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CLINICAL RESEARCH ARTICLE

A quest for a histaminergic or orexinergic biomarker for sudden infant death syndrome

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BACKGROUND: Sudden infant death syndrome (SIDS) occurs predominantly during sleep between 2 and 6 months of age, suggesting impaired maturation of arousal pathways. The neurobiological mechanisms involved remain unclear. We investigated the role of wake-promoting Orexin (Ox) and Histamine (HA) neurons in SIDS.
METHODS: Cerebrospinal fluid (CSF) Ox levels were measured in 61 living controls and 70 Sudden Unexpected Death Infants (SUDI: 38 SIDS, 32 explained deaths (ED)). HA and tele-methylhistamine (t-MeHA) were analyzed in an additional 46 SUDI (34 SIDS, 12 ED) and 42 controls. Immunohistochemistry was performed on hypothalamic tissue from 11 SIDS and 8 ED cases to quantify Ox and HA neuron numbers.
RESULTS: CSF Ox levels did not differ overall between groups but were higher in SUDI infants aged 2–6 months. HA and t-MeHA levels were elevated in SUDI, likely reflecting postmortem release. Ox neuron numbers were increased in rostral hypothalamic region in SIDS compared with EDs, whereas HA neuron numbers were unchanged.
CONCLUSIONS: SIDS is associated with increased Ox neuronal activity during the peak risk period, possibly reflecting a homeostatic upregulation in response to arousal deficit or repeated stress or hypoxia, while the role of HA system remains to be clarified with more sensitive biomarkers.

Pediatric Research; https://doi.org/10.1038/s41390-026-05186-y

IMPACT:

- First study evaluating orexin and histamine systems in SIDS using CSF and postmortem brain tissue.
- Identifies elevated orexin activity in SIDS infants, particularly in the 2–6 month risk window.
- No evidence to date for direct histamine involvement; need for more sensitive biomarkers.
- Suggests orexin system as a potential biomarker for SIDS risk stratification.
- Highlights the importance of combined neurobiological approaches for prevention.

INTRODUCTION

Sudden infant death syndrome (SIDS) is defined as the death of an infant under 1 year of age which occurs during sleep and remains unexplained after a complete evaluation of the death scene, clinical history, and postmortem investigations.[1] To date, SIDS remains the leading cause of post-neonatal mortality in infants aged one month to one year old.[2,3] Epidemiological studies have enabled a better understanding of the risk factors of SIDS, which include prone sleep position, bed-sharing, overheating, infections, prenatal or postnatal cigarette exposure, prematurity, and low birth weight.[2] Awareness campaigns on these risk factors have thus helped dramatically decrease the incidence of SIDS over the last decades.[3,4]

Nevertheless, the pathophysiology of SIDS is still poorly understood. Its occurrence during sleep, within a specific developmental window, especially between 2 and 6 months of age, suggests an association with a dysfunction in the maturation of sleep-wake regulation, especially within the brain arousal systems. Polysomnographic recordings have shown that SIDS victims exhibit altered arousability, including fewer arousals at the

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Received: 9 September 2025 Revised: 29 April 2026 Accepted: 10 May 2026
Published online: 22 June 2026

end of the night and an increased prevalence of incomplete arousals compared to age-matched healthy infants.[5–7] However, the specific arousal systems involved remain unknown.

The orexinergic and histaminergic systems, both located in the posterior hypothalamus, play a distinctive yet synergistic role in promoting wakefulness.[8–10] Dysfunction in either system may impair arousal. Orexin (Ox, also called as hypocretin) has been involved in sleep disorders such as narcolepsy.[10] The Ox system undergoes a maturation process during early infancy, characterized by a progressive increase in cerebrospinal fluid (CSF) Ox levels during the late prenatal period and the first months after birth, followed by a decline around the peak age of SIDS incidence (2–6 months).[11,12] In a previous evaluation of CSF Ox in healthy children aged between 0 and 18 years, we found that infants aged 2–6 months had lower CSF Ox levels than those in other age groups.[12] Moreover, decreased Ox immunoreactivity in both the hypothalamus and brainstem of SIDS victims has been reported,[13] supporting the potential involvement of the Ox system in the pathophysiology of SIDS.

Given the close anatomical and functional interactions between the Ox and histamine (HA) systems, the implication of HA neurons during the critical period of SIDS has also been questioned.[14,15] Impaired HA transmission has been associated with reduced wakefulness and increased sleepiness in both animal models and children with narcolepsy.[16–18] However, findings in adults with hypersomnia are inconsistent.[19] In a recent large-scale study of healthy individuals from birth to adulthood[15] we found elevated CSF levels of HA and its direct metabolite, tele-methylhistamine (t-MeHA), during the first 6 months of life compared to older age groups. This suggests that enhanced HA transmission in early infancy may play a protective role against SIDS.

The present study aimed to investigate whether the Ox and HA systems are altered in SIDS victims. To that end, a first objective was to determine whether CSF Ox and HA levels differ between control infants (living infants who underwent a lumbar puncture for clinical indications and had normal CSF findings) and sudden unexpected death infants (SUDI), which included both SIDS victims and infants who died of other causes (explained death, ED). Analyses were carried out across three developmental age groups (0–2, 2–6, and 6–12 months). A second objective was to assess whether the number of Ox or HA neurons is altered in SIDS victims compared to controls (ED infants). Immunohistochemical staining of hypothalamic sub-regions was performed using antibodies against Ox and histidine decarboxylase (HDC), the enzyme responsible for HA synthesis.

PATIENTS AND METHODS

The present study was carried out on 5 different cohorts of infants referred to the university hospital Hôpital Femme Mère Enfant in Lyon, France. The number of infants in each cohort for either the CSF study or the immunohistochemical study is detailed in Fig. 1.

First part: cerebrospinal fluid study

Orexin levels in cerebrospinal fluid

Infants: CSF samples for Ox measurements were collected from 61 control infants and 70 SUDI: 38 SIDS and 32 infants with ED (Fig. 1). For SUDI children, the clinical information recorded included circumstances of death, delay between CSF puncture and death, and known risk factors of SIDS (prone position, $n = 10$; bed-sharing, $n = 8$; smoke exposure, $n = 25$; infection, $n = 27$). Infection was defined as any clinically and/or microbiologically documented infection identified at the time of evaluation, based on virological PCR and/or bacteriological investigations. In the SIDS cohort, infections were predominantly upper respiratory tract infections, most commonly associated with rhinovirus and adenovirus, and less frequently with parechovirus or coxsackievirus A; HHV-6 and CMV were rarely detected. Occasional viral co-infections were observed. In a small number of cases, viruses were identified in stool samples. SIDS were included only if no explanation for death was found after the regular clinical and paraclinical examinations and autopsy. Five SIDS infants (13.2%) were born prematurely (median 32.5 gestational age (GA) weeks (range from 29 to 36 weeks). The 32 ED infants died from cardiopathy ($n = 5$), fibroelastosis ($n = 1$), asphyxia ($n = 12$), epileptic encephalopathy ($n = 1$), child abuse ($n = 4$), inhalation ($n = 1$), intoxication ($n = 1$), traumatism ($n = 1$), volvulus ($n = 1$). In infants who died of infectious causes, the identified pathogens were mainly viruses, including enteroviruses (e.g., coxsackievirus), rotavirus, and adenovirus. One infant died from bacterial sepsis due to Streptococcus pyogenes (group A streptococcus). Pathogens were identified in cerebrospinal fluid (CSF), blood, or in the tissues involved in the infectious process (meningeal, cardiac, pulmonary, or gastrointestinal) during pathological examination, with associated inflammatory changes. These findings were consistent with established causes of death, including meningitis ($n = 1$), septicemia due to Streptococcus group A ($n = 1$), pulmonary infection ($n = 1$), and gastroenteritis ($n = 1$). Nine (29%) of these infants were born prematurely at a median of 34 weeks GA (range from 27 to 36 weeks).

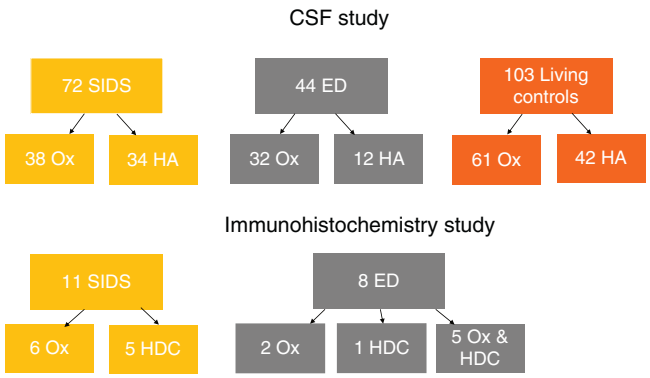


Fig. 1 Study flowchart depicting the different cohorts for both the cerebrospinal fluid and immunohistochemistry studies. All infants were aged under 1-year-old. For the CSF study, the control group was composed of living infants who underwent a lumbar puncture for clinical indications and had normal CSF findings.For the immunohistochemistry study, the control group was composed of ED infants.SIDS sudden infant death syndrome, ED explained death, Ox orexin, HA histamine, HDC histidine decarboxylase.

