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Circumscribed numerical deficit of dorsal raphe neurons in mood disorders

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ABSTRACT

Background. Neurocircuits comprising limbic, striato–pallidal and thalamo–cortical brain areas are assumed to be involved in the pathophysiology of mood disorders. All these brain regions receive serotonergic afferents arising from the rostral raphe, mainly the dorsal raphe. Although serotonergic systems appear to be involved in the pathology of mood disorders, there is uncertainty as to whether structural alterations in raphe nuclei exist alongside a functional dysregulation of the serotonergic system.

Methods. In the brains of 12 patients with mood disorders (major depressive disorder $N = 6$, bipolar disorder $N = 6$) and 12 normal subjects we performed a morphometric post-mortem study on neuronal morphology in all subnuclei of the dorsal raphe nucleus using Nissl stained 20 μm axial serial sections of the brainstem.

Results. The number of neurones of the ventrolateral subnucleus of the dorsal raphe was reduced by 31% in patients with mood disorders compared with non-psychiatric control subjects. Ventrally located subnuclei of the rostral dorsal raphe (ventrolateral, ventral, interfascicular) taken together also showed a smaller number of neurones. Neurone numbers of the dorsal and the caudal subnucleus and volumes of all single subnuclei appeared to be unchanged. Analysis of morphological neuronal types revealed a smaller number of triangular neurones in the ventrolateral subnucleus. Numbers of ovoid and round neurones in the ventrolateral subnucleus also showed a trend to reduction. No correlation was found between neurone numbers in any subnucleus of the dorsal raphe and duration of illness. Neurone numbers did not differ in any subnucleus between patients with unipolar and those with bipolar affective disorder.

Conclusions. Results indicate that patients with primary mood disorders have a circumscribed numerical neuronal deficiency in the dorsal raphe. This structural deviation may contribute to impaired serotonergic innervation of brain regions which are involved in the pathology of mood disorders.

INTRODUCTION

In recent years, several morphological studies on mood disorders indicated structural alterations in limbic–striatal–pallidal–thalamo–cortical loops with a focus in prefrontal cortical areas and striatal–pallidal basal ganglia (for review see Soares & Mann, 1997; Baumann & Bogerts, 1999). Less attention has been paid to brainstem nuclei, which give rise to efferents targeting many structural components of these networks. Together with the median raphe, the dorsal raphe (DR) provides the main source of ascending serotonergic innervation virtually of the whole brain (Wiklund *et al*. 1981). DR lesions have been shown to result in a significant

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reduction of serotonin content in the medial prefrontal cortex (PFC) and the nucleus accumbens (Morrow & Roth, 1996). These brain regions have not only been implicated in neuroanatomical models of depression (Baumann & Bogerts, 1999), it has also been shown in animal models that decreased serotonin release in the nucleus accumbens and the prefrontal cortex is associated with depression-like symptoms (Jaffe *et al*. 1993). Moreover, fewer binding sites for the serotonin transporter recently have been demonstrated in orbital and ventral regions of the PFC in depressed suicides (Mann *et al*. 2000), and this observation can be interpreted as a compensation for a deficit of serotonin secretion in these brain regions. Reports of reduced neuronal size in orbital prefrontal areas can be seen in parallel to this finding since reportedly they are pronounced in the cortical lamina II (Rajkowska *et al*. 1999). Lamina II shows a high density of pre- and post-synaptic serotonergic receptors, which are changed in depressed suicides (Arango *et al*. 1995). Taken together, these data indicate an alteration of serotonergic input into prefrontal regions and it is conceivable that diminished serotonergic afferents arising from the DR contribute to structural and functional disturbances in the prefrontal serotonergic system. This assumption is in keeping with neuroimaging data of decreased serotonin receptor 1A binding in multiple brain regions of depressed individuals with most prominent reductions in the midbrain raphe. The pattern of these changes suggests histopathological changes involving rostral raphe nuclei (Drevets *et al*. 1999). Reports of altered ultrasound echogenicity of the midbrain raphe in patients with primary or secondary depression (Becker *et al*. 1995, 1997) also suggest an association between pathomorphology of raphe nuclei and mood disorders.

Recently, we have demonstrated a volumetric deficit in striatal–pallidal basal ganglia, which appears to be most pronounced in the nucleus accumbens (Baumann *et al*. 1999*a*). Beside other monaminergic afferents, serotonergic innervation of basal ganglia may be disturbed and this may be causally related to structural changes in these nuclei. Thus, it is conceivable that a pathomorphology of the DR contributes to alterations in brain regions that may be relevant to mood disorders.

