Supplementary

Investigating molecular mechanisms in ischemic preconditioning-induced resiliency to severe acute global cerebral ischemia using a mouse model of chronic cerebral hypoperfusion

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Running Title: *CCH induced ischemic resiliency*

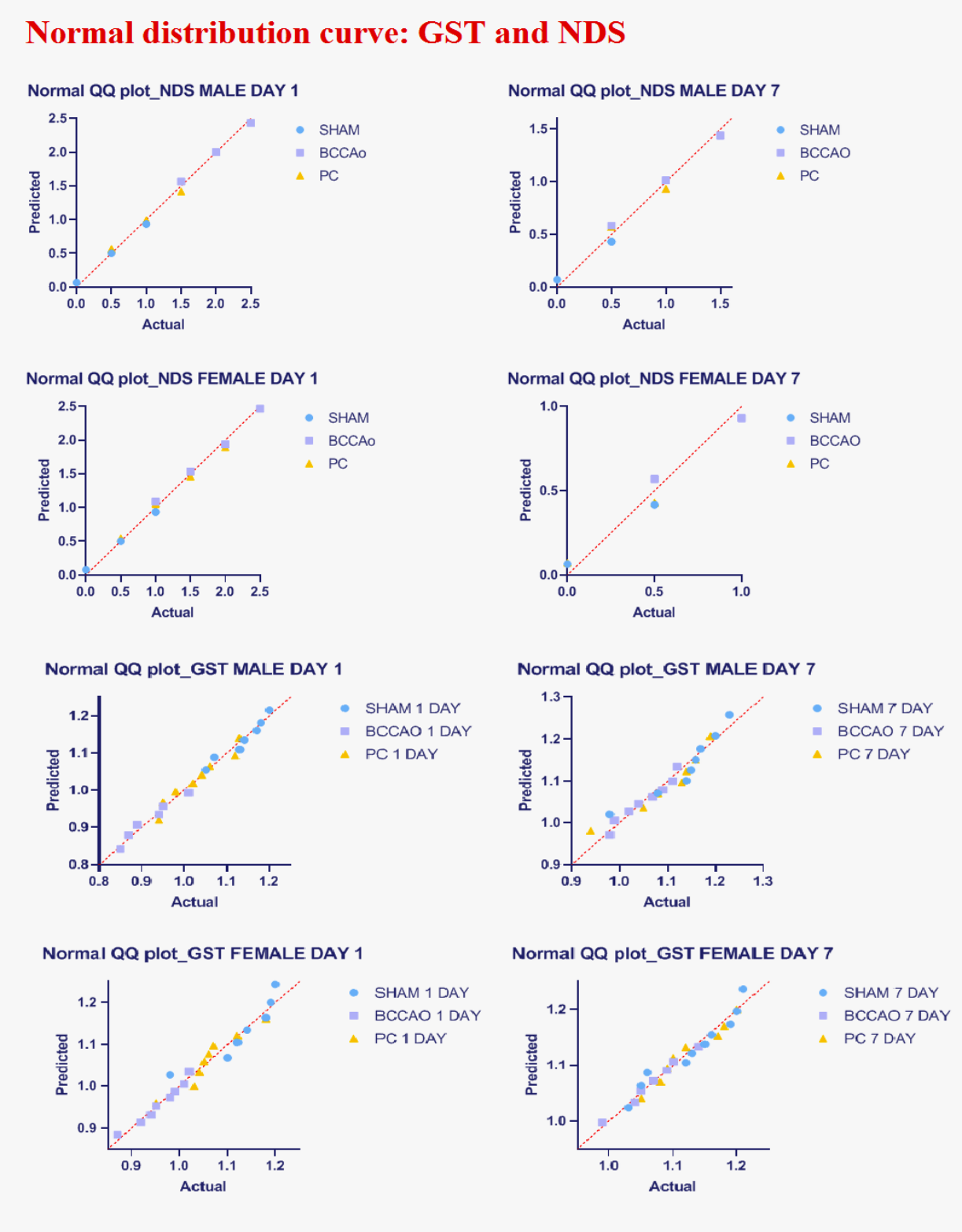

Fig 1. **Quantile-Quantile (Q-Q) plots** illustrating the comparison of observed data quantiles to theoretical normal distribution quantiles for neurodeficit scoring (NDS) and grip strength in male and female mice: The plots represent NDS and grip strength measurements for both sexes at two time points Day 1 and Day 7.

