Background: The involvement of serotonin in depression and suicide has been proposed, because major depression is successfully treated by medications that specifically block the serotonin transporter, and there is evidence for a decrease in serotonin transporters in major depression and suicide. The midbrain dorsal raphe nucleus (DR) has been implicated as a site for diminished serotonergic activity in that suicide victims with major depression have a significant increase in serotonin-1A autoreceptors in the DR.

Methods: \(^3\)H]Paroxetine was used to label the serotonin transporter in the subnuclei of the DR at several rostral-to-caudal levels of the midbrain in ten pairs of suicide victims with major depression and age-matched psychiatrically normal control subjects.

Results: There was a significant increase in serotonin transporters in the entire DR progressing from rostral-to-caudal levels in both normal control subjects and suicide victims with major depression and age-matched psychiatrically normal control subjects.

Results: There was a significant increase in serotonin transporters in the entire DR progressing from rostral-to-caudal levels in both normal control subjects and suicide victims with major depression and age-matched psychiatrically normal control subjects. At comparable rostral-to-caudal levels, there were no significant differences in \(^3\)H]paroxetine binding between depressed suicide victims and normal control subjects in either the entire DR or its constituent subnuclei.

Conclusions: The pathophysiology of serotonin mechanisms in suicide victims with major depression does not appear to involve alterations in the binding of \(^3\)H]paroxetine to the serotonin transporter in the midbrain DR.


Key Words: Dorsal raphe nucleus, serotonin transporter, midbrain, major depression, suicide
(Arango et al 1995; Joyce et al 1993; Laruelle et al 1993; Leake et al 1991; Stanley et al 1982). Alternatively, several reports suggest that altered serotonin or norepinephrine function in major depression and suicide may be detected at the sites of origin of these monoamine neurons in the brainstem (Arango et al 1996; Malison et al 1998; Ordway et al 1994; Stockmeier et al 1998; Zhu et al 1999).

The dorsal raphe nucleus (DR) in the brainstem contains the cell bodies of many of the serotonergic neurons that project to the forebrain and these neurons appear to be involved in the pathophysiology of major depression and suicide. Evidence for involvement of the serotonergic brainstem in depression has emerged from reports of a significant decrease in radioligand binding to the serotonin transporter in major depression (Malison et al 1998), increased radioligand binding to the serotonin-1A autoreceptors in the DR in suicide victims with major depression (Stockmeier et al 1998), or decreased serotonin-1A receptor binding potential in patients in the depressed phase of familial mood disorder (Drevets et al 1999). Recently, morphological evidence has surfaced to suggest that the number and density of serotonin neurons is higher in the DR in suicide victims, most of whom had major depression (Underwood et al 1999).

In animal experiments, chronic treatment with antidepressant medications such as SSRIs or monoamine oxidase inhibitors appears to enhance net serotonergic neurotransmission via mechanisms in the midbrain DR (Blier and deMontigny 1994; Invernizzi et al 1994). Therefore, the serotonergic system in the brainstem may be diminished in major depression and/or suicide, whereas conversely, antidepressant medications appear to enhance that system.

This study was designed to test the hypothesis that serotonin transporters are decreased in the midbrain DR of suicide victims with major depression. The midbrain was sampled at several rostral-to-caudal levels, and quantitative receptor autoradiography was used to measure the serotonin transporter. The suicide victims met clinical criteria for a current episode of major depression and the age-matched control subjects were determined to be without psychiatric illness.

Methods and Materials

Tissue Collection and Preparation

Brain tissue was obtained at autopsy at the Coroner’s Office of Cuyahoga County, Cleveland. The study was performed in accordance with an approved Institutional Review Board Protocol and written consent was obtained from the next-of-kin. As previously described (Stockmeier et al 1996), midbrains were collected and dissected from 10 suicide victims and 10 age-matched comparison subjects dying of natural or accidental causes. The causes of death were certified by the Coroner and are listed in Table 1. Tissues were frozen in isopentane cooled by solid CO2 and stored at −80°C prior to sectioning. Both the tissues and the resultant autoradiograms from the matched pairs of subjects were processed and analyzed in parallel, and laboratory personnel were blinded to the identities of the subjects.