In this study we performed a morphometric analysis of the DR neurones in post-mortem brains of non-elderly patients with mood disorders. The DR, in contrast to the median raphe, shows a topographic organization. Efferents and afferents of the DR reflect a pattern that follows a regional distinction between the dorsal wings and the central, i.e. ventral part of the fountain-like shaped figure of the rostral DR in sections perpendicular to the longitudinal axis (Peyron *et al*. 1998; Janusonis *et al*. 1999). Neuronal projections to the PFC and combined projections to the nucleus accumbens and the PFC arise predominantly from ventral and rostral subnuclei of the DR (O'Hearn & Molliver, 1984; Wilson & Molliver, 1991; Kazakov *et al*. 1993; Van Bockstaele *et al*. 1993). A ventral *versus* dorsal pattern in the rostral part of the dorsal raphe also is indicated from neurochemical and physiological investigations (McLaughlin *et al*. 1996; Kakeyama *et al*. 1997; Peyron *et al*. 1998). We hypothesized that a structural deficit exists in the ventral tier of the DR. Such a deficit could contribute to the above mentioned changes in ventral striatal and prefrontal regions.

METHOD

Subjects and tissue samples

Brains of 12 human subjects (3 males, 9 females) suffering from mood disorders according to DSM-III-R (major depression $N = 6$, bipolar disorder $N = 6$) and of 12 sex-matched control individuals without a history of neuropsychiatric disorder were investigated. Six subjects with mood disorders died of natural causes, the other six were suicide victims. This study was performed in compliance with policies of the ethic commission of the University of Magdeburg and written consent was obtained from the next-of-kin. Information for clinical diagnoses was obtained by the careful study of clinical records and by structured interviews of persons who either lived with, or had frequently contact with, the subjects before death. Structured interviews were carried out to collect information to determine the presence or absence of psychiatric disorders. Data on lifetime and current mental illness were gathered with a Lifetime version of a Schedule for Affective Disorders and Schizophrenia (Spitzer & Endicott 1978). Final Axis I

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Table 1. *Characteristics of patients and controls*

No./Sex/Age (yrs)	Fresh brain weight (g)	Post-mortem delay (h)	Cause of death	Illness duration (yrs)	AXIS I Diagnosis (DSM-III-R)
Patients					
1/f/53	1280	47	Suicide by electrocution	2	MD 296.33
2/m/47	1670	24	Suicide by stabbing	9	BDD 296.53
3/f/39	1400	48	Suicide by overdose of medication	n.a.	MD 296.23
4/f/46	1410	48	Suicide by hanging	n.a.	MD 296.24
5/f/46	1300	4	Suicide by overdose of medication	13	BDD 296.54
6/f/63	1120	17	Pulmonary embolism	n.a.	MD 296.34
7/m/42	1320	17	Suicide by hanging	16	BDD 296.54
8/f/62	1300	72	Pulmonary embolism	11	BDD 296.54
9/f/60	1140	15	Bronchopneumonia	10	BDD 296.54
10/m/39	1520	14	Pulmonary embolism	1·5	BDM 296.62
11/f/61	1240	70	Bronchopneumonia	11	MD 296.34
12/f/41	1210	20	Pulmonary embolism	2	MD 296.34
Mean ± S.D./ 49·9 ± 9·4 yrs	1326 ± 157	33·0 ± 32·0		8·4 ± 5·3	
Controls					
1/f/48	1170	48	Acute asthmatic attack		None
2/m/40	1550	70	Myocardial infarction		None
3/m/64	1310	35	Cardiac insufficiency		None
4/f/48	1200	26	Pulmonary embolism		None
5/f/65	1100	24	Cardiac insufficiency		None
6/f/30	1500	48	Pulmonary embolism		None
7/m/63	1200	48	Cardiac insufficiency		None
8/f/38	1200	24	Pulmonary embolism		None
9/f/61	1350	24	Cardiac insufficiency		None
10/f/67	1210	25	Sudden cardiac death		None
11/f/63	1050	24	Sudden cardiac death		None
12/f/39	1210	24	Heat stroke after sun exposure		None
Mean ± S.D./ 52·2 ± 13·1	1254 ± 149	37·1 ± 21·3			

MD, Major depression; BDD, bipolar disorder depressed; BDM, bipolar disorder mixed.

