Morphometry of the Dorsal Raphe Nucleus Serotonergic Neurons in Suicide Victims

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Background: *The serotonin deficiency hypothesis of suicide has been important heuristically. Few studies have directly examined the brainstem dorsal raphe nucleus (DRN) serotonin neurons. We determined the number and morphometry of DRN serotonergic neurons in suicide victims (*n *= 7) compared to controls (*n *= 6).*

Methods: *Brainstems were collected at autopsy, fixed and cryoprotected. Tissue was sectioned, stained for Nissl and processed with an antiserum that cross-reacts with tryptophan hydroxylase. All DRN neurons were identified, counted and analyzed every 1000 μm. Neuron morphometry was characterized by soma area (μm^2), sphericity, perimeter, length and density (neurons per mm^3).*

Results: *Neuron number and density was higher in suicide victims (1,780 ± 127 neurons/mm^3) than controls (1,349 ± 68). The DRN volume did not differ between groups (66 ± 9 mm^3 for controls vs. 67 ± 5 mm^3 for suicides). Mean neuronal area and sphericity did not differ between suicides and controls. The total number and the density of DRN neurons did not correlate with age.*

Conclusions: *The finding of an increased number of neurons indicates that impaired serotonergic transmission found in association with serious suicide attempts is not due to fewer neurons.* Biol Psychiatry 1999;46:473–483

Key Words: Suicide, mental illness, serotonin, brainstem, quantitation, neuromorphometry

Introduction

Suicidal behavior seems to be associated with a deficit in brain serotonin (5-HT) neurotransmission. A reduction in the concentration of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (CSF) of suicide attempters with major depression was first reported by Åsberg et al (1976). Subsequently, several studies (Ågren 1980, 1983; Åsberg et al 1976; Banki et al 1984; Brown et al 1982; Mann and Malone 1997; Ninan et al 1984; Roy et al 1986; Träskman et al 1981; van Praag 1983; Virkkunen et al 1989), though not all (Roy et al 1990) have confirmed the original observation. The serotonergic deficit seems to be related to the suicidal behavior and not to psychiatric illness (e.g., major depression) because serotonin indices are also reduced in suicides with schizophrenia (see Holden 1995; Mann et al 1996b; Stockmeier and Meltzer 1995) or personality disorder (Coccaro et al 1990; Montgomery 1987). Furthermore, lower CSF 5-HIAA concentrations seem to be more strongly associated with suicidal behavior than with major depression (Roy and Pollack 1994), and the degree of metabolite reduction is correlated with the lethality of the most lethal lifetime attempt (Mann and Malone 1997); patients who have made the most lethal attempts have the lowest concentrations of CSF 5-HIAA.

In postmortem brain tissue, presynaptic and postsynaptic serotonin receptor alterations have been observed in prefrontal cortex and brainstem that are consistent with reduced serotonergic function in suicide completers independent of diagnostic group (Mann et al 1989). Maximum binding to the serotonin transporter (B_{max}) is found to be reduced by some (Arango et al 1995; Arató et al 1987, 1991; Crow et al 1984; Joyce et al 1993; Laruelle et al 1993; Lawrence et al 1990b; Mann et al 1996a; Stanley et al 1982), but not all (Andersson et al 1992; Arora and Meltzer 1989a, 1991; Gross-Isseroff et al 1989; Hrdina et al 1993; Lawrence et al 1990a, 1990b; Meyerson et al 1982; Owen et al 1986) investigators, particularly in ventral and lateral prefrontal cortical regions. Increased binding to postsynaptic 5-HT_{1A} (Arango et al 1995; Matsubara et al 1991) and 5-HT_{2A} (Arango et al 1990; Arora and Meltzer 1989b; Hrdina et al 1993; Mann et al 1986; Stanley and Mann 1983) receptors in prefrontal cortex of suicide victims has been reported by many but not all (Cheetham et al 1990; Crow et al 1984; Dillon et al 1991; Gross-Isseroff et al 1990; Owen et al 1983) studies.

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Similarly, second messenger systems for transducing the 5-HT action on receptors also seem to be impaired in depressed suicide victims (Cowburn et al 1994; Pacheco et al 1996).

Taken together, the data suggest that reduced serotonergic input, perhaps particularly to the ventral prefrontal cortex, constitutes a critical element in the vulnerability to suicidal behavior, regardless of the associated psychiatric illness. Serotonin synthesizing neurons innervating the prefrontal cortex are located primarily in the dorsal and median raphe nuclei in the brainstem (e.g., Bobillier et al 1975; Conrad et al 1974; Pierce et al 1976; Sakai et al 1977). The finding of reduced serotonin transporter binding in the prefrontal cortex of suicide victims (Arango et al 1995; Arató et al 1987, 1991; Crow et al 1984; Joyce et al 1993; Laruelle et al 1993; Lawrence et al 1990b; Mann et al 1996a; Stanley et al 1982) raises the possibility that there is reduced serotonergic innervation. The functional capacity of serotonergic neurons may be reduced because of inadequate innervation of the target brain region or a reduction in the number of serotonin transporter sites synthesized. To date, however, there have been almost no direct studies of the serotonin synthesizing neurons in suicide victims. In the present study we therefore sought to estimate the total number and the morphometric characteristics of serotonergic neurons in the dorsal raphe nucleus (DRN) from suicide victims and a group of nonpsychiatric controls.

