Eur Arch Psychiatry Clin Neurosci (1999) 249 : 212–219 

ORIGINAL PAPER

**Bruno Baumann · Peter Danos · Silvia Diekmann**
**Dieter Krell · Hendrik Bielau · Christian Geretsegger**
**Cornelius Wurthmann · Hans-Gert Bernstein**
**Bernhard Bogerts**

# Tyrosine hydroxylase immunoreactivity in the locus coeruleus is reduced in depressed non-suicidal patients but normal in depressed suicide patients

Received: 29 January 1999 / Accepted: 27 May 1999

**Abstract** Noradrenergic neurons of the locus coeruleus (LC) have been implicated in the neurobiology of depression and suicidal behavior. The current postmortem study determined numbers of noradrenergic neurons by immunostaining the synthesizing enzyme tyrosine hydroxylase in the LC of 12 non-elderly depressed patients with a mood disorder as compared to 12 age- and sex-matched normal controls. Six patients were suicide victims, the other six patients died of natural causes. Non-suicidal patients had fewer neurons immunoreactive for tyrosine hydroxylase (TH-ir) than suicide victims or controls. No difference appeared between the number of TH-ir neurons in suicide patients and controls. Numbers of pigmented LC neurons were equal in patients and controls. The differences of TH-immunoreactivity could neither be attributed to drug influences nor to polarity of depressive disorder (i.e., unipolar/bipolar). Numbers of TH-ir neurons correlated positively with mean doses of tri- or tetracyclic antidepressants. Results of this study suggest a presynaptic noradrenergic deficit of the LC in depressed non-suicidal patients. Indirect evidence is provided that suicide is not related to decreased noradrenergic function and that traditional antidepressants may enhance noradrenergic activity of the LC in depressed patients.

**Key words** Tyrosine hydroxylase · Noradrenaline · Locus coeruleus · Major depression · Suicide

This study was presented at the CINP Symposium in Glasgow 1998

Dr. B. Baumann (✉) · P. Danos · S. Diekmann · D. Krell
H. Bielau · C. Wurthmann · H.G. Bernstein · B. Bogerts
Department of Psychiatry, University of Magdeburg,
Leipziger Strasse 44, D-39120 Magdeburg, Germany
e-mail: Bruno.Baumann@Medizin.Uni-Magdeburg.DE

C. Geretsegger
Department of Psychiatry, Christian-Doppler-Clinics Salzburg,
Ignaz Harrer Strasse 79, A-5020 Salzburg, Austria

## Introduction

The locus coeruleus (LC) comprises the largest group of noradrenaline containing neurons in the mammalian brain (Dahlström and Fuxe 1964) and provides a widespread noradrenergic innervation of the central nervous system (for review see Jones 1991). There exists evidence from preclinical and clinical studies that noradrenergic systems are disturbed in mood disorders (Anand and Charney 1997). As yet, only one recent controlled postmortem study was performed to investigate brain presynaptic noradrenergic levels in mood disorders; the authors found reduced levels of the NA transporter in patients with major depression and interpreted this result as consistent with the noradrenaline deficit hypothesis (Klimek et al. 1997).

Suicide is one of the most frequent causes of death in the developed countries (Lopez 1990; MMWR MORB MORTAL WKLY REP 1997). Therefore, beside diagnostic aspects, extensive work has been focused on the neurobiology of suicide, in particular on the significance of noradrenergic systems (Arranz et al. 1997; Arango et al. 1997). Two postmortem studies investigated tyrosine hydroxylase (TH), the key enzyme of noradrenaline-synthesis, in suicide victims. The results, however, are contradictory. TH protein was found to be elevated in one study (Ordway et al. 1994), whereas another one demonstrated a lower immunohistological staining intensity of LC neurons for TH, while the number of LC neurons immunoreactive for TH was equal as compared to normal controls (Biegon and Fieldust 1992).

Although suicidality is a core symptom of depression and though noradrenergic systems have been related to both suicide and depression, surprisingly few postmortem studies analyzed noradrenergic systems in suicide victims with a well documented diagnosis of a mood disorder. Therefore we investigated TH immunoreactive (TH-ir) neurons of the locus coeruleus in depressed patients who died by suicide or by natural causes, as compared to normal controls. The present study focused on two questions: first, is there a deficit of noradrenaline synthesis in mood

**Table 1** Characteristics of depressed suicide patients

| Patient No./ Sex/Age (yrs.) | Fresh Brain Weight (g) | Psychotropic medication in last 4 weeks | Cause of Death | Psychiatric Diagnosis *(DSM-III-R)* |
|---|---|---|---|---|
| 1,m,47 | 1670 | No | Acute loss of blood after suicide by stabbing | Bipolar Disorder Depressed 296.53 |
| 2,f,39 | 1400 | Amitryptiline (150 mg/d); diazepam 5 mg/d, Lithium-carbonate (900 mg/d) | Suicide by overdose of medication | Major Depression 296.23 |
| 3,f,46 | 1410 | Maprotiline (200 mg/d); Haloperidol (5 mg/d) | Suicide by hanging | Major Depression 296.24 |
| 4,m,42 | 1320 | Lithiumcarbonate (900 mg/d), flunitrazepam (2 mg/d), amitryptiline (2 25/d), chlorprothixen (150/d) | Suicide by hanging | Bipolar Disorder Depressed 296.54 |
| 5,f,46 | 1300 | Amitriptyline (200 mg/d), haloperidol (15 mg/d), diazepam (5 mg/d), Lithiumcarbonate (900 mg/d) | Suicide by overdose of medication | Bipolar Disorder Depressed 296.54 |
| 6,f,47 | 1300 | Amitriptyline (150 mg/d), nitrazepam (5 mg/d) | Suicide by overdose of medication | Major Depression 296.34 |
| Mean ± SD 44.5 ± 3.27 (age) | 1400 ± 141 | | | |

disorders and thereby a presynaptic transmitter deficit as suggested by Schildkraut (1965) and Bunney and Davis (1965) and, second, is there an association of suicidality and numbers of locus coeruleus TH-ir neurons?