Table 2. *Psychotropic drugs in the last* 90 *days of life* (*mean daily doses*)

Patient No.	Antidepressants* (amitriptyline equivalents)	Neuroleptics† (chlorpromazine equivalents)	Benzodiazepines (diazepam equivalents)	Lithium (mg)
1	67	0	0	0
2	93	0	3·1	560
3	10	0	0	0
4	124	109	0	0
5	133	327	3·3	558
6	50	0	0	0
7	95	47	18·3	565
8	0	110	17·6	0
9	52	109	10·9	0
10	0	280	0	0
11	30	111	16·5	0
12	17	501	0	0

* All subjects treated with antidepressants received tricyclics except for case 3 (maprotiline) and case 11 (viloxazine).
† None of the patients received atypical neuroleptics.

diagnoses were assigned in consensus meetings of two psychiatrists using all available information from informants and clinical charts. The final diagnosis was compatible with the Diagnostic and Statistic Manual of Mental Disorders-Revised (DSM-III-R; American Psychiatric Association, 1987). In the same way, neuropsychiatric disorders were excluded in the

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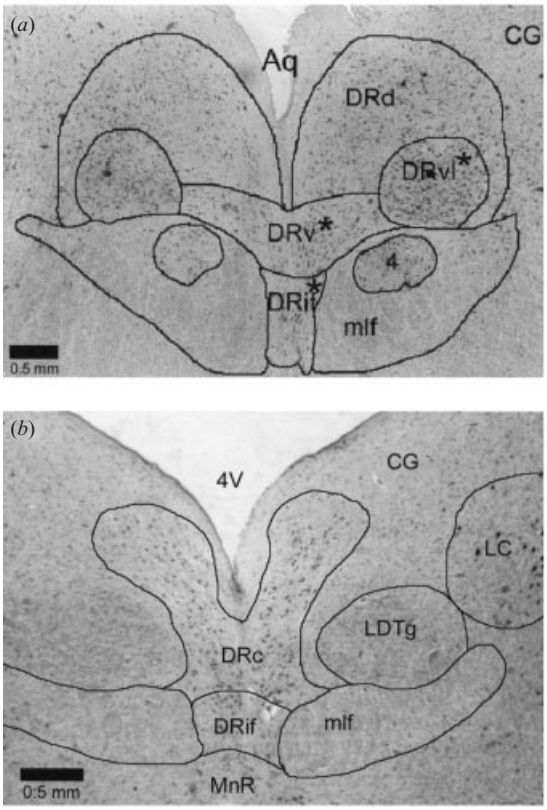


FIG. 1. Sections showing the dorsal raphe at the level of the trochlear nucleus (A) and the rostral locus coeruleus (B) (cresyl-violet). *Ventrally located subnuclei in which a numeric deficit of neurones was determined in patients with mood disorders. DRif, interfascicular; DRv, ventral; DRvl, ventrolateral; DRd, dorsal; DRc, caudal subnucleus of the dorsal raphe; 4, trochlear nucleus; 4V, fourth ventricle; mlf, medial longitudinal fasciculus; CG, central grey matter; Aq, cerebral aqueduct; LDTg, lateral dorsal tegmental nucleus; LC, locus coeruleus; MnR, median raphe.

control subjects. There was no current or lifetime psychoactive substance disorder (abuse or dependence according to DSM-III-R) in any of the subjects. Qualitative neuropathological changes due to neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Pick's disease), tumours, inflammatory, vascular or traumatic processes were ruled out by an experienced neuropathologist. For demographical, histological, and clinical data see Table 1 and 2.

Histology

Brains were removed within 4 to 72 hours after death and processed as described previously (Baumann *et al.* 1999*b*). The brainstem was isolated by a transverse cut along a line from the rostral border of the superior colliculus to the exit point of the oculomotor nerve. A second transverse cut was performed at the caudal level of the medulla. After embedding in paraffin, serial 20 μm thick transverse sections were cut along the whole rostral–caudal axis of the brainstem and mounted. Every 50th section was Nissl-(cresyl-violet-) stained for determination of total neurone numbers. Thus, distance between the sampled sections was 1 mm.

Morphometry

A computerized image analysis system (Digitrace®; Imatec, Germany) was used, it was attached to a high resolution videocamera on a Leica microscope. The microscope was equipped with a motorized scanning-stage. For identification and delineation of DR subnuclei, that is the interfascicular, ventral, ventrolateral, dorsal and caudal subnucleus, panorama images of cross sectional areas were created by composing microscopic images serially taken at higher magnification using a $\times 5$ objective. The composed image then was adapted for size of the videoscreen. Evaluation of images at higher magnification as well as on reduced panoramic images showing the entire cross-sectional area of the DR allowed for delineation of subnuclei as well as classification of morphological neurone types following the cytoarchitectural and cellular description of the DR given by Baker and colleagues (1990) (Figs. 1, 2). Classification of neurone types, i.e. round, ovoid, fusiform and triangular neurones, was based on neuronal shape, size and staining characteristics. Neurones were determined as round from shape and light staining of Nissl substance. Their mean area was 372 ± 62 μm^2. Neurones were counted as ovoid primarily according to their shape. Since differentiation of round and ovoid neurones sometimes proved to be unsure, an additional numerical algorithm was used by means of a form factor (4π (area/perimeter^2)). This form factor would be 1 for a perfect circle, while it would approach 0 for long, thin objects. All neurones which appeared as round or ovoid from morphological criteria were categorized as round if the form factor was $> 0{\cdot}95$. Fusiform neurones were determined by shape, weak-staining and dorso-ventral orientation of the elongated fusiform neurones, and occurence of