Methods and Materials

All procedures for collection of brain tissue were approved by the Institutional Review Board for Human Use Considerations of the University of Pittsburgh and the New York State Psychiatric Institute.

Subject Characteristics

Control subjects died suddenly of accidental death ($n = 2$) or natural causes ($n = 4$) and had no evidence of drug abuse, neuropathology or psychopathology based on psychological autopsies, toxicological screens, autopsy findings and data obtained by the Coroner's or Medical Examiner's staff from relatives, friends and other sources (Table 1). Control cases ($n = 6$) ranged in age from 17 to 74 years (38.5 ± 9.4 years) and had a male:female ratio of 4:2. A determination of suicide was made by the Coroner or Medical Examiner and verified by the investigators based on the available records. The method of suicide was hanging ($n = 3$), gun shot wound ($n = 2$), jumping ($n = 1$) and overdose ($n = 1$). The suicide group ($n = 7$) did not differ significantly in age from controls (14–84 years; 45.7 ± 9.9 years; $t = .52$; $p = .61$), and the male:female ratio was 5:2 (Table 1). The postmortem interval (PMI) for all cases was 16.5 ± 1.9 hours (range 12–24 hours) and PMI was not different between groups ($t = -.726$, $p = .48$).

Tissue Collection

Brainstems ($n = 13$) were collected at autopsy from the Pittsburgh Coroner's Office or the New York City Medical Examin-

Table 1. Comparison of Suicide Victims and Controls: Demographics and Serotonin Neuron Indices

	Sex[a] (M:F)	Age (yr)[b]	PMI[c]	Race[d] (W:B)	Cause of death[e]	Diagnosis[f]	DRN Length (mm)	DRN Volume (mm^3)	Neuron density ($\#/mm^3$)
Control group	Male	22	24	W	CV	None[g]	12	21	1,566
	Male	56	12.5	W	CV	None	11	77	1,193
	Male	43	17.5	B	CV	NA	22	92	1,266
	Male	19	14	W	Explosion	None	19	79	1,272
	Female	74	12	B	CV	NA	18	63	1,199
	Female	17	19	W	MVA	None	7	64	1,596
Mean SEM	**4:2**	**39**	**16.5**	**4:2**			**14.8**	**65.9**	**1,349**
		9	**1.7**				**2.1**	**9.1**	**68**
Suicide group	Male	84	20	W	GSW	NA	17	73	1,398
	Male	78	13	W	GSW	MD	21	60	1,798
	Female	34	4	W	Hanging	MD	7	57	1,611
	Male	23	16	B	Hanging	MD	19	60	1,917
	Female	43	12	W	Overdose[h]	MD	19	99	1,343
	Male	14	16	B	Hanging	MD	12	59	2,358
	Male	44	20	W	Jumped	SZ	16	62	2,036
Mean SEM	**5:2**	**46**	**14.4**	**5:2**			**15.9**	**67.1**	**1,780**
		9	**1.9**				**1.7**	**5.1**	**127**

[a]Sex: M, male; F, female.
[b]Age: age in years,values are mean ± SEM.
[c]PMI: postmortem interval (time from death to fixation in hours).
[d]Race: B: black; W: white.
[e]Cause of death: CV: cardiovascular; MVA: motor vehicle accident; GSW: gun shot wound.
[f]Diagnosis: DSM III-R (American Psychiatric Association Task Force 1987) Axis I diagnosis determined by psychological autopsy; NA: not available; MD: major depression; SZ: schizophrenia.
[g]Seizure disorder.
[h]Overdose by a mixture of diazepam, fluoxetine and acetamiophen.

er's Office. All subjects were free of gross neuropathology and had negative toxicology screens in blood, urine and bile for psychoactive and neurotoxic drugs. Only the suicide victim who died by overdose had a positive peripheral toxicological screen for diazepam and acetaminophen.

Upon removal of the brain from the cranium, the cerebellum was removed and the brainstem separated with a transverse cut at the anterior border of the superior colliculi. The brainstem was then fixed in formalin (10%) for two weeks. After initial fixation in formalin, the brainstem was manually sectioned into 3 cm blocks. Tissue blocks were then infiltrated for 5–7 days each in increasing concentrations (10–30%) of cryoprotectant sucrose in formalin. A final 5-day infiltration in 30% sucrose was done before freezing and storing tissue at −80°C. Blocks were then sectioned on a sliding microtome (Microm Model HM400, Heidelberg) and two 50 μm sections from every 1000 μm were used for this study (one for Nissl and one for PH8 immunostaining).

