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ORIGINAL PAPER

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# Tyrosine hydroxylase immunoreactivity in the locus coeruleus is elevated in violent suicidal depressive patients

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**Abstract** Our postmortem study aimed to determine the impact of suicide on the number of noradrenergic neurons of the locus coeruleus (LC) in suicidal depressive patients. Noradrenergic neurons were shown by immunostaining tyrosine hydroxylase in the LC of 22 non-elderly patients with mood disorders compared to 21 age- and sex-matched normal controls. Eleven patients were suicide victims and the other eleven died of natural causes. Seven violent suicide victims revealed an increased number of tyrosine hydroxylase immunoreactive (TH-ir) neurons compared with non-violent suicide victims and controls. No difference was found between the number of TH-ir neurons in all suicidal patients and controls and between non-suicidal patients and controls. The differences of TH-immunoreactivity could neither be attributed to medication nor to the polarity of depressive disorder (unipolar/bipolar). The numbers of TH-ir neurons in suicidal patients correlated negatively with the mean doses of antidepressants. The study suggested a presynaptic noradrenergic dysregulation in the LC related to the level of self-aggression. Traditional antidepressants may, therefore, regulate noradrenergic activity of the LC in suicide patients, however, without demonstrating the suicide-preventing effect.

**Key words** tyrosine hydroxylase · noradrenalin · locus coeruleus · depression · suicide

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## Introduction

The locus coeruleus (LC) comprises the largest group of noradrenalin (NA) containing neurons in the mammalian brain [22] and provides a widespread noradrenergic innervation to the central nervous system (for a review see [10]). According to preclinical and clinical evidence, mood disorders are associated with abnormalities in the noradrenergic system, which is supported by the multitude of regulatory functions of this system, especially evident in stress response and arousal (for reviews see [3, 55]). Only a few controlled postmortem studies investigated brain presynaptic noradrenergic levels in mood disorders, which suggested a LC-NA diathesis in depression [9, 37, 69].

Depression is the main cause of suicide and suicide is one of the most frequent causes of death in the developed countries [68]. Therefore, in addition to the diagnostic aspects, extensive research work has been focusing on the neurobiology of suicide in general and on the significance of the noradrenergic system in particular [5, 6, 35]. De Paermentier and colleagues [23, 24] emphasised the impact of the level of self-aggression when committing suicide on the abnormalities observed in the noradrenergic system postmortem.

Few postmortem studies investigated tyrosine hydroxylase (TH), the key enzyme of NA synthesis, in suicide victims. The results, however, are contradictory. One study demonstrated elevated TH protein levels [49], while another one showed a lower

immunohistochemical staining intensity of LC neurons for TH accompanied by an equal number of TH-immunoreactive (TH-ir) neurons of the LC compared to the number found in mentally healthy controls [11]. A later study also suggested a normal number of such neurons in depressive suicidal patients [9].

Although suicide is a core symptom of depression and the noradrenergic system has been implicated in both suicide and depression, no postmortem studies have analysed TH-ir neurons of the locus coeruleus in relation to the level of self-aggression in suicide victims with a well documented diagnosis of a mood disorder. We have, therefore, investigated this neuronal population in depressive patients who committed suicide by violent or non-violent means compared to normal controls.

Our study focused on two questions: firstly, is depression and/or suicide related to the number of TH-ir neurons in the LC and secondly, is the level of self-aggression related to this parameter in depressive suicidal patients?

## Materials and methods

### Subjects

The study was approved by the local ethics committee of the University of Magdeburg as being compliant with the Declaration of Helsinki of 1964 and the applicable EU and German laws.

Postmortem brains from 22 depressive subjects with a clinical diagnosis of major depressive disorder (MDD) or bipolar disorder (BD) according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) were obtained from autopsies performed in several pathological and forensic medicine institutes. Eleven of the 22 subjects died by suicide (4 men and 7 women, age range 26–59 years old). The methods of suicide comprised medication overdose (four cases, from them two of benzodiazepine-class and two of chlorpromazine-class overdose) and more violent measures, such as hanging (four), incision of the radial artery (one), self-stabbing (one), fall from the height (one). A toxicology screen on blood and urine for ethanol, other substances of abuse, many antidepressant and antipsychotic drugs, as well as their metabolites was performed at each of medico-legal autopsy.

