

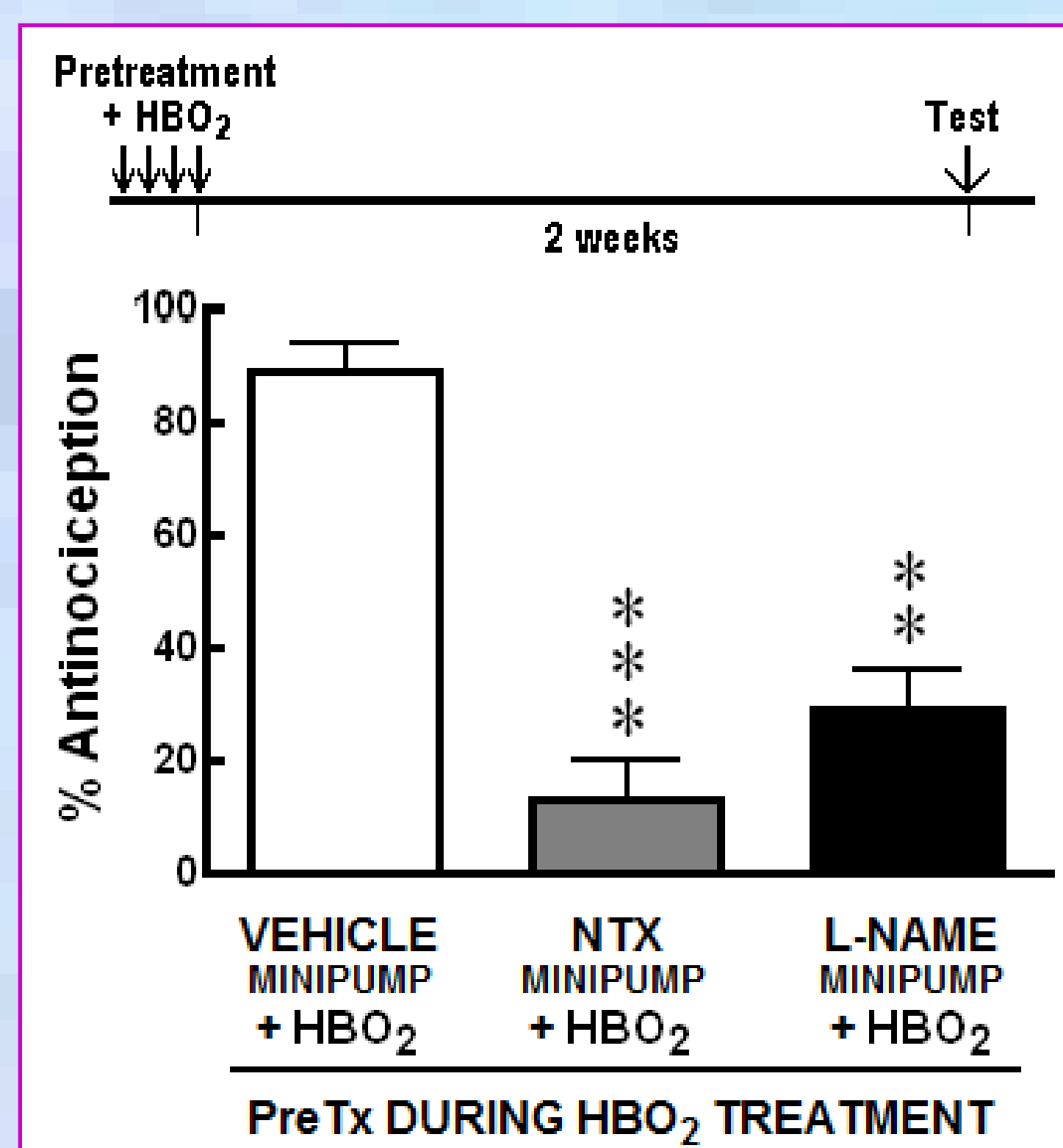
Does hyperbaric oxygen (HBO₂) cause gene mutations that are responsible for the late-phase antinociceptive response in mice?

