Pathophysiology of the human locus coeruleus complex in fetal/neonatal sudden unexplained death

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Objectives: We investigated the locus coeruleus complex in the brainstems of 78 subjects aged from 24 gestational weeks to 8 postnatal months, who died of unknown (sudden unexplained fetal and infant deaths) and known causes (controls). The goals of this study were: (1) to obtain basic information about the morphology of the locus coeruleus complex and the expression of different biological parameters (tyrosine hydroxylase, neuromelanin and apoptosis) during the first phases of human nervous system development; (2) to evaluate possible alterations of this structure in victims of sudden death; and (3) to verify any correlation with risk factors.

Methods: All the victims were subjected to a complete autopsy, including an in-depth histological examination of the autonomic nervous system and in particular of the locus coeruleus complex, the target of this study. Adrenergic neurons were identified by tyrosine hydroxylase (TH) immunohistochemistry and neuromelanin-containing neurons were specifically visualized by the application of Lillie's method. In addition, the activation of programmed cell death (apoptosis) was studied by investigating DNA fragmentation (TUNEL-positive cells).

Results: Alterations of the noradrenaline system, decreased neuromelanin, hypoplasia, in addition to a high neuronal death rate, were observed almost exclusively in the locus coeruleus complex of fetal and infant sudden deaths, and were significantly correlated to maternal smoking.

Discussion: The developmental defects found in the locus coeruleus complex in victims of sudden unexplained fetal and infant death imply alterations of the vital activities related to the widespread brain connections arising from this neuronal center, including coordination of the sleep–waking cycle and control of the cardio-respiratory system.

Keywords: Locus coeruleus, Neuropathology, Brainstem, SIDS, SIUDS, Thyrosine hydroxylase, Neuromelanin

Introduction

The locus coeruleus (LC), known to be the major producer of noradrenaline in the brainstem,[1,2] is a well-delineated cluster of neurons located in the rostral dorsolateral pons at the anterior end of the fourth ventricle.[3,4] The simplicity of its cellular arrangement belies an extremely complex innervation pattern. Projections from this nucleus are, in fact, responsible for more than half of the noradrenergic terminals throughout the brain, including the neocortex, thalamus, amygdala, hippocampus, hypothalamus, cerebellum, and spinal cord.[5–7] The fact that nearly all levels of the central nervous system are innervated by this relatively small set of neurons implies that the LC subserves important physiological functions, including coordination of the sleep–waking cycle[8,9] and control of the cardio-respiratory system.[10,11]

The biosynthesis of noradrenaline, like that of other catecholamines (adrenaline and dopamine), occurs via the amino acid tyrosine by means of the tyrosine hydroxylase (TH) enzyme.[12,13] High concentrations of TH were already detected by immunohistochemistry, about 30 years ago,[14,15] in the LC of rats as from the early stages of prenatal development.

Another byproduct deriving from the tyrosine catabolism is neuromelanin (NM), a dark polymer pigment present in specific populations of catecholaminergic neurons in the brainstem.[16,17] In humans, the amount of NM increases during ontogenesis, even if there is a wide range of reported appearances, from 5 months of gestation to 3 years of age.[18,19] In

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general, mature noradrenergic neurons of the LC contain considerable amounts of NM pigment.

Interest in the LC has seen an increase in recent years because of a hypothesized link between a vulnerability of NM- and/or noradrenaline-containing neurons of this nucleus and neurodegenerative diseases, including Parkinsonism and Alzheimer's diseases.[20–23]

In a previous study, we focused upon the TH expression in the LC neurons of sudden intrauterine unexplained death syndrome (SIUDS) and sudden infant death syndrome (SIDS) victims (18 cases in all),[24] showing a decreased noradrenaline content in these pathologies. Before our study only Obonai *et al.*[25] had investigated the catecholaminergic system in subjects who died of SIDS, but they did not find the presence of noradrenergic neuronal alterations in the LC.

The aim of the present study was to extend the examination of the LC to a larger number of SIUDS and SIDS victims, and to evaluate whether the previously reported TH disorders may be associated to alterations of the NM pigment, as a related byproduct in catecholamine synthesis. We examined the LC in 78 brainstems of subjects aged from 24 gestational weeks to 8 postnatal months, who died of both unknown (SIUDS and SIDS) and known (controls) causes. The study protocol included morphological analysis of the LC in routinely stained sections and the application of specific histochemical and immunohistochemical procedures to highlight the tyrosine byproducts (TH and NM). In addition, the TUNEL method was applied to individuate apoptotic cells, given their role as indicators of hypoxic damage.[26]

Material and Methods

Study subjects

The study sample included a total of 78 cases, grouped into three subsets of victims: a SIDS group, a SIUDS group, and a control group.

SIDS and SIUDS victims

The SIDS cases included 30 infants, 12 females, and 18 males, aged from 1 to 8 postnatal months (median age: 3.7 months). The SIUDS cases included 24 unexplained ante-partum deaths, 11 females, and 13 males, aged 25–41 gestational weeks (median age: 38 weeks).

This was a selected set of cases, sent to our Research Center and diagnosed according to the application of the guidelines stipulated by Italian Law no. 31/2006 'Regulations for Diagnostic Post Mortem Investigation in Victims of SIDS and Unexpected Fetal Death'. This law decrees that all infants with suspected SIDS who died suddenly in Italian regions within the first year of age, as well as all fetuses who died after the twenty-fifth week of gestation without any apparent cause (SIUDS), must undergo an in-depth anatomo-pathological examination, particularly of the autonomic nervous system.