Twenty-one brains from sex- and age-matched control subjects without detectable neuropsychiatric disorders were included. According to the German autopsy laws, informed consent for autopsy and dissections of the brains was obtained from the relatives of all the patients and controls. Antemortem DSM-IV diagnoses were obtained by psychological autopsies involving a careful study of clinical records and the performance of structured interviews with a physician involved in the treatment and at least one person who either lived with or had frequently contact with the subject before death, according to the Structured Clinical Interview for DSM-IV Axis I Disorders. The mean doses of psychotropic medication in the last 90 lifetime days (Table 1) were established according to the clinical records. No patients were treated with atypical antipsychotics or mood stabilizers. The DSM-IV Axis I diagnosis of MDD and BD was established in consensus meeting by two independent psychiatrists (Hendrik Bielau and Johann Steiner). The same procedure was followed to exclude neuropsychiatric disorders in controls. Stressors which occurred in the last year before death were determined on DSM-IV Axis IV. The distribution of smokers and non-smokers was similar in suicidal and non-suicidal patients as well as in controls. Neither patients nor controls demonstrated qualitative neuropathological changes suggestive of neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Pick's disease), tumours, inflammation, vascular disorders or posttraumatic lesions in the opinion of an experienced neuropathologist. Patients or control subjects with a history of alcohol or drug abuse were excluded on the basis of documentary evidence, toxicology screens and liver histology.

Subjects who underwent electroconvulsive therapy (ECT) in the last year before death were also excluded, as ECT has been shown to elevate TH levels [15, 41]. The age ranges in the patients and the controls were 26–69 and 30–67 years old, respectively. All BD patients were in depressive phase of their illness. The patients' and the controls' demographic data are summarised in Table 2.

**Table 1** Psychotropic drugs in the last 90 days of lifetime (mean daily doses)

| Patient no. | Antidepressants (amitriptyline equivalents, mg) | Neuroleptics (chlorpromazine equivalents, mg) | Benzodiazepines (diazepam equivalents, mg) | Lithium (mg) |
|---|---|---|---|---|
| 1 | 50 | 0 | 0 | 0 |
| 2 | 0 | 110 | 17.6 | 0 |
| 3 | 52 | 109 | 10.9 | 0 |
| 4 | 0 | 280 | 0 | 0 |
| 5 | 30 | 111 | 16.5 | 0 |
| 6 | 17 | 501 | 0 | 0 |
| 7 | 0 | 221 | 0.8 | 740 |
| 8 | 0 | 0 | 6.8 | 0 |
| 9 | 0 | 0 | 1.6 | 280 |
| 10 | 0 | 0 | 0 | 0 |
| 11 | 93 | 117 | 3.9 | 0 |
| 12 | 93 | 0 | 3.1 | 560 |
| 13 | 133 | 327 | 3.3 | 558 |
| 14 | 47 | 0 | 0 | 0 |
| 15 | 112 | 140 | 10 | 0 |
| 16 | 20 | 0 | 0 | 0 |
| 17 | 124 | 109 | 0 | 0 |
| 18 | 0 | 0 | 0 | 0 |
| 19 | 0 | 0 | 0 | 0 |
| 20 | 95 | 47 | 18.3 | 565 |
| 21 | 0 | 0 | 0 | 0 |
| 22 | 0 | 0 | 0 | 0 |