## Materials and methods

### Subjects

Postmortem brains from 12 subjects with the clinical diagnosis of a mood disorder (DSM-III-R) were obtained from autopsies performed in the time between 1988 and 1991 in several German and Hungarian neuropathological institutes and medical examiners offices. Similarly, 12 brains from sex- and age-matched control subjects without a detectable neuropsychiatric disorder were included. According to German autopsy laws informed consent was obtained for autopsy and dissections of the brains by the relatives of all patients and control individuals.

Antemortem diagnoses according to DSM III-R were obtained by psychological autopsies referring to the careful study of clinical records and/or interviews of at least one family member or physician involved in the treatment of the patients.

Diagnosis of a mood disorder was established by two independent psychiatric examiners. By the same way neuropsychiatric disorders were excluded in control individuals. Stressors occurred in the last year before death were determined on axis 4 of DSM-III-R. Distribution of smokers and non-smokers was similar in suicide victims and non-suicidal patients as well as in controls (one smoker in each group). Neither in patients nor in controls were qualitative neuropathological changes indicating neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Pick's disease), tumors, inflammatory, vascular or traumatic processes seen by an experienced neuropathologist. Patients or control subjects with a history of alcohol or drug abuse were excluded by anamnesis, toxicological screens, and liver histology. Furthermore, subjects with ECT treatment in the last year were excluded, since ECT reportedly elevates TH levels (Masserano and Takimoto 1981, Brady et al. 1994). Age ranged from 39 to 63 yrs. (patients), and 30 to 67 yrs. (controls). Demographic data of patients and controls are presented in Tables 1–3.

### *Histological procedure*

Brains were removed within 4 to 72 hours after death. Mean postmortem intervals were 36.33 hours for brains of suicide patients, 36.17 hours for those of non-suicidal patients and 35.6 hours for controls. Brains were fixed in toto in 8% phosphate-buffered formaldehyde for at least 2 months (pH = 7.0, T = 15–20 °C). Frontal and occipital poles were separated by frontal sections anterior to the genu and posterior to the splenium of the corpus callosum. The brainstem was isolated by a cut perpendicular to its longitudinal axis at the level of the leaving oculomotor nerve. After embedding all parts of the brains in paraffin, 20 µ thick serial sections of the brainstem were cut perpendicular to the longitudinal axis in a rostro-caudal direction on a Leica Polycut microtome and mounted. Each 50th section was deparaffinized, hydrated, and Nissl- (cresyl violet-) stained. Adjacent sections to each third Nissl stained section were selected along the rostro-caudal axis of the LC (nucleus proper) and immunocytochemically stained for TH. The first (most rostral) section for immunostaining of TH was randomly chosen from the first three rostral Nissl-stained sections. Thus, selection of sections was in accordance with the Cavalieri theorem of systematic sampling (Cavalieri 1966). Distance between the Nissl-stained sections was 1 mm and between TH immunostained sections 3 mm. Thereby three to five sections of each LC were investigated for TH immunoreactivity.

### *Immunocytochemical procedure*

Mounted sections were deparaffinized, hydrated, and neuromelanin pigment of the locus coeruleus was bleached by hydrogen peroxide for 48 hrs. To immunolocalize TH a polyclonal antibody raised against TH in rabbits was used. The specificity of the anti-

**Table 2** Characteristics of depressed nonsuicidal patients

| Patient No./ Sex/Age (yrs.) | Fresh Brain Weight (g) | Psychotropic medication in last 4 weeks | Cause of Death | Psychiatric Diagnosis *(DSM-III-R)* |
|---|---|---|---|---|
| 7,f,62 | 1300 | Lorazepam (5 mg/d), flupentixoldecanoate (20 mg/14d), haloperidol (20 mg/d) | Pulmonary embolism | Bipolar Disorder Depressed 296.54 |
| 8,m,39 | 1520 | Haloperidol 15 mg/d (week 1–3), last week: 3 mg fluphenazine + 2 mg benperidol for 4 days. Last 3 days no psychotropic medication | Pulmonary embolism myocardial infarction | Bipolar Disorder Mixed 296.62 |
| 9,f,61 | 1240 | Amitriptyline 90 mg/d, haloperidol 5 mg/d, nitrazepam 2,5 mg/d except for last 4 days | Bronchopneumonia | Major Depression 296.34 |
| 10,f,60 | 1140 | Trimipramine (150 mg/d) for 3 days the last week, chlordiazepoxide (20 mg/d) for 3 days in the last week | Bronchopneumonia | Bipolar Disorder Depressed 296.54 |
| 11,f,41 | 1210 | Amitriptyline (100 mg/d), haloperidol (20 mg/d), levomepromazine (320 mg/d). No psychopharmacological medication the last 2 days before death | Pulmonary embolism | Major Depression 296.34 |
| 12,f,63 | 1100 | Amitriptyline (150 mg/d) | Pulmonary embolism | Major Depression 296.34 |
| Mean ± SD 54.33 ± 11.17 (age) | 1252 ± 149 | | | |