Ethics approval for this study was granted by the Italian Health Ministry in accordance with Italian Law no. 31/2006. Parents of all subjects (SIUDS, SIDS, and controls) provided written informed consent to both the autopsy and the anatomopathologic study, under the protocols approved by Milan University, the Lino Rossi Research Center institutional review board.

For every case, a complete clinical history was collected. Additionally, mothers were asked to complete a questionnaire on their smoking habit, detailing the number of cigarettes smoked before, during, and after pregnancy. Twenty-two of the 54 SIUDS/SIDS mothers (40%) were active smokers before and during the pregnancy, smoking more than 3 cigarettes/day. The remaining 32 mothers (60%) admitted no history of cigarette smoking.

Controls

This group included 24 victims of sudden death (12 infants — three females, nine males, aged from 1 to 8 postnatal months, median age: 3.4 months and 12 fetuses — six females, six males, aged 17 to 40 gestational weeks, median age: 35 weeks) in whom a complete autopsy and clinical history analysis established a precise cause of death. The related infant death diagnoses were: congenital heart disease ($n=5$), severe bronchopneumonia ($n=3$), myocarditis ($n=1$), pulmonary dysplasia ($n=2$), and mucopolysaccharidosis type I ($n=1$). Specific diagnoses among the fetal deaths included: chorioamnionitis ($n=7$) and congenital heart disease ($n=5$).

Seven of the 24 mothers of the control group (29%) reported a smoking habit. The remaining 16 mothers (71%) were not smokers.

Anatomopathologic protocol

All the victims of the study were subjected to a complete autopsy, including examination of the placental disk, umbilical cord and membranes in fetal deaths. In all cases an in-depth histological examination of the autonomic nervous system was made, in accordance with the protocol of the 'Lino Rossi Research Center for the Study and Prevention of Unexpected Perinatal Death and SIDS' of Milan University.[27,28]

In detail, after fixation in 10% phosphate-buffered formalin, the brainstems were processed and embedded in paraffin. Examination of the brainstem included sampling of three specimens, as shown in Fig. 1 (in red), that allow to analyze the main nuclei and structures checking the vital functions.