The control infants were aged under 1-year-old and had CSF collected for clinical indications. During the lumbar puncture, a spare tube was usually collected for possible further analyses. This extra CSF sample was used as control if the results of the CSF analysis were normal in terms of cell count, glucose, proteins, and lactic acid. For each infant, clinical information was collected: date and time of lumbar puncture, date of birth, delivery and birth circumstances, medical history and reason for the lumbar puncture. No control infant had a preterm delivery. Most lumbar punctures were performed to rule out meningitis in a context of fever. The final diagnoses were acute pyelonephritis ($n = 13$), gastroenteritis ($n = 3$), bronchiolitis ($n = 5$), viral meningitis ($n = 4$), other infections ($n = 4$), and unexplained fever/virosis ($n = 21$). Other lumbar punctures were performed for epilepsy ($n = 1$), Hirschsprung disease ($n = 1$), portal cavernoma ($n = 1$), bad weight growth ($n = 3$), bulging fontanel ($n = 1$), without access to the medical file for 4 children.

Orexin measurements. CSF were stored at −80 °C. Orexin-A (Ox-A) was measured from CSF samples as previously described,[20] in the Hôpital Guide-Chauliac (Montpellier, France). All measurements were performed blind to the group of infants. Ox-A was measured in duplicate using the Orexin A/Hypocretin-1 Human Iodine-125- RIA kit (ref. RK-003-30, Phoenix Pharmaceuticals, Inc., Burlingame, CA) according to the manufacturer's recommendations. The detection limit was 10 pg/ml and intra-assay variability was less than 10%. All values were back-referenced to Stanford reference samples (HHMI Stanford University Center for Narcolepsy, Palo Alto, CA).

Histamine levels in the cerebrospinal fluid

Infants: The CSF samples for HA measurements were collected from different infants than those included in the Ox-A CSF study: 42 control infants and 46 SUDI infants (34 SIDS and 12 ED; Fig. 1). For SUDI children, the clinical information recorded included circumstances of death and

Table 1. Demographic characteristics of infants who underwent cell counts on orexin- and histidine decarboxylase-immunoreactive neurons

	All ***N* = 19**	**SIDS** ***N* = 11**	**Controls (ED)** ***N* = 8**
	n (%) or median (min–max)	*n* (%) or median (min–max)	*n* (%) or median (min–max)
Sex (boys)	11 (58%)	7 (64%)	4 (50%)
Age (months)	4.0 (0.5–9.0)	3.5 (1.0–9.0)	6.0 (0.5–7.5)
Corrected age (months)	3.5 (0.0–8.5)	3.5 (0.0-8.5)	5.5 (0.0–7.5)
Gestational age (weeks)	38.5 (27.5–40.0)	38.5 (27.5–40.0)	37.0 (33.0–40.0)
Postconceptional age (months)	12.6 (8.8–17.5)	11.4 (9.3–17.5)	13.8 (8.7–16.7)
Preterm babies	6 (31.8%)	2 (18.2%)	4 (50%)

SIDS sudden infant death syndrome, *ED* explained death.

known risk factors for SIDS. Among SIDS cases, the following risk factors were reported: prone sleeping position ($n = 3$), smoke exposure ($n = 6$), inappropriate bedding ($n = 8$), co-sleeping ($n = 1$), prematurity ($n = 2$), and recent viral infection ($n = 10$). The 12 ED infants died from cardiopathy ($n = 3$), pericarditis ($n = 1$), fibroelastosis ($n = 1$), asphyxia ($n = 2$), encephalopathy ($n = 1$), child abuse ($n = 1$), intoxication ($n = 1$), septicemia ($n = 1$), pulmonary infection ($n = 1$). Among explained deaths (ED), the reported risk factors included prone sleeping position ($n = 1$), smoke exposure ($n = 5$), inappropriate bedding ($n = 5$), co-sleeping ($n = 1$), and prematurity ($n = 2$). There were no preterm babies in the group of control infants. The indications for lumbar puncture were available for 30 control infants and were fever ($n = 24$), febrile seizures ($n = 2$), acute gastroenteritis with dehydration ($n = 2$), and respiratory distress by meconium. All CSF and laboratory results were subsequently found to be normal.

Measurements of histamine and its direct metabolite. The HA and t-MeHA assays were carried out at Bioproject-Biotech (Saint Gregoire, France), using the technique developed by Croyal et al.[21] for the simultaneous analysis of CSF HA and its main stable metabolite t-MeHA. All measurements were performed blind to the group of infants. The CSF samples were immediately preserved at −80 °C after sampling until use. Blood contamination was checked visually and specimens of the CSF with abnormal color were excluded from the study. These measurements rely on derivatization of primary amines using 4-bromobenzenesulfonyl chloride and subsequent analysis by ultra-performance liquid chromatography with mass spectrometric detection.[21]

Second part: neuronal immunohistochemistry study

Postmortem brain material. For this analysis, 19 infants were included: 11 SIDS and 8 ED infants who served as controls (Fig. 1). Among SIDS infants, there were 2 preterm babies (27 and 30 weeks GA), 7/11 (63.6%) were boys and the median corrected age at death was 3.5 months (range 0–8.5 months). Among the ED infants, there were 4 preterm babies (33, 34, 34, and 36 weeks), 4/8 were boys (50%) and the median corrected age at death was 4.5 months (range from 0 to 7.5 months; Table 1). The eight infants died from known causes (cardiopathy ($n = 1$), fibroelastosis ($n = 1$), asphyxia ($n = 4$), meningitis ($n = 1$), gastro-enteritis ($n = 1$).

Orexin-A and histidine decarboxylase immunohistochemistry. The Ox-A and HDC immunohistochemistry staining were performed by automated Ventana BenchMark XT system (Roch, Ventana Medical Systems Inc., Tucson). For each infant, 1 block of brain tissue (0.8–1 cm thickness) across the posterior hypothalamus was cut to constitute 2 sets of 15–20 sections (at 3 µm thickness) to perform the Ox and HDC immunoreactions. The first set of sections was deparaffinized in xylene and rehydrated through a graded alcohol series. After washing in aquadest (2 × 5 min), slices were briefly washed (3 min) and microwave-treated with 0.01 M sodium citrate in phosphate buffer saline (PBS), pH = 6.0; 2 ×5 min) for antigen retrieval. A one-hour pre-incubation step in 5% PB-milk (0.05 M Tris, 0.15 M NaCl, pH = 7.6) at room temperature (RT) was included to reduce background staining. Sections were incubated in rabbit-IgG, anti- Ox-A (Catalog no. 003-30, Phoenix Pharmaceuticals, Inc., Belmont, CA) at a 1:4000 concentration in supermix-5% milk (0.25% gelatin, 0.5% Triton in PBS) at RT for an hour, followed by overnight incubation at 4 °C in the moist chamber. Followed by a 1-h secondary antibody incubation in biotinylated anti-rabbit IgG at a concentration of 1:400 at RT, antigen signal amplification was achieved by a 1-h incubation in ABC-complex (Vectastain®, Avidin-Biotin complex kit). Visualization of the signal was reached by a 20 min incubation of the hypothalamic sections in PBS containing 3.3′-diaminobenzidine (DAB; Sigma) and 0.01% vol/vol H_2O_2 (Merck, Whitehouse Station, NJ), which resulted in a brown cytoplasmic precipitate, clearly visible under low- and high-magnification optical microscopy (Fig. 2).

The second set of sections underwent exactly the same immunohistochemical procedure using a rabbit-IgG, anti-HDC (EUD 2601, Acris) at a dilution of 1:4000 (Fig. 3).

Both Ox-A and HDC antibodies stained unambiguously and distinctively posterior hypothalamic neuronal populations. To test the antibody specificity, both Ox-A and HDC antibodies were tested on Ox- and HDC-knockout (KO) mouse brains. As already demonstrated in previous studies, the immunolabeling of Ox-A and HDC in the mouse brains was generally dense and clearly contrasted with the background, with well-defined somata, proximal dendrites, and axons approximate to the perikaryal. In contrast, incubation with the Ox-A antibody resulted in no staining in the Ox-KO brains and that of the HDC produced no labeling in the tuberomammillary nucleus (TMn) and adjacent areas in HDC-KO mice[14,16] attesting the selectivity of the antibodies used in the human hypothalamic tissue.