Information regarding medications and prescriptions for the subjects was collected where available, and the toxicological records from the coroner’s office were excerpted (Table 1). The sources regarding medications included one or more of the following: the next-of-kin, physician or hospital records for the subjects, and an inventory of medications brought to the coroner’s office by investigators at the scene of the death. Not all next-of-kin were aware of medications used by the subjects, some requests for medical records were not granted, and not all prescriptions were filled. Current prescriptions (within last month of life, drugs printed boldface in Table 1), and lifetime prescriptions are both recorded in Table 1. Issues of compliance within the last month of life with prescriptions received for psychotropic medications were assessed by discussions with the next-of-kin, assessment counts of remaining pills from the coroner’s records, and examination of results from the toxicology report. Where medications prescribed within the last month of life were not detected in samples of blood or urine, and compliance information from the next-of-kin and counts of remaining medications would support the conclusion, it is assumed that subjects were not compliant with medications (e.g., subject with recent nortriptyline and perphenazine prescriptions). The toxicology laboratory of the Cuyahoga County Coroner’s Office examined blood and urine samples from the subjects. Qualitative and quantitative assays were used to detect classes of drugs as previously described (Stockmeier et al 1996). The results of the toxicology tests are given in Table 1.

Retrospective Psychiatric Assessments

Retrospective psychiatric assessments were performed as previously described to identify suicide victims with a current diagnosis of major depression and comparison subjects that were psychiatrically normal (Stockmeier et al 1997). Kelly and Mann (1996) have shown there is good agreement between informant-based retrospective psychological assessments of deceased subjects and diagnoses by clinicians treating the same subjects before their deaths. For each subject in our study, a trained interviewer conducted a structured interview with a knowledgeable informant about 3 months after the subject died. Knowledgeable informants either lived with or interacted with the subjects several times a week. Data on lifetime and current mental illness were gathered with a modified Schedule for Affective Disorders and Schizophrenia: lifetime version (SADS-L; Spitzer and Endicott 1978). Diagnoses for Axis I disorders were independently assessed by a clinical psychologist and a psychiatrist, and consensus diagnosis was reached in conference using information from the knowledgeable informants, the coroner’s office, and inpatient and outpatient records, where available. The final diagnosis was compatible with the DSM-III-R (American Psychiatric Association 1987). The psychiatric diagnoses are summarized in Table 1.
Table 1. Characteristics of Subjects

