

Early neuropathological and neurobehavioral consequences of preterm birth in a rabbit model

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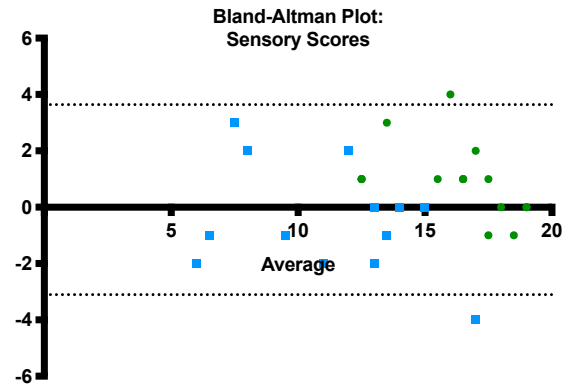
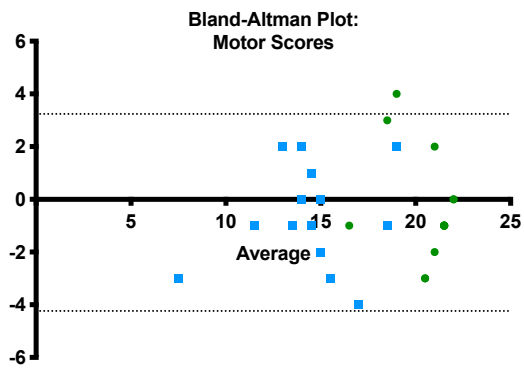
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Rabbit kit: Early neurobehavioral assessment PN+1d				Date of evaluation: __ __ __ __			
Rabbit ID: __ __ __ __		Weight (g):		PN day: +		Video ID:	
During adaptation: __ Sleepy __ Awake Some activity __ Active and exploring							
NEUROMOTORIC				NEUROSENSORY			
A. Posture				G. Surface Righting Reflex (5 attempts) (2)			
0	Lays supine				no. of times turned to prone position within 2 sec seconds when placed in supine position		
1	Lays on side/minimal movement						
2	Cannot maintain prone position (roll over > 50% time)						
3	Prone position with legs coiled (roll over < 50% time)			H. Sensation - Facial/Whisker touch response			
B. Gait (2)				0	No Response		
0	Not able to move/uses whole body to move			1	Subtle (none directed reaction after ≥ 3 stimuli)		
1	Crawls, trunk touching ground, unstable > 50% time			2	Mild (slow or directed response after 2 stimuli)		
2	Walks, trunk low/touching ground, unstable <50% time			3	Immediate avoidance or brushes side of head		
3	Walks but can't propel body using hind legs synchronously			I. Sucking and swallowing			
4	Walks, runs, and hops (synchronous) and coordinated			0	No movement of jaw, milks dribbles out completely		
C. Locomotion(4) [all criteria must be met]				1	Some jaw/neck movement, most milk dribbles out		
0	Not able to move/uses whole body to move			2	Definite suck and swallow, some milk in nose		
1	Some movement, single steps, slight hops, <15cm			3	Good suck and swallow, no milk in nose		
2	Good ROM, 2-3 continuous steps, 1-2 hops, 15-45cm			J. Head turning during feeding			
3	Entire ROM, >3 continuous steps, >3 hops, >45cm			0	No movement		
	Maximum number of continuous steps			1	Slow, occasional movement of head		
	Total distance travelled (cm)			2	Distinct movement of only head		
	Total number of squares crossed (n)			3	Rapid forceful movements of head and body		
				K. Odour Aversion Test		Ethanol	
				0	No Response (>20s)		
D. Motor Activity				1	Subtle (6-20sec)		
	Head	Fore limbs	Hind limbs	2	Low (2-5sec)		
0 No Movement				3	Rapid/Normal (<2sec)		
1 Slight Movement				L. Pain sensation – response to pin prick on limbs			
2 Distinct Movement				0	No Response within 20 seconds with >3 pricks		
3 Rapid/Purposeful Movement				1	Subtle (>2seconds, response with >2 pricks)		
E. Duration of activity during observation				2	Mild (<2 seconds, rapid response with >1prick)		
0	No movement			3	Rapid/immediate, repetitive movement		
1	Active for < 20 seconds						
2	Active for 20-40 seconds						
3	Active for > 40 seconds						
F. Limb Tone (Hind limbs) (5)							
0	Limb rigid in flexion or extension						
1	Tone considerably increased, passive movement difficult						
2	Marked increase in tone but limb is easily flexed						
3	Slight increase in tone when limb is moved						
4	No increase in tone						

