0.6, 1.2, 2.5, 5, 7.5, and 10 n*M*; specific activity, 29.8 Ci/mmole) (New England Nuclear) and 35 μl of buffer with or without 10 μ*M* desipramine. After 60 minutes 100 μl of the incubate was diluted in 5 ml of ice-cold buffer and filtered through Whatman GF/F glass filters with a filter manifold (Hoefer Scientific Instruments). Filters were washed three times with 5 ml of buffer, dried, placed in scintillation vials with Aquasol, and counted. Specific binding was defined as that which was displaced by 10 μ*M* desipramine, and represented 60 to 70 percent of total binding at the concentrations used in these experiments.

Scatchard analysis of the data showed no difference in [$^3$H]imipramine binding affinity ($K_d$) between suicides and controls. However, the mean number of binding sites ($B_{max}$) for suicides (330 fmole per milligram of protein) was approximately 44 percent lower than for controls (587 fmole per milligram of protein) (Fig. 1). The difference is significant at $P < .01$ (two-tailed Wilcoxon matched-pairs test). This finding is consistent with the reports of investigators who noted a similar reduction in the number of receptors in platelets from patients with a diagnosis of major affective disorder (*1, 2*). In addition, our results support the hypothesis that peripheral changes in [$^3$H]imipramine binding are indicative of changes in central binding and are, therefore, consistent with reports that [$^3$H]imipramine binding is similar in platelets and the brain (*4, 5*) and that both kinds of binding sites are functionally related (*6*).

A number of human and animal studies have suggested that [$^3$H]imipramine binding is associated with the neuronal uptake mechanism for serotonin. Animals given lesions of the dorsal raphe nucleus have reduced serotonin concentrations as well as a decreased number of [$^3$H]imipramine binding sites (*9*). Use of the irreversible ligand 2-nitroimipramine leads to reduced [$^3$H]imipramine binding and serotonin uptake in platelets (*10*).

The importance of serotonergic systems in affective disorders has been emphasized in both in vivo and postmortem studies. A number of investigators have found that the concentrations of serotonin and its principal metabolite, 5-hydroxyindoleacetic acid (5-HIAA), are significantly lower in the brains of suicides than in control brains (*11*). Similarly, lower levels of 5-HIAA have been reported in the cerebrospinal fluid of patients who were diagnosed as depressed or who had recently attempted suicide (*12*).

In conclusion, it appears that the reduced [$^3$H]imipramine binding found in the frontal cortex of suicides indicates a functional decrease in serotonergic neuronal activity, which may in turn be related to a diagnosis of a major affective disorder. Further, because imipramine binding is associated with serotonergic neurons it would be of interest to determine whether changes in the concentration of serotonin and 5-HIAA or in serotonergic receptors occur in the same population.

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## Tonotopic Organization of the Human Auditory Cortex

Abstract. *Neuromagnetic measurements of responses to auditory stimuli consisting of pure tones amplitude-modulated at a low frequency have been used to deduce the location of cortical activity. The evoked field source systematically increased in depth beneath the scalp with increasing frequency of the tone. The tonotopic progression can be described as a logarithmic mapping.*

In humans, there is a topological correspondence between the periphery and the primary sensory cortex in both the somatic and visual sense modalities. Evidence from the cat (*1*), squirrel (*2*), and monkey (*3*) suggests that there may also be a human tonotopic mapping as well, but thus far evidence of such mapping is lacking. We report evidence from neuromagnetic studies indicating the existence of an orderly projection of frequencies onto the human auditory cortex.

Previous measurements of magnetic fields after auditory stimulation have revealed transient fields outside the human scalp (*4–6*). The equivalent sources of these fields may be modeled as current dipoles in the vicinity of the auditory cortex of each hemisphere oriented normal to the lateral sulcus. The direction of current flow producing these fields is opposite to the flow associated with corresponding components of auditory evoked scalp potentials. Consequently, the most likely source of the evoked field is the net flow of intracellular currents within the cortex forming the floor of the lateral sulcus rather than the volume currents that are associated with the evoked potentials.