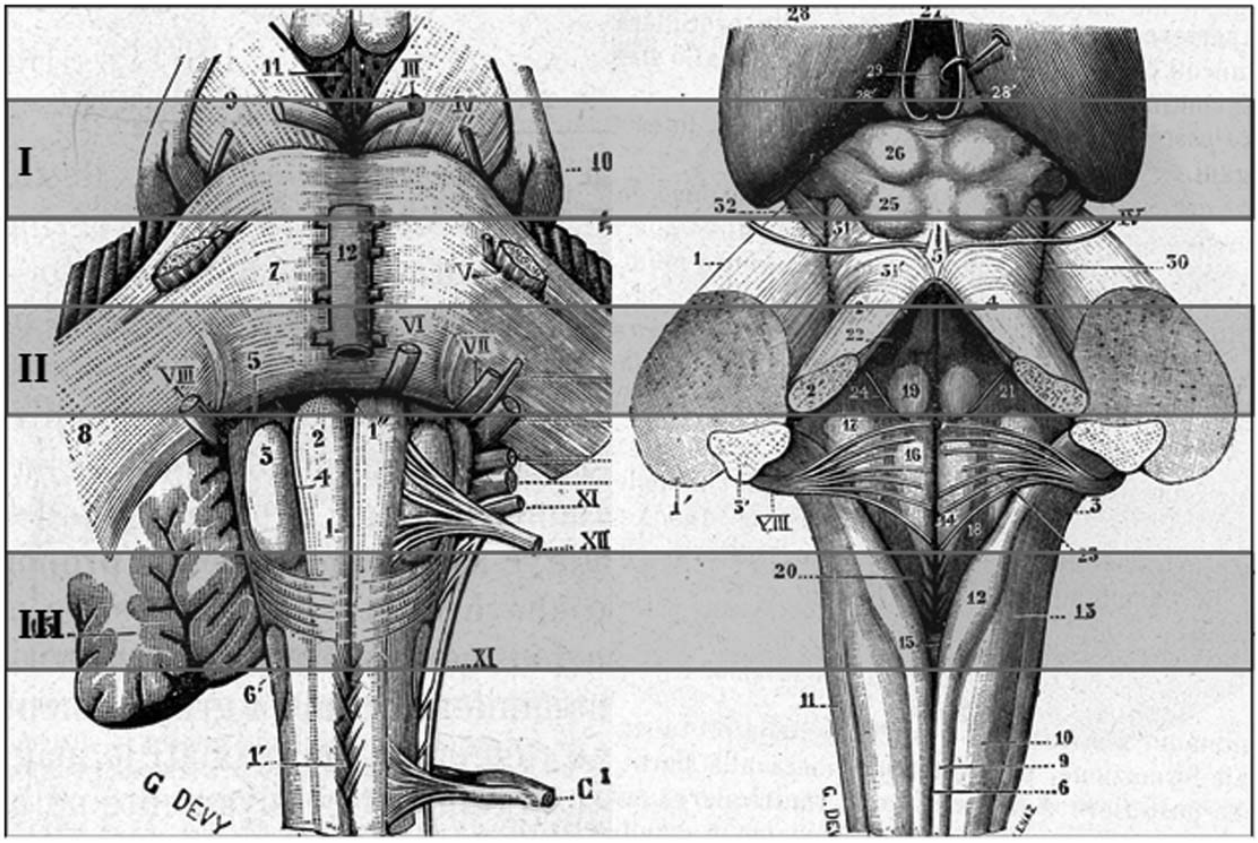

Figure 1 Sampling of the brainstem: ventral (left) and dorsal (right) surface (modified from L. Testut, 'Anatomia Umana', Unione Tipografico-Editrice Torinese, Torino, 1923). Areas to be sectioned are in red. (I) Cranial block, extending from the caudal mesencephalon to the upper part of the pons; (II) intermediate block, including the upper medulla oblongata and the adjacent caudal pons; (III) caudal block, with the obex as reference point (2–3 mm above and below the obex).

The first specimen ponto-mesencephalic (I), includes the upper third of the pons and the adjacent portion of mesencephalon. The second (II) extends from the upper medulla oblongata to the adjacent caudal portion of the pons. The third (III) specimen takes as reference point the obex and extends 2–3 mm above it and below it.

To make the in-depth analysis of the LC, the target of this study, serial histological sections were selected in every case from the entire first specimen (I), which includes the nucleus in its complete extension. These sections were then stained by histochemical and immunohistochemical techniques. Histological examination was performed using routine hematoxylin and eosin, and Klüver–Barrera stains and, at times when useful, Bielschowsky's silver impregnation technique.[29] Two populations of LC neurons were specifically examined in consecutive sections: catecholaminergic neurons identified by TH immunohistochemistry and NM-containing neurons, visualized by Lillie's method.[30] Also, the Bielschowsky's method was used for the demonstration of NM, since it is blackened by acid silver nitrate solution.[29] In further sections, the specific immunohistochemical method for apoptosis (TUNEL) was applied.

For comparative analysis of the LC among the different cases, we examined its cytoarchitecture and the TH, NM, and apoptotic expression at the same levels, namely at the most cranial histological sections of the pons.

Immunohistochemistry

TH immunohistochemistry

The selected sections were rinsed three times in 0.1 m Trizma buffered saline (TBS) followed by a 48-h incubation at 4°C with a 1/500 dilution of primary rabbit antiserum to TH (Novocastra Laboratories, Newcastle, UK). The dilutions were prepared with a solution of 1% normal goat serum (NGS) and 0.25% Triton X-100 in 0.1 m Tris-saline. This was followed by a 2.5-h incubation with biotinylated goat anti-rabbit immunoglobulin G (Vector Laboratories, Burlingame, CA, USA) diluted 1/200 with 1% NGS in Tris-saline. The tissue was then incubated for 2 h with the avidin–biotin complex diluted 1/100 with 1% NGS in Tris-saline (Vector). Between each incubation, the sections were rinsed three times with 1% NGS in Tris-saline. The sections were then treated for 6 minutes with a 0.05% solution of 3,3′ diaminobenzidine and 0.01% hydrogen peroxide, rinsed in

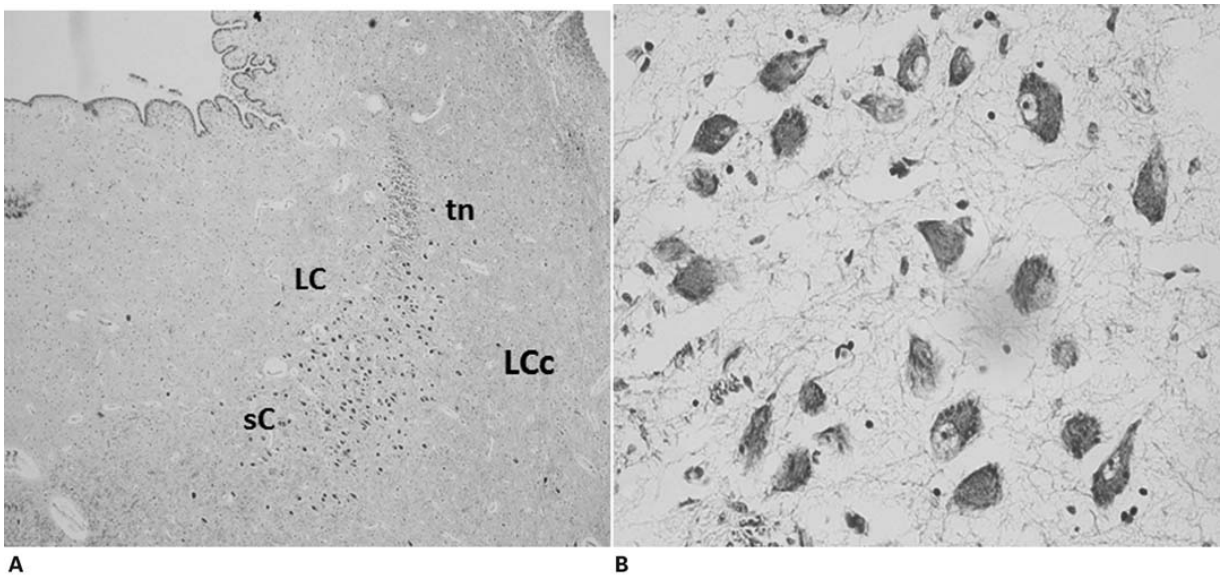

Figure 2 (A) Histological section of human rostral pons (3-month-old control) showing the localization (around the mesencephalic tract of the trigeminal nerve) and morphology of the LCc, including the LC nucleus proper and the sC nucleus. Klüver–Barrera stain. Magnification: ×10 (tn: trigeminal nerve). (B) Neurons of the LC nucleus. Klüver–Barrera stain. Magnification: ×40 (LC: locus coeruleus; LCc: locus coeruleus complex; sC: subcoeruleus; tn: trigeminal nerve).

phosphate buffer, mounted on gel-coated slides, cleared in xylene and coverslipped with Depex mounting medium.