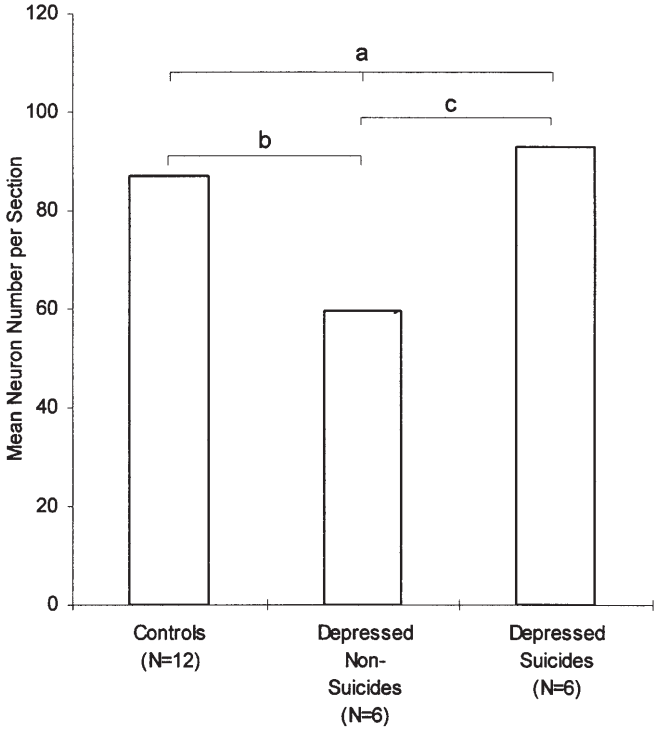
**Table 3** Characteristics of psychiatrically normal control subjects

| Control No./Sex/ Age (yrs.) | Fresh Brain Weight (g) | Cause of death |
|---|---|---|
| 1,m,64 | 1310 | Cardiac insufficiency |
| 2,f,67 | 1210 | Sudden cardiac death, coronary heart disease |
| 3,f,63 | 1050 | Sudden cardiac death, coronary heart disease |
| 4,f,50 | 1055 | Rupture of aortal aneurysm |
| 5,f,65 | 1100 | Cardiac insufficiency, aortal stenosis, insufficiency of mitral valve |
| 6,f,38 | 1200 | Pulmonary embolism |
| 7,m,40 | 1550 | Myocardial infarction |
| 8,f,30 | 1500 | Pulmonary embolism |
| 9,f,39 | 1210 | Heat stroke after sun exposure |
| 10,m,54 | 1430 | Fulminant pulmonary embolism |
| 11,f,61 | 1350 | Cardiac insufficiency, coronary heart disease |
| 12,f,61 | 1160 | Cardiac insufficiency |
| Mean ± SD 52.67 ± 12.85 (age) | 1260 ± 168 | |

serum has been confirmed by the supplier performing Western blotting and immunocytochemistry (Eugene Tech International Inc., New Jersey, USA). After the preincubation of the sections with methanol/H2O2 to depress endogenous peroxidases and repeated washing with phosphate-buffered saline (PBS), the TH antiserum was used at a dilution of 1 : 1000 for 24 hrs. at 4 °C after preadsorbation with natural melanin (Sepia officinalis, Sigma, St. Louis, MO, 0.1 mg/ml for 60 minutes) to remove possible cross reactivity with neuromelanin. Further immunocytochemical protocol involved the incubation with donkey anti-rabbit IgG serum (Amersham, Freiburg, Germany) and the application of the avidin-biotin technique (Amersham). The chromogen 3,3′-diaminobenzidine was used to visualize the reaction product. For purposes of control the primary antiserum was either replaced by buffer or normal serum. Sections without the specific primary antiserum did not show any immunostaining. Immunostaining was abolished by preadsorbation of the primary antiserum with homogenated and centrifugated adrenal human tissue, which is rich in TH. Two further TH antisera (Sigma Biosciences. St. Louis, Missouri; Camon, Wiesbaden, Germany) were applied for immunostaining resulting in a comparable neuronal staining pattern in the LC.

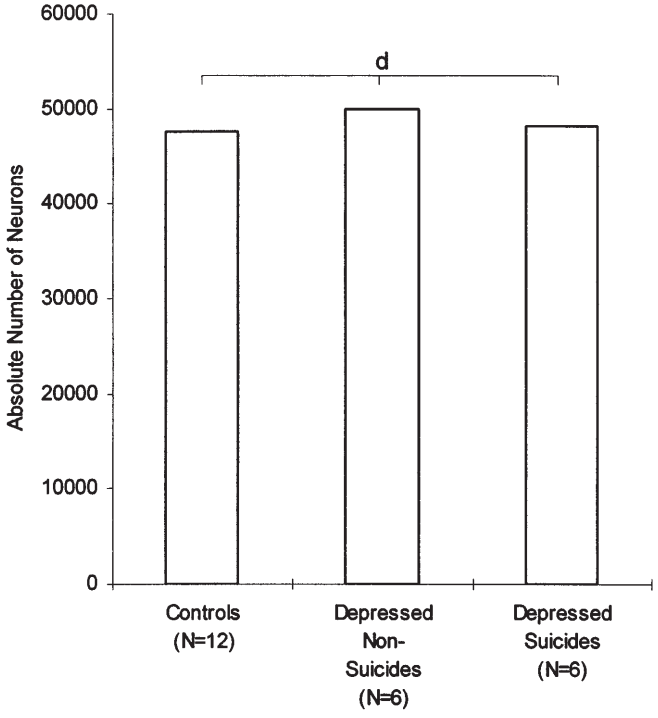
*Morphometry*

To estimate the total number of LC neurons, all Nissl-stained neurons in the LC on both sides were counted in each section with the aid of a video-based computed system (DIGITRACE®) connected with a microscope which was equipped with a motorized stage. Immunoreactive neurons were identified visually on the video-screen and traced by aid of a computer mouse. Only sections with more than three LC neurons on each side were included. The volume of the LC was estimated by integrating cross sectional areas from the most rostral to the most caudal section. Given the volume of the LC in a single section by the product of cross sectional area multiplied by the section thickness, neuronal numerical densities could be calculated for each section. To remove overestimation of neuron numbers by counting cell profiles (Coggeshall and Lekan 1996), the unfolding procedure after Cruz-Orive (Cruz-Orive 1978; Weibel 1979) with a correction for section thickness was applied in order to derive neuron numbers from profile counts.




