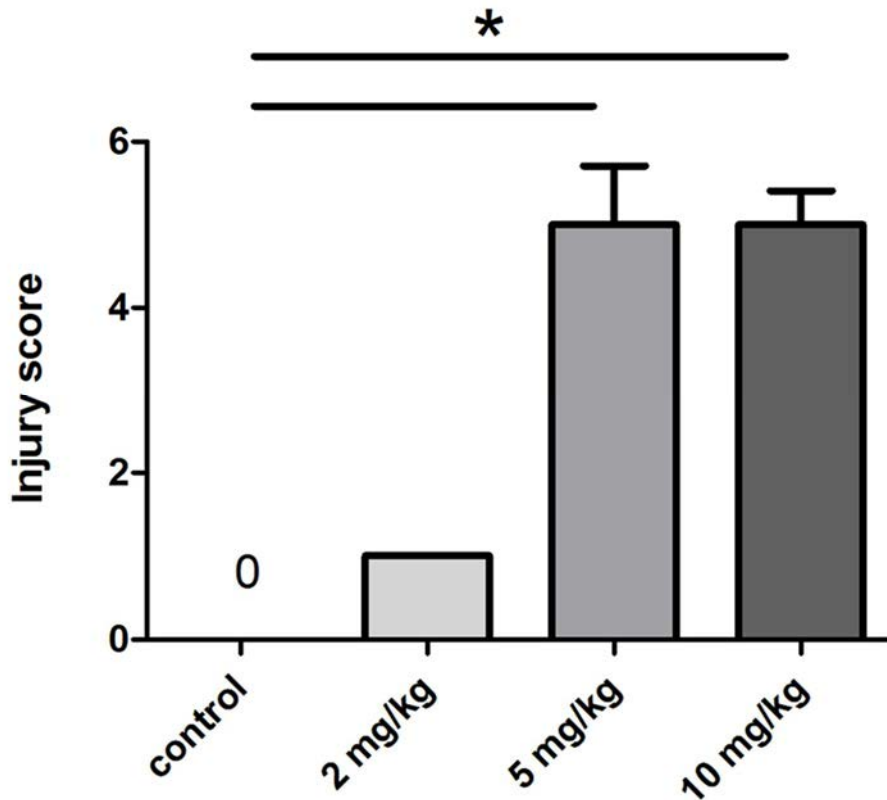


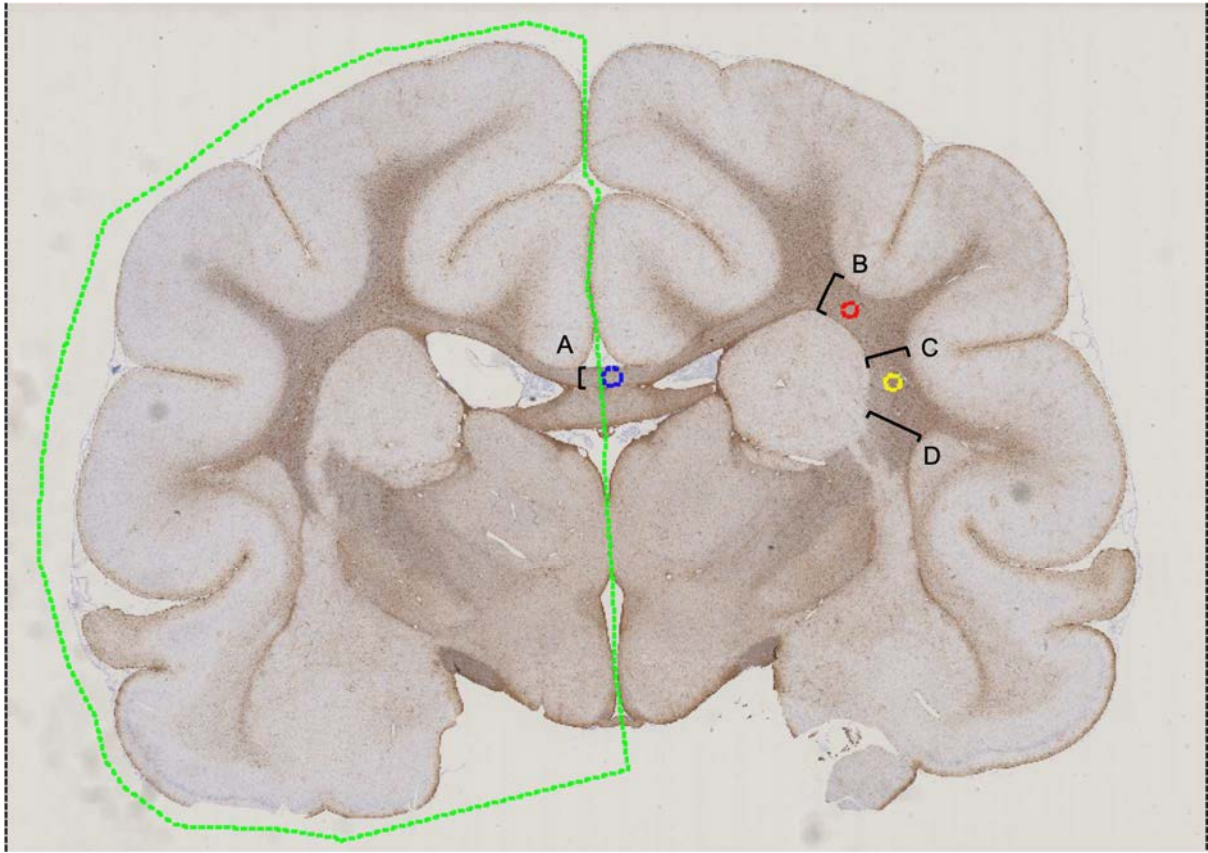
A Ferret Model of Encephalopathy of Prematurity

