Does Human Chorionic Gonadotropin Act Directly or Indirectly to Promote Neuroregeneration and Improve Cognitive Performance in Adult Male Rats Following a Moderate to Severe Traumatic Brain Injury?

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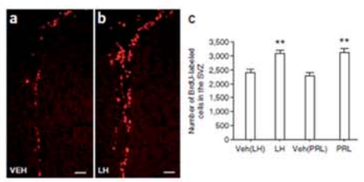
ABSTRACT

At last years IOA Colloquium we demonstrated that the hormone human chorionic gonadotropin (hCG), which induces the proliferation of neural stem cells as well as the synthesis of progesterone, improves functional outcomes in young (i.e., 6 month old) adult male rodents with focal penetrating controlled cortical impact (CCI) injury. The current study is a follow-up investigation aimed at determining if hCG acts directly, or synergistically or indirectly via hCG-induced upregulation of progesterone synthesis and signaling, to improve functional performance in adult male rodents following a CCI injury. To examine the role of progesterone signaling in mediating hCG's neuroregenerative effects, 6–month old male rats with a TBI or without a brain injury were administered the progesterone receptor (PR) blocker RU-486 (mifepristone) ± hCG or vehicle every other day over a 4-week period and changes in histopathology (i.e., lesion size), functional performance (vestibular balance and coordination), and cognition (Morris water maze performance) determined. TBI is known to produce cystic infarcts (large lesion cavities) in the brain, which, are themselves implicated in loss of major functions based on the brain region that is impacted. We speculate that blocking PR signaling during hCG therapeutic intervention will diminish the ability of hCG to decrease lesion size and promote functional recovery in our rodent model of TBI.

INTRODUCTION

- Reproductive hormones drive neurogenesis during embryogenesis and adulthood and may therefore be useful candidates for the promoting neuroregeneration following a TBI.
- The pregnancy hormone human chorionic gonadotropin (hCG) is a potent mitogen, promoting the proliferation of stem cells and their differentiation into neural stem cells (Gallego et al. 2010).
- LH, the adult equivalent of hCG that binds the same receptor as hCG, promotes "neurogenesis" during adulthood (Bryan et al., 2010).



The Effect of LH on Neurogenesis. Photomicrograph of proliferating cells in SVZ. of overectomized rats given Vehicle (a) or LH (b). The graph to the right (c) depicts the quantification of BrdU labeled cells in response to Vehicle, LH, or Prolactin.

- hCG treatment promotes cognitive and motor skill improvement in rats following a TBI.
- LH/hCG promotes the production of progesterone (P_4).
- P_4 production also has been demonstrated to:
  - promote neuronal differentiation (Gallego et al, 2010).
  - attenuate lesion size and cognitive decline in young adult male rats following a TBI (Geddes et al., 2014).

This study was designed to determine if the beneficial effects of hCG on cognition are mediated directly, or indirectly, through the production and signaling of P_4

HYPOTHESIS

We hypothesize that suppression of P_4 signaling will diminish the beneficial effects of hCG on TBI-induced tissue loss (lesion size) and on cognitive and motor deficits in young adult male rats.

METHODS & MATERIALS: EXPERIMENTAL DESIGN, INJURY MODEL, DRUGS & TIMELINE

Groups and Sample Sizes (n= number of rats)

Treatment groups / Surgery type	Vehicle (Veh.)	hCG	RU-486 + Vehicle	RU-486 + hCG
NSC rats	7	7	-	-
Sham rats	5	7	6	6
CCI rats	9	11	5	6
Total (n) rats	*23*	*25*	*11*	*12*

CCI Injury Coordinates in the Brain
A/P = +2.5 mm from Bregma (β)
M/L = 0.0 mm from midline
D/V = -3.0 to -3.5 mm from brain surface
*under 2-5% isoflurane/O_2 gas anesthesia (30-40 mins. long)

Subjects: 5-6 month old adult male Sprague Dawley rats (>450 grams) at beginning of experimental study.

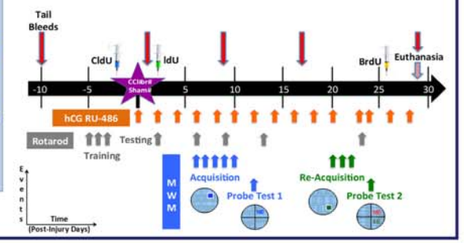
hCG: 1,000 IU/ml solution of Pregnyl® at 400 IU/kg or equimolar volume of saline or vehicle solution was injected every 48h (i.m.) up to 28 day (4 weeks).

RU-486: 100 mg/ml solution of mefiprestone® at 40 mg/kg/body weight was given (i.p.) 15-20 minutes before every hCG injection.

Lactate Ringer solution: 5 ml was injected (s.c.) after every survival surgery procedure.