[a]Significant group effect (F=4.63, df=2, 21, p=0.02)
[b]Significant post-hoc test
[c]Significant post-hoc test

**Fig. 1 A** Tyrosine hydroxylase-immunoreactive neurons in the locus coeruleus




[d]Insignificant group effect (F=0.23, df=2, 21, p=0.80)

**Fig. 1 B** Pigmented neurons in the locus coeruleus

The total numbers of Nissl stained LC neurons were computed by multiplying the mean numerical cell density by the volume of the LC. Mean numbers of TH-ir neurons per section were calculated using the same unfolding procedure as referenced above.

*Statistics*

Differences between groups were analyzed by ANOVA and post hoc least significant difference test. Effects of confounding variables such as age, postmortem delay, time of tissue fixation, duration of illness, and age at onset of disease were investigated by means of Pearson's linear correlations. Composition of groups with respect to qualitative or categorical parameters such as sex or diagnosis was tested by the Chi-Square test or Fisher's exact test. Statistical analyses were performed by use of SPSS 7.5.

## Results

Patients (depressed non-suicidal patients + depressed suicide victims) did not differ from controls with respect to age, brain weight and postmortem delay ($F < 1.0$, df = 1,22, $p > 0.33$). Similarly, except for age, suicide victims showed no significant differences to non-suicidal patients ($F < 3.2$, df = 1,10, $p > 0.10$). There was a non-significant trend towards higher age in non-suicidal patients ($F = 4.3$, df = 1,10, $p = 0.07$).

ANOVA showed a significant difference among non-suicidal patients, suicide victims and controls with respect to mean numbers of TH-ir neurons per section ($F = 4.6$, df = 2.21, $p = 0.02$) (Fig. 1 A). Because of possible confounding effects of age and mean doses of tri- or tetracyclic antidepressants given in the last 7 days before death, these two parameters were included in the ANOVA as covariates without changing the degree of significance. Post hoc least significant difference tests showed the number of TH-ir neurons significantly lower in non-suicidal patients (59.5 ± 20.6) as compared to controls (86.9 ± 20.8) or suicide victims (93.1 ± 21.6), while this parameter did not differ between suicide victims and controls (Figs. 1 A and 2).

ANOVA yielded no significant difference of mean numbers of TH-ir neurons per section between patients with bipolar disorder, unipolar depression or controls ($F = 0.8$, df = 2,21, $p = 0.46$). Total numbers of Nissl-stained LC neurons showed no changes between suicide victims, non-suicidal patients or controls (Fig. 1 B).

Neither age at death nor age at onset of disease correlated with total numbers of Nissl stained LC neurons ($r = 0.03$, $n = 12$, $p = 0.93$; $r = 0.05$, $n = 9$, $p = 0.90$, respectively) or mean number of TH-ir neurons per section ($r = –0.11$, $n = 12$, $p = 0.73$; $r = 0.37$, $n = 9$, $p = 0.32$, respectively). Duration of depressive illness did not correlate with total numbers of Nissl stained neurons ($r = –0.08$, $n = 9$, $p = 0.83$), but there was a significant positive correlation of illness duration with mean number of TH-ir neurons per section ($r = 0.77$, $n = 9$, $p = 0.02$). Furthermore, the latter parameter showed a positive correlation with mean doses of (tricyclic or tetracyclic) antidepres-