Cell counts on orexin-A- and histidine decarboxylase-immunoreactive neurons. The human atlas for appropriate age[22,23] was used as a reference to define the exact levels of the tissue sections for ease of comparison across cases and studies. Based upon the counterstaining by Nissel, the medial lateral and the TMn borders were roughly defined as the distance between the most rostral and the most caudal sections that contained three or more typical TMn neurons, as well as the descending position of fornix, while the borders were subsequently determined more precisely by immunocytochemistry for Ox-A and HDC. In each section, all immunopositive neurons with their typical cell profiles and a visible nucleolus and developed perikarya were counted using light microscopy at a magnification of 40x. For quantification of cell counts, the rostral-caudal length of the TMn regions and the perifornical areas of the dorso- and medial- parts of the lateral hypothalamus were subdivided into 3 parts, thereby distinguishing 3 sub-regions: rostral, central (where the most numerous labeled cells were found), and caudal parts. Not all infants had tissue available for all three hypothalamic sub-regions; the number of infants who had Ox- or HDC-immunoreactions in each sub-region is indicated in Supplemental Table S1. For Ox immunoreactions 1–10 measures per sub-region per infant were performed and for HDC immunoreactions, 1–5 measures per sub-region per infant were performed (details in Supplementary Table S2). When more than one measure per sub-region per infant was available, the mean count for the sub-region was calculated. Two experienced researchers performed the cell count blinded to the nature of the tissue.

Statistical analysis

Cerebrospinal fluid study: Fisher's exact chi-square and Kruskal–Wallis tests were used for descriptive analyses. Mixed models were performed to study the Ox ranks and HA ranks according to the corrected age at CSF sampling (0–2 months, 2–6 months, 6–12 months) in the three groups of infants (SIDS, ED, controls). Groups were also compared 2 by 2 and *p*-values were corrected for multiple comparisons. Statistical analyses were

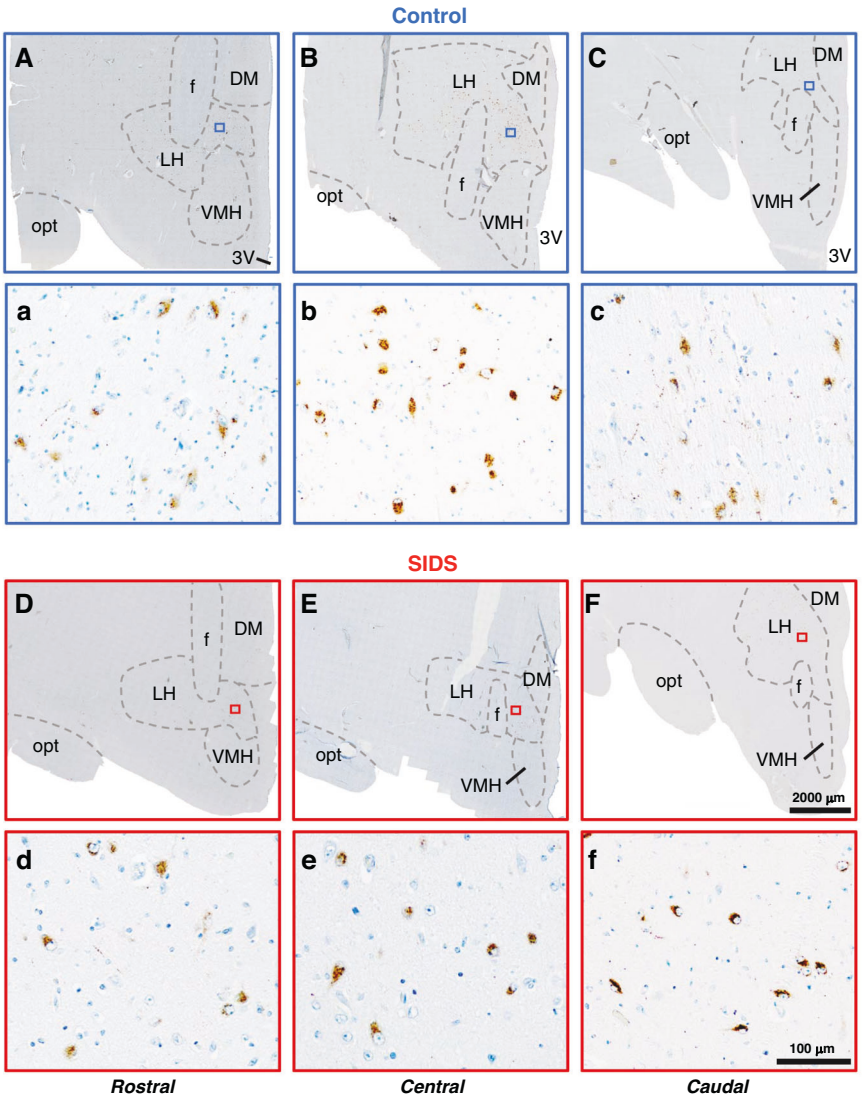


Fig. 2 Photomicrographs of orexin immunoreaction in the hypothalamus of control infants (with explained death) and those with sudden infant death syndrome. Photomicrographs from representative immunoreacted coronal sections illustrating orexin (Ox) **A**-immunoreactive neurons across the dorsal part of the posterior hypothalamus in control (upper, **A**–**C**and **a**–**c**) and SIDS (lower, **D**–**F** and **d**–**f**) patients. Sections were counterstained by Nissel (blue). **A**–**F** lower magnification showing the distribution of stained neurons; **a**–**f**, higher magnification from a blue or red inset in **A**–**F**, showing the details of the labelling and morphology of stained neurons in the dorsal part of the posterior hypothalamus. Note the similar distribution and morphology of Ox A-ir neurons between control and SIDS examples. DM dorsal medial hypothalamus, f fornix, LH lateral hypothalamus, opt optic tractus, 3V third ventricle. Scale bars = 2000 µm in (**A**–**F**) and 100 µm in (**a**–**f**).

performed using SAS® version 9.4 (SAS Institute Inc, Cary, NC). In all studies, statistical significance was defined with a level of $p < 0.05$.

Immunohistochemical study: The differences between the groups were evaluated by Mann-Whitney U tests. All values are reported as median and range.

Ethics. Upon admission, parents were informed of the potential use of their child's laboratory samples for research purposes and provided written informed consent. The research was conducted in collaboration with the French National Registry for Sudden Unexpected Infant Death (Observatoire national de la Mort Inattendue du Nourrisson, OMIN). The registry and automated processing of infants' health data received authorization from the French data protection agency (Commission Nationale de l'Informatique et des Libertés, CNIL) and was approved by an ethics committee (Groupe Nantais d'Ethique dans le Domaine de la Santé, GNEDS). A biobank linked to the registry (BioMIN, declaration No. DC-2019-3850) has been established and contains laboratory samples (blood, urine, CSF, hair, feces…) from SUDI cases. The biobank was declared to the French Ministry in charge of research and was approved by an appropriate ethics committee (Comité de Protection des Personnes, CPP Ouest IV; file no. 06/15; March 26, 2020). All data included in this study were collected at the Hôpital Femme Mère Enfant in Lyon, France.

RESULTS

Cerebrospinal orexin, histamine, and tele-methylhistamine levels in SIDS, ED, and control infants

Orexin levels. No difference in CSF Ox levels was observed between SIDS, ED, and controls when compared overall ($p = 0.45$) or 2 by 2 (all $p > 0.47$), even after adjusting for age (all $p > 0.31$). In addition, no significant difference in CSF Ox levels according to sex was found between groups ($p = 0.09$; Supplementary Table S3). However, when analyzing CSF Ox levels according to age (Table 2 and Fig. 4), the SUDI infants in the 2–6 months age group had significantly higher median Ox levels (388 pg/ml, range 179–588 pg/ml for SIDS and 347 pg/ml, range 234–486 pg/ml for

