# [YIA2-5] Anesthetic Agents Induce Protein Misfolding and Affect Carbon Dioxide Response in the Brainstem

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## Introduction

Phox2B transcription factor senses carbon dioxide levels and triggers breathing. Mutations cause Congenital Central Hypoventilation Syndrome (CCHS), causing dysfunction of respiratory control with decreased sensitivity to hypercapnia, requiring lifelong ventilation. Phox2B contains a poly-alanine repeat sequence and CCHS results from expansion of this region. Longer repeats cause earlier disease onset. CCHS has become symptomatic after exposure to anaesthetic drugs, indicating that anesthetics can affect the function of the Phox2B protein. We hypothesize that anesthetic drugs change cellular localization of Phox2B and affect the protein's ability to trigger breathing in response to rising blood carbon dioxide levels.

## Methods

We created five mutants of the Phox2B gene with increasing polyalanine expansions, the largest being the most severe genotype (wild-type, 3ALA, 5ALA, 7ALA, 10ALA and 13ALA). A fluorescent protein (mCherry) was tagged to the Phox2B construct in a mammalian expression vector, creating a fluorescent fusion protein. Each construct was transfected into mammalian cells and tested for expression (HeLa cells) using high-content fluorescent imaging. After imaging, each of the transfections were exposed to anesthetic agents at clinically relevant concentrations (propofol 10uM, morphine 10 uM, isoflurane 1 MAC) and imaged for any change in Phox2B location or cellular morphology.

## Results

- All of the constructs except for 13ALA were located within the nucleus of the cell, consistent with being a transcription factor.
- Disease causing 13ALA was present as misfolded protein in the cytoplasm, preventing transcription of genes associated with carbon dioxide response.
- After exposure to morphine and isoflurane, the small expansion mutants (wild-type, 3ALA, and 5ALA) remained in the nucleus
- The larger expansions (7ALA, 10ALA and 13ALA) were now in the cytoplasm as aggregated, non-functional protein, illustrating the adverse effect of morphine and isoflurane on Phox2B function.
- Propofol had no effect on the location or morphology of Phox2B.
- There were no changes in cellular morphology, indicating that our observations were not a generalized cellular response induced by anesthesia.

Conclusions

Our results show that morphine and isoflurane can induce Phox2B protein misfolding and precipitate the CCHS disease phenotype. Our induced misfolding is known to lead to a loss of carbon dioxide response and permanent brainstem dysfunction. Our results shed light on the induction of CCHS by anesthesia but also show how anesthetic agents can affect protein folding and possibly induce other protein misfolding diseases. Our model will lead to further investigation of anesthetic effects on the cellular Unfolded Protein Response and whether the adverse effects of anesthetics on protein folding, including Phox2B, is preventable.

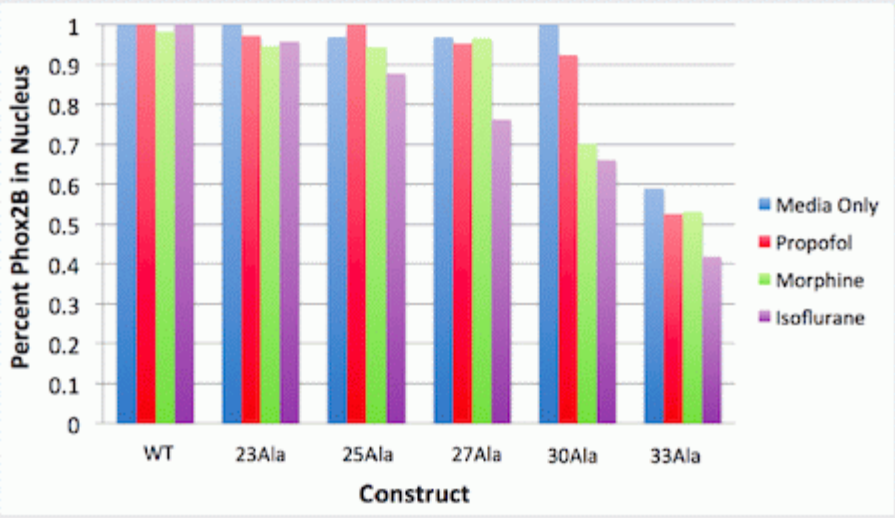


**Fig.2 Percentage of Phox2b protein located in the nucleus for each of the different mutants comparing control and anesthesia exposures. Note the effect of isoflurane on misfolding is proportional to the size of the alanine mutation, and morphine induces misfolding in the 30ala mutant only. Propofol had no effect.**