PCI3000 Precision Cortical Impactor™
Bit diameter = 5 mm

Timeline of Experimental Events



RESULTS: NEUROPATHOLOGY, VESTIBULAR PERFORMANCE & COGNITIVE (SPATIAL) SKILLS

hCG's tendency to reduce size of CCI-induced lesions is independent of progesterone signaling

Gross Lesion Analysis: Percent of Surface Area Damaged

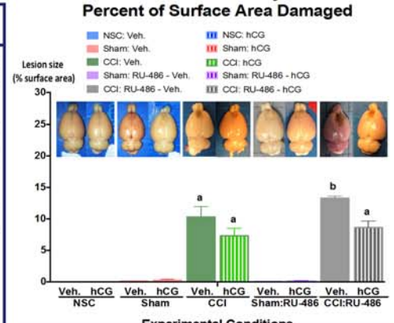
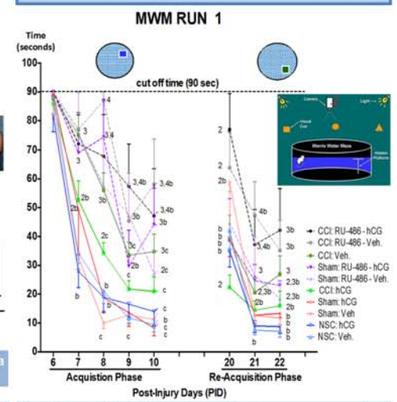


Image J was used to determine gross lesion size as a preliminary indicator of neuropathology.

hCG diminishes and RU-486 pre-treatment exacerbates CCI-induced deficits in medium- and long-term spatial learning

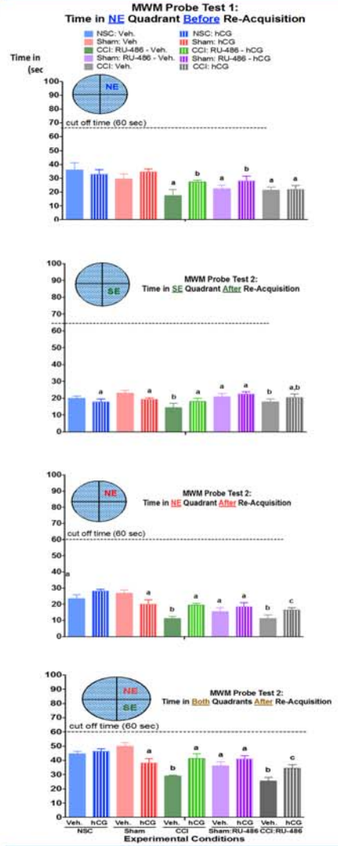
MWM RUN 1



Run 1 on PID 6-10 tests medium-term memory (i.e., occurring every 24-h). Run 1 on PID 20 tests longer-term (i.e., 48 h), after partial extinction (i.e., 1st Probe test).

hCG improves CCI-induced spatial memory deficits, RU-486 is detrimental

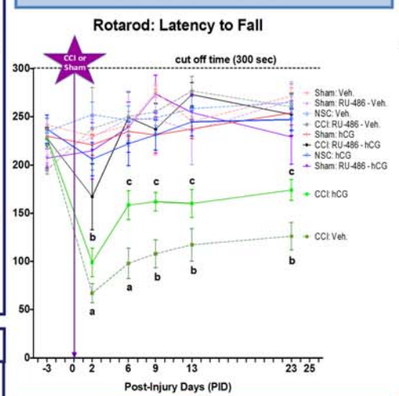
MWM Probe Test 1: Time in NE Quadrant Before Re-Acquisition



During the Probe tests the platform is removed. Medium & longer-term memories are assessed based on time spent in the new & old target (i.e., platform associated) quadrants over 60 sec.

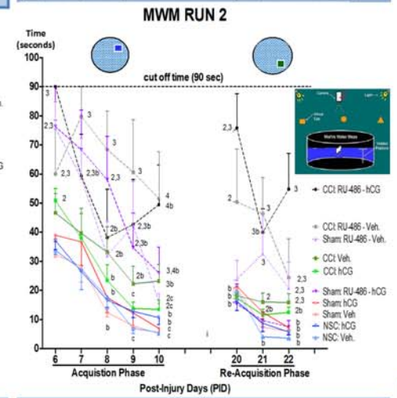
hCG and RU-486 improves CCI-induced decline in vestibular function

Rotarod: Latency to Fall



Rotarod measures a decrease in acquired motor skills. The rod rotates at 1 RPM, then accelerates 1 RPM every 10 sec up to 30 RPM by 5 minutes.

MWM RUN 2



Run 2 always occurs 5 minutes after Run 1, and thus is understood to test short-term memory during both the Acquisition and the Re-Acquisition Phases.

SUMMARY

- **SPATIAL LEARNING & MEMORY**

In young adult male rats following CCI:
- hCG improved short-, medium- and long-term spatial learning
- hCG improved medium- and long-term spatial memory
- RU-486 impaired short-, medium- and long-term spatial learning
- RU-486 reduced hCG's effect on both memory tests

In non-injured young adult male rats:
- RU-486 had an unexpected detrimental impact on short-, medium- and long-term spatial learning
- RU-486 also had a detrimental impact on medium- and long-term spatial memory

- **VESTIBULAR PERFORMANCE**

In young adult male rats following CCI:
- hCG improved vestibular performance ~30%
- RU-486 surprisingly improved vestibular performance back to control levels

- **GROSS LESION SIZE**

In young adult male rats following CCI:
- hCG reduced lesion size independent of P_4 signaling
- RU-486 increased lesion size

CONCLUSIONS

In response to a CCI in young adult male rats:
- hCG's positive effects on spatial learning may be dependent on P_4 signaling, while hCG's impact on memory is effected but less dependent on P_4
- hCG works independently of P_4 to improve motor skills, however suppression of P_4 signaling improves motor performance
- hCG also works independently of P_4 to reduce lesion size, while suppression of P_4 signaling increases lesion size

In non-injured young adult male rats:
- RU-486 had a detrimental impact on both spatial learning and memory

Preventing P_4 signaling may be detrimental to neuropathology (i.e., lesion size) and tasks dependent on spatial learning and memory (i.e., cognition) but not vestibular (i.e., physical) performance.

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