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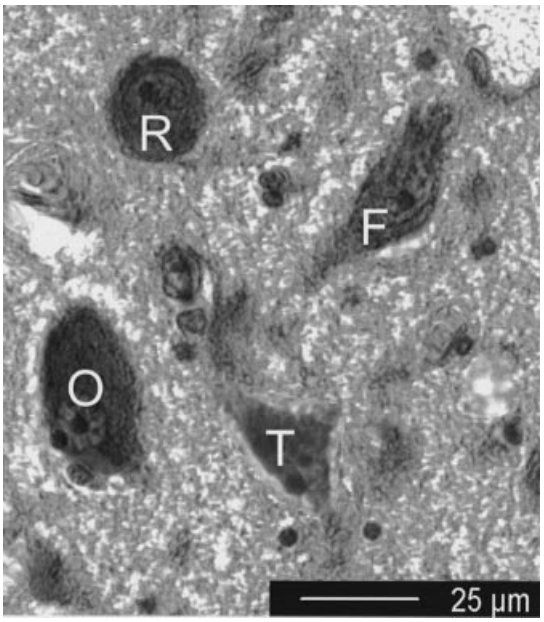


FIG. 2. Types of neurones in the dorsal raphe. R, round; O, ovoid; F, fusiform; T, triangular.

lipofuchsin pigment in the typical fusiform neurones. Triangular neurones were determined by shape, small size (mean area $234 \pm 59\ \mu m^2$) and dark-staining. In contrast to Baker and colleagues we counted all ovoid neurones which were clearly distinguishable from glial cell nuclei.

Glia cells were distinguished from neurones on the basis of their smaller size, the absence of cytoplasm, a darker nuclear membrane, and the presence of a dispersed pattern of nuclear chromatin (Selemon *et al.* 1999).

Like Baker and colleagues (1990) we too identified the rostral pole of the DR in the midbrain at the level of the caudal pole of the oculomotor complex. Neurones of the DR showed darkly-stained Nissl substance and often had eccentrically located nuclei. The midbrain portion of the DR including the B7 group of serotonergic neurones (Dahlström & Fuxe, 1964) contained the interfascicular, ventral, ventrolateral and dorsal subnucleus extending to 1 mm rostral to the level of the isthmus. The caudal subnucleus of the DR was essentially present in the pontine brainstem reaching from 1 mm rostral to the isthmus to the level of the motor trigeminal nucleus in the pons. The caudal pole of the interfascicular nucleus extended to the rostral pons at the level of the rostral border of the locus coeruleus and here it was located ventral to the rostral pole of the caudal subnucleus of the DR and dorsal to the median raphe nucleus. The caudal subnucleus and caudal parts of the interfascicular subnucleus contribute to the B6 group of serotonergic neurones ((Dahlström & Fuxe, 1964). For rostro-caudal identification of corresponding levels between subjects, all sections were referred to the isthmus as an anatomical landmark.

Volumes of all subnuclei were calculated from areas measured for all sections on the video-screen performing morphometrical operations previously described in detail (Bogerts *et al.* 1990). In each section, neurones of the DR were counted by the optical dissector method using a $\times 100$ objective in dissector fields, which were selected using systematic random sampling along the whole cross sectional area of the DR (West, 1993; Coggeshall & Lekan, 1996). Application of the optical dissector made it necessary to measure movements in the *z*-axis, which was performed by aid of a microcator as an integral part of the microscope. Only neurones were counted, in which a nucleolus was clearly visible.

Measurements of volumes and neurone counts were performed by one of the authors (H.B.) blind for the diagnosis. To establish inter-rater (H.B., B.Ba.) and test–retest reliability, repeated measurements for five brains were carried out. Intraclass correlation analyses yielded highly corresponding coefficients for neurone numbers and volumes in all subnuclei with $r = 0{\cdot}87$ to $0{\cdot}95$. Inter-rater reliability for different neuronal types was in the range $r = 0{\cdot}91$ to $0{\cdot}94$.