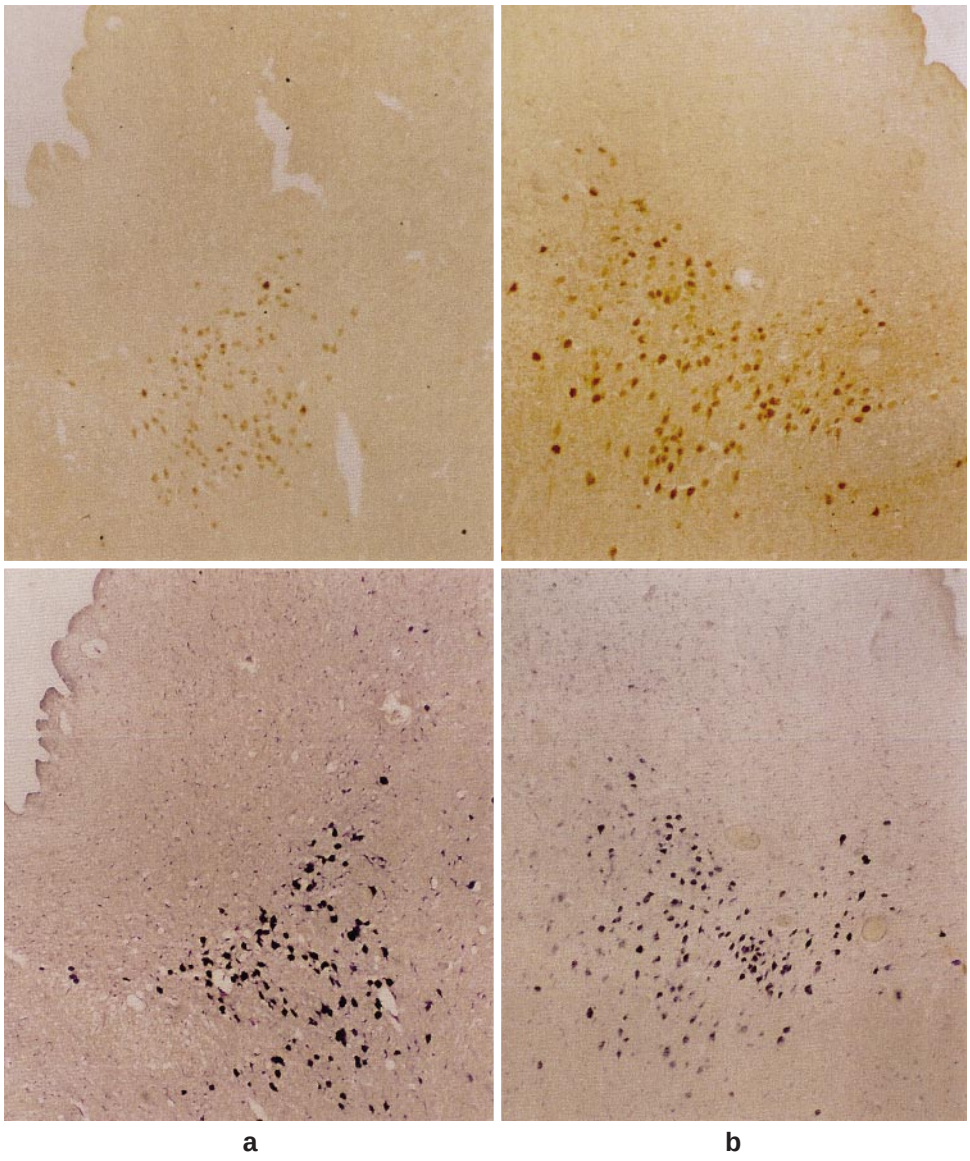



**Fig. 2** Lack of tyrosine hydroxylase-immunoreactive neurons in the locus coeruleus of a non-suicidal[a] as compared to those in a suicidal[b] depressed patient

[a] 41 yr. old women with unipolar depression, died of fulminant pulmonary embolism
[b] 46 yr. old women with unipolar depression, died of suicide by hanging
[a, b] Top: Tyrosine hydroxylase-immunoreactive neurons. Bottom: Nissl-stained neurons in sections adjacent to those shown at the top. Levels shown on the left side ([a]) are comparable to those of the right side ([b]) with regard to the rostro-caudal extension of the LC.
Original magnification: 50×

sants given during the week before death ($r = 0.79$, $n = 9$, $p = 0.01$) (Fig. 3). Postmortem delay and time of fixation showed no correlation with the amount of Nissl stained LC neurons ($r = 0.30$, $n = 12$, $p = 0.34$; $r = 0.15$, $n = 12$, $p = 0.65$, respectively) nor with mean numbers of TH-ir neurons per section ($r = –0.28$, $n = 12$, $p = 0.36$; $r = –0.12$, $n = 12$, $p = 0.73$, respectively). Each group comprised three patients with bipolar disorder and three with unipolar major depression. Similarly, distribution of gender showed no significant differences in these two patient subgroups: suicide victims 2 : 4 (male – female); non-suicidal patients 1 : 5 (male – female). Recent stressors as determined by DSM-III-R did not differ between patient groups and controls ($F = 2,2$, $df = 2.17$, $p = 0.14$). Stressors were not correlated to the amount of TH-ir neurons per section (patients: $r = 0.31$, $n = 11$, $p = 0.35$; controls: $r = 0.13$, $n = 9$, $p = 0.97$).

## Discussion

We found that in patients with mood disorders, who did not commit suicide, the number of locus coeruleus neurons immunoreactive for tyrosine hydroxylase are lower than in controls, while suicide victims with mood disorders did not show this difference. Considering tyrosine hydroxylase as the limiting enzyme of noradrenaline synthesis, this result indicates a noradrenergic deficit in those patients with mood disorders who are actually not prone to suicide. Since only 3 of the patients in the present study were free of tricyclic antidepressants, which are reported to reduce TH levels in rats (Valentino et al. 1990; Brady et al. 1991; Komori et al. 1992; Melia et al. 1992), a noradrenergic deficit in non-suicidal patients could be discussed as a sequel of antidepressive medication. There are, however, some arguments against the assumption that chronic application of tricyclic antidepressants, which occurred in most cases of the present study, decreases levels

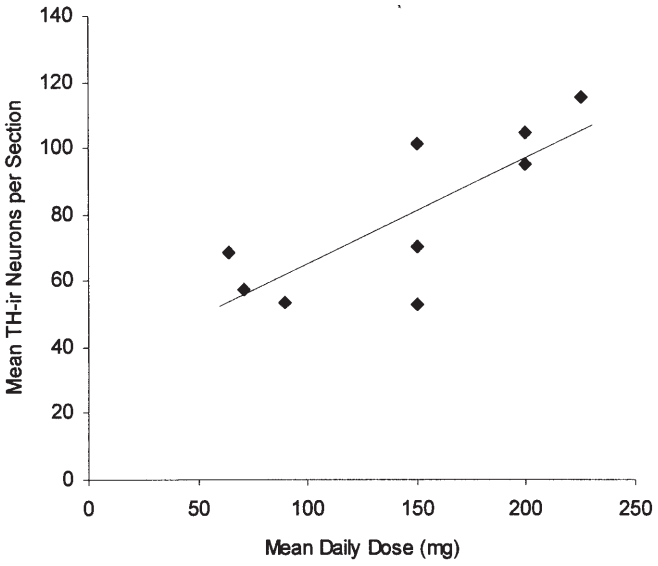



**Fig. 3** Correlation of tyrosine hydroxylase-immunoreactive neurons and medication of tri- or tetracyclic antidepressants in the last 7 days before death of each depressed patient

or activity of TH in depression. Aside the possibility that morphology and physiology of the human LC may not be analogous in every aspect with the one of rodents, effects of antidepressants on the activity of TH might differ state-dependently in depression-like and normal behavior (Komori et al. 1990). In the current study, mean numbers of TH-ir neurons showed a *positive* correlation with mean doses of chronically applied antidepressants. In agreement with this result the patient with the lowest number of TH-ir neurons (# 8) was free of antidepressants for at least three months before death, arguing against the assumption that lower numbers of TH-ir neurons might be a result of antidepressive medication.