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ABSTRACT. Hyperbaric oxygen (HBO₂) is approved by the FDA for a limited number of clinical indications albeit not for chronic pain. Research from our laboratory has demonstrated that HBO₂ treatment produces an antinociceptive effect of unusually long duration that involves nitric oxide (NO) and opioid mechanisms [Zylstra et al., *Exptl Biol Meeting Abst*, abstracts 711.16 and 711.17, 2008]. The objective of the present research is to determine whether the long-lasting effect might result due to changes in genes that regulate NO and opioid mechanisms.

INTRODUCTION. We recently reported that four daily 60-min HBO₂ treatments produce an antinociceptive effect that persists for up to three weeks. There was an early-phase response of 6 hr duration that subsided by 12 hr after HBO₂ treatment; and a late-phase response that emerged at 24 hr and lasted up to three weeks after the last HBO₂ treatment. Continuous treatment with naltrexone (NTX, an opioid antagonist) or L-N^G-nitro arginine methyl ester (L-NAME, a nitric oxide synthase inhibitor) during the four days of HBO₂ treatment significantly inhibited the late-phase antinociceptive effect. (See figure below.)



The purpose of the present study is to employ microarray analysis to test the hypothesis that four-day HBO₂ treatment initiates a sequence or cascade of gene changes---possibly in the NO-opioid pathway---that may be responsible for the late-phase antinociceptive response.

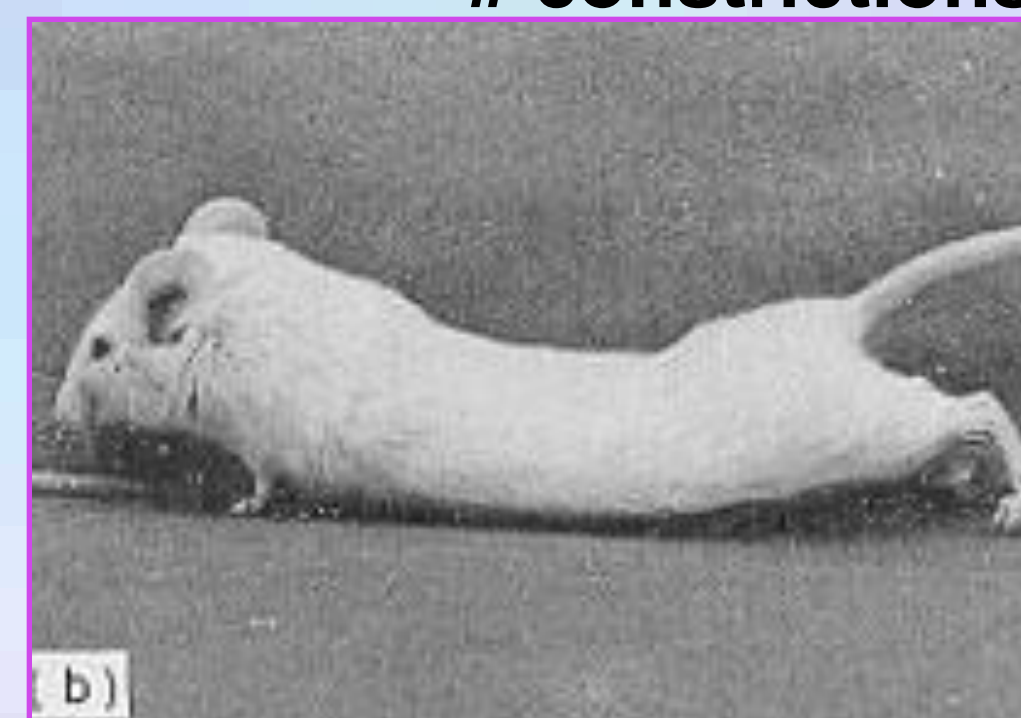
MATERIALS AND METHODS

Animals. Male 10 to 11-week-old NIH Swiss mice, 18-22 g body weight, will be purchased from Harlan Laboratories (Indianapolis, Indiana) and will be used in this IACUC-approved research.

