Effect of recurrent isoflurane anaesthesia during development on the neurochemical profile of the frontal and occipital cortex of adult mice: an *in vivo* ^{1}H MRS study at 14.1 T

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Background: The metabolic profile of the brain is subject to permanent alteration during development [1]. Several psychopathological conditions and disorders of the central nervous system are associated with abnormal brain development [2], and thus possibly also with abnormal brain metabolic profiles. ^{1}H NMR spectroscopy is a particularly suitable method to assess and quantify such alterations. However, the *in vivo* NMR scanning of animals at different stages of development requires recurrent anaesthesia. The present study investigated whether recurrent isoflurane anaesthesia during development has an effect on the cortical neurochemical profile of the adult mice. The mice under study were furthermore subjected to a comprehensive battery of behavioural tasks to investigate whether recurrent anaesthesia interferes with basic motor function, exploratory activity, anxiety-like behaviour, mood and learning capacity.

Methods: C57BL/6 mice (12 males and 6 females) were anaesthetized at P10, P20, P30 and P60 under 2% isoflurane (in oxygen) anaesthesia for 1 hour, the temperature being maintained at 37 °C. Control mice (8 males and 6 females) were not anaesthetized but were separated from the mother for the same period. A battery of behavioural tests was performed between P60 and P90, to test the following capacities: forepaw grip strength, running on the rotarod, escape from a hanging wire, exploration in the open field the elevated platform, and in a Y-maze, coping in the Porsolt swim test, and spatial and object recognition memory. At P90, mice were anesthetized with 2% isoflurane, under spontaneously ventilation, with body temperature maintained at 37 °C. Localised *in vivo* NMR spectroscopy was performed on a 14.1 T, 26 cm VNMRS spectrometer (Varian, Magnex) using a home-built 14 mm diameter quadrature surface coil (used both for RF excitation and signal reception). Field homogeneity was adjusted by FASTMAP [3], and ^{1}H NMR spectra were acquired from VOIs of 5-6 µl placed in frontal or occipital regions of the cortex, using SPECIAL [4,5] with TE of 2.8 ms and TR of 4 s. Typically, spectra were acquired with 640 scans. Metabolite concentrations were estimated using LCModel [6] and data was compared with the two-way ANOVA and Bonferroni's post-test.

Results: Control mice and mice submitted to recurrent anaesthesia periods showed similar performance in behavioural tasks, suggesting that repeated isoflurane anaesthesia during development does not affect mice behaviour. From the ^{1}H NMR spectra acquired at 14.1 T we were able to estimate the concentration of 20 metabolites, in the frontal and occipital cortex, with average Cramer-Rao Lower Bounds of 15±4%. In general, the present results showed that the neurochemical profile in these cerebral regions was not affected to a great extent by recurrent isoflurane anaesthesia (figure 1). The average alteration in the metabolite concentrations in the cortex of recurrently anaesthetized mice relative to controls was 0.9±2.5% in the occipital cortex and 9.2±3.1% for the frontal cortex. The only statistically significant alteration caused by recurrent anaesthesia was a small increase in taurine in the occipital cortex (+12±4%, $P<0.05$). We conclude that short recurrent anaesthesia is without major effect on behaviour and within 10% on the cortical neurochemical profile.

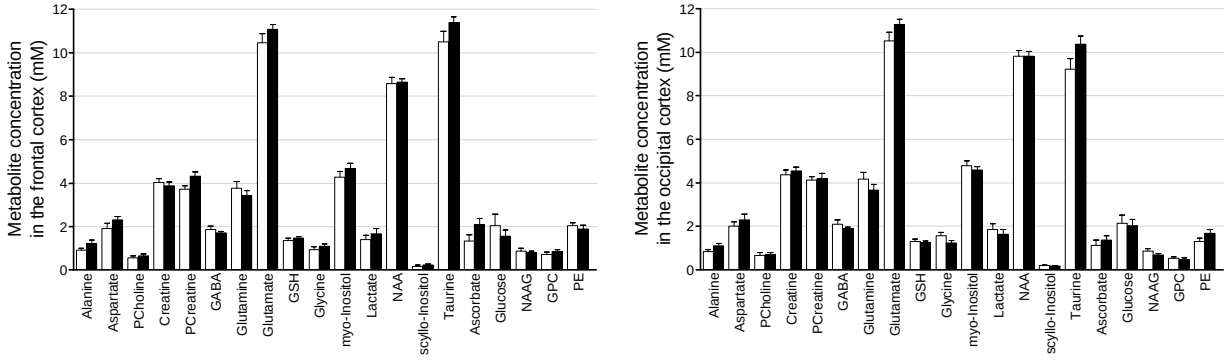

Figure 1. Neurochemical profile at 14.1 T in frontal (left graph) and occipital (right graph) cortical regions of control mice (white bars) and mice exposed to recurrent isoflurane anaesthesia (black bars).

References: [1] Tkác *et al.*, Magn Reson Med 50:24 (2003). [2] Hüppi, Clin Perinatol 29:827 (2002). [3] Gruetter, Magn Reson Med 29:804 (1993). [4] Mlynárik *et al.*, Mag Reson Med 56:965 (2006). [5] Mlynárik *et al.*, J Mag Reson 194:163 (2008). [6] Provencher, Mag Reson Med 30:672 (1993).

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