**Table 2** Characteristics of subjects

| No./Sex/Age (years) | Psychiatric diagnosis (DSM-IV) | Cause of death | PMI (h) | TH-ir neurons total number |
|---|---|---|---|---|
| Controls | | | | |
| 1/M/47 | | CF | 24 | 62,099 |
| 2/M/47 | | Bronchopneumonia | 24 | 78,578 |
| 3/F/64 | | HF | 24 | 82,395 |
| 4/F/50 | | Dissecting aortic aneurysm | 40 | 58,102 |
| 5/M/40 | | MI | 50 | 67,086 |
| 6/M/64 | | HF | 35 | 36,884 |
| 7/F/48 | | PE | 26 | 86,868 |
| 8/F/65 | | HF | 24 | 76,068 |
| 9/F/30 | | PE | 48 | 54,853 |
| 10/F/64 | | HF | 26 | 64,026 |
| 11/M/63 | | HF | 48 | 91,340 |
| 12/F/61 | | SCD | 40 | 130,683 |
| 13/F/38 | | PE | 24 | 69,101 |
| 14/M/39 | | Peritonitis | 50 | 72,800 |
| 15/F/61 | | HF | 24 | 50,741 |
| 16/F/67 | | SCD | 24 | 56,886 |
| 17/M/54 | | RHF | 24 | 63,159 |
| 18/F/63 | | HF | 24 | 73,060 |
| 19/F/39 | | Heat stroke | 48 | 57,010 |
| 20/F/66 | | RHF | 24 | 50,571 |
| 21/F/39 | | MI | 48 | 106,595 |
| Non-suicidal depressives | | | | |
| 1/F/63 | MDD (296.34) | PE | 17 | 127,348 |
| 2/F/62 | BD-D (296.54) | | 72 | 52,741 |
| 3/F/60 | BD-D (296.54) | Bronchopneumonia | 14 | 62,866 |
| 4/M/39 | BD-D (296.53) | PE | 14 | 18,295 |
| 5/F/61 | MDD (296.34) | SCD | 70 | 26,294 |
| 6/F/41 | MDD (296.34) | PE | 20 | 44,805 |
| 7/M/39 | BD-D (296.53) | CF | 56 | 92,817 |
| 8/M/69 | BD-D (296.53) | PE | 48 | 86,634 |
| 9/M/69 | BD-D (296.54) | Bronchopneumonia | 24 | 62,249 |
| 10/F/52 | BD-D (296.53) | PE | 24 | 108,647 |
| 11/F/65 | BD-D (296.54) | HF | 52 | 102,680 |
| Non-violent suicidal depressives | | | | |
| 12/F/39 | BD-D (296.53) | Overdose of medication | 48 | 75,052 |
| 13/F/46 | BD-D (296.54) | Overdose of medication | 38 | 45,399 |
| 14/F/47 | MDD (296.34) | Overdose of medication | 24 | 20,696 |
| 15/F/59 | BD-D (296.54) | Overdose of medication | 38 | 81,805 |
| Violent suicidal depressives | | | | |
| 16/M/47 | BD-D (296.53) | Stab wound | 24 | 64,515 |
| 17/F/46 | MDD (296.24) | Hanging | 48 | 82,283 |
| 18/F/53 | MDD (296.33) | Hanging | 46 | 87,353 |
| 19/F/26 | MDD (296.33) | Fall from the height | 22 | 81,348 |
| 20/M/42 | BD-D (296.54) | Hanging | 17 | 93,016 |
| 21/M/35 | MDD (296.33) | Incision of radial artery | 15 | 112,449 |
| 22/M/36 | MDD (296.34) | Hanging | 42 | 108,713 |

*M* male, *F* female, *PMI* postmortem interval, *MDD* major depressive disorder, *BD-D* bipolar disorder depressed, *CF* coronary failure, *HF* heart failure, *MI* myocardial infarction, *PE* pulmonary embolism, *RHF* right heart failure, *SCD* sudden cardiac death

## Histological procedure

Brains were removed within 14–72 h after death. The mean postmortem intervals for the brains of suicide patients, non-suicidal patients and controls were 32.9, 37.4 and 33.3 h, respectively. Brains were fixed in toto in 8% phosphate-buffered formaldehyde in controlled pH for at least 2.5 months (pH = 7.0, $T$ = 15–20°C). The brainstem was isolated by a cut made perpendicularly to its longitudinal axis at the point of emergence of the oculomotor nerve. The frontal and occipital poles were separated by frontal sections anterior to the genu and posterior to the splenium of the corpus callosum; according to this procedure, the "anterior", the "middle" and the "posterior" blocks of brain hemispheres were obtained. After embedding all parts of the brains in paraffin, 20-μm thick serial sections of the brainstem were cut perpendicular to the longitudinal axis in a rostrocaudal direction on a Leica Polycut microtome and mounted. Each 50th section was deparaffinised, hydrated, and Nissl- (cresyl violet-) stained.

