

# Folic Acid Enhances Early Functional Recovery in a Piglet Model of Pediatric Head Injury

Holly Kinder

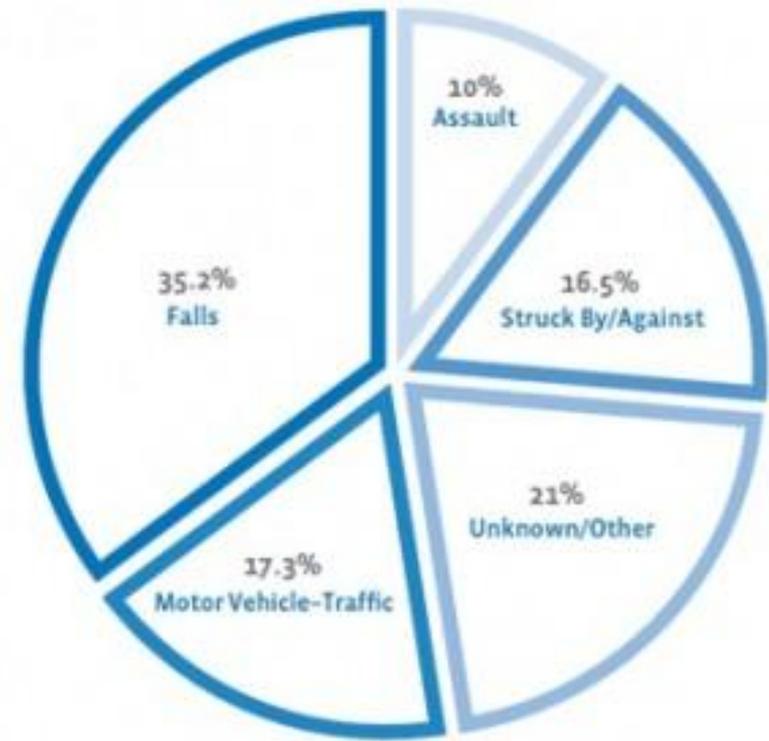
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# Traumatic Brain Injury

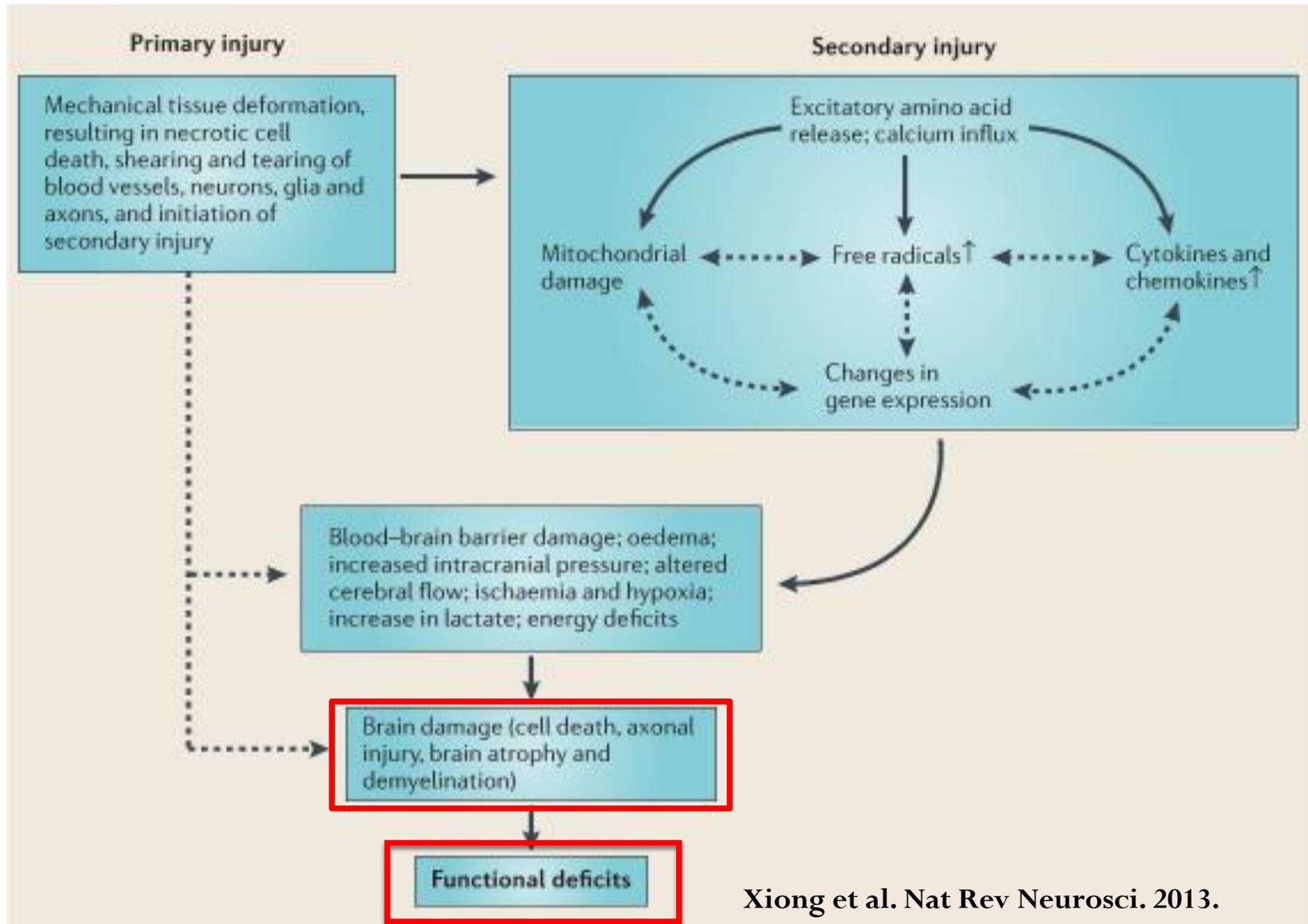
*“A bump, blow, or jolt to the head that disrupts the normal function of the brain”*

**-Centers for Disease Control and Prevention**

- 2.5 million Americans experience a TBI each year
- One third of all injury-related deaths
- 2% of Americans exhibit long-term neurological deficits as a result of TBI
- Direct and indirect costs of TBI estimated \$76.5 billion/year