<table>
<thead>
<tr>
<th>Sample</th>
<th>Age (years)/gender</th>
<th>Cause of death</th>
<th>PMI (hours)</th>
<th>Toxicology</th>
<th>Smoker</th>
<th>Medication</th>
<th>AXIS I diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 27/m</td>
<td>Electrocution by lighting</td>
<td>22</td>
<td>Nothing detected</td>
<td>Yes</td>
<td>No diagnosis, alcohol abuse 7 years ago</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 40/m</td>
<td>Cardiovascular disease</td>
<td>22</td>
<td>Lidocaine</td>
<td>Yes</td>
<td>No diagnosis, alcohol abuse 2 years ago</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 44/m</td>
<td>Cardiovascular disease, aortic aneurism</td>
<td>6</td>
<td>Ephedrine, phenylpropranolol, chlorpheniramine</td>
<td>No</td>
<td>No diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 45/f</td>
<td>Cardiovascular disease</td>
<td>9</td>
<td>Nothing detected</td>
<td>Yes</td>
<td>Famotidine</td>
<td>No diagnosis</td>
<td></td>
</tr>
<tr>
<td>Control 47/m</td>
<td>Cardiovascular disease</td>
<td>17</td>
<td>Nothing detected</td>
<td>Yes</td>
<td>No diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 50/m</td>
<td>Cardiovascular disease</td>
<td>26</td>
<td>Nothing detected</td>
<td>Yes</td>
<td>No diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 57/m</td>
<td>Cardiovascular disease</td>
<td>10</td>
<td>Nothing detected</td>
<td>Hx</td>
<td>Naproxen</td>
<td>No diagnosis</td>
<td></td>
</tr>
<tr>
<td>Control 69/m</td>
<td>Cardiovascular disease</td>
<td>18</td>
<td>Nothing detected</td>
<td>No</td>
<td>No diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 71/m</td>
<td>Cardiovascular disease</td>
<td>23</td>
<td>Chlorpheniramine</td>
<td>No</td>
<td>Nitrogly, warfarin</td>
<td>No diagnosis</td>
<td></td>
</tr>
<tr>
<td>Control 82/m</td>
<td>Cardiovascular disease, aneurism</td>
<td>16</td>
<td>Nothing detected</td>
<td>No</td>
<td>Levophenyltoline</td>
<td>No diagnosis</td>
<td></td>
</tr>
<tr>
<td>Suicide 25/f</td>
<td>Hanging</td>
<td>17</td>
<td>Nothing detected</td>
<td></td>
<td>Nortriptyline, perphenazine, clonidine</td>
<td>Major depression</td>
<td></td>
</tr>
<tr>
<td>Suicide 30/m</td>
<td>SIGSW-chest</td>
<td>18</td>
<td>Ethanol, 0.07%</td>
<td>Yes</td>
<td>Major depression, alcohol abuse 2 years ago</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suicide 42/m</td>
<td>SIGSW-chest</td>
<td>20</td>
<td>Nothing detected</td>
<td>No</td>
<td>Major depression, dysthymia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suicide 43/m</td>
<td>Hanging</td>
<td>21</td>
<td>Nothing detected</td>
<td>No</td>
<td>Major depression, dysthymia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suicide 45/m</td>
<td>Multiple knifing</td>
<td>8</td>
<td>Nothing detected</td>
<td></td>
<td>Major depression, dysthymia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suicide 48/m</td>
<td>SIGSW-chest, slashed wrists</td>
<td>21</td>
<td>Flurazepam</td>
<td>No</td>
<td>Flurazepam, lorazepam</td>
<td>Major depression, alcohol abuse 24 years ago</td>
<td></td>
</tr>
<tr>
<td>Suicide 62/m</td>
<td>Hanging</td>
<td>5</td>
<td>Nothing detected</td>
<td>Yes</td>
<td>Major depression, alcohol abuse 4 years ago</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suicide 70/m</td>
<td>SIGSW-head</td>
<td>23</td>
<td>Phenytoin (acute)</td>
<td></td>
<td>Major depression, alcohol dependence 15 years ago</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suicide 73/m</td>
<td>SIGSW-chest</td>
<td>17</td>
<td>Diazepam, codeine</td>
<td>No</td>
<td>Trazodone, fluoxetine, hydroxyzine, diaz, nitroglycerin, furosemide</td>
<td>Major depression</td>
<td></td>
</tr>
<tr>
<td>Suicide 83/f</td>
<td>Slashed wrists</td>
<td>21</td>
<td>Nothing detected</td>
<td></td>
<td>Temazepam</td>
<td>Major depression</td>
<td></td>
</tr>
</tbody>
</table>

Medications printed in boldface were prescribed in the last month of life. Please see the Methods and Materials section for a description of the collection of information regarding prescription medications.

PMI, postmortem interval; m, male; f, female; SIGSW, self-inflicted gunshot wound; phenylpro, phenylpropranolol; nitrogly, nitroglycerin; Diaz, diazepam.