Adjacent sections to each Nissl-stained section were selected along the rostrocaudal axis of the LC (nucleus proper) and immunocytochemically stained for TH. The first (most rostral) section for immunostaining of TH was randomly selected from the first three rostral Nissl-stained sections. Thus, the selection of sections was in accordance with the Cavalieri theorem of systematic sampling. The distance between the Nissl-stained sections as well as between TH immunostained sections was 1 mm. So a total on the average of 12 sections of each LC was investigated for TH immunoreactivity.

## Immunocytochemical procedure

Mounted sections were deparaffinised and hydrated, and neuromelanin pigment of the locus coeruleus was bleached with hydrogen peroxide for 48 h. The TH-ir of the polyclonal antibody serum was confirmed by the manufacturer (Biotrend Chemikalien GmbH, Cologne, Germany) by Western blotting and immunocytochemistry. After the pre-incubation of the sections with methanol/$H_2O_2$ to inactivate endogenous peroxidases and repeated washing with phosphate-buffered saline, a TH antiserum raised against TH in rabbits was used. The antiserum was used at a 1:1,000 dilution for 24 h at 4°C after pre-adsorption with natural melanin (*Sepia officinalis*, Sigma, St Louis, USA, 0.1 mg/ml for 60 min) to remove any cross reactivity with neuromelanin. Further immunocytochemical protocol involved the incubation with goat anti-rabbit IgG serum (DAKO, Wiesentheid, Germany) and the application of the avidin–biotin technique (Amersham, Freiburg, Germany). The chromogen 3,3′-diaminobenzidine was used to visualise the reaction product. For the purposes of control, the primary antiserum was either replaced by a buffer or a normal serum. Sections without the specific primary antiserum did not show any immunostaining. Immunostaining was abolished by pre-adsorption of the primary antiserum with homogenised and centrifuged human adrenal tissue, which is rich in TH. Two further TH antisera (Sigma, St Louis, USA; Camon, Wiesbaden, Germany) were applied for immunostaining resulting in a comparable neuronal staining pattern in the LC.

## Quantification

TH-ir LC neurons were counted with the aid of a video-based computed system (DIGITRACE®) connected to a LEICA-microscope which was fitted with a motor stage. The composed panorama images of the LC cross sections were taken serially at objective magnification 10×. On these images, the TH-ir neurons profiles were identified visually and traced with a computer mouse. The distance between sections was known, therefore, the total number of TH-ir neurons profiles along the LC was calculated by integrating the number of the profiles obtained for each section bilaterally. The aim of described procedure was not to obtain the absolute numbers of the stained neurons, but to detect the difference in the investigated parameter between analysed groups. The recent study on TH-ir neurons in the LC of schizophrenics revealed that by similar magnification the profiles count is a reliable estimator of neurons count and no need existed to apply the assumption-based methods [19]. Applying the simplified method of quantification, we took into account that each quantitative procedure available in practice leads only to the estimated and not to the absolute value of investigated parameter [17, 26, 28, 67].

The intra- and inter-rater reliability of the profiles count were assessed by an analysis of the measures obtained in triplicate (twice by Tomasz Gos and once by Hendrik Bielau) for five cases selected at random from the series. The parameter showed a high intra- and inter-rater reliability (intra-class correlation coefficient of >0.9 in both cases). The coefficient of error of counting method was 0.05.

## Statistics

Due to the fact that not all groups showed normal distribution, non-parametric tests were used. Three different procedures were applied. Firstly, comparisons with unadjusted Mann–Whitney *U* test were carried out to detect two-way differences (depressives vs. controls); secondly, the Kruskal–Wallis rank-based analysis of variance (KW test) was performed using the diagnostic group as a three-way or four-way independent variable (suicides vs. non-suicides vs. controls, MDD vs. BD vs. controls, violent vs. non-violent suicides vs. controls and violent vs. non-violent suicides vs. non-suicides vs. controls), followed by comparisons of unadjusted values by the Mann–Whitney *U* test.

The KW tests were applied to detect the possible differences between the analysed groups according to sex, age, brain weight, time after death, fixation time, shrinkage factor, smoking status and duration of agony followed by unadjusted *U* test comparisons. The duration and polarity of disease as well as psychiatric medication were analysed in the suicidal and non-suicidal depressive patients and in the violent and non-violent suicidal patients by unadjusted *U* test comparisons.