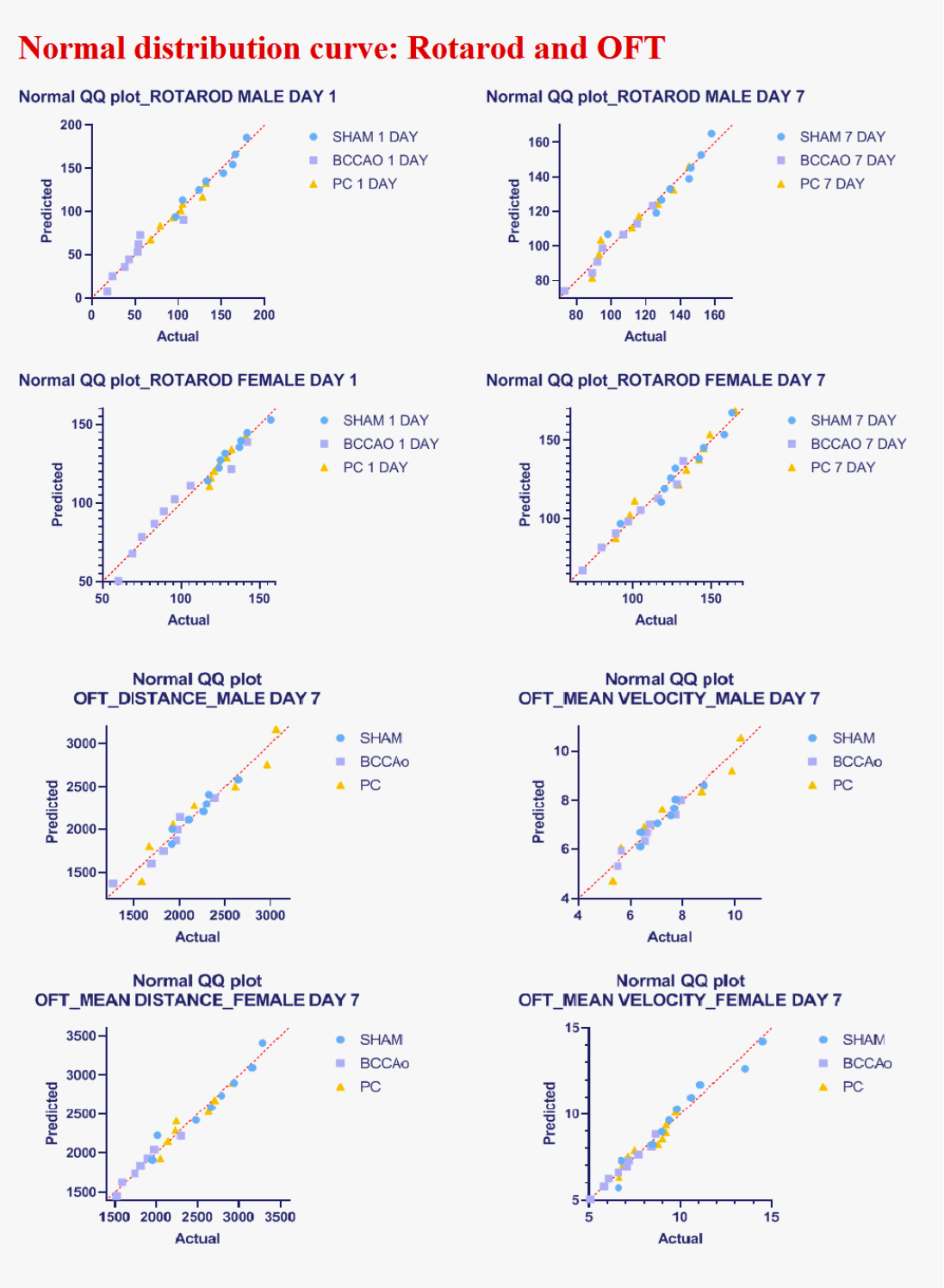

Fig 2 Quantile-Quantile (Q-Q) plots illustrating the comparison of observed data quantiles to theoretical normal distribution quantiles for open field test (OFT) parameters (distance traveled and velocity) and rotarod performance in male and female mice. The plots represent distance, velocity, and rotarod measurements for both sexes at two time points, Day 1 and Day 7.