Statistical analyses

A single-factor analysis of variance (ANOVA) was performed using diagnosis as independent variable. According to the hypothesis of circumscribed structural changes in the DR of patients with mood disorders, neurone number and volume was analysed as dependent variable in the interfascicular, ventral, ventrolateral, dorsal and caudal subnucleus of the DR. Additionally a repeated measures analysis of variance was performed with group and rostro–caudal level as subjects factors (repeated measures). Pearson correlation coefficients were calculated to determine the influence of variables such as brain weight, age at time of death, post-mortem delay, duration of illness or psychiatric medication,

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which might confound results of neurone numbers. P values $< 0{\cdot}05$ were deemed to be statistically significant. Because of the exploratory character of this study no Bonferroni correction was applied to multiple comparisons of raphe nuclei since this approach would be a too conservative.

RESULTS

Neurone numbers and volumes of the dorsal (DRd), the caudal (DRc), the ventral (DRv) and the interfascicular subnucleus (DRif) of the dorsal raphe did not differ between patients with mood disorders and controls. Neurone numbers and volumes in the entire dorsal raphe were unchanged between groups. The ventrolateral subnucleus of the DR (DRvl), however, showed 31% smaller number of neurones in patients compared to controls (Table 3, 4). Due to common projection patterns, the ventrally located subnuclei of the rostral DR, i.e. the interfascicular, the ventral and the ventrolateral subnucleus, were analysed as a complex (DRvc). The DRvc showed a smaller number of neurones and a smaller volume in patients compared to controls ($P = 0{\cdot}01$, $P = 0{\cdot}04$, respectively) (Table 3, 4). An ANOVA with three groups comprising bipolar, unipolar and control individuals also yielded a significant result for the DRvc ($P = 0{\cdot}038$). *Post hoc* least-significant-difference tests revealed a significant reduction of neurone numbers in this area for bipolar patients as compared to controls ($P = 0{\cdot}023$) and a trend for reduction in unipolar patients as compared to controls ($P = 0{\cdot}088$). Neurone numbers did not differ between bipolar and unipolar patients in any subnucleus of the DR (all $P > 0{\cdot}05$). Moreover, neurone numbers were unchanged in each subnucleus between patients who died from suicide and those who died from natural causes (all $P > 0{\cdot}05$). Thus, similar alterations of neurone numbers are found in the ventral tier of the DR of unipolar and bipolar patients as well as of suicide and non-suicide patients.

ANOVA for repeated measures (group, level) yielded a significant main effect of subject group

Table 3. *Neurone numbers in the dorsal raphe nucleus*

Region	Patients ($N = 12$) Mean ± S.D.	Controls ($N = 12$) Mean ± S.D.	Change P *v.* C %	P (ANOVA)
DRif	30654 ± 7894	34972 ± 9637	−12·3	0·24
DRvl	30039 ± 11984	43607 ± 18337	−31·1	0·04*
DRv	37390 ± 7525	42117 ± 10596	−11·2	0·22
DRd	168780 ± 52191	164058 ± 59590	+2·9	0·84
DRc	83092 ± 33900	65370 ± 34595	+27·1	0·22
DRvc	98083 ± 16392	120697 ± 22728	−18·7	0·01*
DR	349955 ± 72963	350125 ± 79396	−0·05	0·99

DRif/DRvl/DRv/DRd/DRc, interfascicular/ventrolateral/ventral/dorsal/caudal subnucleus; DRvc, interfascicular + ventral + ventrolateral subnucleus; DR, entire dorsal raphe; P, patients; C, controls.
* Significant group difference.

Table 4. *Volumes of the dorsal raphe nucleus* (mm^3)

Region	Patients ($N = 12$) Mean ± S.D.	Controls ($N = 12$) Mean ± S.D.	Change P *v.* C %	P (ANOVA)
DRif	4·73 ± 1·17	5·61 ± 2·32	−15·7	0·25
DRvl	4·29 ± 1·26	5·86 ± 2·85	−26·8	0·11
DRv	7·93 ± 2·59	9·06 ± 3·36	−12·4	0·36
DRd	39·14 ± 11·70	39·85 ± 14·43	−1·8	0·90
DRc	18·14 ± 6·30	14·74 ± 8·51	+23·1	0·28
DRvc	16·60 ± 3·78	20·55 ± 5·08	−19·3	0·04*
DR	73·86 ± 17·04	75·14 ± 16·91	−1·7	0·86

For abbreviations see footnote to Table 3.

