



# 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?



Rastafa I. Geddes, Icelle M. Anderson, Quinn C. Bongers, Alex Jensen, Chase Nier, Marlyse M. Wehber, Ryan A. Rauh, Kentaro Hayashi, Sivan Vadakkadath Meethal and Craig S. Atwood

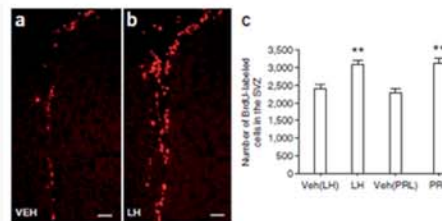
Division of Geriatrics and Gerontology, Department of Medicine, University of Wisconsin-Madison, and Geriatric Research, Education and Clinical Center, Veterans Administration Hospital, Madison WI 53705

## 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 overestomized 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 Proctin.

- hCG treatment promotes cognitive and motor skill improvement in rats following a TBI.
- LH/hCG promotes the production of progesterone (P<sub>4</sub>).
- P<sub>4</sub> 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<sub>4</sub>

## HYPOTHESIS

We hypothesize that suppression of P<sub>4</sub> 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	Vehicle (Veh.)	hCG	RU-486 + Vehicle	RU-486 + hCG
Surgery type				
NSC rats	7	7	6	6
Sham rats	5	7	6	6
CCI rats	9	11	5	6
<b>Total (n) rats</b>	<b>23</b>	<b>25</b>	<b>11</b>	<b>12</b>

**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<sub>2</sub> gas anesthesia (30-40 mins. long)

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

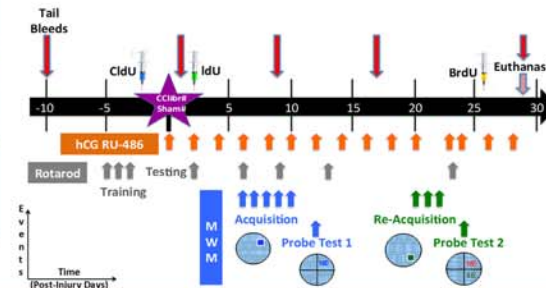
**hCG:** 1,000 IU/ml solution of Pregnyl® at 400 IU/kg or equimolar volume of saline or vehicle solution was injected every 48h (i.m.) up to 28 day (4 weeks).

**RU-486:** 100 mg/ml solution of mifepristone® 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

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

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

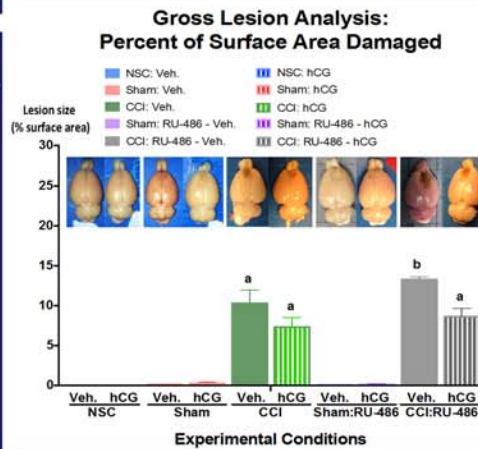
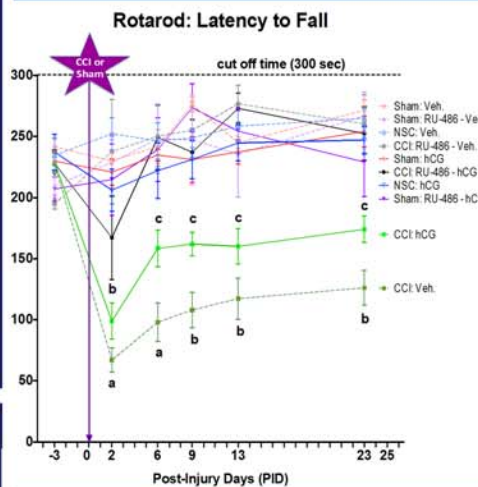
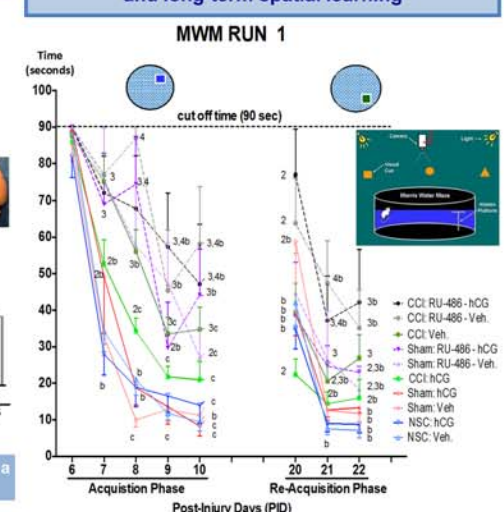


Image J was used to determine gross lesion size as a preliminary indicator of neuropathology.