# Pathophysiology of TBI



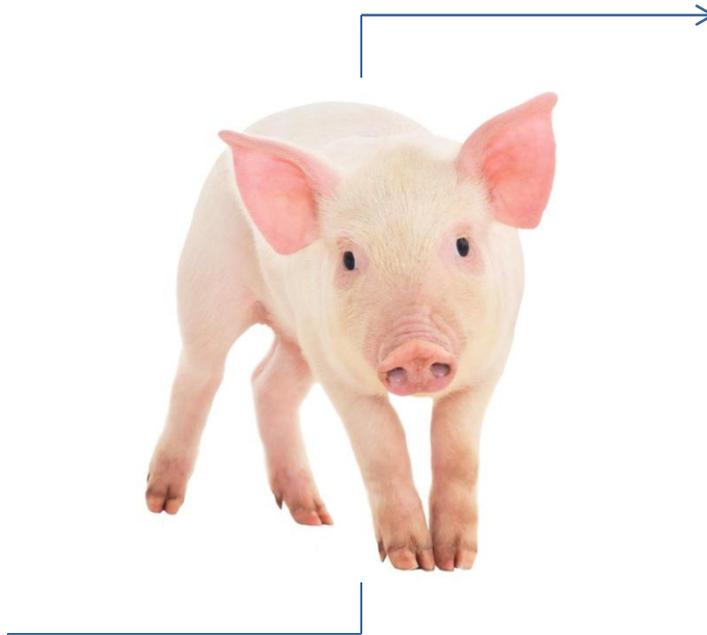
# Current Treatments for TBI

**TABLE 1.**

Families of Neuroprotective Agents and Their Prototype Drugs

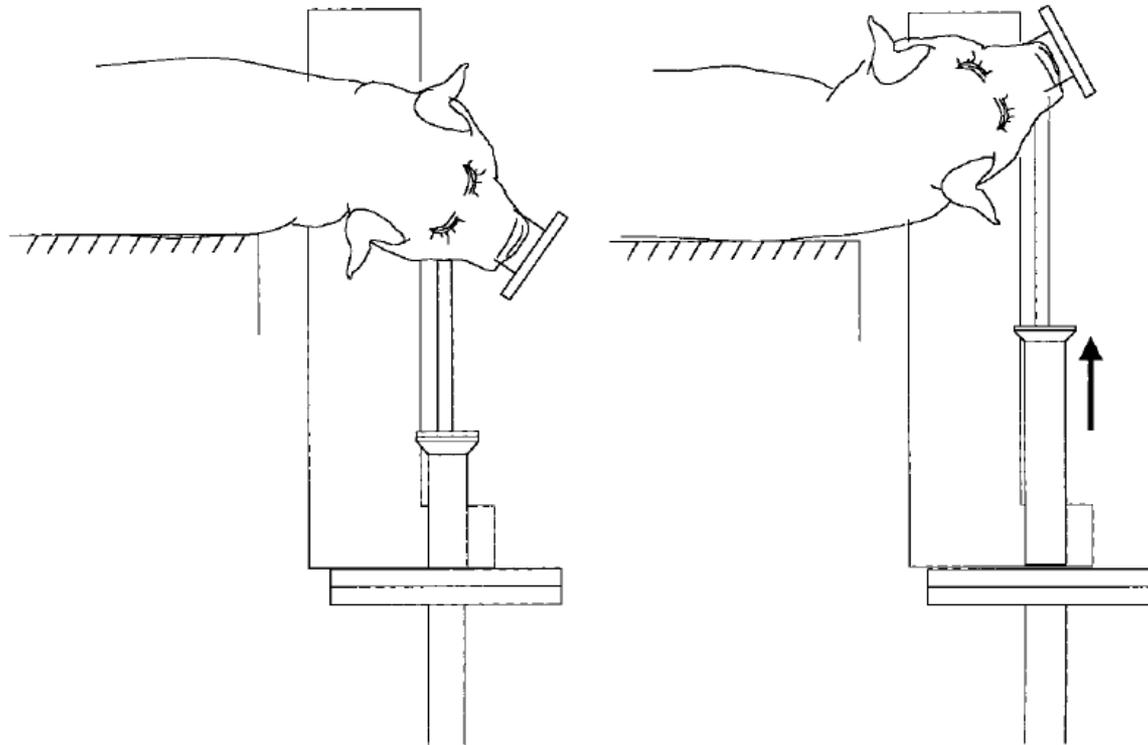
Proposed Mechanism of Neuroprotection	Drugs	Clinical Trials and Results
Glutamate receptor antagonists		
NMDA antagonists	Selfotel (CGS19755)	Complete/no benefit
	Eliprodil	Halted/no benefit
	Aptiganel (Cerestat, CNS1102)	Complete/no benefit
	MgSO <sub>4</sub>	Ongoing/result pending
AMPA antagonist	YM872	Ongoing/result pending
Ion channel modulators		
Calcium channel blockers	Nimodipine	Complete/no benefit
	Flunarizine	Complete/no benefit
Sodium channel blockers	Fosphenytoin	Complete/no benefit
Potassium channel activator	Maxipost (BMS-204352)	Complete/no benefit
Anti-inflammatory agents	Enlimomab	Complete/worsening
	LeukArrest (Hu23F2G)	Halted/no benefit
	rNIF	Halted/no benefit
Free radical scavengers	Tirilazad (U70046F)	Complete/no benefit
	Citicoline (cytidyl diphosphocholine)	Complete/no benefit
	Ebselen	Ongoing/result pending
	NXY-059	Ongoing/result pending

# Animal Models



# Animal Models: Traumatic Axonal Injury (TAI)

TAI AFTER CLOSED HEAD INJURY IN THE NEONATAL PIG



**FIG. 1.** Schematic depicting the linkage set-up of the HYGE device and the position of the piglet on the device. After securing the snout to the bite plate, the bite plate was rotated in the axial plane at an angle of  $110^\circ$ .