MATERIALS AND METHODS (Continued)

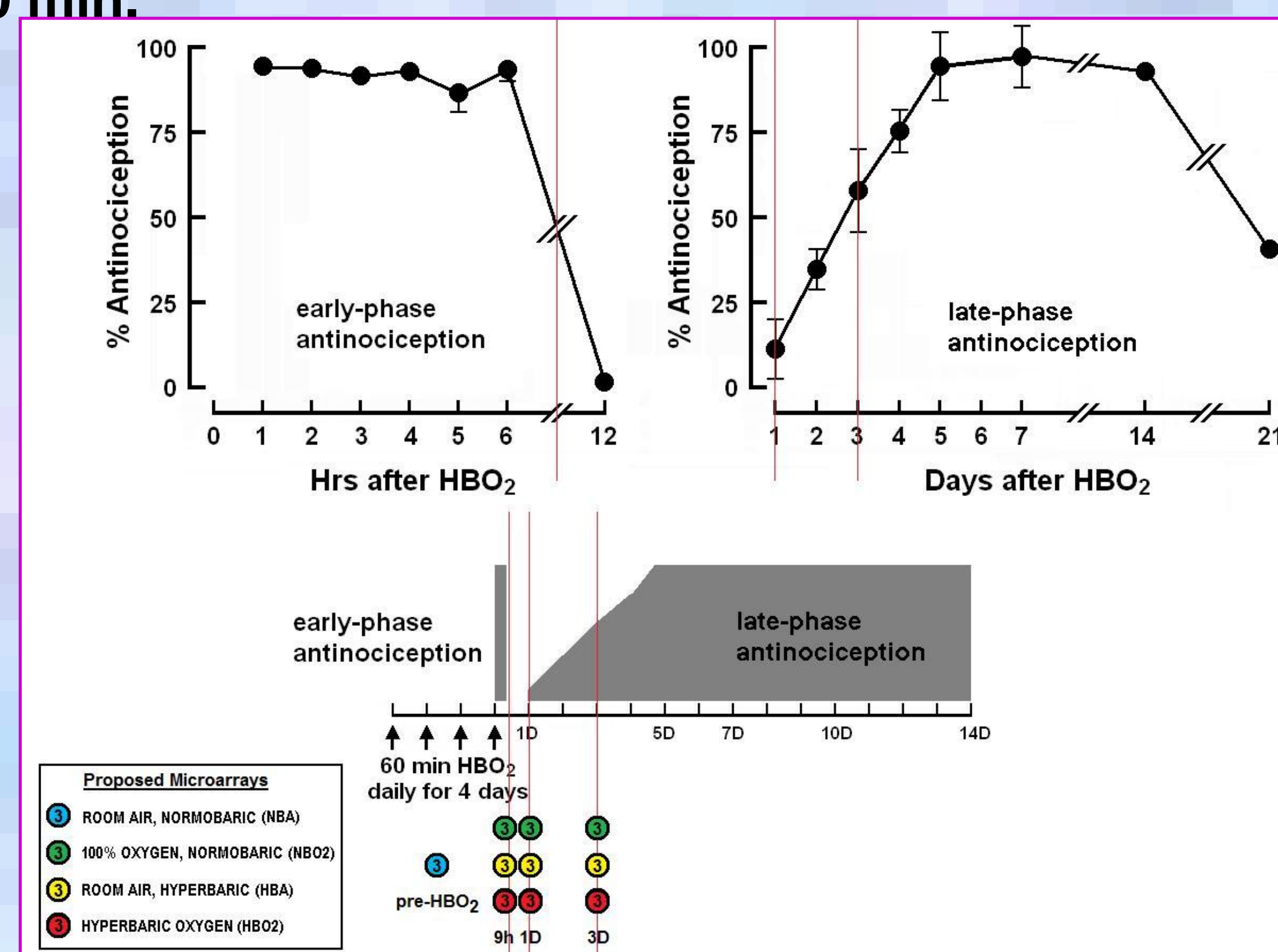
Abdominal Constriction Test. Mice will be treated i.p. with 0.6% glacial acetic acid (0.1 ml/10 g volume of injection). Exactly 5 min later, the number of abdominal constrictions—lengthwise stretches of the torso with concave arching of the back—in each animal will be counted for a 6-min period. The degree of antinociception (inhibition of abdominal constrictions) produced in various treatment groups of mice will be calculated as follows:

$$\% \text{ antinociception} = 100 \times \frac{\# \text{ constrictions in control mice} - \# \text{ constrictions in exposed mice}}{\# \text{ constrictions in control mice}}$$



Example of an abdominal constriction

Exposure to NBO₂, HBA, HBO₂. Different groups of animals will be placed in a B-11 research hyperbaric chamber (Reimers Systems, Inc., Lorton, VA), and subjected to four daily 60-min exposures to normobaric oxygen (NBO₂, 100% oxygen at 1 absolute atmosphere (ATA)), hyperbaric air (HBA, compressed air at 3.5 ATA) or hyperbaric oxygen (HBO₂, 100% oxygen at 3.5 ATA). Animals will be assessed for antinociceptive responsiveness using the acetic acid abdominal constriction test at 9 hr, 24 hr (1 day), and 3 days (3D) following the last HBO₂ treatment. A reference group was exposed to normobaric air (NBA, compressed air at 1.0 ATA) for 60 min.



Induction of early-phase (left) and late-phase (right) antinociceptive responses by repeated HBO₂ treatment (60 min daily for four days). Different groups of mice will be euthanized at 9 hr, 24 hr and 3 days after HBO₂ treatment for extraction of RNA for microarray analysis.

MATERIALS AND METHODS (Continued)

Total RNA Extraction. Mice will be euthanized by decapitation under light isoflurane anesthesia. Whole brains will be immediately removed, flash frozen in TRIzol® buffer (Invitrogen, Carlsbad, CA), and stored at -80 C. Total RNA will be isolated using the RNeasy Lipid Tissue Midi Kit (Qiagen, Valencia, CA).

Microarray Analysis. Total RNA will be prepared for hybridization to the Mouse Gene ST 1.0 microarray, using the WT target labeling from Affymetrix. The amplification and subsequent hybridization will be performed at the Laboratory for Biotechnology and Bioanalysis at Washington State University.

Analysis will contain the follow steps: importing of raw data; quality assurance; preprocessing; transcript filtering; gene-wise statistics; multiple testing correction; and transcript annotation.

DISCUSSION Blockade of opioid receptors or inhibition of NO production in the brain prevents development of the late-phase antinociception. This finding implicates opioid and NO mechanisms in the long duration of antinociception. The microarray analysis will reveal whether the four-day HBO₂ treatment causes changes in genes in opioid and NO function that result in the late-phase antinociception. It is also possible that manipulation of opioid and NO during HBO₂ treatment may cause changes in genes that regulate other systems (e.g., other pathways that mediate pain relief). A better understanding of the mechanism of HBO₂-induced antinociception and identification of the factors that determine its duration of action might portend the clinical application of HBO₂ for long-term treatment of chronic pain conditions.

REFERENCES

- Zylstra CC, Ohgami Y, Chung E, Shirachi DY, Quock RM. 2008 *Exptl Biol Meeting Abst [on CD-ROM]*, abstract 711.16 (2008a)
- Zylstra CC, Zelinski LM, Chung E, Ohgami Y, Shirachi DY, Quock RM. 2008 *Exptl Biol Meeting Abst [on CD-ROM]*, abstract 711.17 (2008b)

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