Immunocytochemistry

Serotonergic neurons cannot be identified in routine Nissl stained material, and serotonin and other catecholamine neurotransmitters dissipate or degrade rapidly after death and therefore, antibodies to neurotransmitters do not work well in human postmortem brain. Dr. Richard G.H. Cotton at the Royal Children's Hospital in Melbourne, Australia has produced and characterized antibodies to phenylalanine hydroxylase (PH8), that has substantial sequence homology to tryptophan hydroxylase. Manipulation of fixation and staining procedures can result in selective labeling of serotonin neurons in human postmortem tissue due to cross-reactivity (Törk et al 1992). The relative specificity of the phenylalanine hydroxylase antibody and cross-reactivity for tyrosine hydroxylase and tryptophan hydroxylase labeling in the raphe, ventrotegmental area and substantia nigra has been previously addressed (Haan et al 1987; Törk et al 1992).

Serotonin neurons were labeled according to the method and modifications of Törk et al (1992). The antibody was generously provided by Dr. Cotton. In brief, brainstems were fixed in formalin solutions and cryoprotected for not less than 5 weeks before sectioning. Incubations and labeling were performed on free floating sections. Sections were treated with Triton X-100 and incubation with the primary antibody was long (1 week) to enhance penetration of the antibody and improve the preservation of the resulting morphometry. The primary antibody was labeled using the avidin-biotin peroxidase method (Hsu et al 1981) with a Vecastain ABC kit (Vector Laboratories).

Counting

Neuron counting (and morphometry) was performed every 1 mm throughout the rostrocaudal extent of the DRN. The distributions were examined with respect to distance from the trochlear decussation, an anatomical landmark that could be located in each case and was therefore suitable as a zero reference point.

Neuron counting was performed using a camera lucida attached to a microscope (Leitz Laborlux). The suggested protocol for estimation of neuronal population by Konigsmark (1970) was followed. The essential features of the protocol include: 1) outlining the territory of the cells to be counted on each slide using the camera lucida and establishing quadrants referenced to the floor of the fourth ventricle, 2) counting all neurons within the defined territory and quadrant (A_1, A_2,. . . A_n, B_1, B_2,. . . B_n, etc.) and 3) calculation of the neuronal population with correction for split cells. In the present study, the boundaries of the DRN and subnuclei were identified at a magnification of 10.24× and defined with the microscope and camera lucida. The immuno labeled DRN neurons were defined at a total magnification of 256×. The formulae used for the calculation of the neuronal population and the correction factor for split cells can be found elsewhere (Konigsmark 1970).

The number of DRN neurons was determined using stereology in 7 cases selected at random to validate the estimates collected using the Abercrombie method. The number of neurons was determined using the fractionator method (Gundersen et al 1988; West 1993). The estimation of the number of DRN neurons compared favorably between approaches as did the coefficient of variation ("Konigsmark": 90,447 ± 41,273; fractionator: 93,839 ± 38,703). These findings suggest that our measurement method yield valid estimates of the total number of neurons.

Morphometry

The morphometric measurement of the size and shape of neurons was performed using video-based, computer-assisted image analysis. The video pixel dimension was calibrated using a microscope slide graticule to provide the linear distance in the x and y axes. All neurons with a clearly defined perimeter (e.g., not overlapping another neuron in a different focal plane) were analyzed. Each neuron was reviewed under the microscope, the video image of the microscope field captured and the neuron measured at an optical magnification of 200× with an additional electronic zoom of the digitized computer image of 4× for optimum clarity of the cell perimeter.

All morphologic measurements were made in a semi-automated fashion by the computer. Indices of size were (for each neuron): neuron area (μm^2), perimeter (μm), maximum chord/length (μm; defined as maximum internal straight length without crossing a cellular boundary) and diameter (μm; defined as the internal distance perpendicular to the curved chord). The measurement for shape was the neuron form factor (form factor = 4π (area)/perimeter^2). All sampling was done by personnel blind to the group assignment of the case.

Data Analysis

Data are presented as mean ± SEM. The aim of this analysis was to identify and quantify differences in DRN serotonergic neuron number and morphometry in controls and suicides. The estimated total number of neurons reported were obtained using the method of Konigsmark (1970). Therefore, neurons for each subject in the two groups were measured on several dimensions and subsequent formal statistical analyses were performed to detect differences in average measurements both within subjects and between groups. For a single neuron, the morphometric characterization comprised seven measurements: area, perimeter, maximum chord length, diameter, form factor, x-feret, and y-feret.

Correlation plots revealed that area, perimeter, maximum chord length, and diameter were highly correlated with each other within subjects; suggesting that these measures represent size of neuron. Therefore, area and perimeter were singled out for further analysis with covariates. Form factor, a dimensionless measure of sphericity, was not correlated with the other measurements and was used as an independent measure of neuron shape. Measurements of orientation, x-feret and y-feret, were not used in this analysis.

Comparisons of means between the suicide and control groups were done using Welch's modified two sample *t*-test. This method works reliably even when the standard deviations between the groups are substantially different. The effect of age on neuron density was assessed using an analysis of covariance, adjusting for group (suicide or control) and its interaction with age.