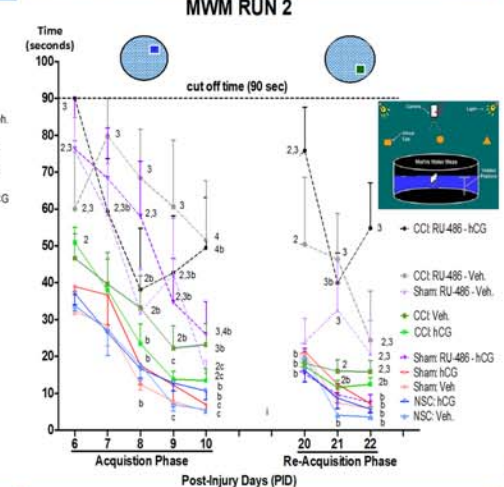
hCG and RU-486 improves CCI-induced decline in vestibular function



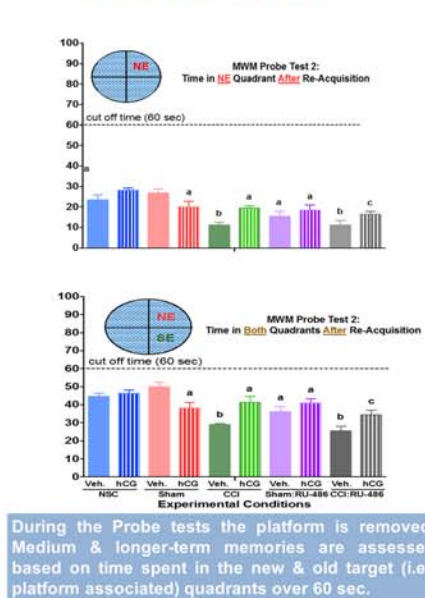
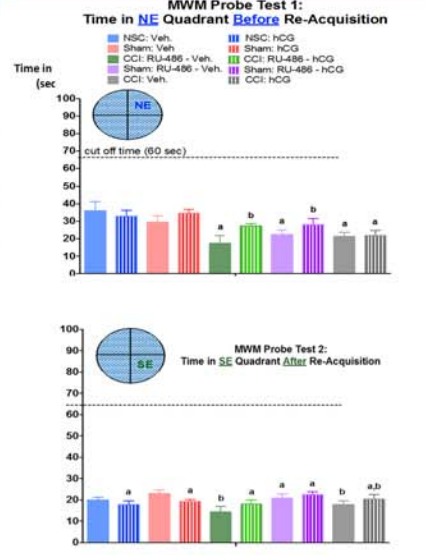
Rotarod measures a decrease in acquired motor skills. The rod rotates at 1 RPM, then accelerates 1 RPM every 10 sec up to 30 RPM by 5 minutes.



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., 1<sup>st</sup> Probe test).



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.



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.

## 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<sub>4</sub> 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<sub>4</sub> signaling, while hCG's impact on memory is effected but less dependent on P<sub>4</sub>
  - hCG works independently of P<sub>4</sub> to improve motor skills, however suppression of P<sub>4</sub> signaling improves motor performance
  - hCG also works independently of P<sub>4</sub> to reduce lesion size, while suppression of P<sub>4</sub> 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<sub>4</sub> 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.

## ACKNOWLEDGMENTS

The research is supported by the Laboratory of Endocrinology, Aging and Disease in the William S. Middleton Memorial Veterans Hospital through a VA Merit Award (CSAR0008). Dr. Geddes is funded by an NIH postdoctoral Biology Of Aging (BOA) Training grant. Special thanks to laboratory members Tina Gonzales and Rachel Krause for their time and individual expertise.

## REFERENCES

Atwood CS and Vadakkadath Meethal S. 2005. Cellular and Molecular Life Sciences 62:257-270.  
 Hsia SM, Yeh CL, Kuo YH, Wang PS, Chiang W. 2007. Effects of Adlay (Coix lachryma-jobi L. var. ma-yuen Stapf.) Experimental Biology and Medicine 232: 1181-1194.  
 Geddes RI, Srinick EA, Sayeed I, Stein DG. 2014. PLoS ONE 9(1): e87252  
 Barha CK, Ishrat T, Epp JR, Galea LAM, Stein DG. 2011. Experimental Neurology 231:72-81.  
 Gallego, MJ, Prashob Porayette, Maria M Kalthcheva, Richard L Bowen, Sivan Vadakkadath Meethal, Craig S. Atwood. 2010. Stem Cell Research & Therapy, 1:28.  
 Bryan KJ, Mudd JC, Richardson SL, Chang J, Lee HG, Zhu XW, Smith MA, Casadesu G. J Neurochem. 2010;112(4):870-881.