The suicide victims met diagnostic criteria for a current history of major depression during the last week of life. One suicide victim with major depression also had a current comorbid diagnosis of dysthymia. No subjects in either groups met criteria for a psychoactive substance use disorder (neither abuse nor dependence) at the time of their deaths. Three suicide victims previously met diagnostic criteria for alcohol abuse at 2, 4, and 24 years prior to death. Another suicide victim met diagnostic criteria for alcohol dependence at 15 years prior to death. Two psychiatrically normal control subjects met diagnostic criteria for alcohol abuse at 7 years and greater than 2 years prior to death. Tissue Sectioning and Immunohistochemistry

Tissue blocks were frozen to pellets with Tissue-Tek O.C.T. Compound (Miles, Elkhart, IN), after which a cryostat microtome was used to collect 20-μm thick sections for receptor autoradiography and 30-μm-thick sections for immunohistochemistry. At 0.5-mm intervals along the midbrain DR, from the initiation of the DR to the decussation of cranial nerve 4, a section was collected for immunohistochemistry and immediately adjacent sections were collected for autoradiography. Sections were thaw-mounted on microscope slides subbed with...
gelatin and chrome-alum, dried under a cool stream of air, and stored at -80°C until the assays were performed.

For each subject, anatomically comparable rostral-to-caudal levels were identified using the immunohistochemical identification of tryptophan hydroxylase (TrpOH) immunoreactive neurons, as previously described (Stockmeier et al 1996, 1998). Depending on inter-individual anatomic variation, generally 10 rostral-to-caudal levels were chosen for each subject. The subnuclei of the DR were identified as described by Baker et al (1990, 1991), and included the dorsal (DRd), interfascicular (DRif), ventral (DRv), and ventrolateral (DRvl). Comparable rostral-to-caudal levels (every 0.5 mm) were located within each pair of subjects as previously described, and each of these levels were used for the autoradiographic experiments (Stockmeier et al 1998).

**Receptor Autoradiography**

Radioligand binding to the serotonin transporter was determined autoradiographically as previously described (Cortes et al 1988; Stockmeier et al 1996). After a 15-min preincubation in 50 mmol/L Tris-HCl (pH 7.4 at room temperature), three adjacent sections from each rostral-to-caudal level were used to determine total binding and incubated for 1 hour at room temperature with [3H]paroxetine (0.35–0.38 nmol/L, 16.6 or 22 Ci/mmol, New England Nuclear, Cambridge, MA) in buffer containing 50 mmol/L Tris-HCl, 150 mmol/L NaCl, and 5 mmol/L KCl (pH 7.4 at room temperature). In duplicate sections immediately adjacent to those used for total binding, nonspecific binding was measured by co-incubating slides with 1 mmol/L fluoxetine-HCl (Lilly, Indianapolis). After the incubation, the sections were washed twice at 4°C for 20 min in nonradioactive Tris/NaCl/KCl buffer (pH 7.4 at 4°C).

Sections were then dipped in ice-cold water, air dried, and stored for 24–48 hours in sealed slide boxes with Drierite capsules. The sections and tritiated plastic standards (American Radiolabeled Chemicals, St. Louis) were exposed to Hyperfilm-3H (Amersham, Arlington Heights, IL) in x-ray cassettes for 12 weeks. Films were developed with Kodak D-19 developer and fixed with Kodak Rapid Fix. The exposure times were selected so the resultant autoradiographic images were quantitated over the optimum range of film sensitivity.

**Quantitation of Radioligand Binding**

Autoradiographic images of radioligand binding were measured in the subnuclei of the DR using the Microcomputer Controlled Imaging Device system (MCID; Imaging Research Inc., St. Catherine’s, Ontario, Canada). The subnuclei were named according to Baker et al (1990, 1991), and their borders were drawn on digital templates constructed from digitized images of TrpOH immunohistochemistry on adjacent slide-mounted sections. The digital templates were superimposed on autoradiographic images by MCID. Images of total and nonspecific binding of [3H]paroxetine were digitized and super-imposed. Nonspecific binding was digitally subtracted from the total binding to determine specific binding. The specific binding of [3H]paroxetine was approximately 70% of total binding. For each section, the serotonin transporter was measured in the entire DR, the entire midline DRif, and the left halves of the other subnuclei. The midline subnuclei were bisected by a line drawn from the most ventral surface of the cerebral aqueduct through the middle of the interfascicular subnucleus. The DR subnuclei on the left half project ipsilaterally to regions of the orbitofrontal and dorsolateral prefrontal cortex where morphometric abnormalities are observed in suicides with major depression (Rajkowska et al 1999; Wilson and Moliver 1991).