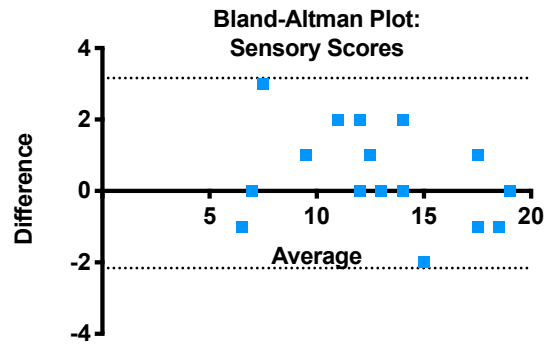
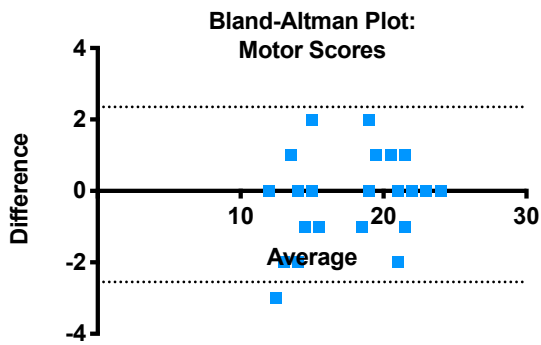
Performed on designated 25 x 25 cm area divided into 5 x 5 cm blocks over a time period of 90sec.