The effect on density of the interaction between subnucleus and group (i.e., the differential effect of group on density in different subnuclei), was assessed using SAS PROC MIXED (Littell et al 1996). Subject was the random factor in the model. A spatial covariance structure for subnuclear density was used in the model (see Chapter 9, Littell et al 1996) with the spatial arrangement of the subnuclei for covariance modeling purposes based on the relative anatomical locations of the subnuclei within the dorsal raphe nucleus.

The dorsal, ventral, ventrolateral, interfascicular and caudal DRN subnuclei were identified based on topographic and cytoarchitectonic characteristics. These subdivisions correspond to those observed by others in tissue similarly immunoreacted with anti-phenylalanine hydroxylase sera (Törk 1990; Törk and Hornung 1990). The ventral subnucleus extends from the anterior pole of the DRN to the rostral appearance of the median sulcus of the fourth ventricle, its cells densely packed and oriented parallel to the midline. Caudal to this point, all DRN neurons are on either side of the floor of the fourth ventricle and none are on the midline. The ventrolateral subnucleus contains predominantly small, multipolar neurons and extends caudally from the central gray to a position just dorsal to the trochlear nuclei. This subdivision has no midline component and extends further caudally than the median subnucleus in the rostral pons. The dorsal subnucleus has loosely arranged medium-sized neurons dorsomedially flanking the dense ventrolateral subgroup. The two wings of this subnucleus are joined in the midline. The caudal subnucleus is made of small to medium-sized neurons in 2 dense strips lateral to the midline and dorsal to the medial longitudinal fasciculus and parallel to the floor of the fourth ventricle.

Results

The distribution of labeled neurons in the DRN and its subnuclei corresponded to descriptions previously reported by others using either Nissl-stained material (Baker et al 1990) or the same antibody (Baker et al 1991; Törk and Hornung 1990; Törk et al 1992).

Most neurons are located in the rostral quarter of the DRN, largely comprising neurons of the dorsal subnucleus (Figures 1, 2), with many fewer neurons located in the caudal three quarters (Figure 2). In contrast to the variation in number of neurons, the density of neurons is more uniform throughout the rostrocaudal extent of the DRN (Figure 3) and the DRN subnuclei (Figure 4).

In controls ($n = 6$), the estimated mean total number of DRN immuno labeled neurons per case is 86,640 $\pm$ 11,081. The *entire* rostrocaudal extent of the DRN was not available in all cases, and therefore the total number of neurons was not available to be counted in all cases. We therefore compared the volume of the DRN and determined the density of neurons because determination of the average density at each level of the DRN does not require availability of all the DRN, and allows direct comparison between groups.

In controls, the DRN is 15 $\pm$ 2 mm in length and has a volume of 66 $\pm$ 9 mm^3. The average density of neurons within the entire DRN is 1,349 $\pm$ 68 neurons per mm^3 (Table 1). In the subnuclei, the range in density is nearly twofold that of the DRN as a whole. It is lowest in the dorsal (906 $\pm$ 433 neurons per mm^3) and caudal (873 $\pm$ 129 neurons per mm^3) subnuclei and highest in the ventral subnucleus with 1,525 $\pm$ 417 neurons per mm^3 (Figure 5).

In the whole DRN of suicide victims there are approximately 35% more labeled neurons compared to controls ($p < .05$). The average density of neurons (1,780 $\pm$ 127) in the whole DRN is also significantly greater in suicides than controls (Figure 4). The volume and length of the DRN in suicides is not different from controls ($p > .05$, Table 1). The difference in the distribution of neuron density between suicides and controls is most apparent in the middle two-thirds of the DRN (Figures 2 and 3). Analysis of the density of labeled neurons in individual subnuclei revealed that the difference in neuron density between controls and suicide victims did not reach statistical significance in the DRN subnuclei (Figure 4).

To determine whether age differences between the two groups or differences in the relationship between age and the number or density of DRN neurons are responsible for the group differences we determined whether neuron number or density is correlated with subject age within groups. No age-related decline in the density of DRN neurons was observed in either the control group or the suicide group (control: $r = -.74$, $p = .09$; suicide: $r = -.57$, $p = .18$). The two groups do not differ significantly ($p > .05$) with respect to mean age (469 vs. 399, $p > .05$, Table 1), suggesting that the group difference in neuron number or density is independent of age. Neither gender nor PMI had significant correlations with either the estimated total number of DRN neurons or the neuron density.

