Ryan S. Herzog's 2025 University of Tennessee Honors Thesis

Increased Cholinergic Activity in the Prefrontal Cortex Attenuates Opioid-induced Respiratory Depression Through Gain Modulation in C57BL/6J Mice

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I am also incredibly grateful for my parents, my brother, and the many friends who have supported me along the way. Their belief in me has pushed me to keep moving forward, but also to take care of myself along the way.

ABSTRACT:

Opioid-induced respiratory depression occurs when opioids inhibit the body's ability to detect and respond to hypercapnia and hypoxia. The potential for opioid-induced respiratory depression is a lethal side effect of opioid overdose and a complication for clinical pain management. The prefrontal cortex is one brain region that mediates the body's detection and response to hypercapnia and hypoxia. The two studies described in this honors thesis investigated cholinergic transmission in the prefrontal cortex as a modulator of opioid-induced respiratory depression. The first study tested the hypothesis that prefrontal cortex administration of neostigmine could attenuate respiratory depression caused by systemically administered fentanyl. The second study tested the hypothesis that the cholinergic modulation of fentanyl-induced respiratory depression could be quantitatively expressed using a gain modulation model. The results show that prefrontal cortex administration of neostigmine did mitigate the fentanyl-induced decrease in respiration. The time course analyses also demonstrate that this mitigation is consistent with prior gain modulation models. Together, the data presented in this honors thesis support the hypothesis that the gain modulation model can describe the effects of the prefrontal cortex in opioid-induced respiratory depression.

INTRODUCTION: Historically, morphine was used as a 'catch-all' drug of choice for any sort of ache or pain. This issue was overlooked until the late 2000s, after opioid prescriptions skyrocketed from 2-3 million prescriptions per year in 1990 to 62 million patients receiving at least one opioid prescription in 2016 (Rummans et al., 2018). In 2014, it was found that as many as 38% of US adults reported using prescription opioids (Lasser, 2017). This drastic rise in legal opioid distribution was encouraged by published reviews downplaying the addictive potential of chronic opioid treatments and deceptive marketing by companies. After the establishment of the systematic pain assessment scale in hospital use and policy, the relevance of opioids increased even more. Acknowledging the danger and uncertainty surrounding opioid use, research initiatives were established to further understand the neurobiology of pain and opioid use disorders. Similarly, new treatments and interventions were sought to limit the adverse side effects of opioids, such as their potential for addiction, sleep disruption, and respiratory depression.

One avenue of opioid research currently being pursued by our lab is the neurobiology underlying opioid-induced respiratory depression. Specifically, my thesis project was inspired by the concept of a "wakefulness stimulus for breathing" and how it is involved in the respiratory depression caused by opioids. The "wakefulness stimulus for breathing" is a construct derived from human studies by Raymond Fink, M.D., to describe the differences in CO_2 sensitivity associated with arousal states in humans (Fink, 1961). These studies revealed that during experimentally induced increases in blood levels of carbon dioxide, awake humans were able to compensate by increasing breathing frequency and tidal volume. In contrast, during sleep or anesthesia, the human respiratory control system failed to respond to the

hypercapnic stimulus. Fink theorized that during wakefulness, cerebral drive sustains a significant proportion of breathing (Fink et al., 1963). Identifying the neural networks and neurotransmitters underlying this cerebral drive to breathe is relevant for developing treatments for opioid-induced respiratory depression (OIRD). OIRD occurs when arterial O_2 and pH decrease while CO_2 increases without the typical compensatory increase in pulmonary ventilation (Yeadon & Kitchen, 1989). Similarly, one mechanism contributing to OIRD is the decreased sensitivity of central chemoreceptors to arterial pH changes (van den Hoogen & Colpaert, 1986). These phenomena are both directly related to the wakefulness stimulus for breathing. The primary cause of death from opioid misuse is diminished drive to breathe, and although the average mortality risk increases at higher opioid doses, no amount of opioid is without risk (Ramirez et al., 2021). The incompletely understood variability in the opioid response between individuals presents a profound barrier to understanding the specific causes of OIRD and forming effective measures to prevent OIRD (Ramirez et al., 2021).