Techniques used in biomagnetic studies have been described (*7*). Our magnetic field sensor consists of a second-derivative gradiometer with 2.4-cm diameter and 3.2-cm baseline between adjacent coils coupled to a SQUID sensor (S.H.E. Corporation). This assembly provides both the sensitivity required to measure evoked fields and a satisfactory reduction of environmental noise without the aid of magnetic shielding. All superconducting circuits and elements were contained in a superinsulated fiber glass Dewar, which permitted placing the pickup coil of the gradiometer as close as 8 mm to the scalp. The Dewar was oriented so that the magnetic field component normal to the scalp was monitored by the gradiometer. The output voltage of the SQUID electronics, which is simply proportional to the net field sensed by the gradiometer, was applied to a bandpass filter tuned to the stimulus modulation frequency with roll-off of 48-dB per octave on the high- and low-frequency sides.

Auditory stimuli were presented binaurally by means of standard airline plastic earphones, the transducers of which had been tested to avoid undesirable magnetic artifacts. The stimuli consisted of pure tones, the amplitudes of which were sinusoidally modulated. The depth of modulation was somewhat less than 100 percent, and the modulation frequency was much lower than that of the carrier frequency. Hence, the Fourier spectrum of the acoustic signal was composed of a carrier frequency and two sidebands shifted from the carrier by an amount equal to the modulation frequency. The acoustic signal was therefore confined to a narrow bandwidth. A 1024-channel signal averager triggered by the oscillator providing the modulation signal was used to average the filtered SQUID output to reveal the steady-state response at the modulation frequency. Four different carrier frequencies were used for both of our two subjects. For subject S.W. they were 200, 600, 2000, and 5000 Hz; because subject C.P. did not respond strongly to the 5000-Hz signal, the frequencies were 100, 200, 600, and 2000 Hz. Since responses with strong amplitudes were obtained at a modulation frequency of 32 Hz in pilot studies, we used that value (*8*). Initially, one stimulus amplitude was set at about 80-dB sound pressure level so that it could be easily perceived above the

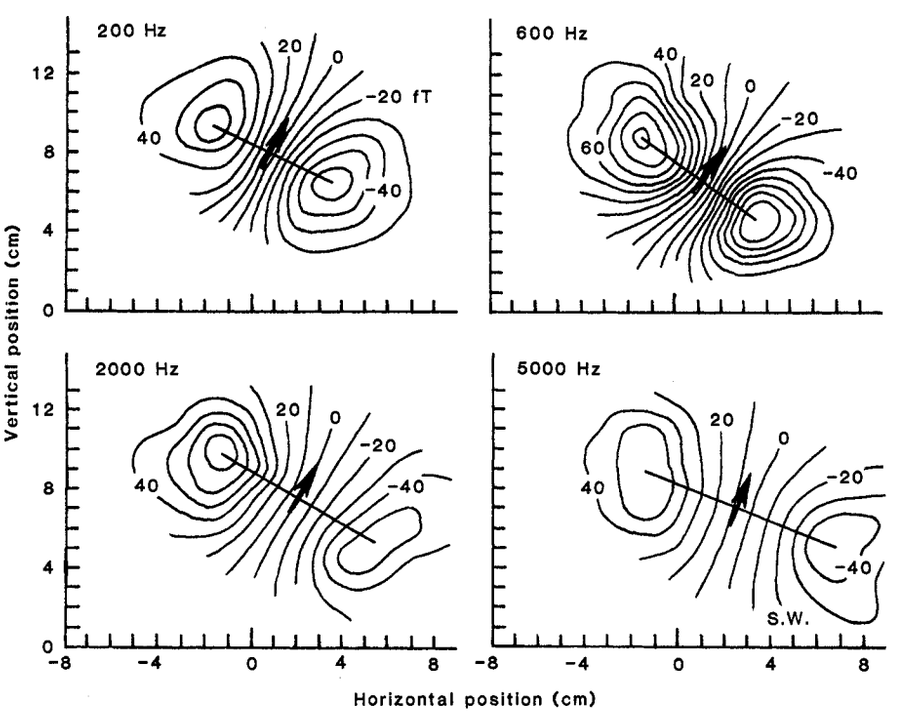


Fig. 1. Isofield contours for the component of the evoked magnetic field normal to the scalp detected over the right hemisphere of subject S.W. The origin is at the ear canal, the corner of the eye lies at position (0, 9), and the vertical axis points to the vertex. Arrows denote the position and the orientation of the equivalent current dipoles for 200-, 600-, 2000-, and 5000-Hz tones. The sense of the arrow is arbitrarily chosen.


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