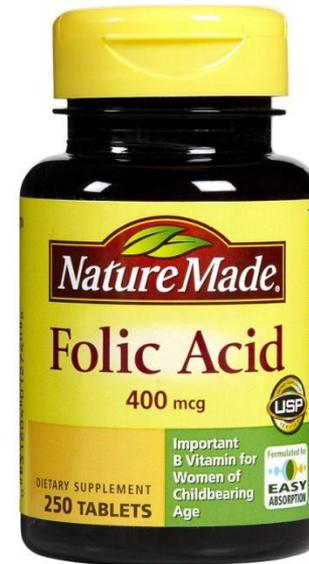
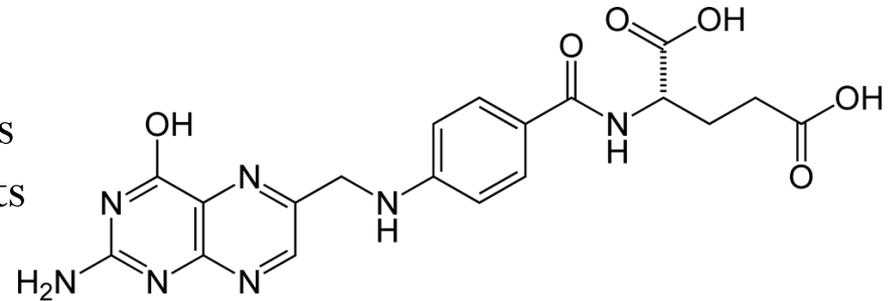
# Possible Treatment: Folic Acid

## • Folic Acid in Development

- Deficiency in pregnant women results in increased risk of neural tube defects in their babies
- Inborn errors in folic acid metabolism in children results in developmental delays

## • Folic Acid as a Neuroprotectant

- Supplementation reduces the incidence of stroke in adults
- Enhances repair processes in adult rat model of spinal cord injury
- Reduces glutamate and NMDA-induced neuronal death in cultured mouse neurons



# Question and Hypothesis

- **Question:** Does folic acid reduce brain injury and improve neurological outcome in a neonatal piglet model of traumatic brain injury
- **Hypothesis:** Folic acid supplementation will decrease the severity of traumatic axonal injury and will enhance early functional recovery following TBI in piglet model

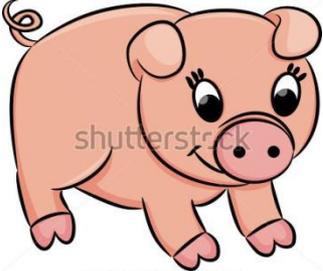


# Methods- Behavioral Testing

- Why behavioral testing?
  - Wide array of behavioral tests
  - Assess different aspects of cortical function relevant to human higher cortical functions
  - Great tool to assess **MOTOR FUNCTION, LEARNING, AND MEMORY!**



# Open Field Testing

## OPEN FIELD:

Present / Absent

- nudging toys
- walking
- sleeping

- zones entered
- lines crossed

## PIG X:

Present / Absent

- sniffing
- head butting
- mounting
- fighting
- social sleeping
  
- interpig distance

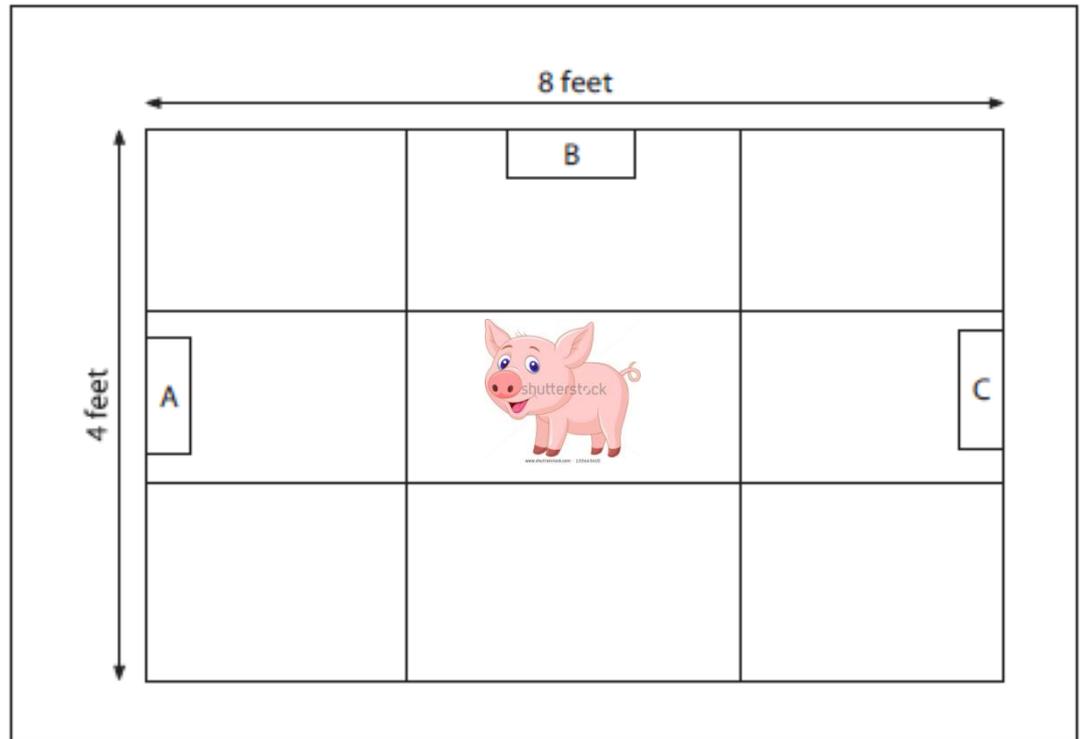
# Mirror Test

## Time spent in front of:

- mirror
- reversed mirror
- null zone

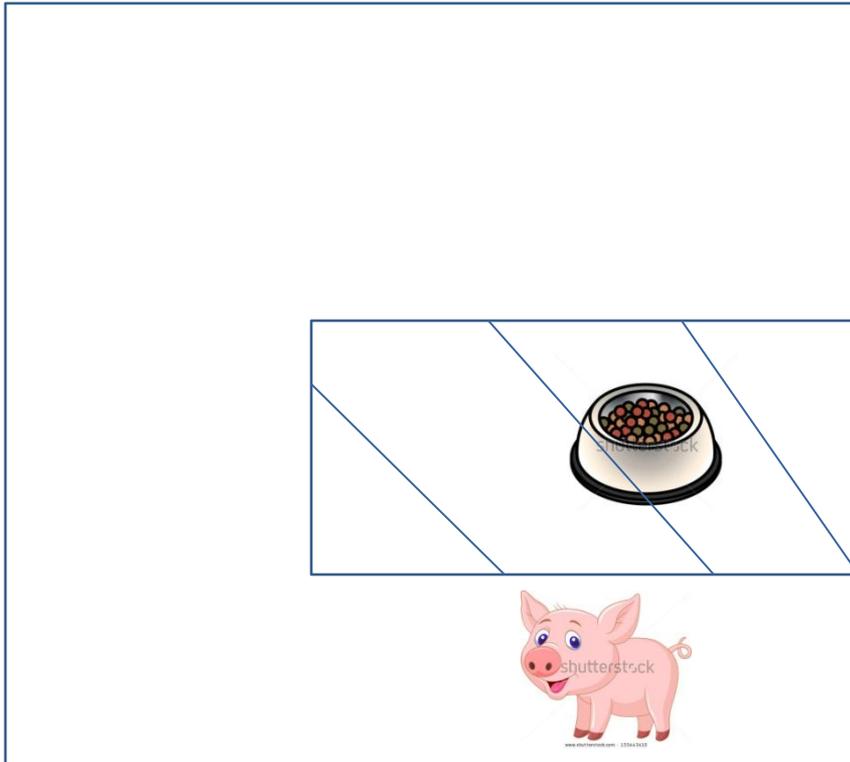
## Exploratory Behaviors:

- snout contact
- pacing
- backing up
- approaching
- body contact



**Fig. 2.** Mirror test. After open-field testing, a mirror (A) was added to the test space, as well as a mirror reversed to expose its non-reflective surface (B). A third area, the null zone (C), was used to compare the frequency of the piglet in the zone of the mirror or reversed mirror. Time in seconds in the zones of the mirror, reversed mirror and null zone as well as specific behaviors were recorded per 1-min epoch for 10 min.

# Glass Barrier Task



-Time to food reward

## Number of :

- nudges at glass barrier
- times piglet walks away from barrier
- failures to reach food

# Food Cover Task



-Time to food reward

## Number of :

- errors
- failures

# Balance Beam Task



- Time to complete task
- Number of failures
- Number of foot errors

# T-Maze Test



## **T-MAZE**

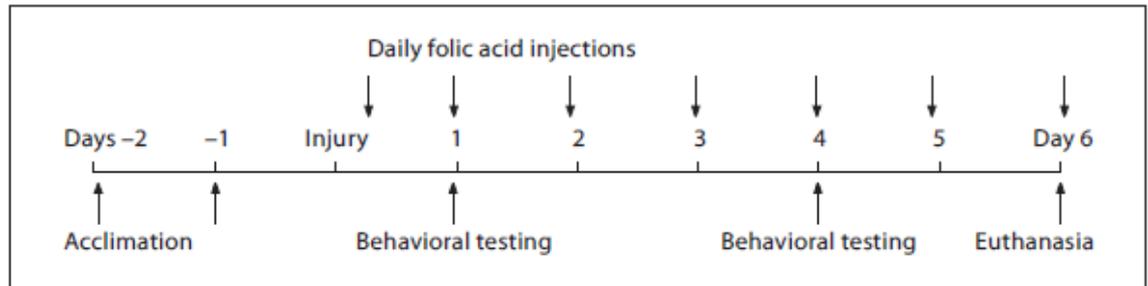
- # of successes
- Success= finding food in <15 sec

## **INTRAMAZE CHALLENGE**

- time to reach food reward
- number of errors
- length of time in contact with pie plate

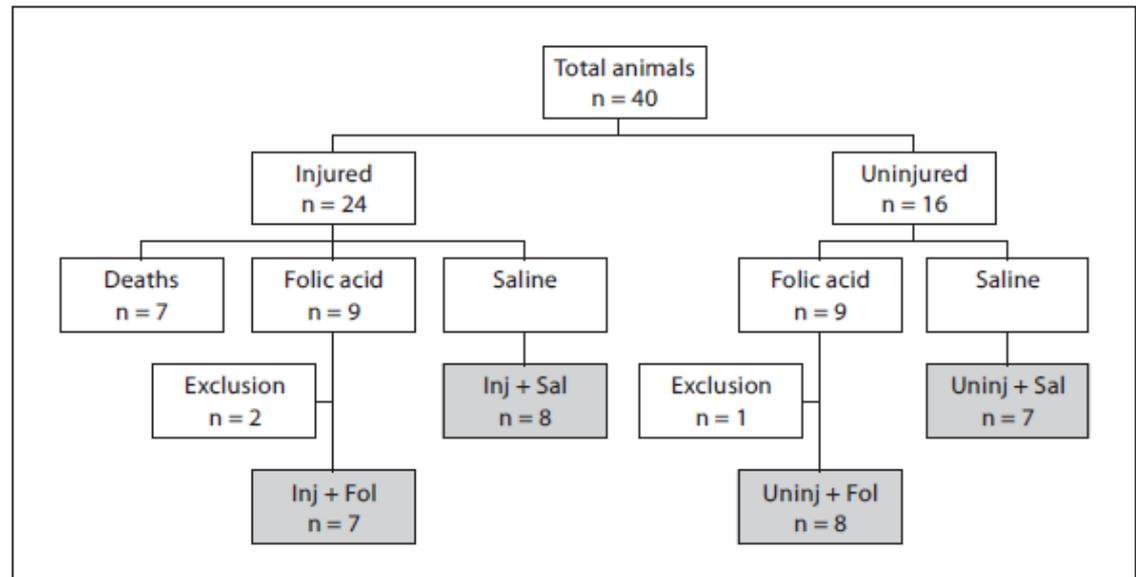
# Figure 1

**Fig. 1.** Time line of study. Piglets underwent 2 days of acclimation to the research staff, followed by injury, behavioral testing on days 1 and 4 following injury, and daily folic acid injections following injury. The animals were euthanized on day 6.



# Figure 3

**Fig. 3.** Flow diagram of study animal selection. There were 7 deaths in the injured animal group, 3 animals were excluded from behavioral analysis. The final numbers of animals used in the behavioral analysis were 15 injured animals and 15 uninjured animals.