Supplement 1. Neurobehavioural assessment protocol

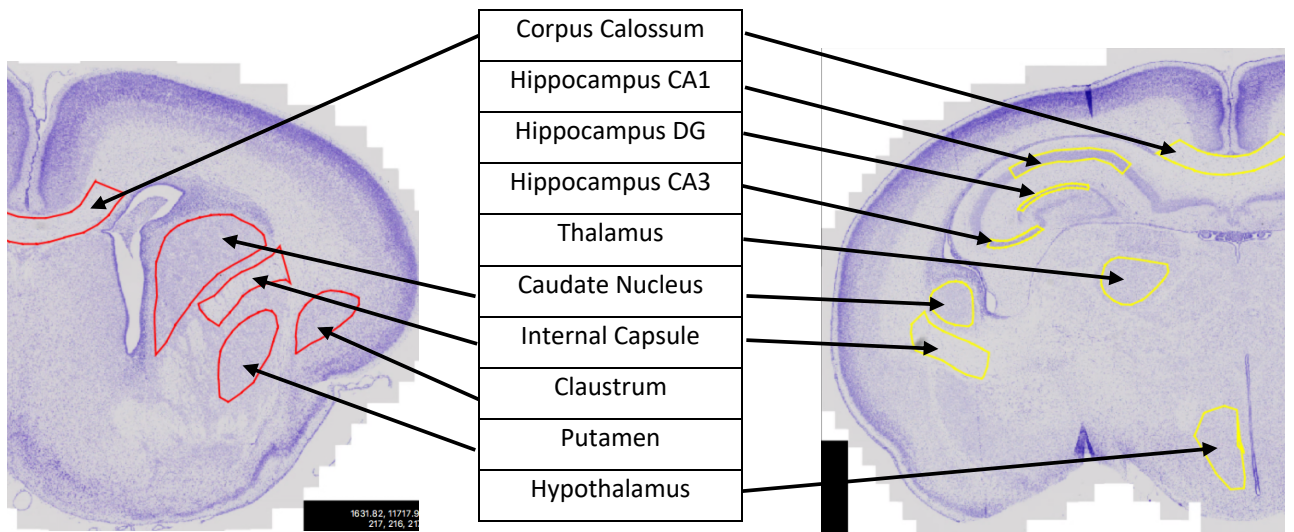
Inter-observer Agreement



Intra-observer Agreement



Supplementary Figure 2: Bland Altman plot for the intra- and interobserver agreement in neuromotor and sensory scores. To test for the reliability of the neurobehavioral assessment, 30 randomly selected neurobehavioral videos were analyzed by 2 observers.



Supplementary Figure 3. Regions of interest annotated on the two levels taken serially from each brain. From the first set of coronal sections, the corpus callosum, caudate nucleus, putamen, internal capsule and claustrum were annotated. On the second set the corpus callosum, hippocampal regions CA1, CA3 and DG, caudate nucleus, the anterior ventral nucleus of the thalamus, internal capsule, claustrum and hypothalamus.

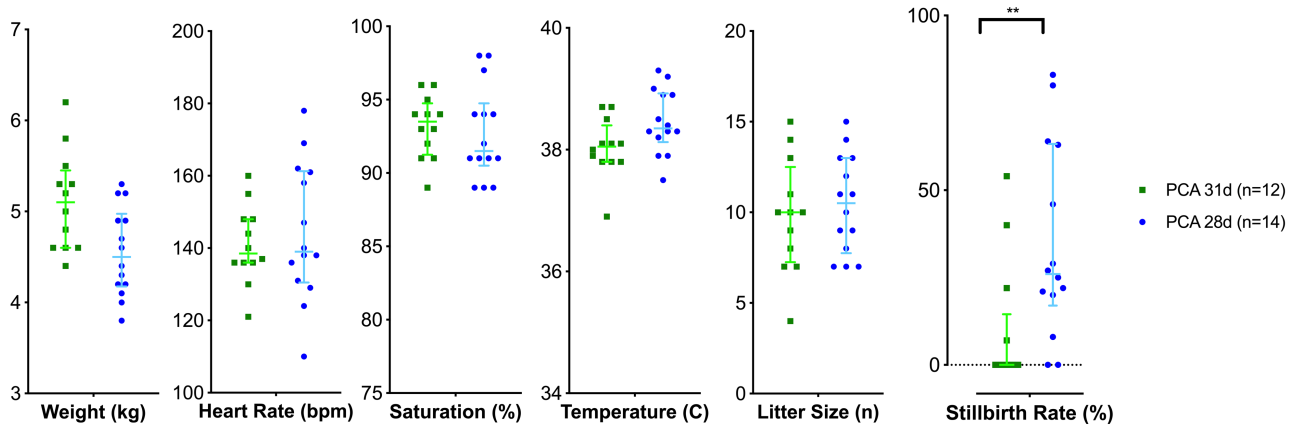


Figure 4a. Maternal characteristics of pregnant rabbit does at the time of delivery. PCA 31d n=12, PCA 28d n=14. Data displayed as median and IQR with significance as * $0.05 \geq p > 0.01$; ** $0.01 \geq p > 0.001$; *** $p < 0.001$.