**Statistical Analysis**

The age and postmortem interval (PMI) of the two groups of subjects were compared using paired t tests. The influence of age or PMI on autoradiographic density values was examined in the subnuclei of the midbrain DR. Pearson correlation coefficients were calculated to evaluate relationships between age or postmortem interval and radioligand binding to the serotonin transporter. Because the specimens were processed as age-matched pairs of control subjects and suicide victims, a paired analysis of variance (ANOVA) was performed, using two within-subjects (repeated measures) factors (i.e., group and rostral-to-caudal level). Statistically significant group-by-level interactions were followed up with paired t tests at each level to determine the rostral-to-caudal location of any group differences.

**Results**

The average age of the suicide group was 52 ± 6 years (mean ± SEM, range 25–83) and the average time between death and freezing of the tissue (PMI) was 17 ± 2 hours (mean ± SEM, range 5–23)(Table 1). The average age of the control group was 53 ± 5 years (range 27–82) with an average PMI of 17 ± 2 hours (range 6–26)(Table 1). There were no significant differences between groups for either age or PMI.

[3H]Paroxetine binding was examined within the DR of the human midbrain. A schematic diagram identifying the DR subnuclei is presented in Figure 1. As described by Cortes et al (1988) and Stockmeier et al (1996), specific binding of [3H]paroxetine is present in the DR. The specific binding of [3H]paroxetine was identified within the subnuclei of the DR on the basis of TrpOH-like immunoreactivity present over presumably serotonin-synthesizing neurons (Figure 1C). Representative autoradiograms of [3H]paroxetine binding at four rostral-to-caudal levels are presented in Figure 2.

The suicide victims with major depression and the normal control subjects were compared with each other at anatomically corresponding levels along the length of the midbrain DR. When comparing the two subject groups, there was no statistically significant main effect of subject group for the entire DR, or any of the subnuclei (Table 2 and Figure 3). In other words, no significant differences in radioligand binding were observed in the DR between...
suicide victims with major depression and psychiatrically normal control subjects.

The distribution of radioligand binding to the serotonin transporter along the length of the midbrain DR is depicted at several rostral-to-caudal levels in Figure 3. There is a significant main effect of rostral-to-caudal level for the entire DR as well as for the dorsal subnucleus (Figure 3 and Table 2). For the entire DR, the autoradiographic density of serotonin transporters nearly doubled along the length of the midbrain (Figure 3A). Closer inspection of the subnuclei revealed a statistically significant greater than doubling of serotonin transporters in the dorsal subnucleus when progressing in a rostral-to-caudal fashion (Figure 3C). There were no rostral-to-caudal differences in transporter binding within the ventrolateral, interfascicular or ventral subnuclei (Figures 3B, 3D, and 3E).

The influence of age or PMI on the serotonin transporter was examined. Age had no significant effect on the autoradiographic density of serotonin transporters in the subnuclei of the DR of either suicide victims with major depression or control subjects. A postmortem interval ranging from 5 to 24 hours had no apparent effect on the radioligand binding to the serotonin transporters in the suicide victims and a very minimal effect in the normal control subjects. In contrast to age or PMI, serotonin transporters in the DR are significantly increased when progressing rostral-to-caudal in the midbrain. These results reveal that the precise rostral-to-caudal level of the midbrain DR is an important variable to standardize when comparing groups of subjects. It appears that neither age nor PMI, albeit over the fairly narrow range of these 10 pairs of subjects, are particularly critical in the interpretation of \[^{3}H\]paroxetine binding to the serotonin transporters in either the entire midbrain DR or its subnuclei. In a companion study of sections taken adjacent to those for this study of serotonin transporters, radioligand binding of an agonist to serotonin-1A receptors was significantly increased in the DRd and DRvl subnuclei (Stockmeier et al 1998). Thus, although the increase in presumably inhibitory serotonin-1A autoreceptors in the midbrain DR may provide evidence for diminished serotonin function in suicide victims with major depression, radioligand binding to serotonin transporters in the midbrain is not significantly altered postmortem in these same subjects.