Spearman's correlation coefficients were calculated to determine the influence of the above demographic, clinical and methodical variables which might confound the results of dependent variables. Intra-rater and inter-rater reliability were assessed by calculating the intra-class correlation coefficients for pairs of measurements.

Generally, *P* values of <0.05 were accepted as statistically significant. When both KW test and multiple post hoc comparisons with *U* test were considered in combination, the *P* values were corrected for multiple comparisons.

# Results

According to the *U* test results, no significant difference was observed between all depressive patients and controls in the number of TH-ir neurons on each side and the total number of these neurons bilaterally. There was also no significant difference in side-related or total numbers of TH-ir neurons between patients with MDD and BD or controls and between non-suicidal patients and suicide victims or controls, and between violent and non-violent suicide victims and non-suicides or controls, as shown by KW test. Contrary, the KW test revealed significant difference between violent and non-violent suicides and controls with respect to the number of TH-ir neurons on the right ($P = 0.018$) and bilaterally ($P = 0.042$). The Mann–Whitney *U* test showed the total number of TH-ir neurons to be significantly higher in violent suicide victims versus controls and non-violent suicide victims. This effect was caused by the significant increase on the right side only (Table 3).

## Confounding variables and correlations

Patients did not differ from controls with respect to sex, age at death, postmortem interval, fixation time, brain weight, shrinkage factor, smoking status and duration of agony. The age at death in suicidal patients, non-suicidal patients and controls were different (KW test, $P = 0.027$). The suicidal patients were significantly younger than controls (*U* test, $P = 0.022$) and non-suicidal patients (*U* test, $P = 0.048$). However, there was no significant difference in age at death in violent suicidal patients, non-violent suicidal patients and controls (KW test, $P = 0.055$).

A tendency to higher dose of antidepressants in the last seven days before death was found in the suicidal subgroup compared to non-suicidal patients (*U* test, $P = 0.059$) but there was no such tendency between violent and non-violent suicidal patients (*U* test, $P = 0.57$). The age at the onset of illness was signifi-

**Table 3** The numbers of tyrosine hydroxylase-immunoreactive neurons in the locus coeruleus of violent versus non-violent suicides and controls

| | C $n = 21$ | NV $n = 4$ | V $n = 7$ | C versus NV $U$ test $P$ values[a] | C versus V $U$ test $P$ values[a] | NV versus V $U$ test $P$ values[a] |
|---|---|---|---|---|---|---|
| Mean total | 70,900 | 55,738 | 89,954 | n.s. | *0.042* | *0.042* |
| SD | 20,901 | 28,210 | 16,604 | | | |
| Mean right | 35,418 | 27,811 | 45,735 | n.s. | *0.018* | *0.024* |
| SD | 10,427 | 16,306 | 7,134 | | | |
| Mean left | 35,482 | 27,927 | 44,219 | n.s. | n.s. | n.s. |
| SD | 10,830 | 13,109 | 10,260 | | | |

*C* controls, *NV* non-violent suicides, *V* violent suicides, *n* number of cases, *SD* standard deviation, *n.s.* non-significant
[a]*U* test *P* values were corrected for multiple comparisons; the significant *P* values are shown in italics

cantly lower in suicidal versus non-suicidal patients ($U$ test, $P = 0.003$) but no difference was found between the violent and non-violent suicidal subgroups ($U$ test, $P = 0.91$). The distribution of polarity and sex showed no significant differences in the subgroups of patients. Recent stressors as determined by DSM-IV did not differ among patient subgroups and controls.

The majority of above potential confounders were not correlated to the number of TH-ir neurons on each side or to their total number bilaterally. Only the mean dose of antidepressants given in last 90 days before death correlated negatively with the number of TH-ir neurons on the right side in suicidal patients ($r = -0.725$, $P = 0.018$).

