

## Effects of maternal melatonin administration on offspring of a precocial species subjected to near-term birth asphyxia

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**Objectives:** To determine if chronic maternal administration of melatonin (MEL) in late pregnancy ameliorates or prevents brain damage caused by birth asphyxia in a small animal species with advanced *in utero* brain development – the Spiny Mouse (*Acomys cahirinus*).

**Methods:** Melatonin (0.1 mg/kg/d) or 0.9% saline (SAL; 12 µl/d) was administered *s.c* to pregnant Spiny Mouse dams for 7 d prior to delivery using Alzet™ osmotic minipumps. Dams were killed by cervical dislocation at 37 d gestational age (term is 38-39 d). Pups underwent control or asphyxiated delivery, as performed by us previously (Ireland et al., AJOG; 198, 2008). Control pups were removed from the uterus immediately following maternal death (<1 min), and revived by clearing the mouth and palpating the chest and abdomen. For pups subjected to asphyxia, the intact uterus was quickly removed from the abdomen and immersed in a warm (37°C) saline bath for 7-8 mins, after which the pups were delivered and resuscitated as above. Pups were cross-fostered to a lactating dam for 24 h until post-mortem. Brains were collected, immersion fixed and used to determine inflammatory and apoptotic reactions using lectin and activated caspase-3 immunohistochemistry respectively. A further group of SAL and MEL treated animals were used to measure MEL in maternal and fetal tissues at delivery (37 d gestation).

**Results:** Maternal MEL administration between 30 and 37 d gestation increased fetal tissue [liver] MEL concentrations approximately 10-fold, and significantly increased the birth weight of pups (5.2±0.1 g) compared to the saline treated group (4.2±0.1 g; P<0.05), but did not significantly affect offspring survival rate following asphyxia (SAL; 10/11 pups, MEL; 12/12 pups). Birth asphyxia increased the number of apoptotic cells in the corpus callosum, and this was significantly less in the MEL group. MEL administration also reduced the number of apoptotic cells in a number of brain regions, irrespective of control or asphyxia delivery (Table).

### Activated caspase-3 immunoreactive cells/mm<sup>2</sup>

	Corpus callosum* <sup>#</sup>	Inferior colliculus*	Hippocampus-CA1*	Hippocampus-CA3*
Cont+SAL	4862.2±912.5	2775.9±435.6	7612.7±719.3	3986.4±359.8
Cont+MEL	3079.4±804.1	1098.5±141.8	5034.3±508.0	2651.9±194.6
Asph+SAL	9289.4±2335.5	1897.8±526.2	6852.1±716.7	3921.5±347.5
Asph+MEL	2093.4±594.6	1364.7± 611.1	5440.8±1144.3	2660.7±742.4

\*P≤0.05; MEL v SAL, <sup>#</sup> P≤0.05; interaction between birth type and SAL or MEL.

Following birth asphyxia the number of macrophages but not microglia, was significantly increased in the inferior colliculus and corpus callosum (P=0.038, cont+SAL; 216.99±32.68, cont+MEL; 100.76±12.54, asph+SAL; 242.34±35.65, asph+MEL; 243.98±52.22 macrophages/

**Conclusions:** Reduction in the number of cells undergoing apoptosis indicates that maternal MEL treatment in late gestation can protect the fetal brain against the stress associated with a Caesarean-type birth and asphyxia. The increased birth weight after MEL treatment was unexpected, and may arise from vasodilator properties of this indoleamine on the utero-placental circulation.