Apoptosis immunostaining (TUNEL method)

The successive 2–3 sections for every case from the same block already used for histological examination and TH immunohistochemistry were deparaffinized and incubated with 20 μg/ml proteinase K (Sigma, St Louis, MO, USA). After blocking the endogenous peroxidase with 3% hydrogen peroxide, deoxynucleotidyl transferase (TdT, 0.3 U/ml) was used to incorporate digoxigenin-conjugated deoxyuridine (dUTP, 0.01 mM/ml) into the ends of DNA fragments. The TUNEL signal was then detected by an antidigoxigenin antibody conjugated with peroxidase (Apoptag Peroxidase *in situ* Apoptosis Detection kit; Oncor, Gaithersburg, MD, USA). Counterstaining was performed by immersing the slides in methyl green for 10 minutes.

Statistical analysis

The statistical significance of direct comparison between the groups of victims was determined using analysis of variance (ANOVA), followed by *post hoc* Bonferroni test. Statistical calculations were carried out with SPSS statistical software (version 11.0; SPSS Inc., Chicago, IL, USA). The selected threshold level for statistical significance was $P<0.05$.

Results

Examination of the LC in controls

In the most rostral transverse sections of the pons, the LC appears subdivided into two distinct adjacent regions: dorsally neurons are packed closely together around the mesencephalic tract of the trigeminal nerve (pars compacta/LC nucleus) whereas ventrolaterally cells are more dispersed [pars dissipata/subcoeruleus (sC) subnucleus] (Fig. 2A). These observations allow us to define the LC as the 'LC complex' (LCc), including the LC proper and the sC.

The examination was performed by two independent and blinded observers. Comparison of the resultant data, performed by employing Kappa statistics (Kappa Index) to evaluate interobserver reproducibility, was very satisfactory (Kappa Index=0.85).

A detailed analysis of the neuronal morphology shows that the LCc neurons are prevalently large, multipolar/round with dense lamellar arrays of rough endoplasmic reticulum (Nissl substance) in the cytoplasm. Small neurons are recognizable, prevalently distributed in the sC. Each cell has usually thin processes with extensive dendritic arborization into the surrounding neuropil (Fig. 2B).

The application of both histochemical staining for NM and immunohistochemistry for the TH enzyme and apoptosis allowed us to make the observations reported below.

With regards to NM:

- in fetal life NM is absent; it begins to appear in the neuronal cytoplasm of several neurons in 2–3 month-old infants, mainly involving the largest neurons;
- from the third to the eighth postnatal month the number of NM-containing neurons in the LC progressively increases. Nevertheless, even in the 'oldest' individuals of the study there are few pigment-containing neurons in the LC (<30%) (Fig. 3).

With regards to TH:

- from the earliest gestational weeks, the LC shows evident TH-positive expression in over 50% of the neurons (Fig. 4A);

