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Whole-brain characterization of apoptosis after sevoflurane anesthesia reveals neuronal apoptosis patterns in the mouse neonatal neocortex

C. Sola*(1), J.Areias(2), J.Pico(3), Y.Chastagnier(4), N.Bouquier(4), C.Dadure(5), J.Perroy(4), V.Szabo(6)

(1) Département d'Anesthésie Réanimation Femme Mère Enfant, CHU Montpellier, Montpellier, France , (2) IGF, University of Montpellier, CNRS, INSERM, IGF, Montpellier, France , (3) Département Anesthésie Réanimation Femme Mère Enfant, Unité d'Anesthésie pédiatrique, CHU Montpellier, Montpellier, France , (4) University of Montpellier, CNRS, INSERM, IGF, Montpellier, France , (5) Département Anesthésie Réanimation Femme Mère Enfant, CHU Montpellier, Montpellier, France , (6) Département Anesthésie Réanimation NSTC, CHU Montpellier, Montpellier, France

**Auteur présenté comme orateur*

Position du problème et objectif(s) de l'étude:
In the last two decades, safety concerns about general anesthesia (GA) arose from studies documenting brain cells death in various drug conditions and animal models. A thorough characterization of anesthesia-induced apoptosis across post-natal developmental stages would help identify and further focus on underlying mechanisms. This study's aim was to provide an unbiased anatomical whole brain mapping and quantification of sevoflurane-induced apoptosis during postnatal brain development in mice.

Matériel et méthodes:
All animal procedures were conducted according to the Council of the European Communities Directive, supervised by the local animal protection unit (A34-172-41). Mice were randomly assigned to the sevoflurane (3% Fi - 3X2h) or control group (100%O2 - 6h), on postnatal (P) days 7 (neonatal), P21 (juvenile), or P49 (adult). Whole brain clarified and immunostained for cleaved caspase-3 (apoptotic cell marker) was imaged and the stained volume was quantified. To characterize the anatomical distribution of c-caspase-3 positive cells, we developed a P7 mouse brain digital atlas for semi-automated registration and segmentation. Finally, we characterized the spatial distribution and cytological nature of cells affected by anesthesia-induced apoptosis in brain slices. For statistical analysis, we used the Mann-Whitney U test (for 2 groups of independent variables) and Friedman ANOVA (for 3 paired conditions). Data are presented as median [IQR]. A p value <0.05 was considered significant.

Résultats & Discussion:
Neonatal mice appeared to be highly sensitive to anesthesia-induced apoptosis: the volume fraction of c-caspase-3 was significantly increased after neonatal exposure to sevoflurane compared with the control group ($p=0.003$). In contrast, after exposure during the juvenile (P21) and adult (P49) periods, the volume fractions of apoptotic cells remained similar to those of unexposed control groups (Figure 1 A.). Use of a novel P7 brain atlas showed that the neocortex was the most affected area, followed by the striatum and the metencephalon (Figure 1 B.). Histological characterization in cortical slices determined that the most affected cell type were post-mitotic neurons (NeuN marker) of which nearly a quarter were GABAergic neurons (GAD67 marker), and that distribution followed inter and intracortical gradients. The maximal apoptosis was reported in superficial layers of the posterodorsal cortex (Figure 1 C.).

Conclusion:
The unbiased whole-brain anatomical mapping used here confirmed the high specificity and sensitivity of neonatal developmental stage to sevoflurane-induced apoptosis. Vulnerability appears brain region dependent with the neocortex particularly affected, and a moderate involvement of the hippocampus.

The identification of neocortical apoptotic gradients is consistent with a maturity-dependent mechanism. Further research could then focus on signaling pathways identification and the interference of sevoflurane with neuronal migration and survival during development.

Figure 1.A.
Whole brain mapping of apoptosis after sevoflurane anesthesia at P7, P21 et P49

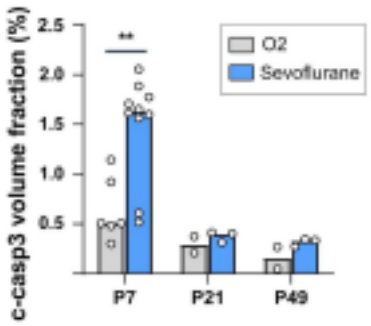


Figure 1.B.
Anatomical distribution of c-caspase-3 positive cells using P7 mouse brain digital atlas

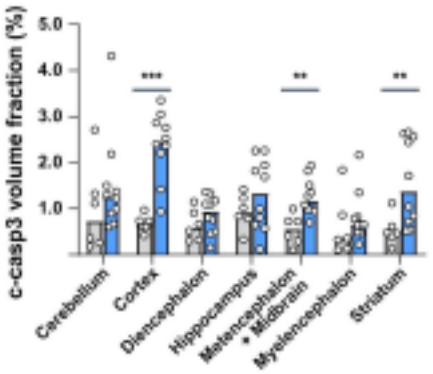
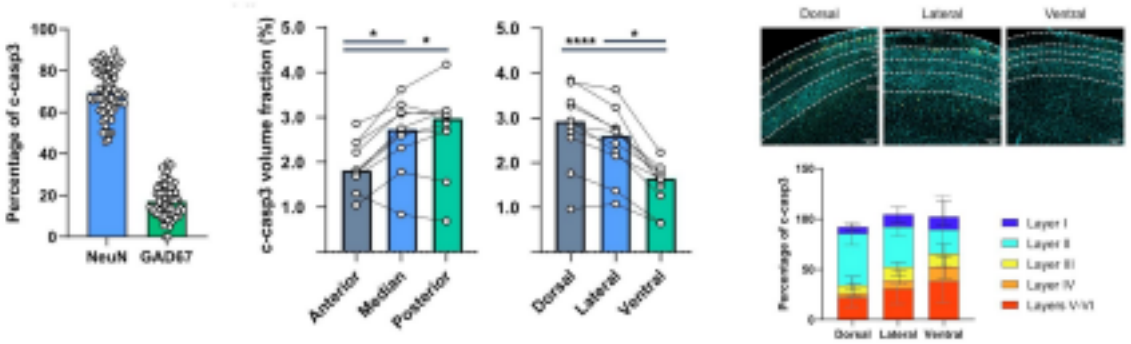


Figure 1.C. Characterization of neocortical apoptosis after sevoflurane anesthesia at P7.



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