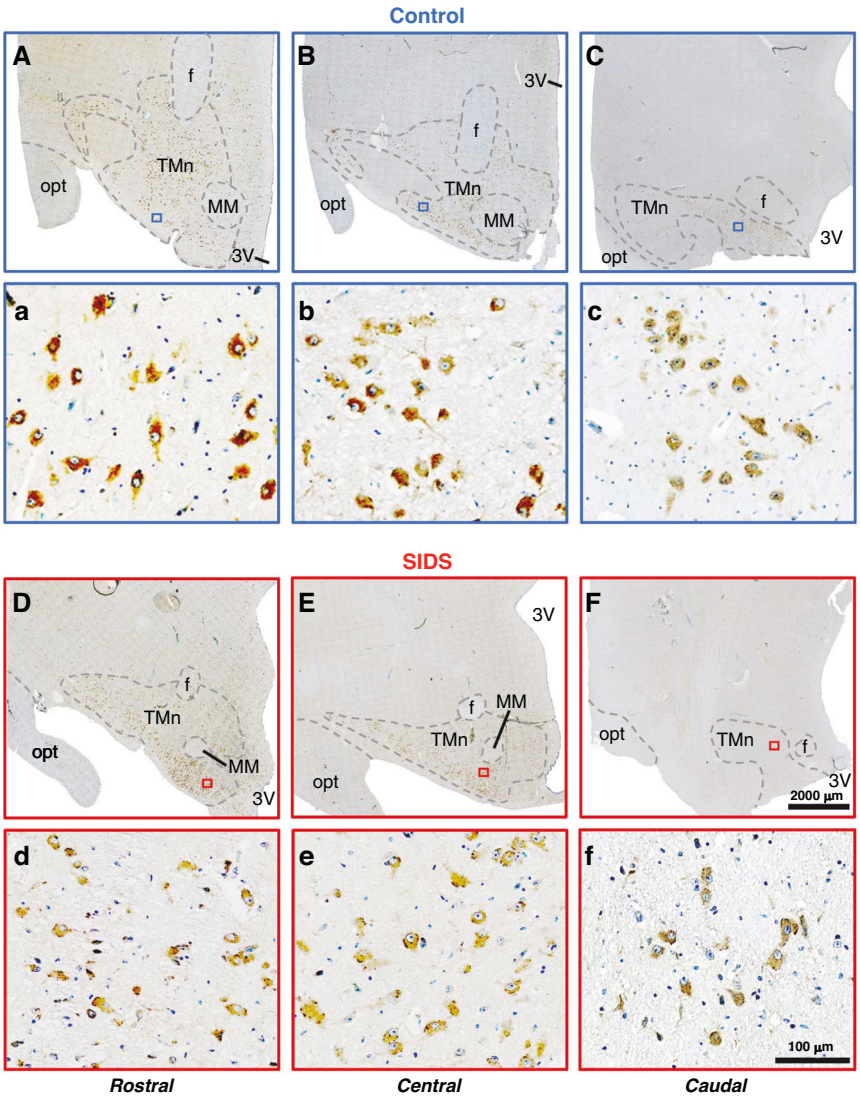


Fig. 3 Photomicrographs of histidine decarboxylase immunoreaction in the hypothalamus of control infants (with explained death) and those with sudden infant death syndrome. Photomicrographs from representative immunoreacted coronal sections illustrating histidine-decarboxylase (HDC)-immunoreactive neurons across the ventral posterior hypothalamus in control (upper, **A**–**C** and **a**–**c**) and SIDS (lower, **D**–**F** and **d**–**f**) groups. Sections were counterstained by Nissel (blue). **A**–**F** Lower magnification showing the distribution of stained neurons; **a**–**f** higher magnification from the part of the tuberomammillary nucleus (TMn) indicated by a blue or red inset in **A**–**F**, showing the details of the labelling and morphology of stained neurons. Note the similar distribution and morphology of HDC-ir neurons between control and SIDS examples. Abbreviations: DM dorsal medial hypothalamus, f fornix, LH lateral hypothalamus, MM mammillary nucleus, 3V third ventricle. Scale bars = 2000 µm in **A**–**F** and 100 µm in **a**–**f**.

ED) compared to controls (276 pg/ml, range 200–673 pg/ml; $p = 0.003$). This was confirmed when tested 2 by 2 (Table 2).

When evaluating the change in Ox levels according to age in each infant group, a significant decrease in Ox levels from 0–2 months to 2–6 months of life was observed in the control group ($p = 0.009$) but not in the SIDS and ED groups.

In SUDI infants, no significant difference in CSF Ox levels was observed according to known risk factors for SIDS, such as prone position, bed-sharing, and smoke exposure. Conversely, CSF Ox levels were higher in SUDI infants who were born prematurely (median 407 $^{225\text{-}588}$ vs. 352 $^{135\text{-}473}$, Kruskal Wallis $p = 0.03$) and in those who had an infection (404 $^{135\text{-}588}$ vs. 348 $^{179\text{-}484}$, Kruskal Wallis $p = 0.003$). In stratified analyses, among ED cases, a borderline difference in CSF orexin levels was observed between premature and non-premature infants (median 406 [226–486] vs. 347 [347–443]; $p = 0.05$), while no difference was found according to infection status (302 [222–459] vs 405 [135–486]; $p = 0.24$). Among SIDS cases, CSF orexin levels did not differ according to prematurity (414 [348–588] vs. 345 [179–473]; $p = 0.10$), whereas infants with infection had significantly higher levels than those without infection (404 [298–588] vs. 347 [179–484]; $p = 0.007$). The time between death and CSF sampling did not differ significantly between ED and SIDS infants (median (Q1–Q3) of 3h40 (3h10–4h35) vs. 2h50 (1h10–12h50), respectively, $p = 0.31$). No association was found between the delay in lumbar puncture and Ox levels in neither group (supplementary Fig. S1).

Histamine and tele-methylhistamine levels. In SUDI infants, no significant difference in CSF HA or t-MeHA levels was observed according to known risk factors for SIDS, such as prone position, bed-sharing, smoke exposure, and infections. Significant sex differences were observed, overall and when groups were compared 2 by 2, but only in infants aged 2–6 months: there were more girls in the ED group compared to the control group

Table 2. Demographic characteristics and cerebrospinal fluid orexin levels in all three groups of infants according to age group.

	Controls	SIDS	ED	Kruskal Wallis *p*-values			
	% (*N*) or median (min-max)	% (*N*) or median (min-max)	% (*N*) or median (min-max)	Overall	SIDS vs. Controls	ED vs. Controls	ED vs. SIDS
0–2 months							
N	40	10	9				
Sex (girls)	50% (20)[a]	50% (5)[a]	44% (4)[a]				
Age (wks)	4.6 (1.6–10.9)	5.0 (2.0–7.0)	6.7 (1.2–16.4)	0.41	0.79	0.44	0.66
Corrected age (wks)	3.6 (0.6–7.9)	3.9 (0.0–6.0)	5 (0.0–7.6)	0.60	0.85	0.84	0.39
Orexin (pmol/L)	376 (159–787)	339.5 (254–466)	360 (222–430)	0.12	0.98	0.29	0.23
≥2–6 months							
N	17	26	12				
Sex (girls)	47% (8)[a]	38% (10)[a]	42% (5)[a]				
Age (wks)	13.3 (8.4–22.0)	17.5 (8.0–28.0)	18.2 (11.1–32.7)	0.07	0.21	0.06	0.76
Corrected age (wks)	11.6 (8.4–22.0)	16.6 (8.0–23.9)	16.5 (8.1–23.3)	0.21	0.31	0.23	0.98
Orexin (pmol/L)	276 (200–673)	388 (179–588)	347 (234–486)	0.002	0.002	0.04	0.69
≥6–12 months							
N	4	3	10				
Sex (girls)	50% (2)[a]	100% (3)[a]	50% (5)[a]				
Age (wks)	26.6 (25.0–36.0)	29.3 (26.0–32.0)	35.9 (28.7–52.4)	0.03	0.76	0.09	0.11
Corrected age (wks)	25.9 (24.4–35.0)	26.0 (25.3–32.0)	33.9 (25.0–48.0)	0.14	0.93	0.23	0.28
Orexin (pmol/L)	315.5 (256–519)	347 (328–414)	386 (135–442)	0.84	0.93	0.84	0.98

SIDS sudden infant death syndrome, *ED* explained death.
[a]Fisher's exact test $p > 0.10$.

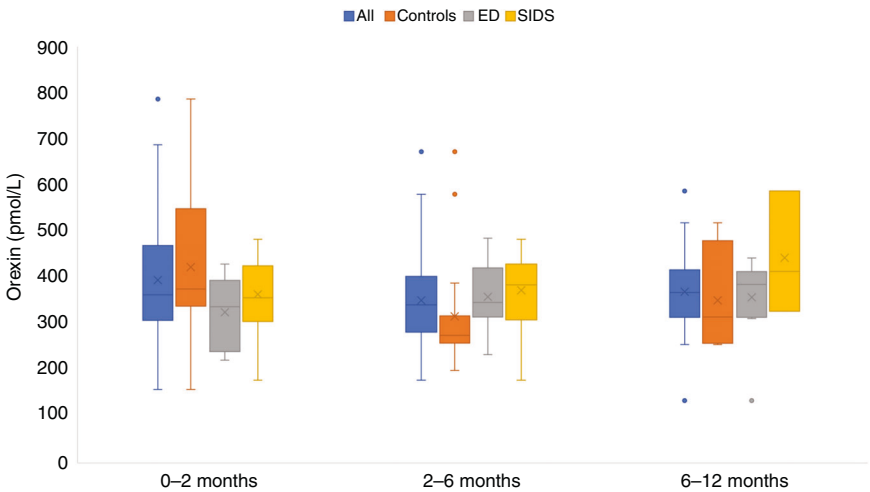


Fig. 4 Orexin levels in the cerebrospinal fluid of all infants (living controls, sudden infant death syndrome, and infants who died of explained death) according to age. Box plots of the Ox CSF values in living controls, SIDS, and ED infants according to age groups (0–2 months, 2–6 months, and 6–12 months). The horizontal solid bars represent the Q1-mean-Q3 values, the X is the median, and the vertical line presents the min-max values. Ox orexin, CSF cerebrospinal fluid, SIDS sudden infant death syndrome, ED explained death.

and fewer girls in the SIDS group compared to the control. In addition, significant age differences were observed within the 2–6 months age group and the 6–12 months age group between the different groups; notably SIDS infants in the 2–6 months age group were significantly older than controls (Table 3). In mixed models accounting for age and sex, SUDI infants presented significantly higher HA levels than control infants and SIDS infants also showed higher t-MeHA levels (Table 3 and Fig. 5a, b). No association was found between the delay in lumbar puncture and HA or t-MeHA levels in ED and SIDS infants (supplementary Figs. S2 and S3).