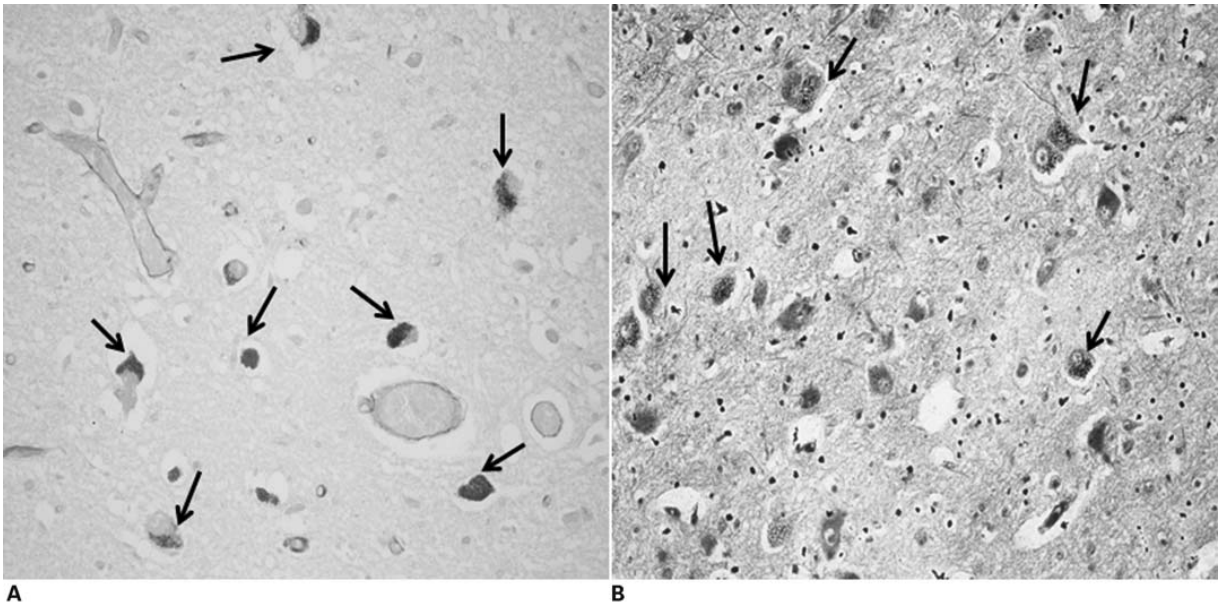

Figure 3 NM histochemistry. Pigmented neurons (see arrows) in the LCc of a control group infant aged 3 months. (A) Lillie's stain. Magnification: ×20. (B) Bielschowsky's silver impregnation technique. Magnification: ×20.

- after birth almost all the LC neurons (mean value: 82%) are noradrenaline-containing neurons.

With regards to apoptosis:

- apoptotic neurons (<20%) showing light chromatin condensation/fragmentation and cytoplasm shrinking are constantly found from the seventeenth gestational week (Fig. 4B).

It is important to note that several cases in the control group differed from the above reported pattern: we detected a decreased TH expression in the LCc neurons of two fetal and three infant deaths and intense apoptosis in both fetal (two cases) and infant deaths (one case).

Examination of the LC in SIUDS/SIDS

The following alterations were found:

- Neuronal loss (LCc hypoplasia) was evident in 33% of both SIUDS and SIDS victims. The decreased number of neurons was mainly limited to the LC pars compacta. The few residual neurons were large, immature, round neurons without Nissl substance and dendrites (Fig. 5).
- Frequently, in both fetal and infant sudden deaths with a normal cytoarchitecture of the LCc (9 SIUDS and 12 SIDS), the neurons showed a lighter TH-immunostaining intensity as compared to the amount of TH in age-matched controls. In addition, TH resulted totally unexpressed in two fetuses and four SIDS victims. Overall, 46% of SIUDS and 60% of SIDS showed a faulty TH immunoreactivity.
- Lacking NM neuronal pigmentation was detectable in 37% of SIDS victims (11 cases, aged more than 2 months).
- The presence of intense TUNEL staining was seen in a high percentage (>40%) of LCc neurons in 11 SIUDS (46%) and 10 SIDS victims (40%).
- A further finding was the frequent, unusual presence of mitotic figures (telophases) in SIDS (10 cases — 33%) (Fig. 6).

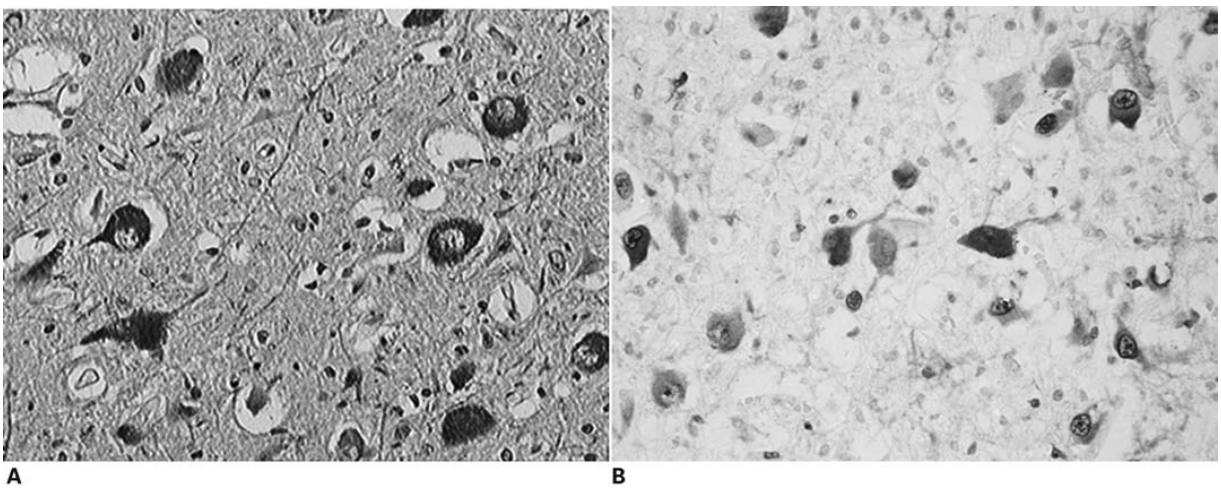

Figure 4 Immunohistochemistry. (A) TH immunohistochemistry. Immunopositive neurons in the LCc of a control fetus (40 gestational weeks). Magnification: ×40. (B) TUNEL immunohistochemical method. Apoptotic cells with chromatin condensation in the LCc of a control group infant aged 3 months. Magnification: ×40.