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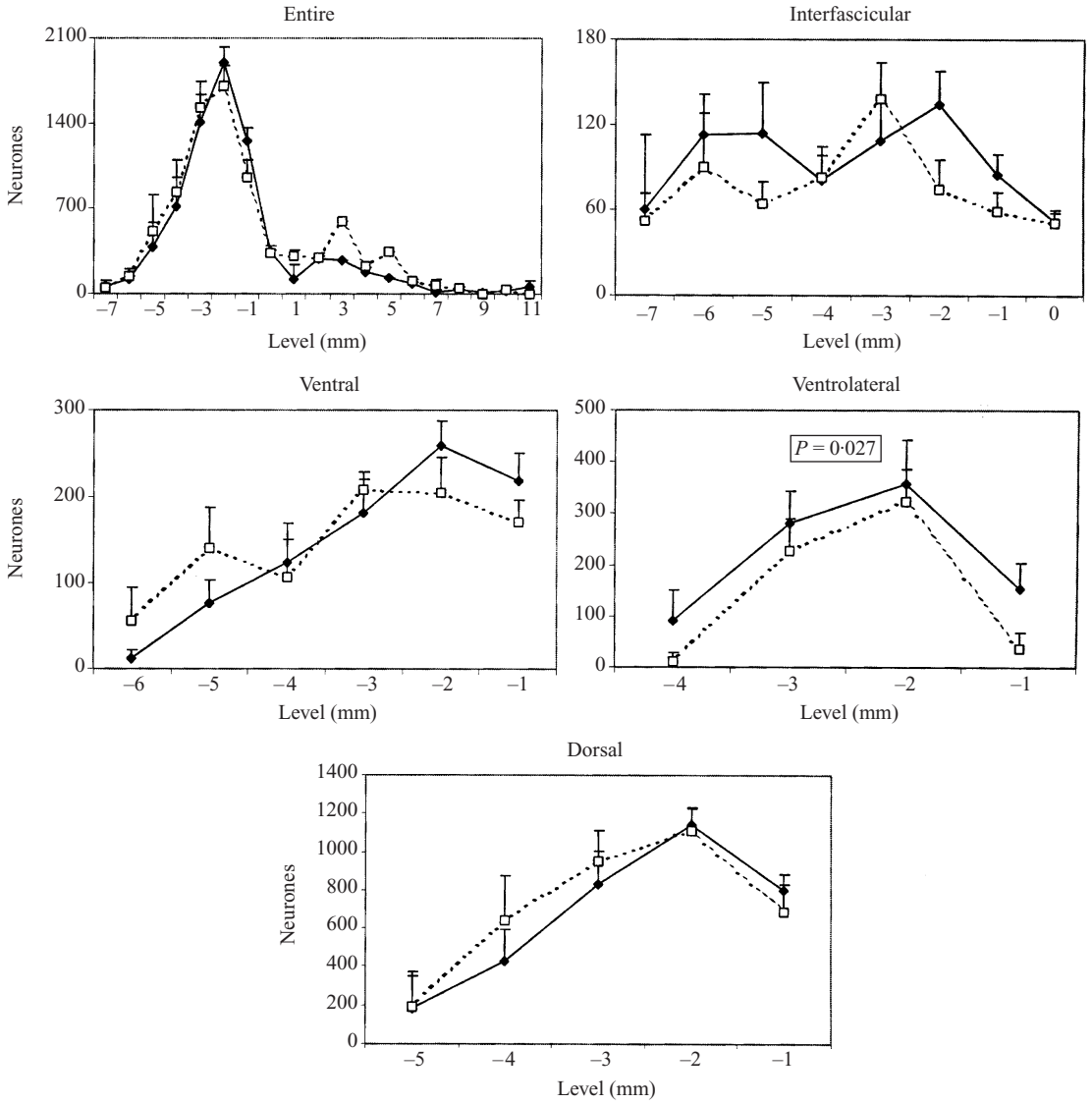


FIG. 3. Distribution of neurones in the entire dorsal raphe and in various subnuclei along rostro-caudal levels with reference to the isthmus (level 0). Levels are given in mm with rostral levels towards the left. The ordinate presents neurone numbers. The most caudal levels of the interfascicular nucleus are not shown. The caudal subnucleus widely corresponds to the right (pontine) part of the figure for the entire dorsal raphe. Neurone numbers are reduced in the ventrolateral subnucleus (significant main effect of subject group), but not in the entire dorsal raphe or in other subnuclei (repeated measures analysis with group and level as repeated measures). (◆—◆, Control subjects; □- - -□, subjects with mood disorders.)

only for the ventrolateral subnucleus ($P = 0{\cdot}027$) but not for other subnuclei or the entire dorsal raphe (all P values $> 0{\cdot}05$) (Fig. 3).