To determine if the difference in the density of DRN neurons is associated with morphometric alterations, we examined neuron size (area and perimeter) and shape (form factor) in both groups. Although mean neuronal size

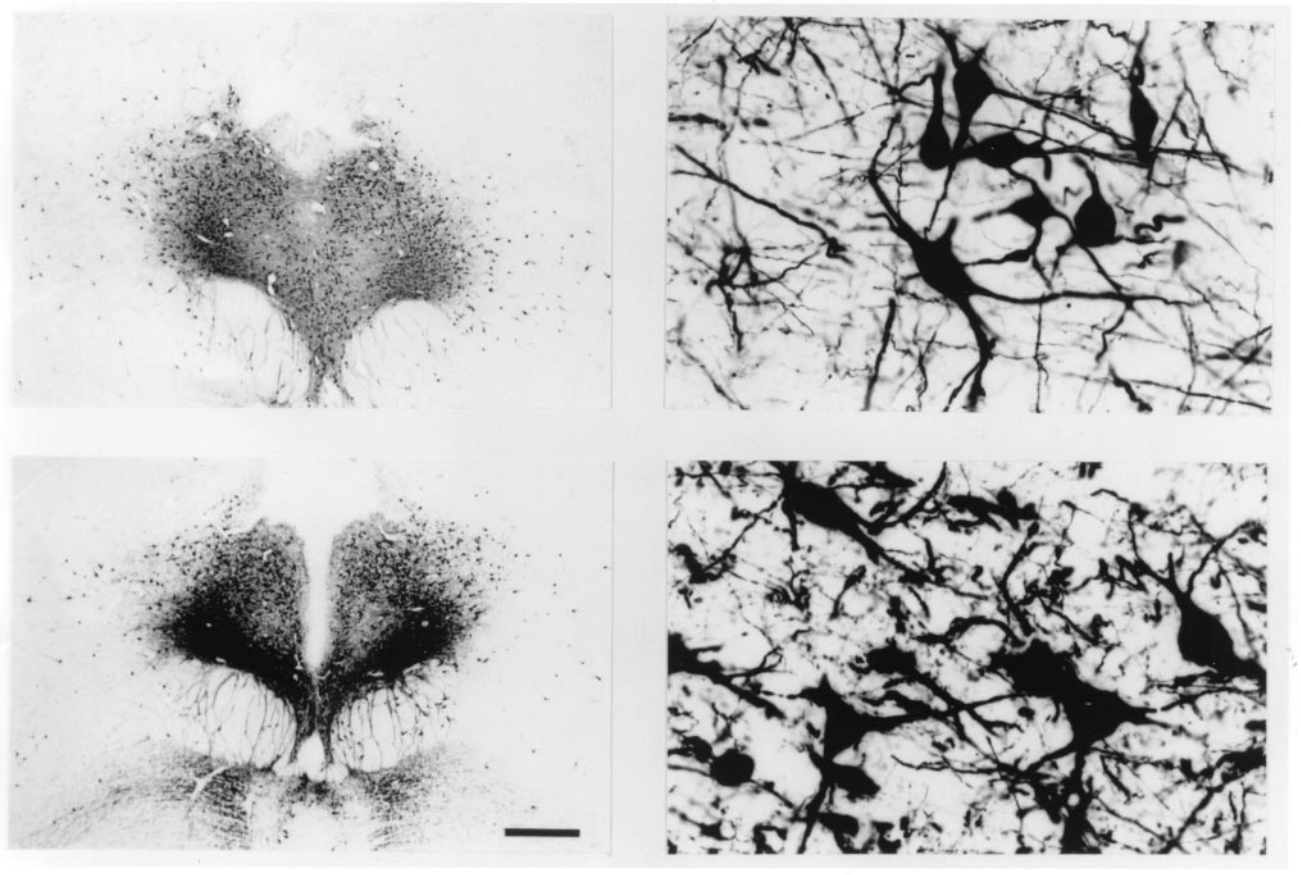

Figure 1. Low magnification (left , 16×) and high magnification (right, 400×) photomicrographs of brainstem sections at a rostral level of the dorsal raphe nucleus (DRN) from a representative control (upper left, upper right; 44 year old white male) and suicide victim (lower left, lower right; 56 year old white male). Serotonin synthesizing neurons were labeled by immunohistochemical staining with an antibody reacting with tryptophan hydroxylase. The high power photographs were taken in the dorsal subnucleus of the DRN. Note that the labeled neurons are confined to the DRN and that the morphology is well preserved. The difference in darkness of the reaction product between control cases and suicides is not an artifact. Six of the seven suicide cases had a more intense (darker) reaction product than the controls. The bar represents 1 mm in the left panels and 80 μm in the right panels.

was less in the suicides throughout the rostrocaudal extent of the DRN (Figure 5), statistically significant differences were not detected. Likewise, neither perimeter nor form factor differed significantly between controls and suicides in the entire DRN or in any of the individual subnuclei.

Discussion

We confirm the number and distribution of DRN serotonergic neurons in normal subjects reported elsewhere (Baker et al 1991; Baker et al 1996). Using a morphometric analysis, we found that suicide victims had 35% greater density and number of serotonin neurons in the dorsal raphe nucleus compared to non-suicide controls. The total volume of the DRN did not differ in the two groups suggesting that there is a difference in the absolute number in DRN neurons because we sampled a comparable volume of DRN. We do not find any difference in neuron size or shape between groups.