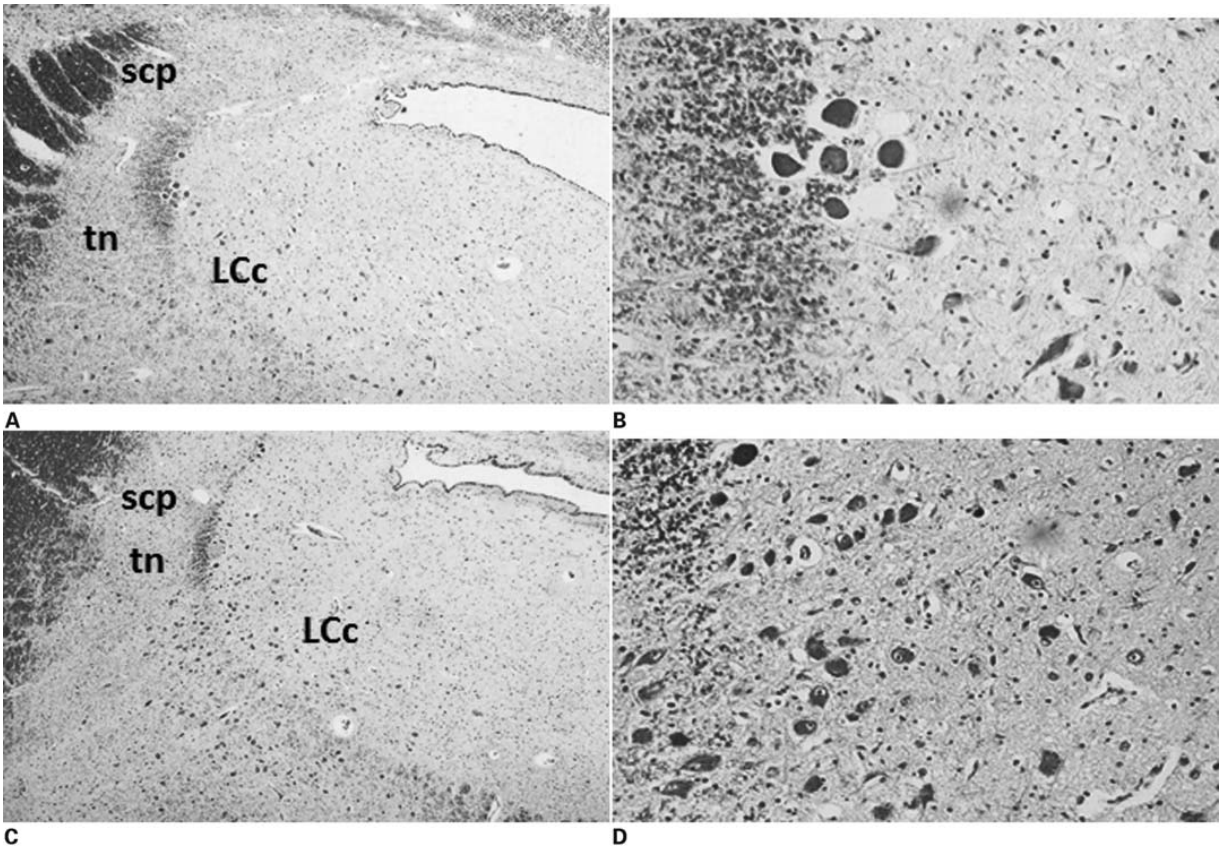

Figure 5 (A) Hypoplasia of the LCc in a subject who died of SIDS at 2 months. (B) Immature neurons at greater magnification. (B, C) Normal images of the LCc and its neurons at greater magnification in an age-matched control. Klüver–Barrera stain. Magnification: (A, C) ×4; (B, D) ×20 (scp: superior cerebellar peduncle; tn: trigeminal nerve).

Table 1 shows the distribution of the main LCc defects found in victims of sudden death and controls. Individual victims displayed some combination of these alterations. The most frequent association was between the presence of decreased TH expression and the absence of NM pigments in SIDS victims.

Overall, SIDS and SIUDS cases show a significantly higher incidence of histological/immunohistochemical alterations of the LCc, as compared with age-matched controls. In fact, 40 of the 54 victims of sudden death (74%), but only 6 of the 24 subjects belonging to the control group (25%) showed LCc modifications ($P<0.01$).

Discussion

It is amply documented that the LC, as it provides the sole central adrenergic innervation to many brain regions, presides over many essential autonomic functions, thanks to its ability to modulate synaptic transmission, membrane potential and the excitability of neurons along its extensive projections.[5–7] It is also known that specific populations of LC noradrenergic neurons contain NM, a dark polymer pigment produced at the same time in catecholamine synthesis.[16,17]

Up to now, the majority of research on this region has been focused on neurodegenerative disorders. In particular, damage to the noradrenergic system, decreased intraneuronal NM, a reduction in cell numbers, in addition to a high neuronal death rate in the LC have been reported in patients with Parkinson's and Alzheimer's diseases.[20–23]

Very similar results were found in this work, the first in-depth examination of the LC pathophysiology in sudden unexplained fetal and infant deaths (SIUDS and SIDS), based on our previously published preliminary data.[24]

Adrenergic neurons were identified by TH immunohistochemistry and NM-containing neurons were specifically visualized by the application of Lillie's method. In addition, the activation of programmed cell death (apoptosis) was studied by investigating DNA fragmentation (TUNEL-positive cells).