Cell counts and morphology of HDC- and Ox-immunoreactive neurons in postmortem brain tissues

On the brain sections from both ED (used as controls) and SIDS infants, Ox-immunoreactive (ir) cell bodies were located

Table 3. Demographic characteristics and cerebrospinal fluid histamine and tele-methylhistamine levels in all three groups of infants according to age group

	Controls ($N = 42$)	**SIDS ($N = 34$)**	**ED ($n = 12$)**	**Kruskal Wallis *p*-values**			
	% (*N*) or median (min–max)	% (*N*) or median (min–max)	% (*N*) or median (min–max)	Overall	SIDS vs. Controls	EDS vs. Controls	ED vs. SIDS
0–2 months	$N = 19$	$N = 10$	$N = 4$				
Sex (Girls)	47% (9)[a]	40% (4)[a]	50% (2)[a]				
Age (wks)	4.0 (1.0–7.0)	5.5 (1.6–15.4)	5.9 (1.1–7.3)	0.70	0.74	0.83	0.99
Corrected age (wks)	4.0 (1.0–7.0)	4.1 (0.0–6.7)	5.6 (0.1–6.7)	0.47	0.51	0.99	0.58
HA (pmol/L)	706 (207–2167)	3271 (457–16,895)	7973 (643–36,283)	0.005	0.02	0.08	0.76
t-MeHA (pmol/L)	6228 (3291–13,377)	29,002 (6202–29,585)	4296	0.05	0.06	0.66	0.33
		(MD = 6)	(MD = 3)				
2–6 months	$N = 15$	$N = 17$	$N = 5$				
Sex (Girls)	60% (9)[b]	12% (2)[b]	80% (4)[b]				
Age (wks)	11.0 (8.0–18.0)	17.1 (8.0–26.9)	18 (13.3–22.4)	0.0007	0.003	0.008	0.85
Corrected age (wks)	11.0 (8.0–18.0)	17.1 (8.0–23.9)	16 (11.3–17.0)	0.002	0.004	0.08	0.42
HA (pmol/L)	716 (183–3208)	3147 (29–20,216)	2453 (511–4833)	0.003	0.003	0.12	0.64
		(MD = 2)					
t-MeHA (pmol/L)	5387 (1293–10,180)	28,938 (3105–59,671)	29,260 (5424–59,823)	0.004	0.01	0.06	0.81
		(MD = 8)	(MD = 1)				
6–12 months	$N = 8$	$N = 7$	$N = 3$				
Sex (Girls)	25% (2)[a]	5 (71%)[a]	67% (2)[a]				
Age (wks)	38.0 (26.0–52.0)	25.1 (10.2–39.0)	33.4 (28.7–43.7)	0.03	0.03	0.98	0.30
Corrected age (wks)	38.0 (26.0–52.0)	26.7 (24.9–39.0)	33.4 (28.7–43.7)	0.03	0.04	0.98	0.20
HA (pmol/L)	601 (343–810)	3193 (1217–4814)	13096 (1742–18977)	0.001	0.003	0.04	0.42
t-MeHA (pmol/L)	3856 (1674–3509)	29,633 (12,784–29,840)	7686	0.01	0.02	0.48	0.33
		(MD = 3)	(MD = 2)				

SIDS sudden infant death syndrome, *ED* explained death, *HA* histamine, *t-MeHA* tele-methylhistamine, *MD* missing data.
[a]Fisher's exact test $p > 0.10$.
[b]Fisher's exact test $p = 0.003$.

dorsorostral to HDC-ir neurons in the posterior hypothalamus. Their perikarya were concentrated on the upper peri-fornical areas of the dorsal part of the posterior hypothalamus. They were distributed in a more scattered and less dense manner than that of HDC-ir neurons. Their distribution and morphology were similar between ED and SIDS brains (Figs. 2 and 3).

On the brain sections from ED infants, numerous HDC-immunoreactive (ir) cell bodies were located in the posterior basal hypothalamus. They were particularly concentrated in the TMn and adjacent areas, which embodied a major part of the ventral posterior hypothalamus. The distribution of HDC-ir cell bodies in the brain sections of SIDS infants was similar to that seen in the ED and no clear distinction could be visualized (Fig. 3).

When counting the number of cells across multiple brain sections within each infant, no overall significant difference was observed in Ox-ir or HDC-ir neuron numbers between SIDS infants and ED (Ox-ir, $p = 0.47$ and HDC-ir, $p = 0.14$; Table 4).

We then subdivided the Ox-ir and HDC-ir neuronal populations into rostral, central, and caudal parts in order to quantify their respective cell numbers and then compare them between SIDS and ED control infants. Regarding Ox-ir neurons, SIDS infants had significantly higher cell counts in the rostral (Kruskal–Wallis $p = 0.04$) parts compared to ED but not in the central part ($p = 0.60$; Fig. 6a). No statistical analysis was performed for the caudal level due to insufficient sample size ($n = 1$ SIDS). No difference was observed in terms of their morphology (Fig. 2).

Regarding HDC-ir neurons, a high variability in cell counts was observed between ED and SIDS infants, especially in the central and caudal parts (Fig. 6b). No significant difference in HDC-ir neuronal counts was found between SIDS and ED infants in neither the rostral ($p = 1.0$), central ($p = 0.67$), nor caudal ($p = 0.45$) parts. In addition, microscopic observation did not reveal any detectable difference between the two groups in terms of neuronal morphology (Fig. 3).

DISCUSSION

SIDS occurs during a critical developmental window and is strongly associated with impaired arousal from sleep.[2,24] In the present study, we investigated two hypothalamic wake-promoting systems—Ox and HA neurons—using complementary approaches combining CSF neurochemistry and postmortem hypothalamic neuronal quantification. The main finding is a selective increase in CSF Ox during the 2–6 month period, the peak age of SIDS vulnerability, whereas no change of the CSF HA and t-MeHA levels

could be specifically attributed to the HA neurotransmission at this state.

Absence of the physiological 2–6-month decline in CSF orexin in SUDI infants

The higher CSF orexin levels observed in SUDI infants compared with living controls are notable because they occur during a developmental period in which CSF orexin levels are normally low in healthy infants, as previously shown by our group and others.[11,12] Indeed, when comparing overall CSF Ox levels across SIDS, ED, and control groups, no significant difference was observed. The age-specific nature of the results thus argues against a non-specific postmortem artifact. This is further supported by our finding of no correlation between the postmortem interval and CSF Ox levels. Thus, rather than reflecting a simple developmental delay, the observed elevation suggests a deviation from the normal maturational trajectory of the Ox system during a vulnerable window for arousal failure.

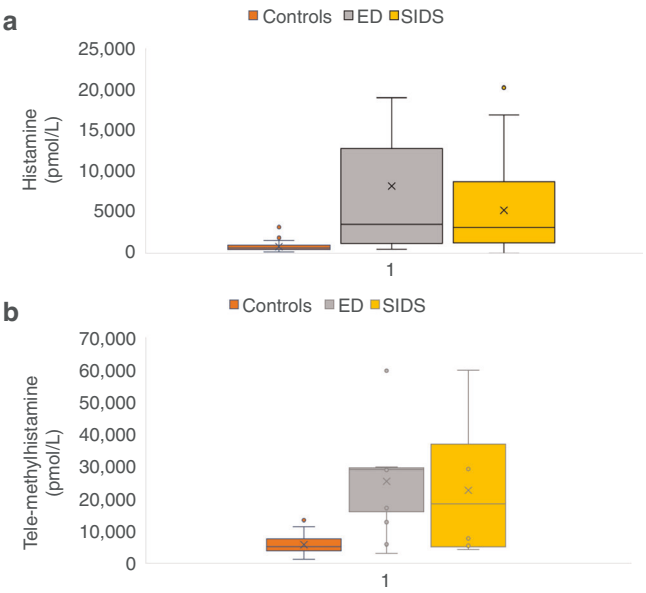


Fig. 5 Histamine and tele-methylhistamine levels in the cerebrospinal fluid of all infants (living controls, sudden infant death syndrome, and infants who died of explained death). a Box plots of HA CSF values in living controls, SIDS, and ED infants. The horizontal solid bars represent the Q1-mean-Q3 values, the X is the median, and the vertical line presents the min-max values. **b** Box plots of t-MeHA CSF values in living controls, SIDS, and ED infants. The horizontal solid bars represent the Q1-mean-Q3 values, the X is the median, and the vertical line presents the min-max values. HA histamine, t-MeHA tele-methylhistamine, CSF cerebrospinal fluid, SIDS sudden infant death syndrome, ED explained death.