It seems that the increase in number and density of DRN neurons is due to the suicide status of the experimental group and not due to other factors. Differences in the volume of the DRN sampled cannot account for the differences in neuron number or density because an equal volume of DRN tissue was sampled in both groups. Furthermore, the two groups did not differ in the mean length of the DRN suggesting there was no sampling bias in the rostrocaudal axis. Moreover, the mean density of serotonergic neurons is also significantly greater in the suicide victims. The differences are not likely due to age differences between the two groups because the number of DRN neurons did not change significantly with increasing age and the two groups did not significantly differ in mean age. The literature on the effect of aging on the serotonin

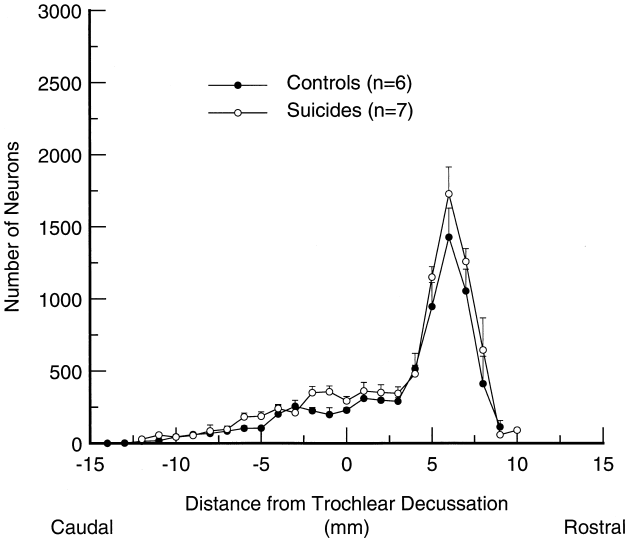

Figure 2. Distribution of the number of serotonergic neurons in the dorsal raphe nucleus (DRN) in suicide victims (open circles) and controls (closed circles). The number of neurons at each rostrocaudal level of the DRN includes neurons from all subnuclei present at that level. Note that suicide victims have more neurons, predominantly in the rostral portion of the DRN. Values for each point are mean ± SEM.

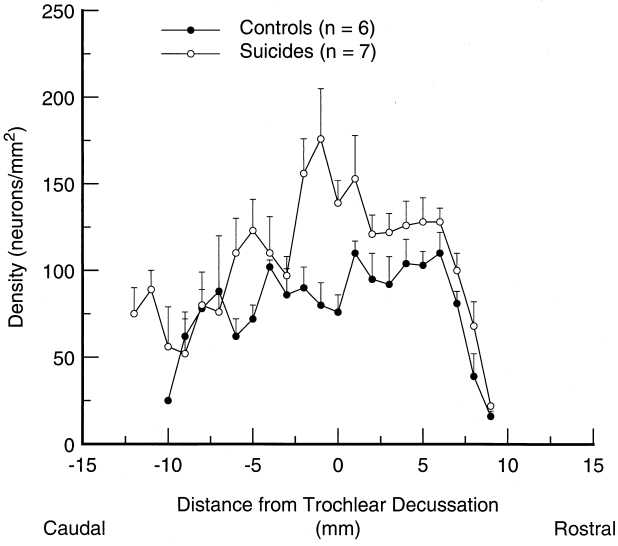

Figure 3. Distribution of the density of serotonergic dorsal raphe nucleus (DRN) neurons in suicide victims and controls. The density of neurons was determined by dividing the number of neurons by the cross-sectional area of the entire DRN. Note that suicide victims (open circles) have a higher density of serotonergic neurons throughout the rostrocaudal extent of the nucleus. Values for each point are mean ± SEM.

system in humans and animals is equivocal (see Amenta et al 1991; Conwell et al 1995; McEntee and Crook 1991 for review). In the event that we did not have enough cases to detect an age-related decline, the group differences could not be attributed to the effect of aging because the suicide group did not differ in age from the control group. In fact, all the suicide cases except one had more DRN neurons than any of the controls.

The findings cannot be explained by the effects of drugs or medications. Drugs such as fenfluramine (e.g., Appel et al 1990; Kleven and Seiden 1989; Molliver and Molliver 1990; Sotelo 1991) or methylenedioxymethamphetamine (MDMA) (Colado et al 1993; De Souza et al 1990; Fischer et al 1995; Johnson and Nichols 1989; Ricaurte et al 1988) are recognized to potentially be toxic to serotonin neurons. It is therefore possible that the presence of drugs could affect the number of neurons. The presence of drugs cannot account for the differences in neuron number in our study because only one of the controls (carbon monoxide) and two of the suicides (carbon monoxide, benzodiazepines) had a positive toxicological screen. Moreover, no evidence of drug abuse was present in any of the cases.

The possibility of a counting bias is unlikely because we validated our method of estimating neuron number using the unbiased stereologic fractionator method (Gundersen et al 1988). Neuron counting and measurements were made by personnel blind to the group assignment. The findings are not due to group differences in gender, race or postmortem interval because these variables were similar in distribution or did not differ between groups.

Finding an increase in the number and density of DRN neurons in suicide victims was the opposite of what we had predicted. On the basis of CSF and brainstem neurochemical findings (see above), we had hypothesized that reduced serotonergic activity would be associated with fewer serotonergic neurons in the DRN.