The developmental defects disclosed in this nucleus, that we define as the 'LCc' given its double composition (LC and sC - pars compacta and pars dissipata, respectively), prevalently detected in SIUDS/SIDS as compared to age-matched controls, were:

1. A significant reduction in noradrenaline levels, with a variable severity from light TH immunostaining to absolute neuronal immunonegativity. This observation suggests that in these pathologies the LCc neurons have an impaired ability to synthesize or store noradrenaline.
2. A lack of NM pigmentation in SIDS victims as from the second month of life, the age in which,

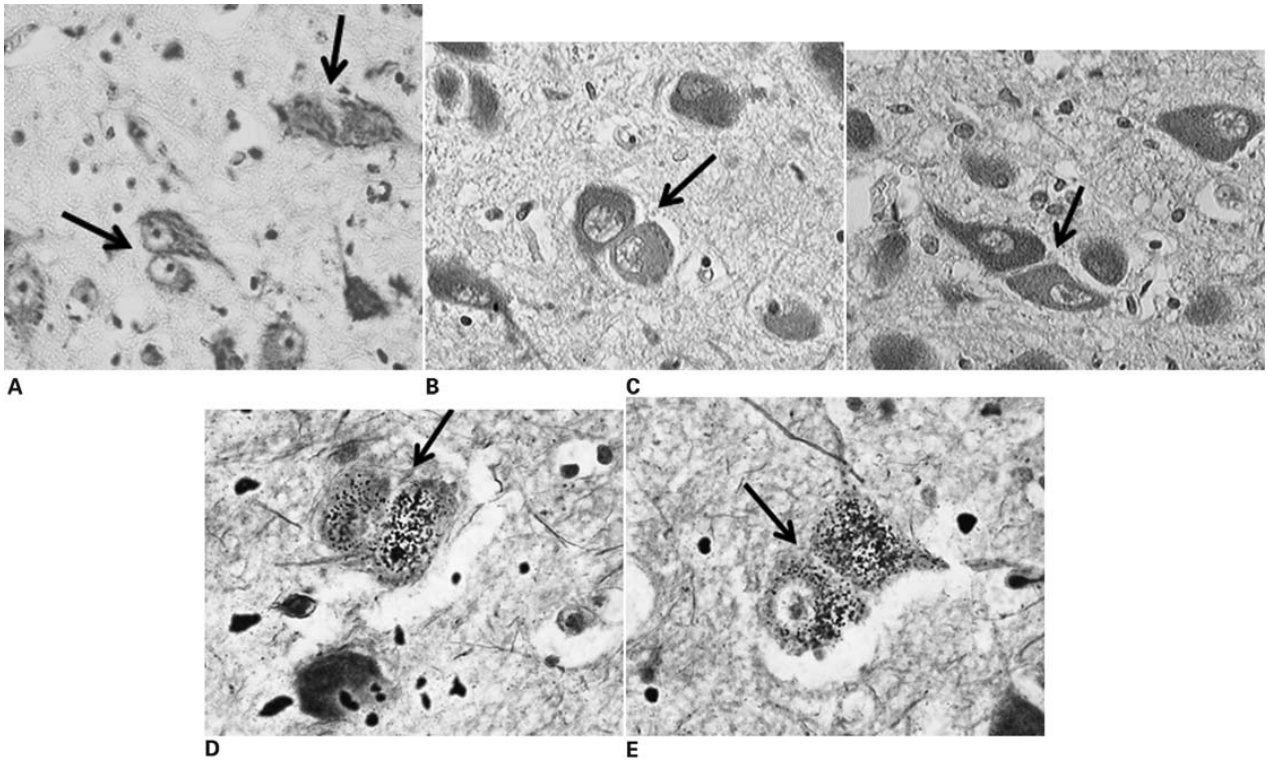

Figure 6 Mitotic figures (telophases) in the LCc highlighted by arrows. (A) Klüver–Barrera stain. (B, C) TH immunohistochemistry. (D, E) Bielschowsky's silver impregnation technique. Magnification: ×100.

according to our findings in control subjects, the pigment begins to appear. This defect was closely correlated with the decrease in TH, as can be easily explained since both NM and TH are tyrosine byproducts.

3. Hypoplasia of the LCc with immaturity of the few remaining neurons. This neuronal reduction constitutes a premature dysfunction which differs from the physiological age-related cell loss. Manaye *et al.*[31] demonstrated, in fact, that in man, from the first to the tenth decade of life, there is a decrease by more than 50% of the LC neuron number, and the cell loss location corresponds to regions featuring more cortically projected neurons.
4. A statistically significant increase in LCc apoptosis, variously associated with the other reported alterations. Although neuronal death plays an important role in normal central nervous system (CNS) development by orchestrating synaptic connectivity,[32] an accentuation of this process can abrogate synaptic organization, leading to developmental dysregulations particularly of the LC, given its neuromodulatory function throughout the wide network of connections.
5. Frequent mitotic figures (telophases) in 33% of SIDS victims. It is postulated that in prenatal CNS ontogeny, neuroblasts from the neuroepithelial matrix undergo mitotic activity and migrate to reach their definitive location, where they differentiate into mature neurons.[33] The hallmark of neuronal terminal differentiation is a permanent exit from the cell cycle, whereby differentiated neurons become stably post-mitotic. Paradoxically, differentiated neurons still express the molecular system necessary for cell proliferation, that can be reactivated under certain conditions when needed.[34] However, the division of differentiated cells rarely occurs, because in post-mitotic stages specific genetic patterns are actively involved in avoiding any re-entry into the cell cycle as this is

Table 1 Number of SIUDS, SIDS and control cases with alterations of the LC

	SIUDS	Fetal control	SIDS	Infant control
LCc alterations*	*N*=24	*N*=12	*N*=30	*N*=12
Morphology				
Hypoplasia	8	–	10	–
Mitotic neurons (telophases)	–	–	10	–
Thyrosine hydroxylase				
Decreased immunostaining	9	2	14	3
Negative immunostaining	2	–	4	–
NM				
Negative pigmentation	–	–	11	–
Apoptosis				
High expression	10	2	12	1

Note: *Individual victims may display any combination of the alterations.

deleterious for the neurons.[35] In fact, the newly formed cells will die in the short term or become permanently arrested at the end of the cycle. Therefore, we can regard the LCc telophases we frequently observed as the last vital signals of neurons that are fated to die.

