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# R04. HIV-1 Glycoprotein 120 Promotes Affective Dysfunction in Mice and Medium Spiny Neuron Necrosis

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# HIV-1 Glycoprotein 120 Promotes Affective Dysfunction in Mice and Medium Spiny Neuron Necrosis

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## Abstract

Neuroendocrine dysfunction is associated with human immunodeficiency virus type 1 (HIV-1) among infected men and women [1]. Glycoprotein 120 (gp120), the HIV-1 envelope protein that is critical for viral infection, also induces neurotoxicity and is therefore thought to contribute to the pathogenesis of HIV-associated neurological disorder (HAND) [2]. However, the mechanisms and degree to which gp120 causes neuroendocrine and related behavioral pathology are poorly understood. Sex differences in gp120 expression have been investigated [3] and our group has observed pregnane steroid metabolites to exert neuroprotective effects over other HIV-1 proteins [4], but it is unknown if this protection extends to actions of gp120. We hypothesized that the expression of gp120 in mice would promote affective behavioral pathology (e.g. increased anxiety- and depression-like behavior) and that fluctuation of gonadal steroids (predominantly estrogens and/or progestogens) across the female rodent estrous cycle would attenuate these effects. Contrary to expectation, gp120 expression decreased anxiety-like behavior on a light-dark transition test (with no difference in depression-like behavior), but also produced neurotoxicity in cell culture. These data may indicate a disruption in the neural networks that promote behavioral inhibition. In support, other neurotoxic HIV-1 proteins have been observed to produce behavioral disinhibition [5]. The neural circuits that may be targeted by gp120 to influence affective behavior are the subject of future investigation.

## Hypotheses

- ***In vivo*, gp120 expression will promote anxiety- and depression-like behavior in mice. These effects will be ameliorated in female proestrous (high-hormone) mice.**
- ***In vitro*, exposure of gp120 will promote necrosis in primary medium spiny neurons.**

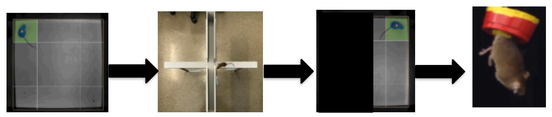
## Methods

Animal Subjects: gp120-tansgenic mice [2] were bred in the vivarium at the University of Mississippi (University, MS). gp120 is expressed under the control of a glial fibrillary acidic protein promoter. Mice were kept in a temperature- and humidity-controlled environment on a 12:12 h light:dark cycle (lights off at 09:00 h) with *ad libitum* access to food and water.

Behavioral Testing:

Mice were assessed in a behavioral battery of tests in the same order.

Open Field  Elevated Plus Maze  Light-Dark  Tail Suspension



Open Field: A measure of general activity and anxiety. A mouse is placed in a the box (brightly-lit center) for 5 min.

Light Dark Test: A measure of anxiety. Mice are placed in a box divided into one well-lit and one dark section and assessed for 5 min.

Elevated Plus Maze: A measure of anxiety. Mice are place in a elevated cross maze with two closed arms and two open arms for 5 min.

Tail Suspension: A depressive measure. Mice are suspended by the tail and immobility/struggling is assessed for 4 min.

Live/Dead Assay: Medium spiny neurons were grown in co-culture with mixed glia cells. All were obtained from C57BL/6N mice as described [4]. Cells were exposed to propidium iodide and Hoechst 33342 to determine dead and total cell counts, respectively.