Serotonin (5-HT) has long been implicated in suicide and depression. Most early studies implicating brainstem serotonergic neurons found reduced concentrations of 5-HT (Pare et al 1969; Shaw et al 1967) or 5-HIAA (Bourne et al 1968) in whole brainstems in suicide victims. The DRN was more specifically examined by Lloyd et al (1974) who found reduced amounts of 5-HT but not 5-HIAA in dissected tissue. The amounts of 5-HT or 5-HIAA were not different between suicides and controls in other raphe nuclei, with the exception of the median raphe where 5-HT was also reduced. The amount of serotonin transporter mRNA in a group of depressed suicide victims was not found to be different from controls in one study (Little et al 1997). To our knowledge, no other study has replicated the finding or otherwise specifically examined morphometrically the serotonin neurons in the raphe nuclei in suicide victims.

Studies of receptors for 5-HT support the hypothesis of reduced serotonergic function in suicide. The serotonin transporter sites located on presynaptic serotonergic axon

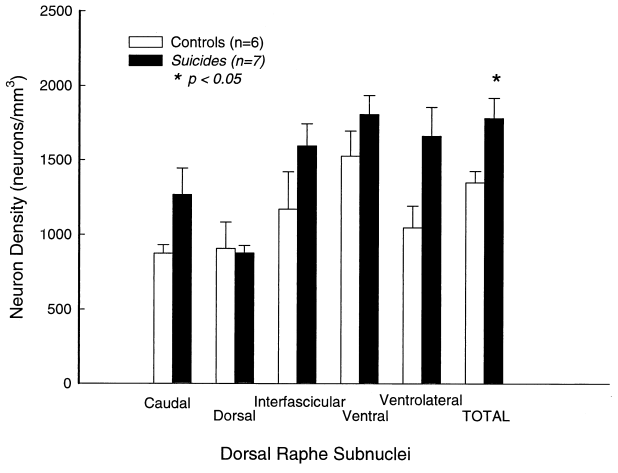

Figure 4. Density of serotonergic neurons in the dorsal raphe nucleus and each of the five subnuclei in suicide victims (filled bars) and controls (open bars). Note that the average density of serotonergic neurons in the entire DRN is significantly greater in suicides than controls. The density in individual subnuclei did not differ significantly between groups. Values are mean ± SEM.

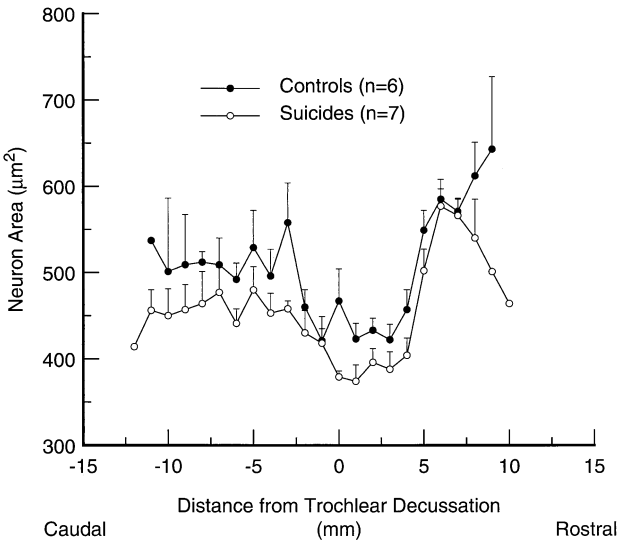

Figure 5. Area of dorsal raphe nucleus neurons in controls (closed circles) and suicides (open circles). All neurons at each rostrocaudal level were averaged to yield the average size of the neuron in each brain. Note that suicide victims have smaller neurons throughout the nucleus, with the most pronounced difference in the caudal two-thirds portion. Values for each point are mean ± SEM.

terminals have been found in some studies to be reduced in the prefrontal or temporal cortex of suicide victims compared to controls (Arango et al 1995; Arató et al 1987, 1991; Crow et al 1984; Joyce et al 1993; Laruelle et al 1993; Lawrence et al 1990b; Mann et al 1996a; Stanley et al 1982). The density of 5-HT_{1A} and $5HT_{2A}$ receptors, postsynaptic in cerebral cortex, have been found by some investigators to be elevated in prefrontal cortex in suicide victims compared to controls, both in homogenates and by autoradiography (Arango et al 1990; Arango et al 1995; Arora and Meltzer 1989b; Hrdina et al 1993; Mann et al 1986; Matsubara et al 1991; Stanley and Mann 1983; Yates et al 1990). Taken together, these studies suggest reduced serotonergic input to prefrontal cortical neurons with possible compensatory upregulation of postsynaptic receptors.