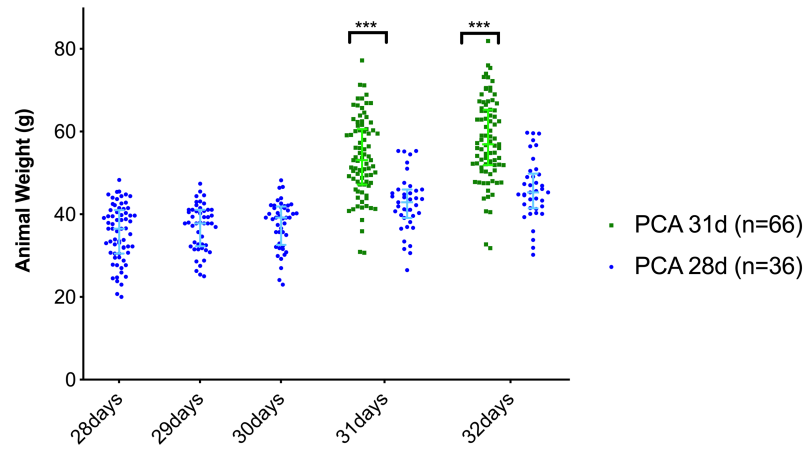


Figure 4b. Postnatal weight of newborn rabbits. PCA 31d n=66, PCA 28d n=36. Data displayed as median and IQR with