The result of lowered TH-ir in the LC of non-suicidal patients is in agreement with the finding of reduced levels of the noradrenaline transporter in patients with major depression (Klimek et al. 1997). These authors interpreted the decrease of the noradrenaline transporter as a counterbalance effect induced by decreased levels of noradrenaline in depression as suggested from animal data (Weiss et al. 1981; Petty et al. 1993). Furthermore, data of the present study are consistent with lowered levels of TH in depression-like behavior of rats under forced running stress (Komori T et al. 1990). In this study also the activity of LC neurons was lowered in the depression-like state, which was associated with lowered neuronal firing rate in the LC (Murase et al. 1987). Therefore it is conceivable that decreased TH-ir in non-suicidal patients reflects a lower activity of LC neurons. This provides a morphological confirmation of the hypothesis formulated as long as more than three decades ago supposing a noradrenergic deficit in depressed patients (Schildkraut 1965; Bunney and Davis 1965). Our data of a positive correlation between TH-ir neurons and antidepressants suggest a normalizing effect of antidepressants with respect to noradrenaline synthesis in depression. In contrast, depressed suicide patients in the current study showed no such noradrenergic deficit which might explain inconclusive results in former studies designed to evaluate the noradrenaline hypothesis but not taking into account the strong impact of suicide on noradrenergic systems and vice versa (for review see Anand and Charney 1997).

The other main result of the current study was the higher number of TH-ir LC neurons in depressed suicide victims as compared to depressed patients who did not commit suicide. Some factors should be considered as confounding variables. The possibility that this difference could be a result of drug treatment can be excluded, since both groups received comparable amounts of antidepressants (five of 6 suicide patients and four of 6 non-suicidal patients). Other psychoactive drugs chronically applied to patients in this study, such as neuroleptics (Cottingham et al. 1990) or benzodiazepines (Nestler et al. 1990) have been shown to be ineffective with regard to tyrosine hydroxylase in rodents. Acute haloperidol administration is reported to elevate firing activity in the LC (Dinan and Aston-Jones 1984), but none of the patients in the present study received such an acute medication. The effect of lithium on TH is a matter of controversy. A recent finding of an increase of TH in cortex, hippocampus and striatum of patients receiving lithium (Chen et al. 1998) is in contrast with a report that lithium does not influence the catecholaminergic system, in particular TH (Ahluwalia and Singhal 1984). Since only three patients investigated in this study received lithium, effects of this drug on the results presented here are unlikely but can not definitely be ruled out.

Other parameters such as age and postmortem delay were in the same range in patient groups and controls. Moreover, age-related effects on TH in the LC occur only in elderly subjects (Chan-Palay and Asan 1989). As found by others (Girault et al. 1989), numbers of TH-ir-LC neurons did not correlate with postmortem delay, nor with times of fixation. Differences in staining intensity were ruled out by using equal numbers of sections from suicide victims, non-suicide victims and control subjects in each staining procedure, keeping the protocol for each staining strictly constant. In none of the investigated individuals a prolonged agonal state was noted. Thus, no hypoxic effects could influence the data. Smoking which is reported to increase TH activity (Hiremagalur and Sabban 1995) was similarly represented in suicide victims, non-suicidal patients and controls indicating no influence of nicotine on results of this study.

A methodological limitation of the current study is given by the relatively large distance of sections investigated for TH immunoreactivity. Thereby the topographical organization of the locus coeruleus with respect to its projections (Mason and Fibiger 1979; Loughlin et al. 1986 a, b) could not be taken into account. This may be of interest for further research since regionally restricted structural changes in the LC have been reported for suicide victims as well as for patients with mood disorders (Arango et al. 1996; Baumann et al. 1999).

The result of similar numbers of TH-ir neurons in the LC of suicide victims as compared to controls is partially in conflict with the finding of Ordway and colleagues (Ord-

way et al. 1994) who described elevated TH protein concentrations in the LC of suicide victims as compared to controls. Variances of neuronal protein concentration as well as differences in the study populations with regard to sex (Babstock et al. 1997), race (Venter and Joubert 1984), underlying psychiatric diagnoses in suicide victims, and pharmacological influences might be responsible for this discrepancy. Aside these problems it can be concluded from the present study as well as from the results of Ordway and coworkers that the risk for suicidal acts in psychiatric patients might increase with TH levels in the LC.

Our data are also in conflict with the opposite finding of decreased TH immunoreactivity in suicide victims reported by Biegon and Fieldust (1992). Similar methodological differences as remarked in the study of Ordway and coworkers (1994), in particular measurement of optical staining intensity instead of the amount of immunoreactive neurons, could explain the contrary findings. However, the result of equal numbers of TH-ir neurons in the LC of suicide victims as compared to controls reported by these authors is in agreement with our data.

The finding of a positive correlation between the number of TH-ir neurons and illness duration is difficult to interprete. One could speculate, that processes of sensitization occuring during recurrent depressive episodes may be responsible for an increasing noradrenergic synthesis in the LC.

The elevated number of TH-ir neurons in the LC of depressed suicide victims as compared to depressed non-suicidal patients suggests that suicidal risk in mood disorders is related to the noradrenaline synthesis. Despite the differences in the number of TH-ir neurons between suicidal and non-suicidal patients, the present study found no numerical alteration of Nissl-stained pigmented LC neurons between these groups. Considering neuromelanin-containing neurons in the brainstem as a catecholaminergic marker (Bogerts 1981), this constellation indicates that varying numbers of TH-ir neurons found in the current study are not due to differences in the total amount of LC neurons capable of noradrenaline synthesis but rather to the present content of TH in these neurons, thus, leading to a changing detectability of TH-ir neurons. This might confirm the number of TH-ir neurons in the LC as a state-dependent marker of suicidality in patients with mood disorder. However, a study reporting distinct alleles of the TH gene associated with suicidal behavior (Persson et al. 1997) might indicate the TH gene as a potential trait marker of vulnerability for suicidal risk.