Serotonergic innervation of the prefrontal cortex arises from neurons located predominantly in the dorsal and median raphe nuclei in rodents (Bobillier et al 1975; Conrad et al 1974; Pierce et al 1976; Sakai et al 1977), non-human primates (Wilson and Molliver 1991a, 1991b) and presumably humans. The finding of reduced 5-HT in the dorsal (and median) raphe nuclei (Lloyd et al 1974) and receptor changes in prefrontal cortex in suicide victims led us to hypothesize that the reduced 5-HT indices may be due to fewer 5-HT synthesizing neurons in the DRN in suicide victims. In the present study, however, we found *more 5-HT neurons* and a *greater density of 5-HT neurons* in a group of suicide victims compared to controls. Other studies have found fewer DRN neurons in diseases such as Alzheimer's (Aletrino et al 1992) and alcoholism (Halliday et al 1993).

One possible explanation of the increased number of 5-HT neurons in suicide victims is abnormal neurodevelopment. In our study, suicide victims had a greater density of DRN 5-HT neurons across the lifespan with each group having a similar trend toward an age-related rate of decline. The observation of a greater density of 5-HT neurons in the suicide group in even our youngest cases further raises the possibility that are greater numbers of serotonin neurons even at a young age in suicide victims. An increased number of neurons may represent a biological predisposition to suicide risk.

The cause of this possible neurodevelopmental abnormality may be genetic. A genetic component has been identified in suicide. Studies in twins (Schulsinger et al 1979; Wender et al 1986) have demonstrated an increased suicide rate in monozygotic relative to zygote twins, regardless of like or different parentage. Genetic linkage studies have similarly shown that suicide can run in families (Egeland and Sussex 1985). Serotonergic indices have been shown in non-human primates to have a strong genetic contribution. The offspring of monkeys with either high or low amounts of 5-HT tend to have amounts similar to the parents (Clarke et al 1995; Higley et al 1993). Rearing practices and early life stress affects serotonergic function in an enduring fashion (Higley et al 1993; Kraemer 1997). Peer raised monkeys reset serotonergic

function at a lower level and this effect persists into adult life. Such an effect could be due to reduced function of existing neurons rather than cell loss. Suicidal behavior is associated with a history of child abuse. It seems improbable that adverse rearing could result in more neurons. Therefore, it is more probable that our observation is the result of genetic or other intrauterine developmental effects. Lower serotonergic function is also associated with externally-directed aggression, and that such aggression may share a common neurochemical substrate with suicide. As such it is of note that aggression in youth is associated with excessive serotonergic activity (Pine et al 1997; Pine et al 1996; Castellanos et al 1994; Halperin et al 1994) and consistent with an increased number of serotonergic neurons. Perhaps adulthood is characterized by the persistence of more serotonin neurons but a loss of function as suggested by low CSF 5-HIAA and the neuroreceptor changes reported in suicide victims.

Regarding neuron function, it must be remembered that the weight of other evidence of serotonergic indices, such as from receptor binding studies postmortem or CSF levels of 5-HT or 5-HIAA in suicide attempters, suggest reduced serotonergic function in suicide. In light of our finding of a greater number and density of serotonergic neurons in suicide victims, we suggest that the 5-HT neurons have a reduced functional capacity. One possible scenario is that during development, the normal apoptotic mechanisms do not occur, resulting in an increased number of 5-HT neurons. Alternatively, reduced serotonergic "capacity" or innervation of target neurons may be "incomplete," resulting in a compensatory reduced rate of serotonergic neuron death by some as yet unknown mechanism. In a rat model of depression employing learned helplessness, an increase in mRNA for the 5-HT_{1B} receptor was found in the dorsal raphe nucleus, raising the possibility that serotonin neurons might have reduced function due to enhanced autoinhibitory activity mediated by the 5-HT_{1B} receptor in the DRN (Neumaier et al 1997).

The anatomy of the differences in the 5-HT neuron density in suicides must also be considered. The DRN neuron density in the suicide group was not significantly different from controls in any of the individual DRN subnuclei. This may reflect a lack of statistical power for subnuclear group measurements. If correct, a broadly distributed reduction would suggest that the difference between the groups is not anatomically restricted. The mean density of 5-HT neurons in the dorsal subnucleus, that contains the largest number of serotonergic neurons was nearly identical between the two groups, suggesting that this subnucleus is not contributing to the differences between groups. The observation of a widespread alteration is no doubt of functional significance as well. The widespread difference is, however, in contrast with receptor binding studies that have found localized changes in prefrontal cortex. Given the many functions in which serotonin is believed to play a role (e.g., feeding, sleep, learning and memory, gender), it is perhaps not surprising to find a widespread defect in the DRN of suicide victims who often exhibit a multitude of symptoms that can accompany the major depression or other psychiatric illnesses.

Research data supportive of the hypothesis of serotonin deficiency in suicide has been remarkably consistent. The observation of more serotonergic neurons in the dorsal raphe nucleus in suicide victims made in this pilot study is counterintuitive to the serotonin deficiency hypothesis. The preliminary finding of more DRN neurons in suicide victims than normal controls may present the first evidence, in postmortem brain tissue, of a neurodevelopmental defect associated with suicide behavior and perhaps suicide risk.

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