# Table 1

**Table 1.** Summary of injury loads and clinical findings

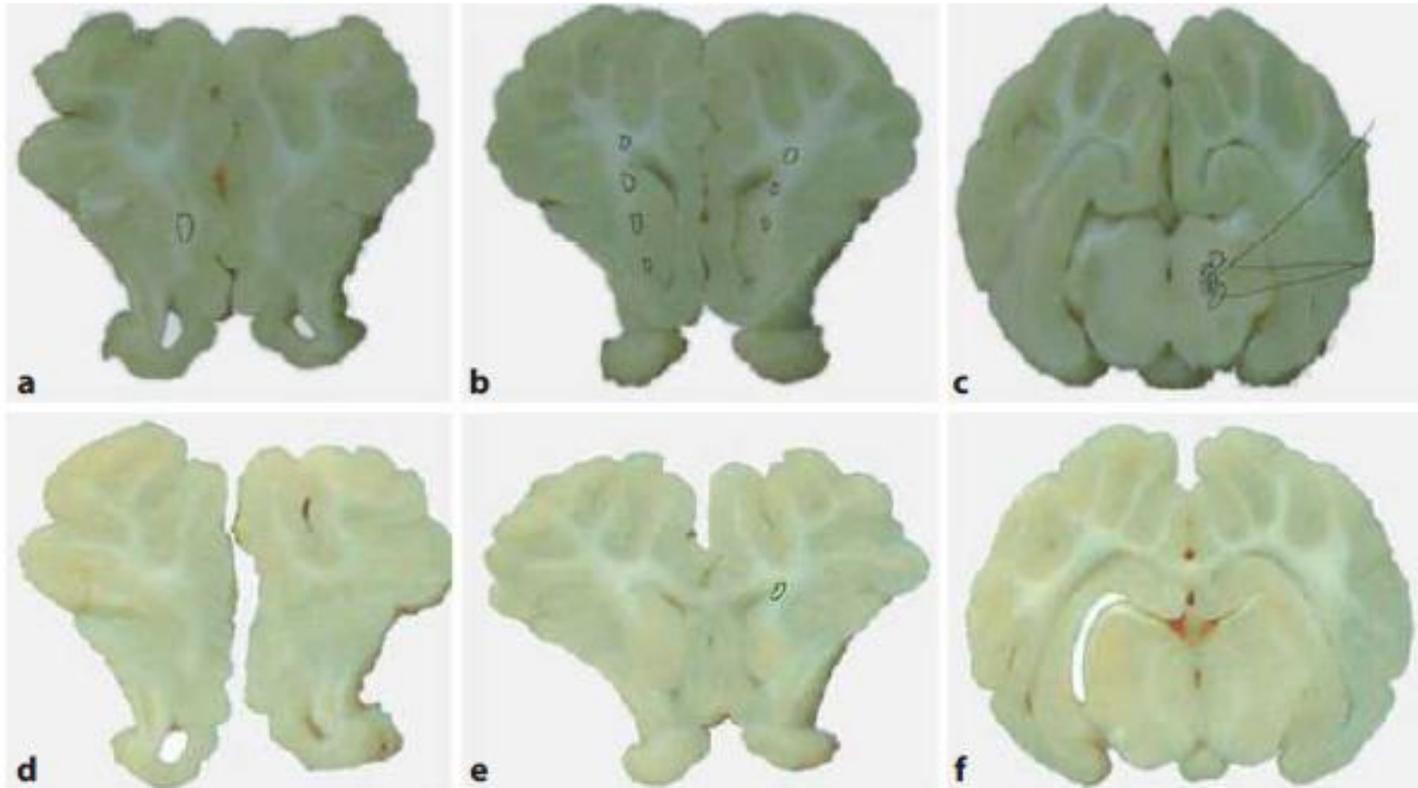
	Angular velocity rad/s	Unconsciousness time min	Folic acid (day 6) ng/ml	Homocysteine (day 6) μmol/l	Preinjury weight kg	Weight (day 6) kg
Inj + Fol	193.29 ± 5.31	6.32 ± 0.12 <sup>a</sup>	21.76 ± 3.5 <sup>1</sup>	6.73 ± 0.8	2.24 ± 0.52	3.12 ± 0.87
Inj + Sal	194.25 ± 8.11	6.23 ± 0.09 <sup>a</sup>	18.68 ± 4.75	6.88 ± 1.72	2.01 ± 0.24	2.95 ± 0.39
Uninj + Fol		4.55 ± 0.1 <sup>b</sup>	24 ± 0 <sup>1</sup>	6.31 ± 2.57	1.8 ± 0.54	2.58 ± 0.79
Uninj + Sal		2.53 ± 0.12	18.08 ± 4.84	5.45 ± 1.86	1.99 ± 0.39	2.47 ± 0.78

Values denote means ± SD. Angular velocities did not differ between injured groups. Durations of unconsciousness were significantly longer: <sup>a</sup> p < 0.05, injured animals compared to uninjured animals; <sup>b</sup> p < 0.05, uninjured animals that received folic acid compared to uninjured animals that received saline. There

were no differences between piglet weights at baseline or on day 6, and all groups gained weight over time.

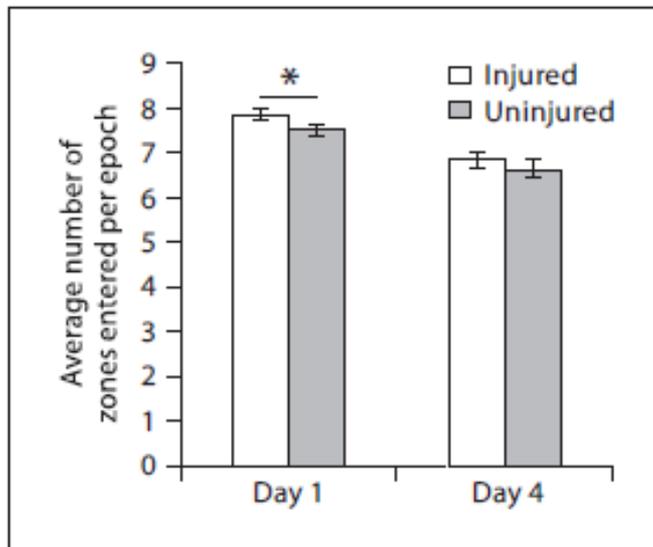
<sup>1</sup> Groups that received folic acid had higher serum folic acid levels on day 6, but not lower homocysteine levels.