Animal models of depression suggest enhanced noradrenergic function of the LC as a response to certain stressors (Komori T et al. 1990; Smith et al. 1991) which can also be assumed for the prodromal phase of a depressive episode in the human. While the depression-like behavior exacerbates, noradrenergic tone in critical brain areas such as the locus coeruleus might return to normal levels as sequel of a stress adaptation (Smith et al. 1991) or even dysregulate to subnormal levels (Weiss et al. 1976; Weiss and Simson 1985; Komori T et al. 1990; Petty et al. 1993; Kitayama et al. 1997). During the phase of remission, noradrenergic innervation might recover and return to normal or temporary elevated levels (Komori T et al. 1990; Kitayama et al. 1997). Considering TH as a marker for suicidality in human depression, increased risk for suicidal acts might occur in the early and in the remissive phase of a depressive state which would be consistent with literature reports suggesting suicidal incidence during recovery from depressive episodes (Schweizer et al. 1988; Goldacre et al. 1993; Shah and Ganesvaran 1997). It is conceivable that suicidal risk is constrained to certain time frames in a depressive episode which are defined by still normal or recovered noradrenergic function. It remains to be established whether antidepressants could temporarily increase suicidality via effects on noradrenergic systems as suggested by some authors (Montgomery et al. 1992; Teicher et al. 1993). In summary, we found decreased numbers of tyrosine hydroxylase-immunoreactive neurons in non-suicidal depressed patients indicating reduced noradrenergic activity. There was no change in suicide patients. The results support the noradrenaline hypothesis of major depression and indicate that treatment with antidepressant drugs increase noradrenergic activity.

**Acknowledgements** This research was supported by the Stanley Foundation, German Research Community (DFG 799/6-1), German Ministry for Research (BMBF 01 ZZ 9510) and the Alfried-Krupp-von-Bohlen-und-Halbach Stiftung. The authors wish to thank Prof. Dr. L. Gerhard (University of Witten-Herdecke, Germany) for qualitative neuropathological investigations of the brains included in this study.

## References

Ahluwalia P, Singhal RL (1984) Comparison of the changes in central catecholamine systems following short- and long-term lithium treatment and the consequences of lithium withdrawal. Neuropsychobiology 12(4):217–223

Anand A, Charney DS (1997) Catecholamines in depression. In: Honig A, Van Praag HM (eds) Depression: Neurobiological psychopathological and therapeutic advances. Chichester, John Wiley & Sons Ltd., pp 147–178

Arango V, Underwood MD, Mann JJ (1996) Fewer pigmented locus coeruleus neurons in suicide victims: preliminary results. Biol Psychiatry 39:112–120

Arango V, Underwood MD, Mann JJ (1997) Biologic alterations in the brainstem of suicides. Psychiatr Clin North Am 20:581–593

Arranz B, Blennow K, Eriksson A, Mansson JE, Marcusson J (1997) Serotonergic, noradrenergic, and dopaminergic measures in suicide brains. Biol Psychiatry 41:1000–1009

Babstock D, Malsbury CS, Harley CW (1997) The dorsal locus coeruleus is larger in male than in female Sprague-Dawley rats. Neurosci Lett 224:157–160

Baumann B, Danos P, Krell D, Diekmann S, Wurthmann C, Bielau H, Bernstein HG, Bogerts B (1999) Unipolar-bipolar dichotomy of mood disorders is supported by noradrenergic brainstem system morphology. Journal of Affective Disorders in press

Biegon A, Fieldust S (1992) Reduced tyrosine hydroxylase immunoreactivity in locus coeruleus of suicide victims. Synapse 10:79–82

Bogerts B (1981) A brainstem atlas of catecholaminergic neurons in man, using melanin as a natural marker. J Comp Neurol 197:63–80

Brady LS, Lynn AB, Glowa JR, Le DQ, Herkenham M (1994) Repeated electroconvulsive shock produces long-lasting increases in messenger RNA expression of corticotropin-releasing hormone and tyrosine hydroxylase in rat brain. Therapeutic implications. J Clin Invest 94 : 1263–1268

Brady LS, Whitfiled Jr HJ, Fox RJ, Gold PW, Herkenham M (1991) Long-term antidepressant administration alters corticotropin-releasing hormone, tyrosine hydroxylase, and mineralocorticoid receptor gene expression in rat brain. Therapeutic implications. J Clin Invest 87 : 831–837

Bunney WE Jr, Davis JM (1965) Norepinephrine in depressive reactions. Arch Gen Psychiatry 13 : 483–494

Cavalieri B (1966) Geometria delqi Indivisibli. Unione Tipografico Editrice, Torino Chan-Palay V, Asan E (1989) Quantitation of catecholamine neurons in the locus coeruleus in human brains of normal young and older adults and in depression. J Comp Neurol 287 : 357–372

Chen G, Yuan PX, Jiang YM, Huang LD, Manji HK (1998) Lithium increases tyrosine hydroxylase levels both in vivo and in vitro. J Neurochem 70 : 1768–1771

Coggeshall RE, Lekan HA (1996) Methods for determining numbers of cells and synapses: a case for more uniform standards of review. J Comp Neurol 364 : 6–15

Cottingham SL, Pickar D, Shimotake TK, Montpied P, Paul SM, Crawley JN (1990) Tyrosine hydroxylase and cholecystokinin mRNA levels in the substantia nigra, ventral tegmental area, and locus ceruleus are unaffected by acute and chronic haloperidol administration. Cell Mol Neurobiol 10 : 41–50

Cruz-Orive LM (1978) Particle shape-size distributions, the general spheroid problem, II: stochastic model and practical guide. J Microsc 112 : 153–167

Dahlstrom A, Fuxe K (1964) Localization of monoamines in the lower brain stem. Experientia 20 : 398–399

Dinan TG and Aston-Jones G (1984) Acute haloperidol increases impulse activity of brain noradrenergic neurons. Brain Res 307 : 359–362

Girault JA, Raisman Vozari R, Agid Y, Greengard P (1989) Striatal phosphoproteins in Parkinson disease and progressive supranuclear palsy. Proc Natl Acad Sci USA 86 : 2493–2497

Goldacre M, Seagroatt V, Hawton K (1993) Suicide after discharge from psychiatric inpatient care. Lancet 342 : 283–286