The influence of age, PMI, and rostral-to-caudal level on the serotonin transporter was examined. Age had no significant effect on the autoradiographic density of serotonin transporters in the subnuclei of the DR of either suicide victims with major depression or control subjects. A postmortem interval ranging from 5 to 24 hours had no apparent effect on the radioligand binding to the serotonin transporters in the suicide victims and a very minimal effect in the normal control subjects. In contrast to age or PMI, serotonin transporters in the DR are significantly increased when progressing rostral-to-caudal in the midbrain. These results reveal that the precise rostral-to-caudal level of the midbrain DR is an important variable to standardize when comparing groups of subjects. It appears that neither age nor PMI, albeit over the fairly narrow range of these 10 pairs of subjects, are particularly critical in the interpretation of \[^{3}H\]paroxetine binding to the serotonin transporter in the human DR.

A number of studies of postmortem brain tissue have investigated the serotonin transporter in projection regions of serotonergic cell bodies in suicide victims with a depressive disorder. Nearly all of these studies examined...
regions of cerebral cortex and the results are somewhat mixed. Early studies primarily focused on suicide victims and used \([^3]H\)imipramine, a less than optimum radioligand for measuring the serotonin transporter. Those studies suggested increases, decreases, or no change in \([^3]H\)imipramine binding to frontal cortex in suicide victims (Arora and Meltzer 1989; Crow et al 1984; Lawrence et al 1998; Owen et al 1986; Stanley et al 1982). More recently, other radioligands including \([^3]H\)paroxetine, \([^3]H\)citalopram or \([^{125}]I\)cyanoimipramine have been identified as superior ligands for measuring the serotonin transporter (Arranz and Marcusson 1994; Gurevich and Joyce 1996). Studies

![Table 2](image)
with these ligands, mostly in subjects with a depressive mood disorder, reveal either significant decreases (Arango et al 1995; Joyce et al 1993; Laruelle et al 1993; Leake et al 1991), or no change (Hrdina et al 1993; Lawrence et al 1990, 1997; Mann et al 1996a) in the serotonin transporter in cerebral cortex. Hence, the issues of a homogeneous psychiatric diagnosis in subjects examined, the radioligand selected to study the serotonin transporter, and the specific region of cerebral cortex examined are variables that may clarify the variance in the studies of the serotonin transporter in postmortem tissues from depressed and suicidal subjects.

There are several potential limitations to this study that necessitate that conclusions be drawn carefully. For example, several of the depressed suicide victims received antidepressant medications at some point during their lives, and some of the subjects have a previous history of a substance use disorder. In animal studies, the effects of chronic treatment with an antidepressant drug on the functional and binding properties of the serotonin transporter have recently been reviewed (Pineyro and Blier 1999). Repeated treatment with imipramine or fluoxetine (but not clorgyline) via osmotic minipumps significantly decreases mRNA hybridization for the serotonin transporter in the midbrain raphe (Lesch et al 1993). Interestingly, functional studies suggest a desensitization of the serotonin transporter in the midbrain DR after repeated administration of paroxetine (El Mansari and Blier, 1996; Pineyro et al 1994); however, repeated treatment with imipramine, clorgyline, fluvoxamine or citalopram via intraperitoneal injections resulted in either no change (Spurlock et al 1994) or an increase (Lopez et al 1994) in mRNA hybridization for the serotonin transporter. Although four of the suicide victims in this study had a history of therapy with antidepressant medications, only one had a prescription for an antidepressant medication in the last month of life. Thus, in this study it does not appear likely that a history of therapy with an antidepressant medication significantly altered the binding of [3 H]paroxetine in the midbrain DR in these subjects.