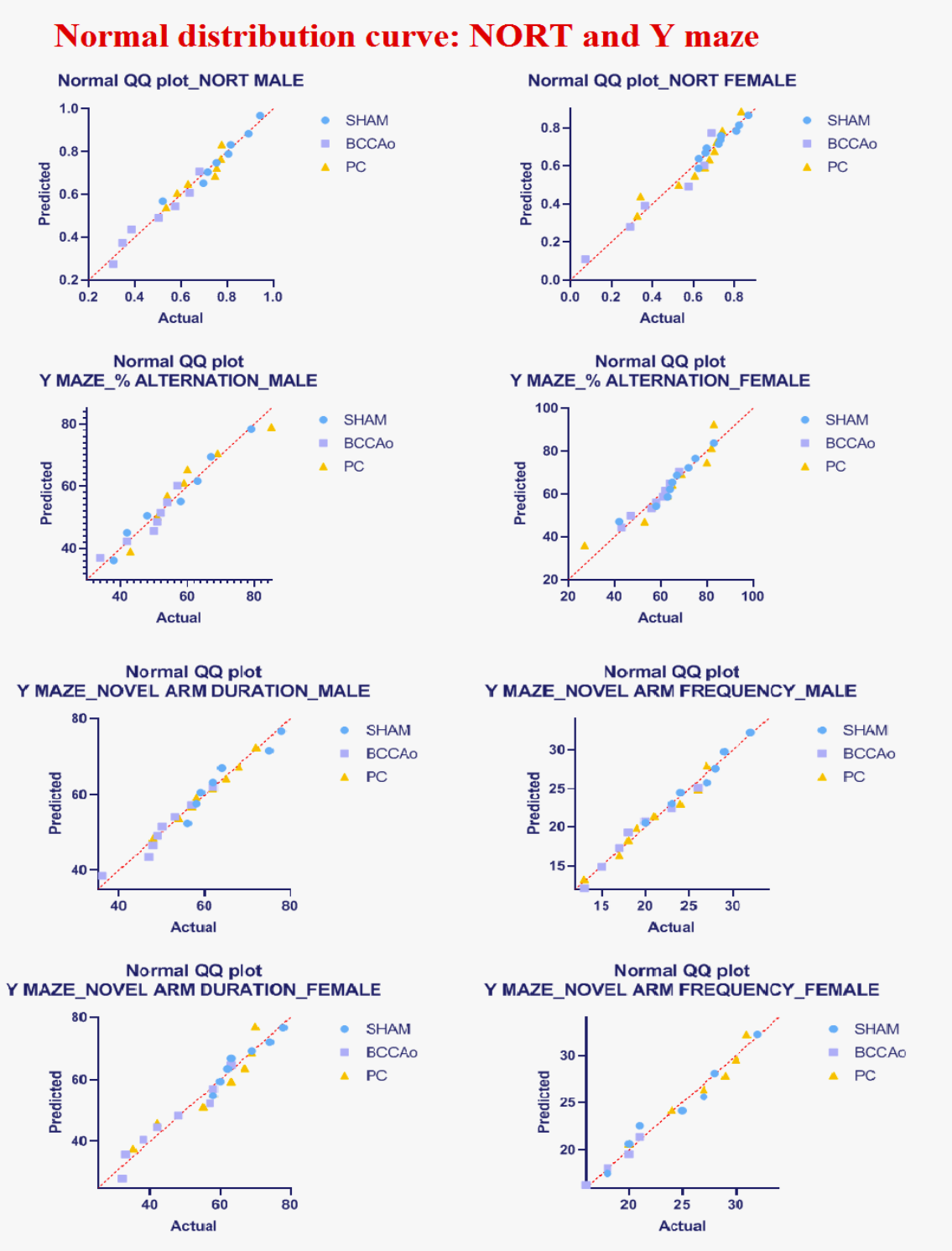

Fig 3. Quantile-Quantile (Q-Q) plots comparing observed data quantiles to theoretical normal distribution quantiles for novel object recognition test (NORT) discriminative index and Y-maze test parameters (percentage alternation, novel arm duration, and frequency) in male and female mice at Day 1 and Day 7.

Table 1: Primer Sequences for genes investigated in the study.

Primers	**Sequences**	
Hif1a	Forward	ACGTGCTTGGTGCTGATTTGTGA
	Reverse	ATTGAGCGGCCCAAAAGTTCTTCC
Il1b	Forward	TGGACCTTCCAGGATGAGGACA
	Reverse	GTTCATCTCGGAGCCTGTAGTG
Becn1	Forward	CAGCCTCTGAAACTGGACACGA
	Reverse	CTCTCCTGAGTTAGCCTCTTCC
Sox2	Forward	AACGGCAGCTACAGCATGATGC
	Reverse	CGAGCTGGTCATGGAGTTGTAC

Table 2: List of Antibodies: Their Catalog Numbers and Dilutions used in the study.