Hiremagalur B, Sabban EL (1995) Nicotine elicits changes in expression of adrenal catecholamine biosynthetic enzymes, neuropeptide Y and immediate early genes by injection but not continuous administration. Brain Res Mol Brain Res 32 : 109–115

Jones BE (1991) Noradrenergic locus coeruleus neurons: their distant connections and their relationship to neighboring (including cholinergic and GABAergic) neurons of the central gray and reticular formation. Prog Brain Res 88 : 15–30

Kitayama I, Yaga T, Kayahara T, Nakano K, Murase S, Otani M, Nomura J (1997) Long-term stress degenerates, but imipramine regenerates, noradrenergic axons in the rat cerebral cortex. Biol Psychiatry 42 : 687–696

Klimek V, Stockmeier C, Overholser J, Meltzer HY, Kalka S, Dilley G, Ordway GA (1997) Reduced levels of norepinephrine transporters in the locus coeruleus in major depression. J Neurosci 17 : 8451–8458

Komori K, Kunimi Y, Yamaoka K, Ito T, Kasahara Y, Nagatsu I (1992) Semiquantitative analysis of immunoreactivities of tyrosine hydroxylase and aromatic L-amino acid decarboxylase in the locus coeruleus of desipramine-treated mice. Neurosci Lett 147 : 197–200

Komori T, Nomura J, Inoue K, Kitayama I (1990) Tyrosine hydroxylase activity in discrete brain regions of depression model rats. Jpn J Psychiatry Neurol 44 : 747–754

Lopez AD (1990) Who dies of what? A comparative analysis of mortality conditions in developed countries around 1987. World Health Stat Q 43 : 105–114

Loughlin SE, Foote SL, Bloom FE (1986 a) Efferent projections of nucleus locus coeruleus: topographic organization of cells of origin demonstrated by three-dimensional reconstruction. Neuroscience. 18 : 291–306

Loughlin SE, Foote SL, Grzanna R (1986 a) Efferent projections of nucleus locus coeruleus: morphologic subpopulations have different efferent targets. Neuroscience. 18 : 307–319

Mason ST, Fibiger HC (1979) Regional topographiy within noradrenergic locus coeruleus as revealed by retrograde transport of horseradish peroxidase. J Comp Neurol 187 : 703–724

Masserano JM, Takimoto GS, Weiner N (1981) Electroconvulsive shock increases tyrosine hydroxylase activity in the brain and adrenal gland of the rat. Science 214 : 662–665

Melia KR, Nestler EJ, Duman RS (1992) Chronic imipramine treatment normalizes levels of tyrosine hydroxylase in the locus coeruleus of chronically stressed rats. Psychopharmacology (Berl) 108 : 23–26

MMWR MORB MORTAL WKLY REP (1997) Regional variants in suicide rates – United States, 1990–1994. (Aug 29)

Montgomery SA, Montgomery DB, Green M, Bullock T, Baldwin D (1992) Pharmacotherapy in the prevention of suicidal behavior. J Clin Psychopharmacol 12 : 27S–31S

Murase S (1987) Electrophysiological study on animal model of depression: Single unit activity of locus coeruleus neurons in rats exposed to long-term severe stress. Mie Medical J 37 : 1–15

Nestler EJ, McMahon A, Sabban EL, Tallman JF, Duman RS (1990) Chronic antidepressant administration decreases the expression of tyrosine hydroxylase in the rat locus coeruleus. Proc Natl Acad Sci USA 87 : 7522–7526

Ordway GA, Smith KS, Haycock JW (1994) Elevated tyrosine hydroxylase in the locus coeruleus of suicide victims. J Neurochem 62 : 680–685

Persson ML, Wasserman D, Geijer T, Jonsson EG, Terenius L (1997) Tyrosine hydroxylase allelic distribution in suicide attempters. Psychiatry Res 72 : 73–80

Petty F, Kramer G, Wilson L, Chae YL (1993) Learned helplessness and in vivo hippocampal norepinephrine release. Pharmac Biochem Beh 46 : 231–235

Schildkraut JJ (1965) The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am J Psychiatry 122 : 509–522

Schweizer E, Dever A, Clary C (1988) Suicide upon recovery from depression. A clinical note. J Nerv Ment Dis 176 : 633–636

Shah AK, Ganesvaran T (1997) Inpatient suicides in an Australian mental hospital. Aust N Z J Psychiatry 31 : 291–298

Smith MA, Brady LS, Glowa J, Gold PW, Herkenham M (1991) Effects of stress and adrenalectomy on tyrosine hydroxylase mRNA levels in the locus ceruleus by in situ hybridization. Brain Res 544 : 26–32

Teicher MH, Glod CA, Cole JO (1993) Antidepressant drugs and the emergence of suicidal tendencies. Drug Saf 8 : 186–212

Valentino RJ, Curtis AL, Parris DG, Wehby RG (1990) Antidepressant actions on brain noradrenergic neurons. J Pharmacol Exp Ther 253 : 833–840

Venter CP, Joubert PH (1984) Ethnic differences in response to beta 1-adrenoceptor blockade by propranolol. J Cardiovasc Pharmacol 6 : 361–364

Weibel ER (1979) Stereological methods: Practical methods for biological morphometry, Orlando, Fla, Academic Press Inc.1, pp 186–196

Weiss J, Glazer H, Pohorecky L: Coping behavior and neurochemical changes in rats: an alternative explanation for the original ‘learned helplessness’ experiments. In: Serban G, Kling A (eds) Animal models in human psychobiology. New York, Plenum Press, 1976, pp 141–173

Weiss JM, Goodman PA, Losito BG, Corrigan S, Charry JM, Bailey WH (1981) Behavioral depression produced by an uncontrollable stressor: relationship to norepinephrine, dopamine and serotonin levels in various regions of rat brain. Brain Res Rev 3 : 167–205

Weiss JM, Simson PG (1985) Neurochemical basis of stress-induced depression. Psychopharmacol Bull 21 : 447–457