Age at time of death was negatively correlated with neurone numbers in the interfascicular and in the dorsal subnucleus ($r = -0{\cdot}41$, $P = 0{\cdot}047$; $r = -0{\cdot}46$, $P = 0{\cdot}02$, respectively). Brain weight showed a positive correlation with neurone numbers in the dorsal subnucleus ($r = 0{\cdot}44$, $P = 0{\cdot}03$). No significant correlation was seen for post-mortem delay and times of fixation with neurone numbers in any subnucleus of the DR (all rho-values between $-0{\cdot}38$ and $0{\cdot}32$, all P values $> 0{\cdot}05$). Duration of illness was not

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correlated with neurone numbers in any subnucleus (all rho values between −0·54 and 0·22, all P values $> 0{\cdot}05$). Neither mean doses of antidepressants, nor of neuroleptics, given in the last 90 days, correlated with neurone numbers in any subnucleus (all rho-values between −0·29 and 0·27, all P values $> 0{\cdot}05$). Neurone numbers in patients treated with lithium ($N = 3$) did not differ from those in patients without lithium medication ($N = 9$) in any subnucleus (all P values $> 0{\cdot}05$). Similarly, neurone numbers in patients treated with sedatives ($N = 6$) did not differ from those in patients without sedatives ($N = 6$) (all P values $> 0{\cdot}05$).

Since the DRvl showed a numerical neuronal deficit, this region was analysed by counting different neuronal subtypes (ovoid, round, triangular, fusiform) separately. Group comparisons yielded significant lower cell numbers only for triangular neurones in patients as compared to controls ($P = 0{\cdot}028$). But there was a nearly significant deficit in depressives also for ovoid and round neurones ($P = 0{\cdot}057$ and $P = 0{\cdot}082$, respectively). When this analysis was performed for the whole DRvc, a similar pattern of group differences emerged. Qualitative investigation of neurones in the DRvl as well as in the entire DR showed no obvious qualitative morphological abnormalities.

DISCUSSION

The main result of the study is a numerical deficit in the ventrolateral subnucleus of the DR in mood disorders. While neurone numbers and volumes of the dorsal subnucleus of the DR are unchanged, these parameters are decreased in a complex of ventrally located subnuclei of the rostral part of the DR that reportedly share some common projection pattern. This deficit cannot be explained by incorrect delineation of subnuclei, since this delineation was carried out according to well-defined cytoarchitectural criteria and anatomical landmarks and retest reliability was excellent for all subnuclei of the DR. The structural deficit of the ventral subdivision of the rostral DR is of major relevance, since efferent projections of this part of the DR terminate mainly in the prefrontal cortex and in striatal basal ganglia (O'Hearn & Molliver, 1984; Wilson & Molliver, 1991; Kazakov *et al.* 1993; Van Bockstaele *et al.* 1993). Reportedly, the latter brain regions also show a structural deficit in patients with major depression or bipolar disorder (Drevets *et al.* 1998; Baumann *et al.* 1999*a*; Rajkowska *et al.* 1999). Whether morphological alterations in the basal ganglia and the prefrontal cortex can be attributed to structural abnormalities in the DR remains to be elucidated.

A structural deficit consistent with lower neurone numbers in the DR has been shown in secondary forms of depression associated with Parkinson's or Alzheimer's disease (Zweig *et al.* 1988; Paulus & Jellinger, 1991). The findings of this study on patients with primary depression differ in two points from these results. First, the neuronal deficit in our patients was restricted to the ventral area of the rostral part of the dorsal raphe (DRvc), in particular to the ventrolateral subnucleus. Secondly, the missing correlation of neurone numbers in the subnuclei of the DRvc with illness duration suggests that a deficit of this part of the DR exists prior to clinical manifestation, probably occurring during brain development.

A developmental aetiology is also supported by the notion of a neuronal disarrangement rather than a general neuronal deficit in the DR. While neurone numbers are lower in the ventrally located subnuclei taken together, neurone numbers are 27% higher in the DRc of patients with mood disorders, although the latter result proved not to be significant. In week 10 of the embryonic period, serotonergic neurones are first detectable in human rostral raphe nuclei (i.e. the DR and the median raphe), which are located in the pons. Then they undergo stages of migration: the DR is first located in the dorsomedial region of the brainstem, then it migrates ventrally (Shen *et al.* 1989; Yew & Chan, 1999). It is tempting to speculate that a disturbance in the manner in which formation of raphe nuclei proceeds might result in a disproportional arrangement of raphe nuclei without a loss of the general cytoarchitectonic characteristics such as they are described for subnuclear organization of the DR (Baker *et al.* 1990).

Since serotonin, the main transmitter produced in the raphe nuclei, serves as a regulating factor in brain development (Whitaker-Azmitia *et al.* 1996), an early structural disturbance of the raphe may result in a

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pathomorphology of other brain regions as reported for the PFC (Drevets *et al.* 1998, Rajkowska *et al.* 1999), or the basal ganglia in mood disorders (Baumann *et al.* 1999*a*). Thus, even a circumscribed deficit of the DR might contribute to neuromorphological abnormalities as they are observed in depressive illness.