Increased CSF orexin levels as a marker of chronic stress in SIDS infants

The increased CSF Ox levels together with an increased Ox-ir neuron number in a hypothalamic rostral subregion would suggest a stronger recruitment of Ox neurons in SIDS victims They might experience more chronic or prolonged challenges compared to infants exposed to more acute stress or hypoxic events (as in ED cases). Although the total number of Ox-ir neurons, remained unchanged, the mild increase observed in peripheral rostral subregion, together with elevated CSF Ox levels during the 2–6 month age window, could be biologically relevant in SIDS victims. Given the tight regulation of Ox during infancy[11] and its excitatory action that can largely impact brain targets controlling arousal, autonomy and respiration,[10,25–28] even small changes in their activity during early development may have functional consequences.[10,26–28] We interpret these findings as reflecting a chronic compensatory or homeostatic upregulation of the Ox system in SIDS infants. This may occur in response to repeated challenges to arousal, such as hypoxia. Indeed, infants who later succumbed to SIDS were often found to experience more frequent obstructive sleep apneas than healthy controls.[6,29,30] Several known SIDS risk factors—such as prematurity and infections[31,32]—which were associated with higher CSF Ox levels in our SUDI group, have also been linked to an increased incidence of obstructive apneas in infants. In our data, the association with infection appeared more pronounced in the SIDS group, suggesting that inflammatory or infectious processes could represent an important trigger for increased orexinergic activity in vulnerable infants. Rather than indicating enhanced arousal capacity, such upregulation may signal chronic physiological stress within the arousal network.

Orexin, developmental maturation, and SIDS

Increased activity of Ox neurons may result from repeated hypoxic episodes prior to death, as suggested by animal studies.[33] However, findings in the literature are not entirely consistent. Both hypoxia and hypercapnia can rapidly suppress Ox expression,[34,35] and experimental models demonstrate that orexin responses vary depending on the pattern of hypoxia (intermittent versus sustained),[33] developmental stage,[36] circadian phase,[27] and regional distribution.[27] Interactions with other arousal systems further modulate these effects.[13] In a previous study, Hunt et al. reported reduced Ox immunoreactivity in the hypothalamus (−21%) and pons (−40–60%) of SIDS victims, without a decrease in cell number.[13] In that cohort, the mean post-conceptional age (PCA) at death was approximately 14 months (13.9 ± 2.9 months in SIDS and 14.5 ± 3.2 months in controls ($p = 0.59$)), whereas in our study the median PCA was 12.0 ± 0.7 months in SIDS and 13.2 ± 1.0 months in ED ($p = 0.32$). As PCA reflects overall biological maturity at death, these differences suggest that maturational stage may contribute to inter-study variability. Consistent with this interpretation, human data indicate that

Table 4. Orexin and histidine decarboxylase immunoreactive neurons in sudden infant death syndrome and explained death (controls) groups

	Ox			HDC		
	N	Median (min–max)	Mean ± SD	*N*	Median (min–max)	Mean ± SD
All infants	13	129 (30–324)	168 ± 101	11	464 (118–6682)	1166 ± 1891
SIDS	6	214 (72–323)	197 ± 101	5	458 (118–520)	393 ± 163
Controls (ED)	7	128 (30–324)	144 ± 102	6	1025 (157–6682)	1810 ± 2456

When more than one measure per sub-region per infant was available, the mean count per sub-region was calculated. The median and mean per infant were then computed.
Fisher's exact test SIDS vs. controls: Ox $p = 0.47$ and HDC $p = 0.14$.
Ox orexin, *HDC* histidine decarboxylase, *SIDS* sudden infant death syndrome, *ED* explained death, *SD* standard deviation.

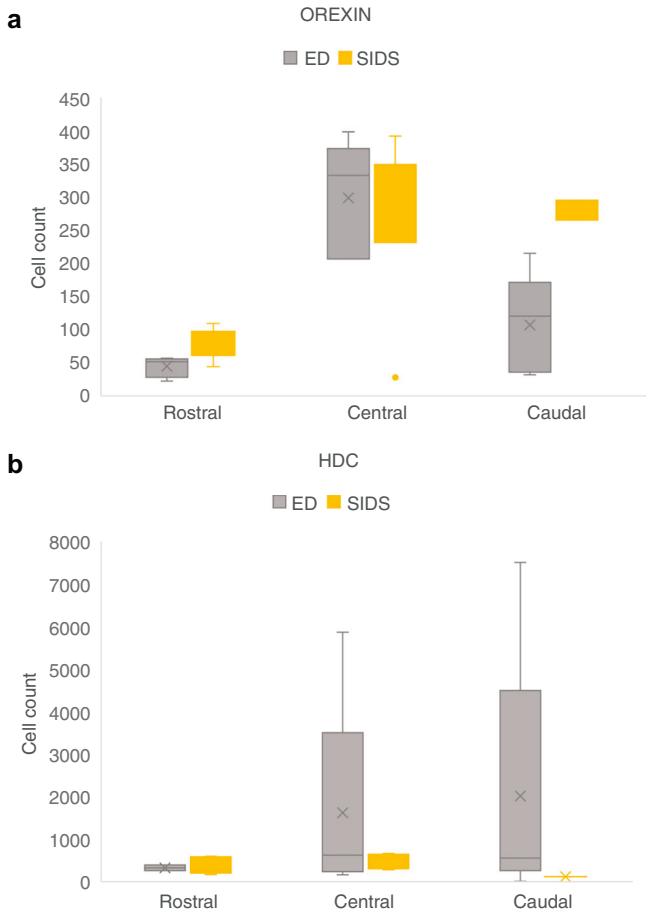


Fig. 6 Cell count quantification of orexin and histidine decarboxylase-immunoreactive neurons in the rostral, central, and caudal sub-regions of the posterior hypothalamus of ED infants (with explained death) and those with sudden infant death syndrome. a Box plots showing cell counts and quantification of the number of Ox-ir neurons from the rostral, central, and caudal subdivisions of the tuberomammillary nucleus and adjacent areas in ED infants and SIDS victims. The horizontal solid bars represent the Q1- mean-Q3 values, the X is the median, and the vertical line presents the min-max values. **b** Box plots showing cell counts and quantification of the number of HDC-ir neurons from the rostral, central, and caudal subdivisions of the tuberomammillary nucleus and adjacent areas in ED infants and SIDS victims. The horizontal solid bars represent the Q1-mean-Q3 values, the X is the median, and the vertical line presents the min-max values. Ox orexin, ir immunoreactive, SIDS sudden infant death syndrome, ED explained death.

normal hypothalamic maturation is associated with modest reductions in neuron number but substantially greater decreases in fiber immunoreactivity.[37] Our findings could therefore reflect enhanced Ox release prior to death, leading to depletion of axonal peptide stores while preserving neuronal somata. In addition, given the known role of Ox in neuroplastic and neurogenic processes,[38] the localized increase in Ox-ir neurons may represent adaptive remodeling within hypothalamic circuits exposed to sustained stress,[39–41] in conditions where other arousal systems are impaired.

Multisystem arousal dysregulation in SIDS

Increased Ox activity may represent an unsuccessful compensatory response to dysfunction in other arousal mechanisms in infants at risk of SIDS. Alterations in multiple arousal systems have been implicated in SIDS pathophysiology. Since the early 2000s, reduced levels of the serotonin metabolite 5-HIAA have been reported in the medullary serotonergic nuclei in approximately 30% of SIDS cases, suggesting impaired serotonergic modulation of autonomic and respiratory control.[42] More recently, evidence has also pointed to involvement of the cholinergic system. Harrington et al. reported significantly lower butyrylcholinesterase (BChE) activity in SIDS infants compared with both ED infants and age-matched living controls, indicating altered cholinergic regulation.[43]

Taken together, these findings support the hypothesis that SIDS may involve dysregulation across multiple interacting arousal systems rather than isolated dysfunction of a single neurotransmitter pathway. In this context, increased Ox-ir neurons could reflect an attempt to compensate for impaired serotonergic or cholinergic signaling within a vulnerable arousal network. Beyond orexin and other monoaminergic systems, the histaminergic (HA) system represents another component of the central arousal network. We therefore examined whether structural or biochemical alterations of HA neurons might also contribute to SIDS vulnerability.