## gp120 Modulates Affective Behavior in Transgenic Mice

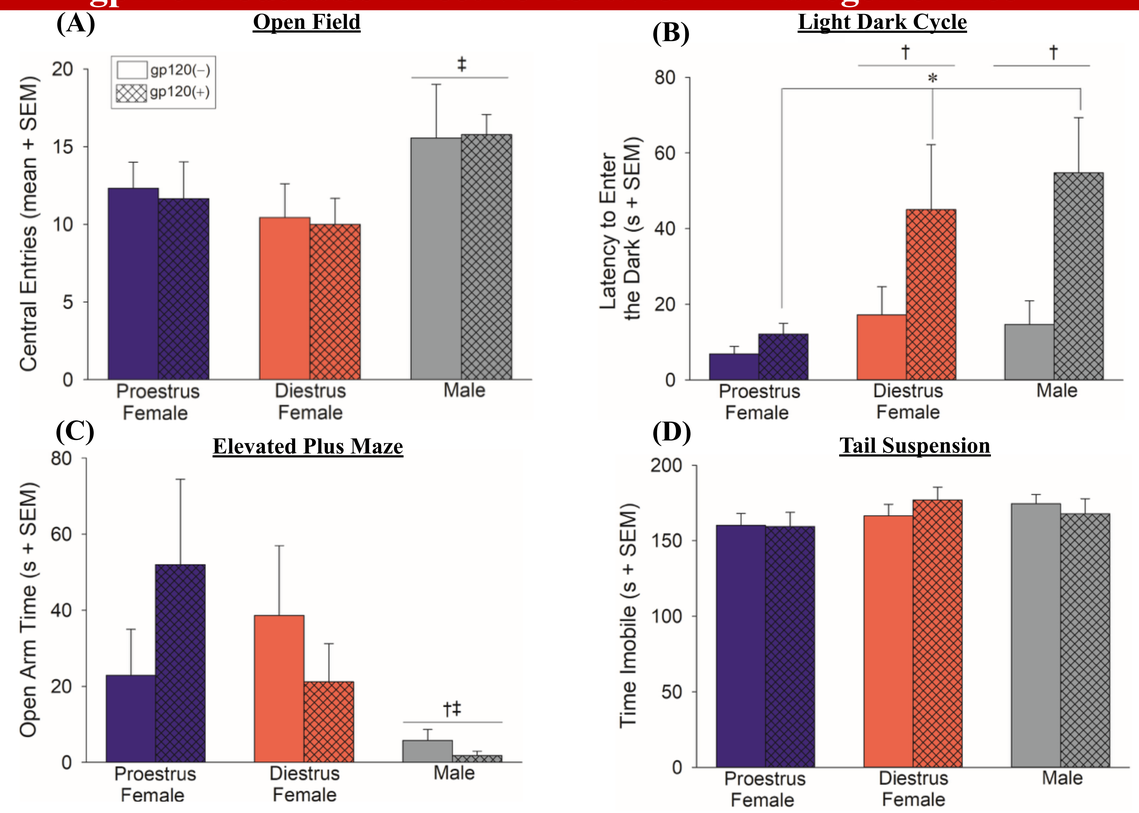
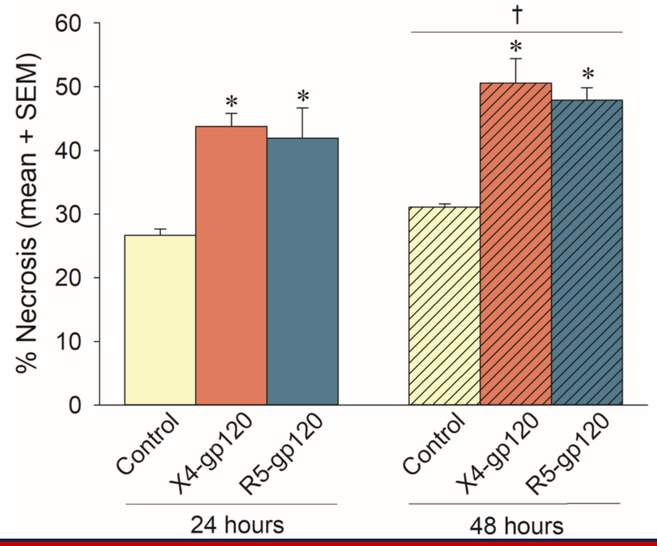



**Figure 1.** Female mice were in the proestrous or diestrous phase of their estrous cycle. Female and male mice that expressed gp120 (gp120+) or did not (gp120−) were assessed in a behavioral battery (n=9/grp). (A): The frequency of entries into the brightly-lit center of an open field; (B) The latency to enter the dark-side in the light-dark transition test; (C) The time spent in the open arms of an elevated plus maze; (D) Time spent immobile in a tail suspension test. ‡ indicates significant difference from diestrous females; † indicates significant difference from proestrous females; ✻ indicates significant effect of gp120, $p \leq 0.05$.

## gp120 Produces Neurotoxicity in Murine Medium Spiny Neurons



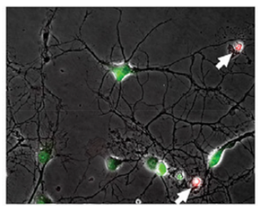



**Figure 2 (Inset).** Representative live/dead image form our prior work using current methodology [4].

←**Figure 2.** Co-cultured medium spiny neurons neurons and mixed glia were obtained from C57BL/6N mice. Cells were exposed to media (control), X4-tropic gp120 (500 pM), or R5-tropic gp120 (500 pM) proteins for 24 or 48 hours.

✻ significant difference from respective control;

† significant difference from 24 h time point, $p < 0.05$.

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## Summary

- Male mice made significantly more center entries than did diestrous females (Fig. 1A).
- Diestrous females and males took significantly less time to enter the dark side of a light-dark apparatus than did proestrus females. In addition, gp120(+)-exposed mice took longer to enter the dark side of the apparatus than did gp120(−) mice (Fig. 1B).
- Males spent less time on the open arms of an elevated plus maze than did proestrous or diestrous females (Fig. 1C).
- No significant differences in the time spent immobile were observed on the tails suspension test (Fig. 1D).
- X4- or R5-gp120 produced significant necrosis in medium spiny neurons after 24- or 48 h (Fig. 2).
- Neuronal necrosis was greater after 48 hours compared to that observed after 24 hours (Fig. 2).

## Conclusions

*The present data demonstrate the capacity for gp120 exposure to decrease anxiety-like behavior* in vivo *while promoting neurotoxic effects* in vitro. *These seemingly paradoxical findings may be the result of a disruption in the neural networks that promote behavioral inhibition. Thus, future investigations may assess prefrontal inhibitory circuitry that may be disrupted by gp120 protein, potentially contributing to HAND.*

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