Aside from the underlying disease, several factors can have an impact on neurone numbers. In the current study, age at time of death, brain weight, and post-mortem delay showed no differences between patients and controls. Furthermore, there was no correlation between neurone numbers in the subnuclei of the DRvc and any of these confounding variables, indicating that these factors were not the cause for the structural differences between both groups. Our analyses also showed that psychotropic drugs had no significant effect on neurone numbers in any subnucleus of the dorsal raphe. Exclusion of individuals with a limetime diagnosis of drug abuse in the current study may result in an unrepresentative sample since it is known that a considerable number of patients with mood disorders show drug abuse. This may be a point of limitation of this study. Future investigations should include a sample of patients with mood disorders and co-morbid drug abuse.

Since an immunohistochemical study pointed out that only 70% of the human DR neurones might be serotonergic (Baker *et al.* 1991), the reduced neurone number in the DRvl could indicate a lack of serotonergic neurones, but other neurone types might be involved too. As shown by our quantitative morphological analysis, small triangular neurones also show a numeric reduction in the DRvl. Baker and colleagues (1991) found that virtually all of these smaller neurones are non-serotonergic and may produce other neuromodulators. When excluding triangular neurones, the difference of the remaining neurones (i.e. ovoid, round and fusiform) in the DRvl as well as in the whole DRvc remained significant between groups ($P = 0{\cdot}05$, $P = 0{\cdot}01$, respectively). According to the findings of Baker and colleagues (1991), these non-triangular neurones are mostly serotonergic. Thus, the neuronal deficit in the DRvl and in the whole DRvc appears to be largely a deficit of serotonergic neurones in this region. Studies using tryptophan hydroxylase as a marker for serotonergic neurones may confirm this assumption but such studies will have to consider state-dependent regulative effects.

Alterations on the neurohistological level including lower neurone numbers in the midbrain raphe provide an explanation for reduced ligand binding sites as shown in a PET study of the serotonin 1A receptor (Drevets *et al.* 1999) and the serotonin transporter in the midbrain (Malison *et al.* 1998). Reduced binding for receptors on serotonergic neurones in the raphe might simply result from a circumscribed numerical neuronal deficit. Such a neuronal deficit in the raphe would also be in line with a reduction of serotonergic efferents targeting certain brain areas as is suggested by the smaller neuronal size in prefrontal areas that receive serotonergic innervation mainly from the DR (Rajkowska *et al.* 1999).

A report of increased binding to the serotonin 1A receptor in the DR in depressed suicide victims seems to be contradictory to the data discussed here, but this post-mortem result might be attributable to suicide rather than to depression, or to an interaction of suicide and depression (Stockmeier *et al.* 1998). Divergent changes in the DR of depressed and suicidal subjects are also suggested by a finding of Underwood and colleagues who observed an increased number of tryptophan hydroxylase immunoreactive neurones in the DR of suicide victims (Underwood *et al.* 1999*a*, *b*). This finding can hardly be compared with the current study, since expression of tryptophan hydroxylase might be confounded by state dependent influences such as drugs, psychopathology and suicidal behaviour present in the time before death.

A histological pathology in the brainstem raphe in depression could have a considerable impact on our understanding of the association between the serotonergic system and depressive disorder. Given an association between an impaired serotonergic system and depressive symptomatology, a circumscribed pathomorphological change in the raphe – the crucial source of serotonergic transmission in the brain – might be regarded as a trait factor that contributes to increased vulnerability for the development of depressive syndromes. Strong evidence has been provided by lesion studies that destruction of the raphe nuclei leads to behavioural alterations

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and severe disturbances of the serotonergic (Morrow & Roth, 1995; Koprowska & Romaniuk, 1997; Romero *et al*. 1998) and other monaminergic systems (Morrow & Roth, 1995; Bolte Taylor *et al*. 1998). As mentioned above, involvement of the DR in neurodegenerative disorders is accompanied by mood impairment. A recent study indicating projections of the midbrain DR to autonomic centres in the medulla suggests that changes in the DR might also contribute to autonomic disturbances frequently observed in depressive disorder (Underwood *et al*. 1999*a*). Thus, crucial clinical signs as well as dysregulation of neurotransmitter systems seen in depressive illness might be attributable to structural alterations of the DR.

In summary, the current data indicate a morphological abnormality of the DR in affective illness. Further characterization of raphe pathology and its pathophysiological consequences might be an important approach to the neurobiology of mood disorders.

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