significance as * $0.05 \geq p > 0.01$; ** $0.01 \geq p > 0.001$; *** $p < 0.001$.

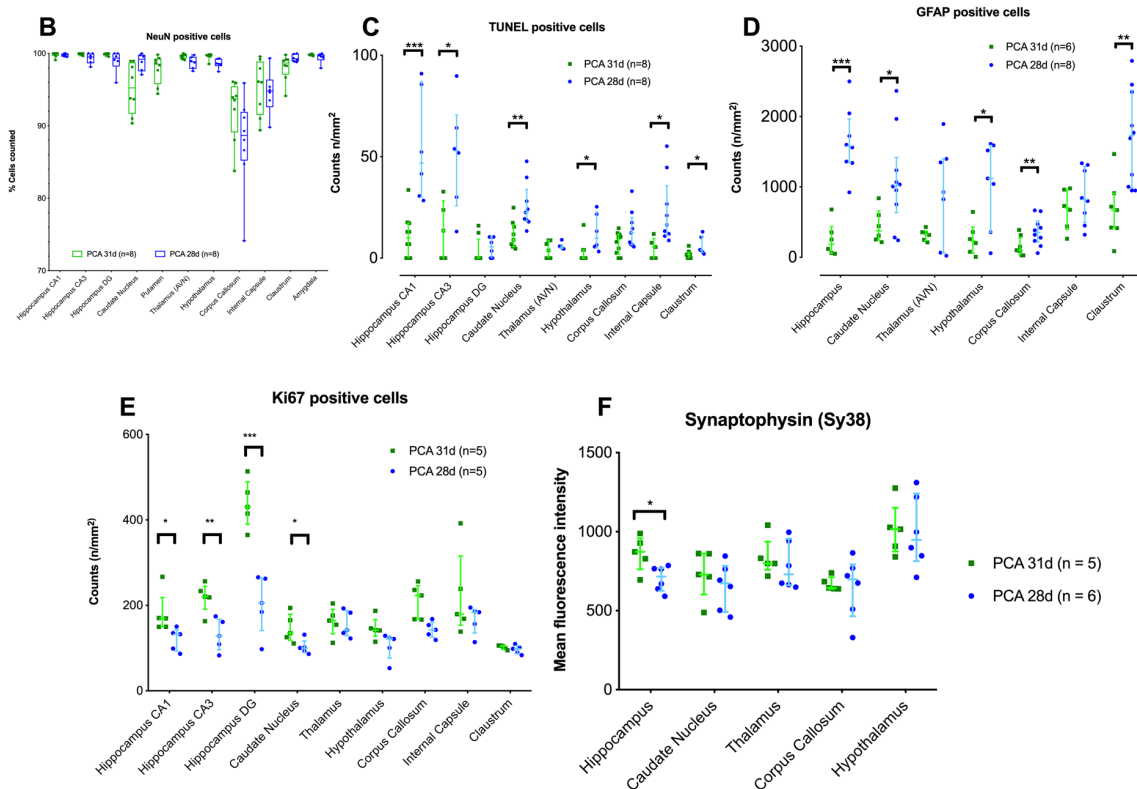
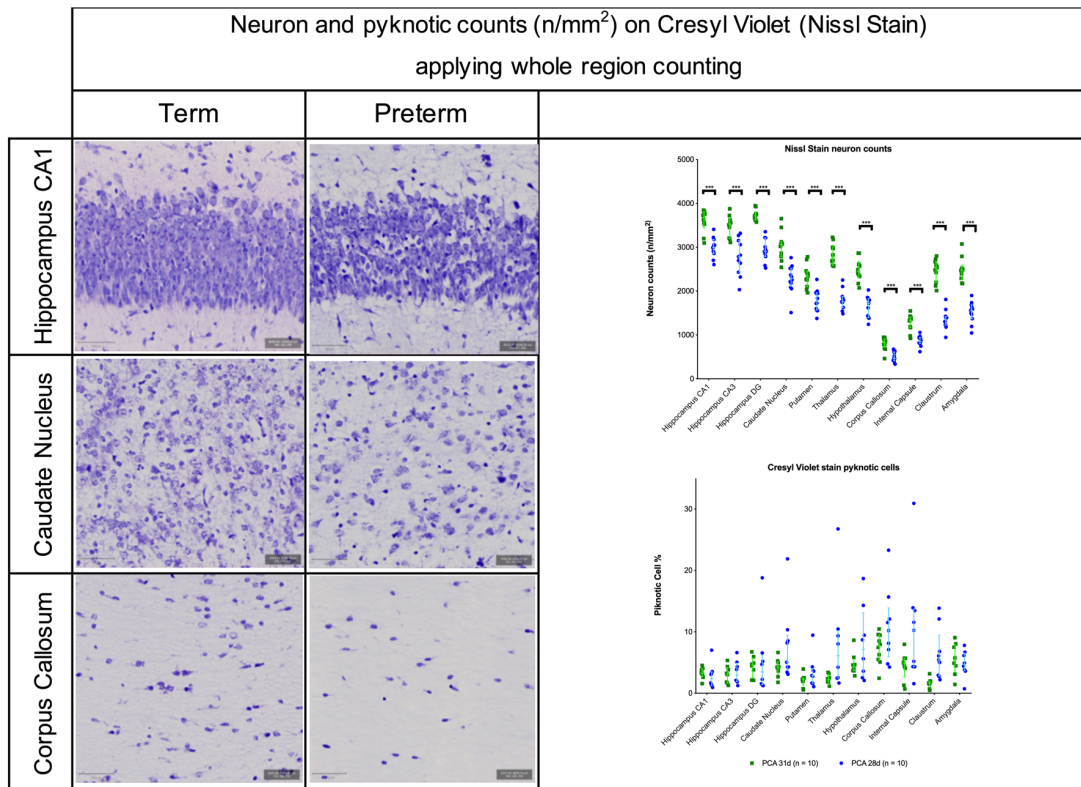