# Figure 4

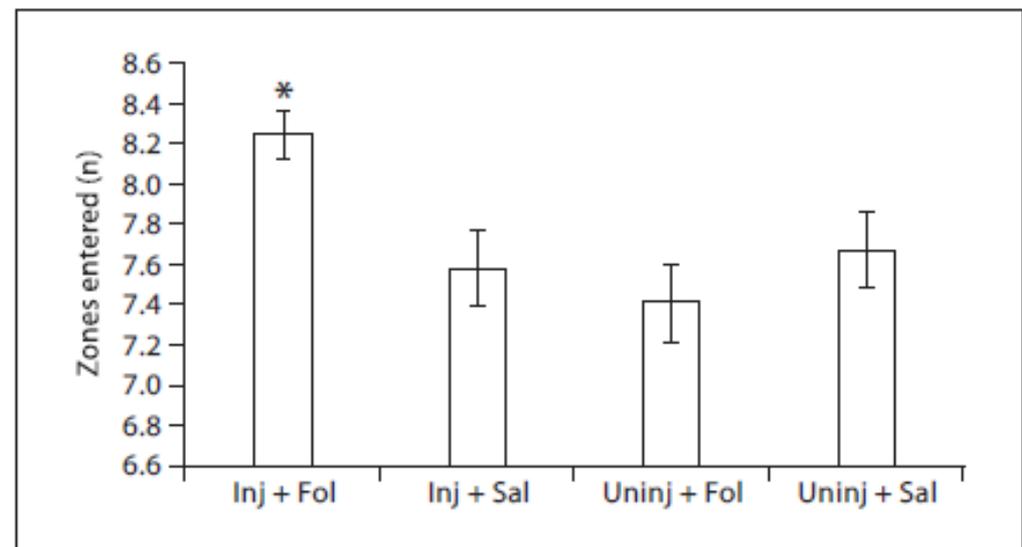


# Figures 5 & 6

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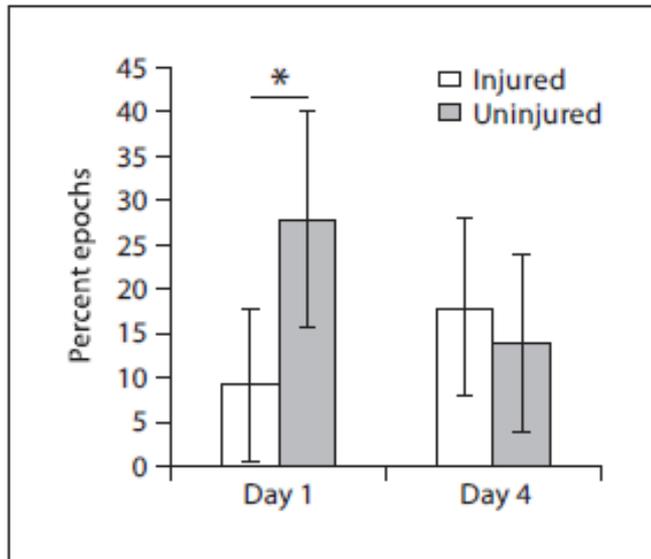


**Fig. 5.** Zones entered in open-field testing. On day 1, injured animals entered more zones compared to uninjured animals during the 10 min of open-field testing. This difference was not seen on day 4 of testing. \*  $p < 0.05$ .

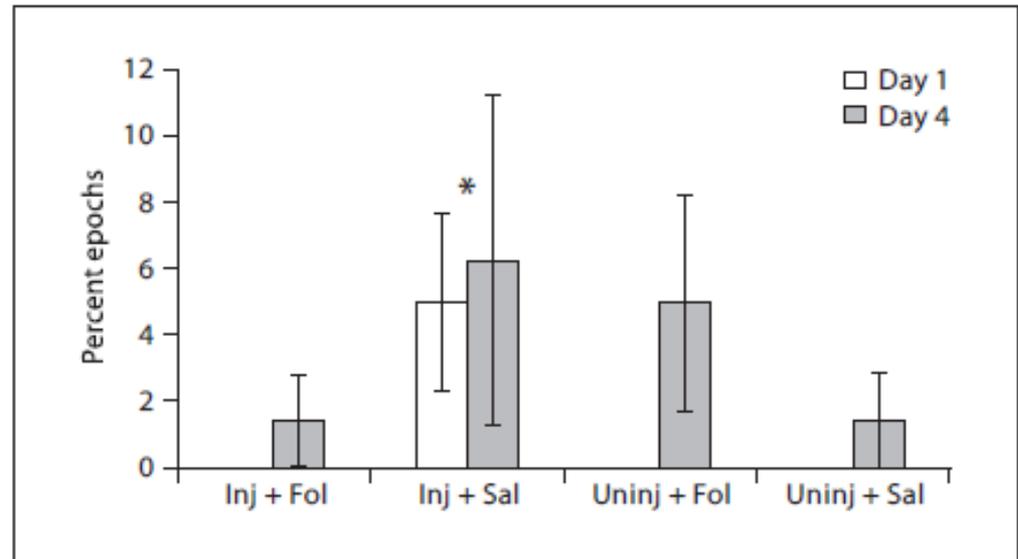
**Fig. 6.** Zones entered in open-field testing. On day 1 of testing, injured animals that received folic acid entered significantly more zones than all other groups. \*  $p < 0.05$ .

# Figures 7 & 8

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**Fig. 7.** Open-field testing behaviors. Uninjured animals ran significantly more on day 1 of open-field testing compared to injured animals. This difference was not seen on day 4. \*  $p < 0.05$ .

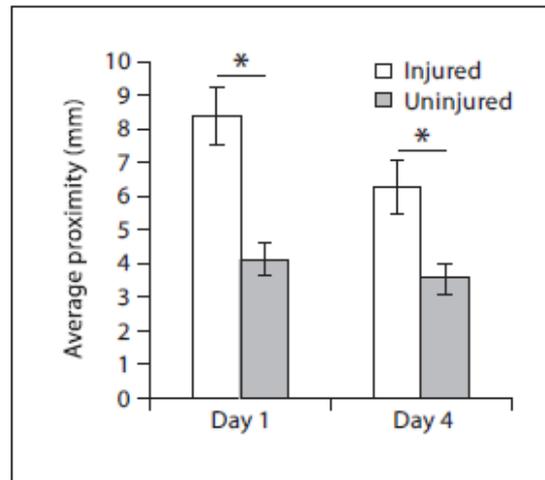
**Fig. 8.** Open-field testing behaviors. Inj + Sal animals spent significantly more time laying down on day 1 of open-field testing, indicating less interest in exploring the test space, compared to the other groups, who did not lie down at all during the first day of testing. All animals spent time laying down on day 4 of testing, indicating habituation to the test space; however, there were no significant differences between groups. \*  $p < 0.05$ .