## Discussion

We found that in patients with mood disorders who commit violent suicide, the number of locus coeruleus neurons immunoreactive for tyrosine hydroxylase is higher than in controls or non-violent suicide victims in a side-specific manner. On the other hand, no difference was found between all depressive patients and controls, and between non-suicidal patients, suicide victims and controls. Of the potential confounding demographic, clinical and methodical variables, only the age at death and the age at the onset of illness were significantly different between the compared groups. However, similarly to the majority of other potential confounding variables, no correlation was found between these variables and the number of TH-ir neurons. Therefore, there existed no variables which should be considered as confounding variables or analysed as covariates. Moreover, the applied non-parametric procedure excluded the analysis of covariance with confounding variables as covariates.

It should be especially pointed out that no confounding effects of autolysis and fixation time (as methodical variables) or polarity, recent stressors and smoking status (as clinical variables) were found between compared groups. The level of TH-ir in the LC of long-term cigarette smokers is lower compared with non-smokers [38], therefore, the smoking status was considered confounding variable. Moreover, the difference between suicidal and non-suicidal patients could not be considered as an artefact related to the cause of death, i.e., overdose in the latter group, because no drugs are known which attenuate the expression of TH in LC neurons after acute administration [45, 50, 56, 64]. On the other hand, no patients were treated with mood stabilizers or atypical antipsychotics which are known to increase TH expression after chronic treatment [50, 56, 64]. Therefore, the effect observed in violent suicidal subgroup could not be related to the medication. The possible impact of acute central stress reaction related to the cause of death should be considered additionally in violent suicides because LC is one of key components of this reaction (for a review see [10]). However, the experimental data do not support the suggestion that acute stress could simultaneously produce an increased TH-protein expression in the LC neurons [18, 25, 32, 42, 51]. To sum up, the effect observed in violent suicidal subgroup should not be considered as related to the mentioned, possibly confounding variables.

The higher number of TH-ir neurons in the LC of violent suicide victims versus controls is consistent with the finding of Ordway and colleagues [49], who described elevated TH protein concentrations in the LC of suicide victims versus controls. It is also consistent with the study by Zhu and colleagues [69], who found elevated TH-immunoreactivity in LC in major depression patients. The majority of suicide victims in the former and of depressive patients in the latter study were violent suicide victims. On the other hand, our data are inconsistent with the decreased TH immunoreactivity in suicide victims reported by Biegon and Fieldust [11]. These contrasting findings could be explained by the variances of neuronal protein concentration as well as differences in the study populations with regard to sex [8], race [63], underlying psychiatric diagnoses and the form of suicidal act in suicide victims, pharmacological influences and, in particular, the fact that the optical staining intensity was measured rather than the number of immunoreactive neurons (Fig. 1).

The former studies suggest that the varying numbers of TH-ir neurons are not a result of differences in the total number of LC neurons capable of NA synthesis but rather the current content of TH in these

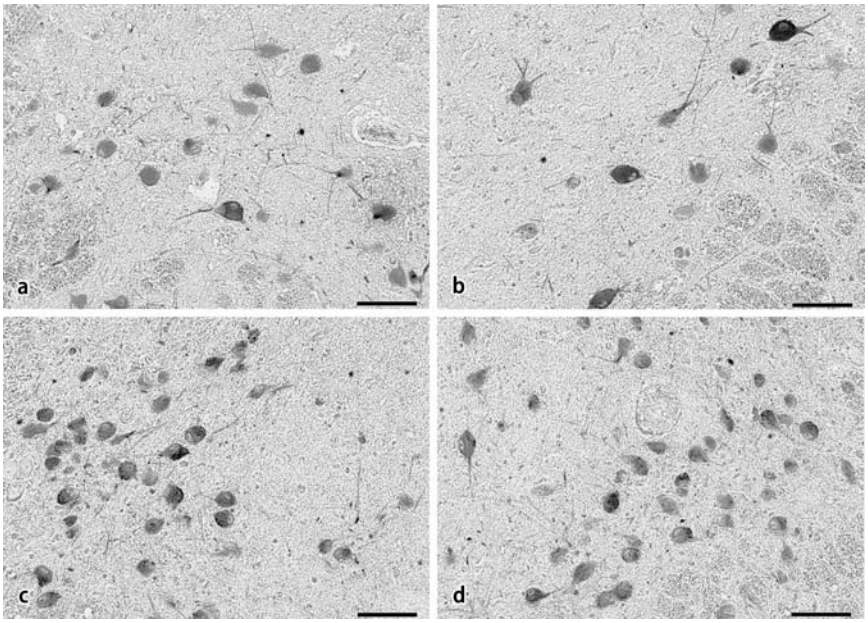


**Fig. 1** TH-ir neurons in the LC of control subject (**a** right side, **b** left side) compared with violent suicidal depressive patient (**c** right side, **d** left side); *scale bars* 100 μm