Figure 5. (A) Neuron and pyknotic cell counts (n/mm²) on Cresyl Violet (Nissl Stain) applying whole region counting. Representative fields from main regions of interest. Scale bar 50µm. (B) % NeuN positive cells in each region of interest. (C,D,E) Total number of positive cells for TUNEL, GFAP and Ki67 stains per region of interest. (F) Sy38 positive cells mean fluorescence intensity in selected region of interest. Data displayed as median and IQR with significance as * 0.05 ≥ p > 0.01; ** 0.01 ≥ p > 0.001; *** p < 0.001.

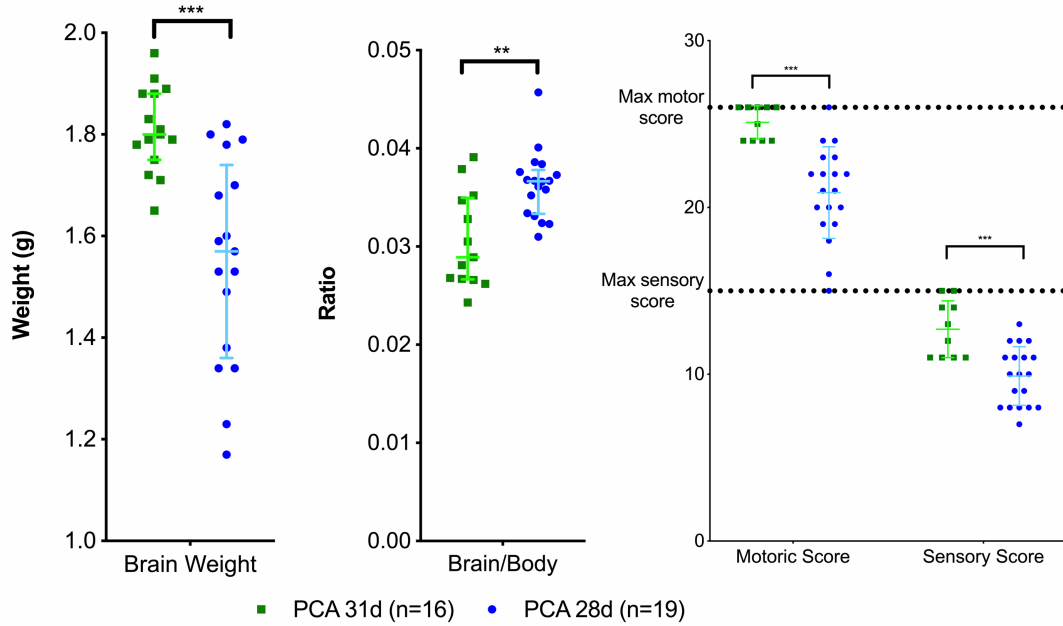


Figure 6a. Characteristics of the subjects that underwent MRI. Data displayed as median and IQR with significance as * $0.05 \geq p > 0.01$; ** $0.01 \geq p > 0.001$; *** $p < 0.001$.

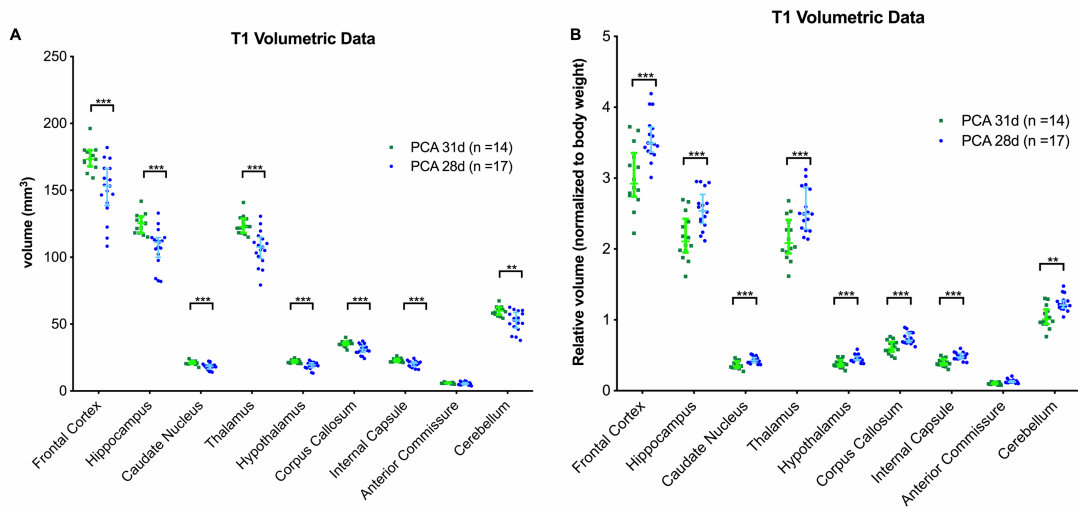


Figure 6b. T1-weighted volumetric data at postconceptional 32days. **(A)** Absolute volumes **(B)** Relative volume normalized to body weight of the animal. Data displayed as median and IQR with significance as * $0.05 \geq p > 0.01$; ** $0.01 \geq p > 0.001$; *** $p < 0.001$.