# Figures 9 & 10

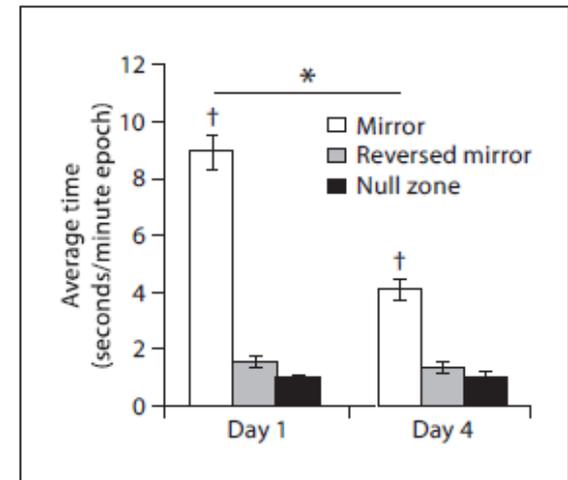
**Fig. 9.** Socialization with male sibling (Fig-X). Injured animals were further away from Fig-X compared to uninjured animals during the period of socialization testing on days 1 and 4 of testing. \*  $p < 0.05$ .

**Fig. 10.** Mirror test. All animals spent more time in front of the mirror compared to the reversed mirror and null zone on days 1 and 4. In addition, all animals spent more time in front of the mirror on day 1 compared to day 4, indicating habituation to the mirror over time. †  $p < 0.05$  compared to reversed mirror and null zone; \*  $p < 0.05$  between day 1 and day 4.

9



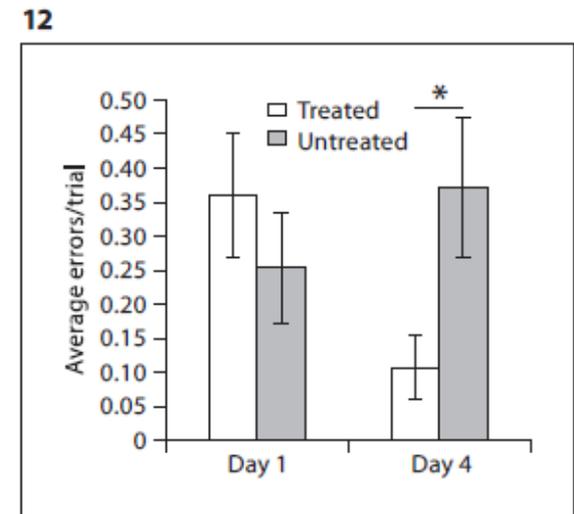
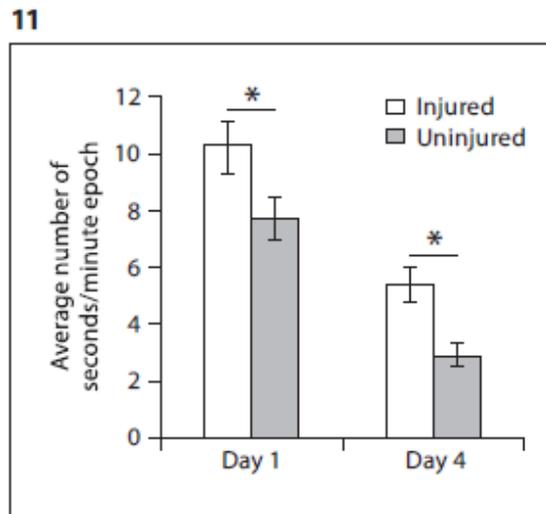
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# Figures 11 & 12

**Fig. 11.** Mirror test. Injured animals spent more time per minute epoch in front of the mirror compared to uninjured animals on both days 1 and 4. \*  $p < 0.05$ .

**Fig. 12.** Glass barrier testing. Animals that received treatment with folic acid made less errors per trial on day 4 compared to untreated animals. This difference was not seen on day 1 of testing. \*  $p < 0.05$ .

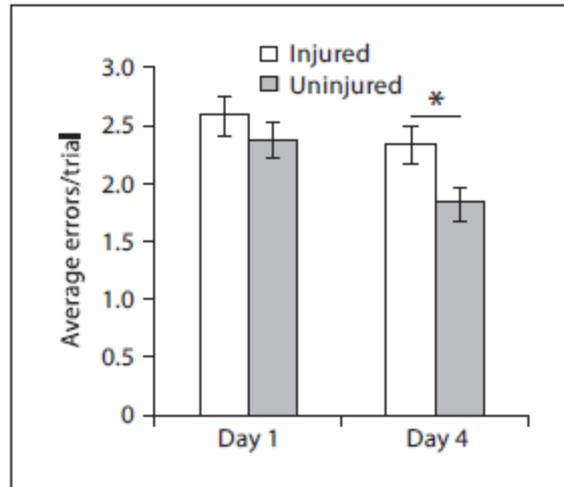


# Figures 13 & 14

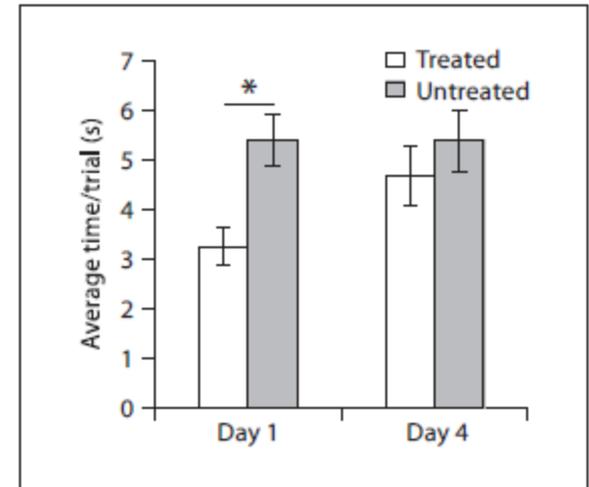
**Fig. 13.** Food cover testing. Injured animals made more errors per trial on day 4 compared to uninjured animals. This difference was not seen on day 1 of testing. \*  $p < 0.05$ .

**Fig. 14.** Beam testing. Treated animals completed the beam test quicker than the untreated animals on day 1. This difference was not seen on day 4. \*  $p < 0.05$ .