Supplemental Material

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Supplemental Figure 1. Injury after escalating LPS doses. Ferrets treated with three doses of 2 mg/kg (n=3), 5 mg/kg (n=4) and 10 mg/kg (n=4) every 12 h at P9-P10 before hypoxia/hyperoxia were examined histologically (H&E staining) at P12. Increased inflammation within the meninges and to a lesser degree the neuropil was observed in all dose groups compared to untreated controls (n=4). Score comparison revealed significant differences between 5 mg/kg LPS and control and 10 mg/kg LPS and control, but no significant difference between 5 mg/kg and 10 mg/kg doses of LPS (Figure 2D). At 5 mg/kg and 10 mg/kg, rare acute microhemorrhages characterized by small areas of extravascular red blood cells were noted.



Supplemental Figure 2. Quantitative immunohistochemistry. Representative GFAP image showing regions of interest as taken to analyze MBP, Olig2, and GFAP staining intensity for a total hemisphere (**green**), corpus callosum (**blue**), internal capsule 1 (**red**), and internal capsule 2 (**yellow**). For white matter thickness, the corpus callosum (**A**), and three levels of the internal capsule (**B-D**) were measured.

Supplemental Table 1. Iterative injury protocols. Development of the model involved the iterative increase in LPS dose, changes in dose timing, and manipulating the delay between LPS doses and hypoxia. Initially, LPS alone was used. Subsequently a period of hypoxia was added, then a period of hyperoxia after hypoxia and, finally, a second period of hypoxia with continuous rectal temperature monitoring during the insult period. Data from the penultimate row is depicted in Supplemental Figure 1. The current protocol is detailed in the final row.

Age at insult	LPS	No. of LPs doses	Delay (last LPS dose to hypoxia)	Hypoxia	Hyperoxia	2nd Hypoxia	Waterbath temp during insult (°C)	Rectal temp during insult (°C)
P9-P12	10mcg/kg/24h	4	N/A	N/A	N/A	N/A	N/A	N/A
P9-P12	50-100mcg/kg/24h	3	24h	8% for 4 h	N/A	N/A	35.7-37.5	N/A
P9-P11	100mcg/kg/24h	3	None	6% for 6h	N/A	N/A	35.7-37.5	N/A
P10	0.5-1mg/kg	1	None	6% for 4h	N/A	N/A	37	N/A
P9-P10	1mg/kg/12h	1 vs 3	None	6% for 45min	100% for 6h	N/A	37	N/A
P9-P10	2 vs 5 vs 10mg/kg/12h	3	None	10% for 2h	80% for 2h	10% for 2h	40	37 (hypoxia) 38 (hyperoxia)
P10-P11	5mg/kg	1	4h	9% for 2h	60% for 2h	9% for 2h	40	37 (hypoxia) 38 (hyperoxia)