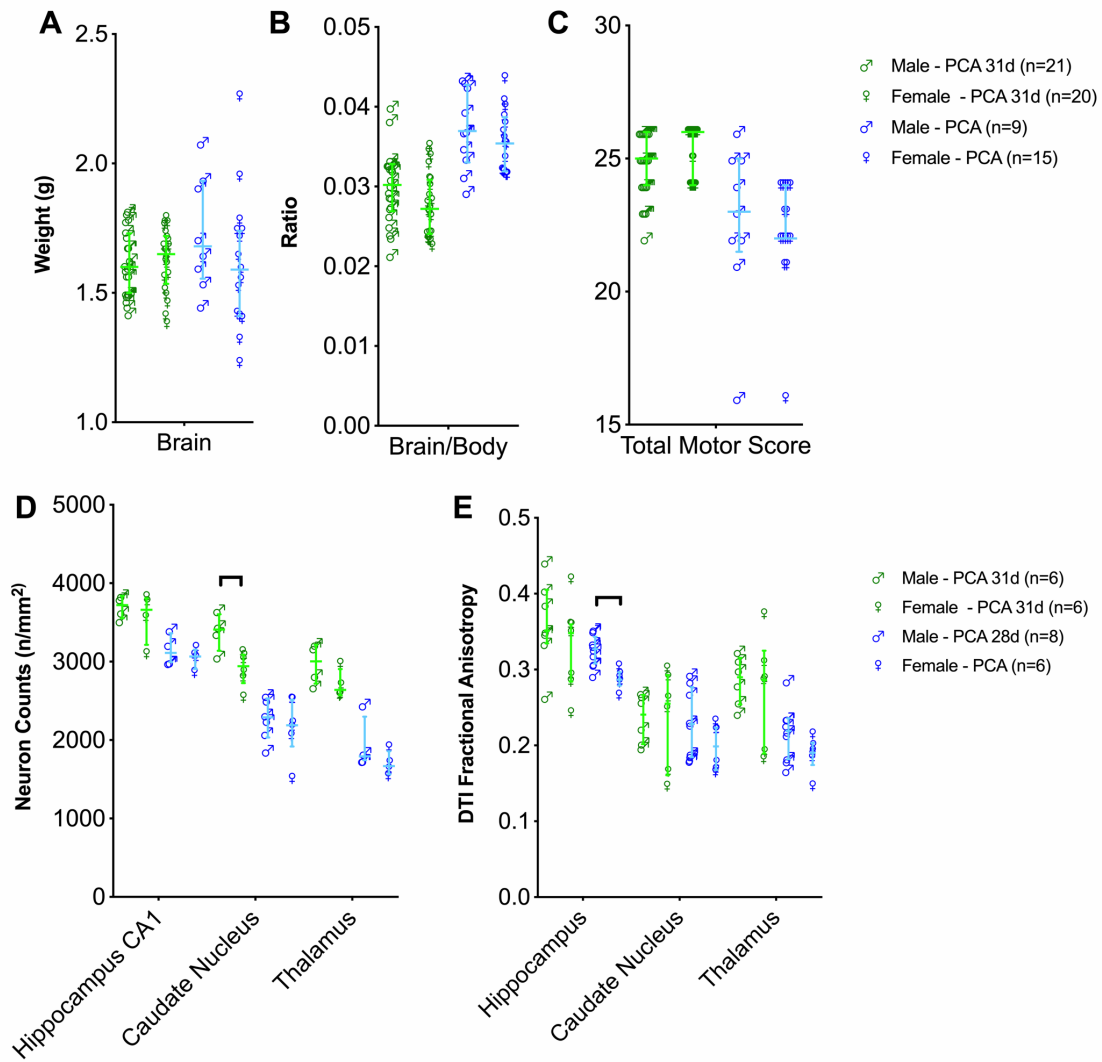


Figure 7. Sex subgroup differences in relation to the brain weight (**A**), brain to body ratio (**B**), motor score (**C**), neuron densities per selected region of interest (**D**) and DTI Fractional Anisotropy per region of interest (**E**). Data displayed as median and IQR with significance as * $0.05 \geq p > 0.01$; ** $0.01 \geq p > 0.001$; *** $p < 0.001$.