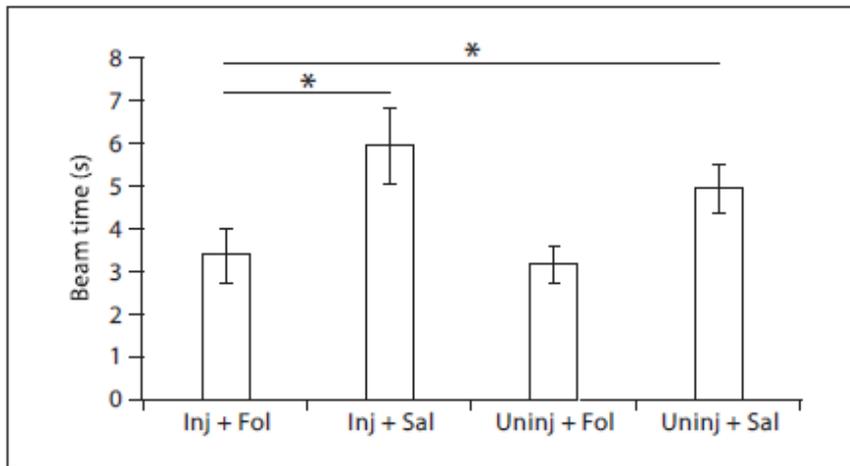
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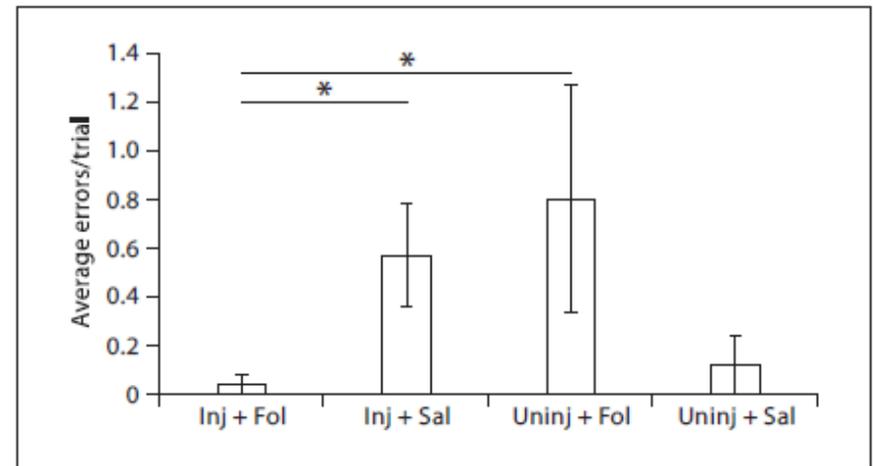
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# Figures 15 and 16



**Fig. 15.** Beam testing. On day 1 of testing, Inj + Fol animals had significantly shorter beam completion times compared to Inj + Sal and Uninj + Sal animals. \*  $p < 0.05$ .



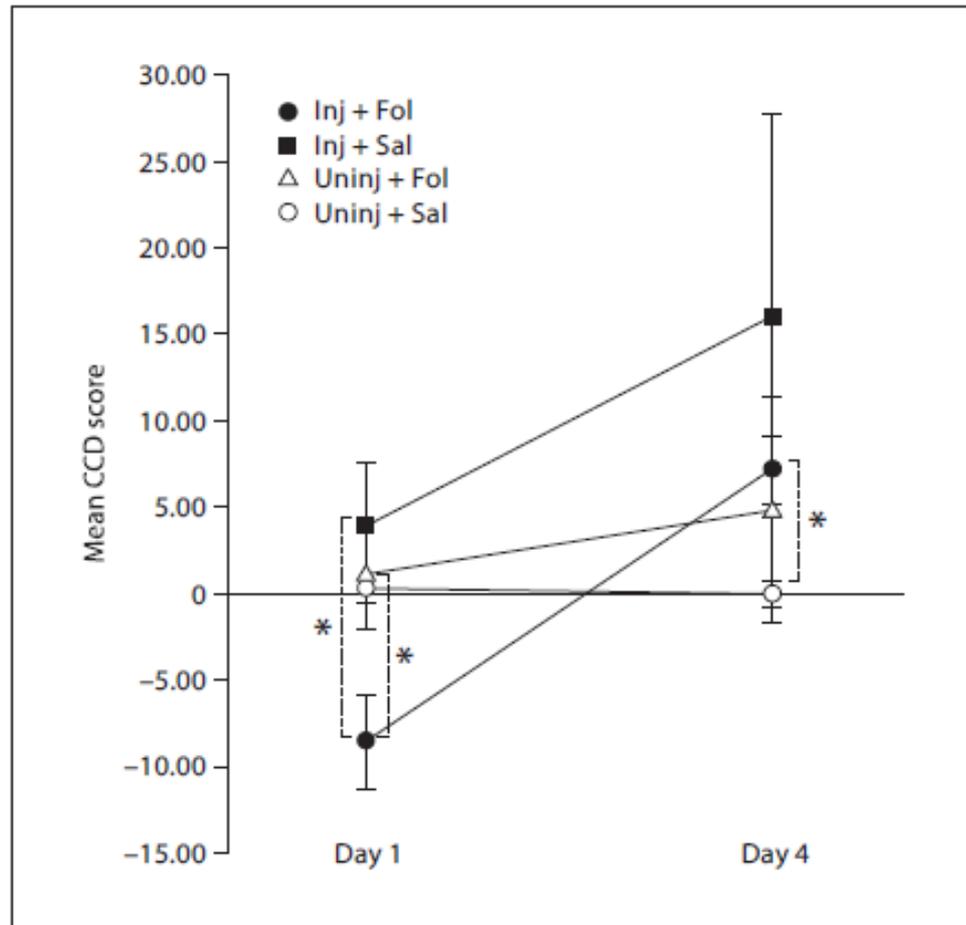
**Fig. 16.** T-maze testing (normal trials). On day 1 of testing, Inj + Fol animals made significantly less errors on normal trials than Inj + Sal and Uninj + Fol animals. \*  $p < 0.05$ .

# Figure 17

**CCD Score** - a measure of the overall neurobehavioral performance of a piglet

**Includes:**

- T-maze training failure rate, -T-maze intramaze change time in contact with novel object
- time to food reward for T-maze normal trials and T-maze reversal trials,
- sniffing the walls on open-field testing.



**Fig. 17.** CCD. On day 1 following injury, Inj + Fol animals had a significantly lower CCD score compared to Inj + Sal and uninjured animals (Uninj + Fol, Uninj + Sal). On day 4, although Inj + Fol animals had a lower CCD score compared to Inj + Sal animals, this was not statistically significant, but was significantly higher than that of uninjured animals. Error bars = standard error. \*  $p < 0.05$ .

# Conclusions

- Serum folic acid levels were significantly higher in both Fol groups on day 6.
- 1 day following injury, the Inj + Fol group showed significantly more exploratory interest, better motor function, learning, and problem solving compared to the Inj + Sal group
- Inj + Fol animals had a significantly lower cognitive composite dysfunction score compared to all other groups on day 1.
- Folic Acid may enhance early recovery after TBI