Even if the heterogeneity of our findings suggests different pathways of damage to LCc neurons, on the whole these alterations imply failings of the vital activities related to the wide brain connections. Above all after birth, LCc alterations result in breathing dysfunctions that can easily lead to sudden infant death. The LC, that has been identified as a central CO_2 chemosensory site,[36,37] participates, in fact, in postnatal respiratory control, promoting a compensatory increase of ventilation when necessary. Recently, Biancardi *et al.*[38] have shown that a reduction by approximately 80% of LC noradrenergic neurons was associated with a large decrease in the response to CO_2 by approximately 64%, indicating that this nucleus exerts an important influence on CO_2-driven breathing.

Hypoxia is known to be a critical risk factor for brainstem alterations.[26,39] So, we believe that the catecholamine changes and the loss of noradrenergic neurons in the LCc of SIUDS/SIDS may be caused by chronic hypoxia, all the more so as noradrenergic neurons are particularly vulnerable to hypoxia, as shown in experimental studies. Robinson *et al.*[40] demonstrated that the noradrenaline concentration in the LC of rats is reduced after cerebral artery occlusion. A reduced number of noradrenergic neurons was also reported by Buller *et al.*[41] in experimental hypoxia induced by carotid artery resection in an immature animal model (rat pup).

Prominent among the biochemical neuronal features determined by hypoxia is the loss of cellular ATP, resulting in increased intracellular Na^+ and Ca^{2+}, and decreased intracellular K^+. These ionic imbalances can contribute to oxidative stress, with a net increase in reactive oxygen species.[42] Chen *et al.*[43] reported that oxidative stress may cause degeneration of the LC, as observed in patients with CNS degenerative diseases. One of the major cell pathologies after brainstem oxidative injuries is apoptosis. In fact, deprivation of the glucose and oxygen supply and the subsequent cascade of biochemical events including damage to lipids, proteins, and DNA, lead to severe cell dysfunction and ultimately to cell death.[42]

The high percentages of LCc apoptotic neurons observed in this study validate the involvement of hypoxic events in the pathogenetic mechanism of sudden death in human fetuses and newborns.

Nicotine, a neuroteratogen present in tobacco, is the main candidate agent of hypoxia inducing injury during pregnancy. Experimental studies have, in fact, demonstrated that nicotine infusions in pregnant rats cause fetal acute hypoxia through disruption of the oxidative metabolism, resulting in developmental alterations of the catecholaminergic systems.[44,45]

Alterations of NM may also result in this way. Like peripheral melanins, NM may function *in vivo* to attenuate the effects of damage stimuli. Among several possible suggested mechanisms, the ability of NM to mediate intracellular oxidative mechanisms has received particular attention.[43] Recent data on NM in the Parkinson's disease brain suggest that this function may be impaired, thus rendering pigmented neurons vulnerable to oxidative damage in this disorder.[46]

Another aspect of the protective role of NM has been shown by Simon *et al.*[47] They demonstrated that NM is able to effectively bind metal ions through its carboxylate and phenolic hydroxyl groups. Thus, it may serve to sequester potentially toxic agents and to attenuate damaging stimuli, protecting the rest of the cells. This theory is supported by the fact that in Parkinson's disease the loss of NM is frequently accompanied by an increase in iron levels in the brain.[46]

In accordance with these data, we suggest that the protective function of NM may be impaired in SIUDS/SIDS, causing a consequent increase of the vulnerability of pigmented neurons, very probably as a result of prenatal exposure to tobacco smoke, given the well-known affinity of nicotine with melanin-containing tissues.[48,49]

Nevertheless, we cannot exclude more global environmental factors. Air pollution, for example, can contribute to severe disruption of the oxidative metabolism and consequently to morphological and/or physiological developmental abnormalities of many brainstem centers, including the LCc, whose neurons are known to be particularly vulnerable to toxic agents.[50] The fact that many victims included in this study are from Lombardy, a highly polluted Italian region in which the mean $PM_{2.5}$ and P_{10} levels are recognized to contribute in a substantial way to perinatal mortality, supports this hypothesis.

In conclusion, we suggest that the LCc is particularly vulnerable in SIUDS/SIDS victims and that the numerous developmental defects highlighted in this complex may result from exposure to several combinations of both genetic and environmental risk factors. The extreme outcome of this interaction is sudden death in perinatal life. Less severe consequences can affect cognitive activities during development in childhood, since the LC provides many of its extensive adrenergic projections to the forebrain and in particular to the prefrontal cortex, that has an essential role in the regulation of attention, learning and memory.[51,52]

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