Histaminergic system findings in SIDS

In contrast to Ox, we observed no differences in HA neuron number between SIDS and control cases, despite the known sensitivity of these neurons to hypoxia,[44] hypercapnia,[45] and arousal failure.[46] No significant variation in the number of HDC-ir neurons was detected across hypothalamic subregions. In the central nervous system, HA originates from both tuberomammillary nucleus neurons and mast cells. Under physiological conditions, CSF HA levels exhibit circadian variation and fluctuate across the sleep–wake cycle.[47] Altered CSF HA concentrations have been reported in disorders such as childhood narcolepsy and excessive daytime sleepiness and can be enhanced by H3-receptor inverse agonists, supporting a predominantly neuronal origin.[18,48,49]

However, while CSF levels of neuropeptides such as Ox can be reliably quantified in many conditions, including after death,[50,51] biogenic amines like HA undergo rapid postmortem changes. The wide variability in CSF HA levels observed in our ED and SIDS cases likely reflects multiple factors, including postmortem interval, temperature and interindividual differences in premortem stress. Additional mechanisms, such as unpredictable mast cell degranulation, cessation of neuronal HA turnover, and rapid enzymatic degradation may further contribute. It remains to be clarified whether the highly elevated CSF HA levels observed in infants with SIDS reflect increased neuronal release or mast cell degranulation,[52–54] and whether they contribute to SIDS pathogenesis or instead represent postmortem changes. On the one hand, the markedly elevated t-MeHA levels support a neuronal origin, as neuronal HA is characterized by rapid metabolism and high turnover, whereas mast cells exhibit minimal HDC activity and low metabolic rates.[55] On the other hand, the higher levels of HA and t- MeHA observed in both SIDS and ED compared with controls, together with the absence of significant differences between SIDS and ED groups, suggest that these elevations may largely reflect postmortem processes rather than SIDS-specific mechanisms.

Methodological considerations and limitations

Several limitations should be acknowledged when interpreting these findings. First, the relatively small number of postmortem samples reduces detection power (post-hoc 20%) and our possibility to account for confounding factors. Therefore, larger sample sizes will be necessary in future studies. In addition, the absence of significant associations in some stratified analyses on CSF orexin levels —particularly for prematurity in the SIDS group and infection in the ED group—should be interpreted cautiously, as it may partly reflect limited statistical power due to the small

number of cases within these subgroups. The integration of neurochemical, genetic, and imaging data (e.g., via the Biominrisk project, NCT06244433)[56] will also be essential to understanding the pathophysiology of SIDS. Second, Ox and HA were not measured in the same individuals, precluding paired analyses and limiting inference regarding interactions between these systems. This was due to practical constraints, including the limited volume of CSF obtained, which prevented measurement of both analyses from the same sample. Additionally, for logistical reasons, it was not possible to obtain both CSF and postmortem brain tissue from the same subject. Third, while the HDC antibody used is highly specific in the rat, cross-reactivity with other monoaminergic neurons cannot be totally excluded in other species.[57] Nevertheless, this did not confound cell counts in the posterior hypothalamus,[58,59] as the antibody does not stain tuberomammillary neurons in HDC knockout mice[14] and the morphology and distribution of HA neurons in SIDS were comparable to previous studies using HA-specific antibodies in human brains.[60] Finally, our study used standard formalin-fixed, paraffin-embedded tissue, which allows immunostaining of HDC, the HA-synthesizing enzyme, but not HA itself, the most specific and sensitive marker for HA neurons[17,60,61] HA immunostaining requires special fixation with carbodiimide (EDCDI), which is not routinely used in hospitals. Future studies with HA immunohistochemistry would improve the detection of HA neuron alterations with greater sensitivity. Additionally, CSF control samples were obtained from infants undergoing lumbar puncture for clinical indications, which may introduce variability related to illness or stress, potentially reducing contrast with deceased groups.

CONCLUSIONS

In summary, this study provides evidence of altered orexin system activity in infants who die during the peak SIDS risk period, characterized by increased CSF Ox levels and a localized increased in Ox-ir neurons without evidence of neuronal loss. These findings likely reflect compensatory upregulation in response to impaired arousal or repeated stress rather than primary hyperarousal. In contrast, the HA system does not appear to be selectively involved at the current state. Taken together, these data raise the possibility that Ox dysregulation may represent a developmental biomarker of vulnerability to SIDS. This underscores the importance of age-specific and integrative neurobiological investigations to better understand how interacting arousal systems contribute to failure of protective responses in at-risk infants.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

REFERENCES

1. Krous, H. F. et al. Sudden infant death syndrome and unclassified sudden infant deaths: a definitional and diagnostic approach. *Pediatrics***114**, 234–238 (2004).
2. Moon, R. Y., Horne, R. S. & Hauck, F. R. Sudden infant death syndrome. *Lancet***370**, 1578–1587 (2007).
3. de Visme, S. et al. National variations in recent trends of sudden unexpected infant death rate in Western Europe. *J. Pediatr.***226**, 179–185.e174 (2020).
4. Blair, P. S., Sidebotham, P., Berry, P. J., Evans, M. & Fleming, P. J. Major epidemiological changes in sudden infant death syndrome: a 20-year population-based study in the Uk. *Lancet***367**, 314–319 (2006).
5. Schechtman, V. L., Harper, R. M., Wilson, A. J. & Southall, D. P. Sleep state organization in normal infants and victims of the sudden infant death syndrome. *Pediatrics***89**, 865–870 (1992).
6. Kahn, A. et al. Sleep and cardiorespiratory characteristics of infant victims of sudden death: a prospective case-control study. *Sleep***15**, 287–292 (1992).
7. Kato, I. et al. Incomplete arousal processes in infants who were victims of sudden death. *Am. J. Respir. Crit. Care Med***168**, 1298–1303 (2003).
8. Sakurai, T., Saito, Y. C. & Yanagisawa, M. Interaction between orexin neurons and monoaminergic systems. *Front. Neurol. Neurosci.***45**, 11–21 (2021).
9. Lin, J. S., Anaclet, C., Sergeeva, O. A. & Haas, H. L. The waking brain: an update. *Cell Mol. Life Sci.***68**, 2499–2512 (2011).
10. Mieda, M. & Sakurai, T. Overview of orexin/hypocretin system. *Prog. Brain Res.***198**, 5–14 (2012).
11. Aran, A., Shors, I., Lin, L., Mignot, E. & Schimmel, M. S. Csf levels of hypocretin-1 (orexin-a) peak during early infancy in humans. *Sleep***35**, 187–191 (2012).
12. Lancien, M. et al. Low cerebrospinal fluid hypocretin levels during sudden infant death syndrome (SID) risk period. *Sleep. Med.***33**, 57–60 (2017).
13. Hunt, N. J., Waters, K. A., Rodriguez, M. L. & Machaalani, R. Decreased orexin (hypocretin) immunoreactivity in the hypothalamus and pontine nuclei in sudden infant death syndrome. *Acta Neuropathol.***130**, 185–198 (2015).
14. Melzi, S. et al. Histamine in murine narcolepsy: what do genetic and immune models tell us? *Brain Pathol.***32**, e13027 (2022).
15. Plancoulaine, S. et al. Cerebrospinal fluid histamine levels in healthy children and potential implication for SIDS: observational study in a French Tertiary Care Hospital. *Front. Pediatr.***10**, 819496 (2022).
16. Anaclet, C. et al. Orexin/hypocretin and histamine: distinct roles in the control of wakefulness demonstrated using knock-out mouse models. *J. Neurosci.***29**, 14423–14438 (2009).
17. Parmentier, R. et al. Anatomical, physiological, and pharmacological characteristics of histidine decarboxylase knock-out mice: evidence for the role of brain histamine in behavioral and sleep-wake control. *J. Neurosci.***22**, 7695–7711 (2002).
18. Franco, P. et al. Impaired histaminergic neurotransmission in children with narcolepsy type 1. *CNS Neurosci. Ther.***25**, 386–395 (2019).
19. Dauvilliers, Y. et al. Normal cerebrospinal fluid histamine and tele-methylhistamine levels in hypersomnia conditions. *Sleep***35**, 1359–1366 (2012).
20. Barateau, L. et al. Cerebrospinal fluid monoamine levels in central disorders of hypersomnolence. *Sleep***44**, zsab012 (2021).
21. Croyal, M. et al. Histamine and tele-methylhistamine quantification in cerebrospinal fluid from narcoleptic subjects by liquid chromatography tandem mass spectrometry with precolumn derivatization. *Anal. Biochem.***409**, 28–36 (2011).
22. Ernst, L. M., Ruchelli, E. & Huff, D. S. (eds.) *Color Atlas of Fetal and Neonatal Histology*. (Wiley, 2011).
23. Altman, J. a. B., S. *Atlas of Human Central Nervous System Development* (CRC Press, 2004).
24. Franco, P. et al. Arousal from sleep mechanisms in infants. *Sleep Med.***11**, 603–614 (2010).
25. Fung, S. J., Yamuy, J., Sampogna, S., Morales, F. R. & Chase, M. H. Hypocretin (Orexin) input to trigeminal and hypoglossal motoneurons in the cat: a double-labeling immunohistochemical study. *Brain Res.***903**, 257–262 (2001).
26. Yokota, S., Oka, T., Asano, H. & Yasui, Y. Orexinergic fibers are in contact with kolliker-fuse nucleus neurons projecting to the respiration-related nuclei in the medulla oblongata and spinal cord of the rat. *Brain Res.***1648**, 512–523 (2016).
27. Spinieli, R. L., Ben Musa, R., Cornelius-Green, J., Hasser, E. M. & Cummings, K. J. Orexin facilitates the ventilatory and behavioral responses of rats to hypoxia. *Am. J. Physiol. Regul. Integr. Comp. Physiol.***322**, R581–R596 (2022).
28. Corcoran, A. E., Richerson, G. B. & Harris, M. B. Functional link between the hypocretin and serotonin systems in the neural control of breathing and central chemosensitivity. *J. Neurophysiol.***114**, 381–389 (2015).
29. Kato, I. et al. Developmental characteristics of apnea in infants who succumb to sudden infant death syndrome. *Am. J. Respir. Crit. Care Med.***164**, 1464–1469 (2001).
30. Ducloyer, M. et al. The ogival palate: a new risk marker of sudden unexpected death in infancy? *Front. Pediatr.***10**, 809725 (2022).
31. Huang, Y. S. et al. Sleep-disordered breathing, craniofacial development, and neurodevelopment in premature infants: a 2-year follow-up study. *Sleep. Med.***60**, 20–25 (2019).
32. Blackwell, C. Infection, inflammation and SIDS. *FEMS Immunol. Med. Microbiol.***42**, 1–2 (2004).
33. Yamaguchi, K. et al. Intermittent but not sustained hypoxia activates orexin-containing neurons in mice. *Respir. Physiol. Neurobiol.***206**, 11–14 (2015).
34. Qiao, Y., Liu, Z., Yan, X. & Luo, C. Effect of intermittent hypoxia on neurofunctional recovery post brain ischemia in mice. *J. Mol. Neurosci.***55**, 923–930 (2015).
35. Du, M. K., Hunt, N. J., Waters, K. A. & Machaalani, R. Cumulative effects of repetitive intermittent hypercapnic hypoxia on orexin in the developing piglet hypothalamus. *Int J. Dev. Neurosci.***48**, 1–8 (2016).
36. Spinieli, R. L., Ben Musa, R., Kielhofner, J., Cornelius-Green, J. & Cummings, K. J. Orexin contributes to eupnea within a critical period of postnatal development. *Am. J. Physiol. Regul. Integr. Comp. Physiol.***321**, R558–R571 (2021).
37. Hunt, N. J., Rodriguez, M. L., Waters, K. A. & Machaalani, R. Changes in orexin (Hypocretin) neuronal expression with normal aging in the human hypothalamus. *Neurobiol. Aging***36**, 292–300 (2015).