neurons [4, 9, 14]. This might confirm the number of TH-ir neurons in the LC as a state-dependent marker of violent suicidal behaviour in patients with mood disorders. It is conceivable that violent suicidal risk is constrained to certain time frames during a depressive episode which are defined by noradrenergic dysfunction [36, 54, 65, 66]. However, a study reporting distinct alleles of the TH gene associated with suicidal behaviour [53] might suggest the TH gene as a potential trait marker of vulnerability for suicidal risk.

Given the fact that tyrosine hydroxylase as the key enzyme of NA synthesis, our findings might suggest an elevated production of NA with consequently increased noradrenergic neurotransmission in those patients with mood disorders who are actually prone to the escalated self-aggression. This interpretation would be in line with the data from many animal studies of stress and aggression (for reviews see [10, 29]). However, no simple correlation exists between the increased number of TH-ir in LC neurons and the increased noradrenergic neurotransmission. On the contrary, experimental data suggests that the increased TH expression in LC could also result from the synaptic NA depletion corresponding with the decreased NA transporter affinity in LC [20, 43]. The findings of postmortem investigations of LC of suicidal and depressive patients were similar to the finding mentioned above and what is more, violent suicidal cases prevailed in these studies [37, 49, 69]. The decreased concentration of the major NA metabolite, methoxy-4-hydroxyphenylglycol, was found in the cerebrospinal fluid in depressive patients with violent suicidal tendencies [1] and in plasmas of depressive violent suicide attempters [62]. Many of the NA depletion studies suggest that this depletion may in itself be sufficient to lead to severe depression in some individuals (for reviews see [3, 55]). Independently, concerning the relevance for noradrenergic neurotransmission, the increased expression of TH in LC we observed could be related to the activating effect of corticotropin-releasing hormone on LC [7, 10, 12, 34, 61].

Because LC innervates predominantly the ipsilateral prefrontal cortex (PFC) [10], the abnormality of the right LC found in our study is consistent with the reported association of negative affect with the right hemisphere [13, 30, 40, 47, 48, 52] and the arousal caused by disinhibition of the right ventromedial PFC [58]. Thus the disturbed LC and PFC function could be related in a feedback manner accordingly to their reciprocal connections [10].

The negative correlation between the mean dose of antidepressant medication given in the last 90 days and the number of TH-ir neurons in suicidal patients may be seen as a consequence of this medication according to experimental reports [16, 25, 27, 36, 39, 46, 60]. However, the putative reducing effect of antidepressants given for longer periods before death on the number of TH-ir neurons could not prevent suicidal acts. Moreover, the trend to higher dose of antidepressants given shortly before death was found in suicidal patients compared with non-suicidal group. This deleterious phenomenon could be partially explained by the effect of antidepressants on the serotonergic system [2, 57], but the first days and weeks after starting antidepressant treatment could be in general a high risk period for suicidality in a subgroup of patients also through its effects on the noradrenergic system [31, 33, 44, 59].

A major limitation of our study is small case number. A further limitation of this study is given by the lack of data on drug exposure across the whole life span, since we could only collect data on psychotropic

medication in the last 3 months prior to death. The possible impact of unipolar–bipolar dichotomy of mood disorders on the obtained results should be also considered, although the statistical evaluation of our collection has not supported such notion. Moreover, the body of data suggests that depressive episode in MDD and BD are similar phenomena (for a review see [21]). Additionally, for a comparison between MDD and BD patients within analysed groups the sample size is too small. Therefore, for further studies case numbers from both diagnostic groups should be increased.

In summary, we found an increased number of TH-ir neurons in depressive patients committing violent suicide whereas no change was observed in non-violent suicidal and other depressive patients. These findings suggest an LC dysfunction specific for the escalated self-aggression. The antidepressant medication tends to normalise the number of TH-ir LC neurons in suicidal subgroup of depressive patients. However, it is not an effect that could prevent suicidal acts.

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