	Antibody	**Catalog No.**	**Dilutions**
1	Anti PSD95 (anti mouse)	MA1-046	1:1000
2	Anti BDNF (anti rabbit)	Ab226843	1:1000
3	Anti Syn (anti mouse)	MAB368	1:1000
4	Anti α tubulin (anti rabbit)	Ab 15246	1:5000
5	Goat anti-mouse secondary	G21040	1:5000
6	Goat anti-rabbit secondary	G21234	1:5000
7	GFAP (anti rabbit)	mAb#3670	1:200
8	Iba1 (anti rabbit)	mAb#17198	1:200
9	Alexa fluro 555	4413s	1:500

Hematoxylin and eosin (H&E)

Striatal sections from 1 day post surgery were processed for hematoxylin and eosin (H&E) staining following a standard protocol. Briefly sections were deparaffinized in xylene for 1 minute each and rehydrated through a graded ethanol series. After staining with hematoxylin for 5 minutes, sections were gently rinsed under running tap water to remove excess stain, followed by counterstaining with eosin for 45 seconds. Dehydration was performed through a reverse ethanol series and sections were cleared in xylene for 1 minute before mounting with DPX.

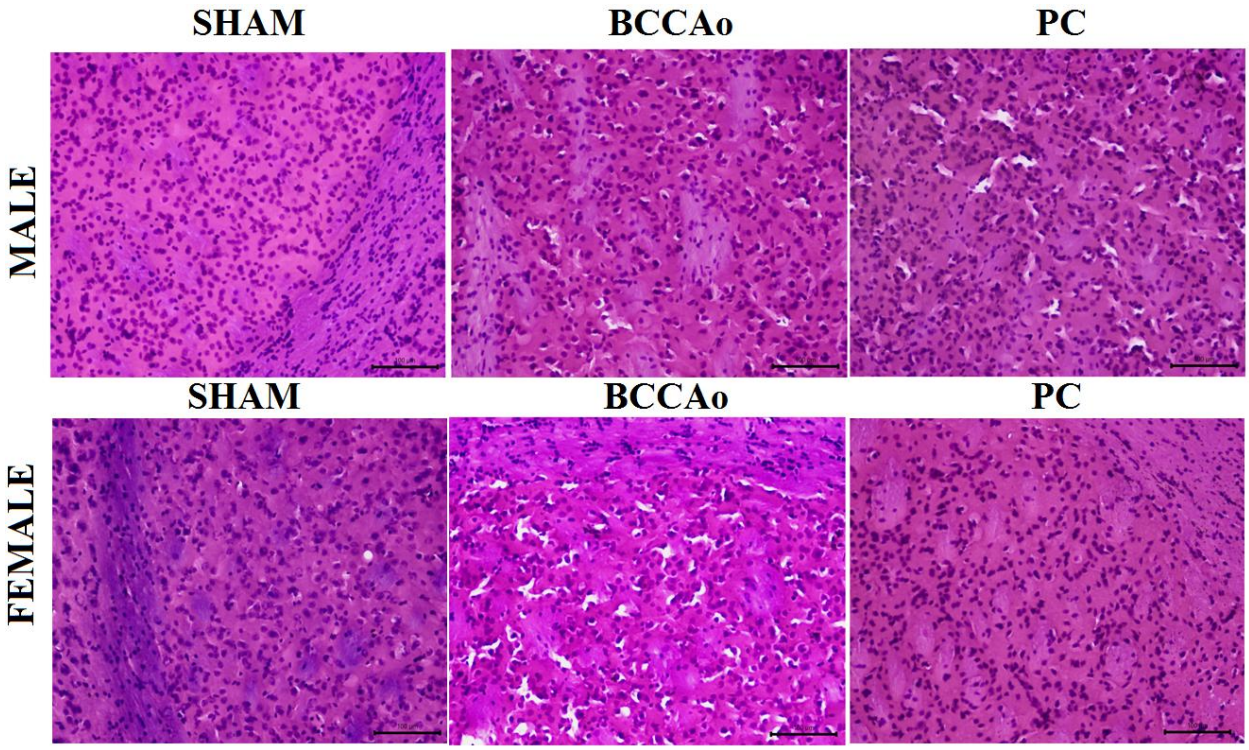

Fig 4: Hematoxylin and eosin (H&E) staining of brain sections from sham, BCCAo, and PC groups in male and female mice, highlighting histological differences across conditions.

Hematoxylin and eosin (H&E) staining highlights tissue architecture by staining nuclei blue with hematoxylin and cytoplasm and extracellular matrix pink with eosin. Representative H&E-stained images of striatal brain sections from sham-operated, bilateral common carotid artery occlusion (BCCAO), and preconditioning (PC) groups in both male and female mice after one day are shown. The sham group exhibits intact histological structure, while the BCCAO group shows subtle structural changes, indicating ischemic damage. The PC group demonstrates slight improvements compared to the BCCAO group, suggesting early protective effects of preconditioning. These findings provide a preliminary comparison of structural alterations across experimental groups and sexes.