38. Chieffi, S. et al. Orexin system: the key for a healthy life. *Front. Physiol.***8**, 357 (2017).
39. Ito, N. et al. I.C.V. administration of orexin-a induces an antidepressive-like effect through hippocampal cell proliferation. *Neuroscience***157**, 720–732 (2008).
40. Ren, B. et al. Mechanism by which low-intensity focused ultrasound promotes angiogenesis and neurogenesis after traumatic brain injury in a rat model via the oxa/mapk signaling pathway. *Neural Regen. Res*. **21**, 4297–4310 (2025).
41. Stoyanova, I. I., Rutten, W. L. & le Feber, J. Orexin a in cortical cultures: expression and effect on synaptogenesis during development. *Cell Mol. Neurobiol.***32**, 49–57 (2012).
42. Duncan, J. R. et al. Brainstem serotonergic deficiency in sudden infant death syndrome. *JAMA***303**, 430–437 (2010).
43. Harrington, C. T., Hafid, N. A. & Waters, K. A. Butyrylcholinesterase is a potential biomarker for sudden infant death syndrome. *EBioMedicine***80**, 104041 (2022).
44. Fan, Y. Y. et al. Activation of the central histaminergic system is involved in hypoxia-induced stroke tolerance in adult mice. *J. Cereb. Blood Flow. Metab.***31**, 305–314 (2011).
45. Kernder, A., De Luca, R., Yanovsky, Y., Haas, H. L. & Sergeeva, O. A. Acid-sensing hypothalamic neurons controlling arousal. *Cell Mol. Neurobiol.***34**, 777–789 (2014).
46. Lozeva, V., Tuomisto, L., Tarhanen, J. & Butterworth, R. F. Increased concentrations of histamine and its metabolite, tele-methylhistamine and down-regulation of histamine H3 receptor sites in autopsied brain tissue from cirrhotic patients who died in hepatic coma. *J. Hepatol.***39**, 522–527 (2003).
47. Zeitzer, J. M. et al. Time-course of cerebrospinal fluid histamine in the wake-consolidated squirrel monkey. *J. Sleep. Res.***21**, 189–194 (2012).
48. Kanbayashi, T. et al. Csf histamine contents in narcolepsy, idiopathic hypersomnia and obstructive sleep apnea syndrome. *Sleep***32**, 181–187 (2009).
49. Soya, A. et al. CSF histamine levels in rats reflect the central histamine neurotransmission. *Neurosci. Lett.***430**, 224–229 (2008).
50. Narita, N., Yamada, R., Kakehi, M. & Kimura, H. Diurnal fluctuations of orexin-a and -B in cynomolgus monkey cerebrospinal fluid determined by a novel analytical method using antiadsorptive additive treatment followed by nanoflow liquid chromatography-high-resolution mass spectrometry. *ACS Chem. Neurosci.***14**, 609–618 (2023).
51. Grady, S. P., Nishino, S., Czeisler, C. A., Hepner, D. & Scammell, T. E. Diurnal variation in CSF orexin-a in healthy male subjects. *Sleep***29**, 295–297 (2006).
52. Edston, E., Gidlund, E., Wickman, M., Ribbing, H. & Van Hage-Hamsten, M. Increased mast cell tryptase in sudden infant death - anaphylaxis, hypoxia or artefact? *Clin. Exp. Allergy***29**, 1648–1654 (1999).
53. Holgate, S. T. et al. The anaphylaxis hypothesis of sudden infant death syndrome (SIDS): mast cell degranulation in cot death revealed by elevated concentrations of tryptase in serum. *Clin. Exp. Allergy***24**, 1115–1122 (1994).
54. Bonelli, A., Bacci, S., Vannelli, B. & Norelli, A. Immunohistochemical localization of mast cells as a tool for the discrimination of vital and postmortem lesions. *Int. J. Leg. Med.***117**, 14–18 (2003).
55. Schwartz, J. C., Arrang, J. M., Garbarg, M., Pollard, H. & Ruat, M. Histaminergic transmission in the mammalian brain. *Physiol. Rev.***71**, 1–51 (1991).
56. Ducloyer, M. et al. Identification of novel genetic, neurobiological and radio-anatomical biomarkers for risk stratification of sudden unexpected death in infancy and early childhood: the biominrisk study protocol. *BMJ Open***15**, e101811 (2025).
57. Mizuguchi, H., Yabumoto, M., Imamura, I., Fukui, H. & Wada, H. Immuno-cross-reactivity of histidine and dopa decarboxylases. *Biochem Biophys. Res. Commun.***173**, 1299–1303 (1990).
58. John, J. et al. Greatly increased numbers of histamine cells in human narcolepsy with cataplexy. *Ann. Neurol.***74**, 786–793 (2013).
59. Valko, P. O. et al. Increase of histaminergic tuberomammillary neurons in narcolepsy. *Ann. Neurol.***74**, 794–804 (2013).
60. Panula, P., Airaksinen, M. S., Pirvola, U. & Kotilainen, E. A histamine-containing neuronal system in human brain. *Neuroscience***34**, 127–132 (1990).
61. Lin, J. S., Hou, Y., Sakai, K. & Jouvet, M. Histaminergic descending inputs to the mesopontine tegmentum and their role in the control of cortical activation and wakefulness in the cat. *J. Neurosci.***16**, 1523–1537 (1996).

AUTHOR CONTRIBUTIONS

Jian-Sheng Lin and Patricia Franco conceived and designed the study. Clara Inocente, Aurore Guyon, Marion Comajuan, Beatrice Kugener collected the data. David Meyronnet, Anne Jouvet performed the neuropathological analyses. Yan Zhao, Guang-Fu Cui, Yi-Ping Hou, Marine Bersault performed biochemical analyses. Yves Dauvilliers, Manuela Lotierzo, Philippe Robert performed the OX and HA measurements in CSF Marine Thieux and Sabine Plancoulaine performed the statistical analyses. Yan Zhao, Jian-sheng Lin, Sabine Plancoulaine and Patricia Franco drafted the manuscript. All authors critically revised the manuscript and approved the final version. We thank Verena Landel for her proofreading.

FUNDINGS

Supported by Laboratory of Integrative Physiology of the Brain Arousal System, INSERM U628 and U1028, Université Claude Bernard Lyon1, ANR NarConX, and ANR Histawake. Work involving GF Cui and YP Hou was supported by the National Natural Science Foundation and the Talent-Introducing Project of Foreign Experts Affairs of China (X2017008) ^{81771426}. We thank Dr. Ling Shan for his help with the postmortem histological study and for the scientific discussion.

COMPETING INTERESTS

The authors declare no competing interests.

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ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41390-026-05186-y.

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