A psychoactive substance use disorder can alter the binding of [ 3 H]paroxetine in human brain. For example, prolonged and serious alcohol abuse is associated with a decrease in binding to the serotonin transporter site in the hippocampus but not the frontal cortex or dorsal raphe (Chen et al 1991; Little et al 1998). Cocaine dependence or abuse is associated with a decrease in radioligand binding to the serotonin transporter in the human dorsal raphe (Little et al 1998). In the current study, two control subjects had an old history of alcohol abuse and the suicide victims with major depression had old histories of abuse (three subjects) or dependence (one subject); however, none of the control subjects or suicide victims met diagnostic criteria for any psychoactive substance disorder (abuse or dependence) at the time of death, and their times of abstinence from alcohol were 2, 4, 7, 15, and 24 years. A recent imaging study using single photon emission computed tomography (SPECT) described potential alterations in the serotonin transporter in major depression. Malison et al (1998) reported a decrease in the availability of the serotonin transporter in the brainstem in living subjects with major depression. The subjects met criteria for major depression at the time of the imaging and were free of antidepressant medications for at least 3 weeks. The decrease in availability of the serotonin transporter was interpreted as a decrease in the density of the serotonin transporter and as further evidence for a serotonergic alteration in major depression. Because of issues of spatial resolution, it is possible that the digitally summed transaxial brainstem slices analyzed by Malison et al

Figure 3. The distribution of [3 H]paroxetine binding to the serotonin transporter in the entire dorsal raphe (DR) or various subnuclei along rostral-to-caudal levels of the midbrain from 10 pairs of suicide victims with major depression and age-matched psychiatrically normal control subjects. The abscissa represents 0.5-mm levels through the midbrain with rostral levels located to the left. The ordinate represents [3 H]paroxetine binding (fmol/mg protein) to the serotonin transporter. *There was a significant effect of rostral-to-caudal level in the entire DR (A, p = .013) and dorsal subnucleus (C, p = .001).
(1998) included serotonin transporters located in the substantia nigra, ventral tegmental area, median raphe nucleus, as well as the dorsal raphe nucleus. One other postmortem study examined serotonin transporters in the midbrain of depressed suicide victims. Using the same radiolabeled cocaine derivative as in the SPECT study, Little et al (1997) reported no significant change in radioligand binding to the serotonin transporter in the midbrain substantia nigra or ventral tegmental area, and no significant change in mRNA for the serotonin transporter in the midbrain DR or in the median raphe nucleus. Thus, the present study with \[^3\text{H}\]paroxetine is the first study to examine the serotonin transporter specifically in the midbrain DR subnuclei of suicide victims with major depression and to find no change in transporters as compared to control subjects that were psychiatrically normal.

It would be of interest to use SPECT or positron emission tomography to examine serotonin transporters in living subjects with major depression and a history of suicide attempts as compared to subjects with major depression and no history of suicidality. The imaging study revealing changes in serotonin transporters in major depressives that are not suicidal may reflect a neuropharmacological state that is unique in comparison to suicide victims with major depression.

There is evidence in the brainstem for the involvement of monoaminergic systems other than serotonin in major depression and suicide. For example, suicide victims have fewer pigmented neurons in the locus coeruleus (Arango et al 1996). In major depressives, most of whom committed suicide, there are increased amounts of tyrosine hydroxylase in the locus coeruleus (Ordway et al 1994; Zhu et al 1999), and decreased levels of the norepinephrine transporter in the locus coeruleus (Klimek et al 1997). The decrease in radioligand binding to the norepinephrine transporter in the locus coeruleus in major depression may reflect a compensatory down-regulation of the norepinephrine transporter protein in response to an insufficient availability of norepinephrine at the synapse.

In future studies using postmortem tissues, measurements of the serotonin and norepinephrine transporter throughout the brainstem DR should provide additional information on the involvement of these neurotransmitters in the pathophysiology of major depression and suicide. In particular, these studies will need to examine individuals with major depression dying of natural causes and suicide victims not meeting criteria for major depression.

The authors gratefully acknowledge the contributions of Herbert Y. Meltzer, M.D., and James C. Overholser, Ph.D., in the retrospective psychiatric diagnoses. The authors thank Jinrong Wei for assistance in determining rostral-to-caudal levels within the midbrains. The editorial assistance of Lisa M. Kempfer is also greatly appreciated.

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