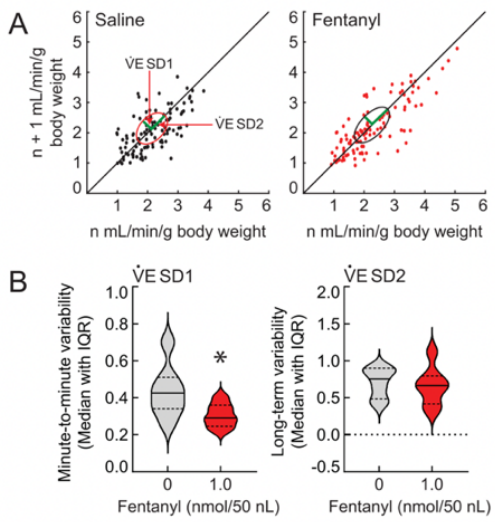
Studies of OIRD using mice circumvent the fact that it is unethical to administer opioids to humans for study purposes. In addition, the strong genetic homology between mice and humans (Breschi et al., 2017) supports the probability that research using mice can enhance understanding of human respiratory control. For example, systemic administration of opioids to mice has been found to diminish neurobehavioral arousal and to induce a dissociated state of wakefulness that can include EEG delta waves (0.5 to 4 Hz) that normally occur during sleep (O'Brien et al., 2021). Neuroanatomical studies have reported high densities of mu-opioid receptors in the prefrontal cortex (PFC) and suggest that the PFC may contribute to opioid-induced changes in arousal and OIRD (Giacchino & Henriksen, 1998). Additional

neuroanatomical data provide evidence for direct neuroanatomical connectivity between regions in the medial PFC and brainstem respiratory nuclei (Terreberry & Neafsey, 1987; Van Eden & Buijs, 2000). Key studies in rodents have also shown bidirectional interactions between the PFC and brainstem in both breathing and arousal (Hassan et al., 2013; Yackle et al., 2017).

One key aspect of this connectivity is the importance of PFC signaling in transforming sensory input into relevant behavioral changes (Le Merre et al., 2021). One important instance of this connectivity is related to the continuous, multi-faceted stream of sensory input received by the respiratory control centers in the brain stem. This complex sensory input must be accurately and effectively incorporated into behavioral changes in breathing to maintain homeostasis and prevent hypoxia (van den Bosch et al., 2021). When OIRD threatens respiratory homeostasis, this encoding of sensory input by the respiratory nuclei in the brain stem becomes more relevant. One potential mechanism for how PFC feedback signaling could alter this encoding is through a gain modulation model. For this thesis, gain modulation was evaluated as a model to describe the effect of PFC neostigmine on respiratory depression caused by systemic administration of fentanyl. Gain modulation is the process by which a neural circuit's responsiveness is modulated by an outside, separate neural circuit (Ferguson & Cardin, 2020). Gain modulation enables neural networks to respond flexibly to changes in internal state and external drives by transforming how the network processes and encodes input.

Neurochemical studies provide functional data showing that descending cholinergic input from PFC to the brainstem promotes behavioral and EEG arousal (Van Dort et al., 2009). Recent studies from our laboratory have confirmed that the PFC of intact, behaving mice

(Glovak et al., 2022) contributes to OIRD. These studies produced four major findings illustrated by the following figures.



Figs. 1A&B. From (Glovak et al., 2022). Microinjection of fentanyl (1 nmol; 530 ng/50nL) into the lateral PFC significantly decreased variability in minute ventilation (mL/min/g body weight) in 19 mice. Variability in cardiopulmonary control systems is a marker of health commonly used clinically to indicate how well a patient can respond to respiratory stressors. The distribution of points in the Poincaré plots (Fig. 1A) illustrates the decrease in minute-to-minute (short-term or breath-to-breath) variability in ventilation (SD1) and long-term variability during 15 min of recording minute ventilation (SD2). Fig. 1B shows that PFC fentanyl significantly decreased minute-to-minute ventilation variability.

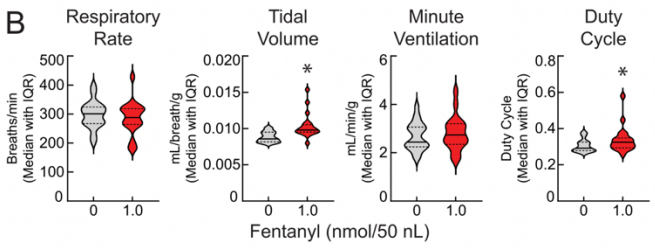


Fig. 2. From (Glovak et al., 2022). In all mammals, the temporal organization of each breath is comprised of phases devoted to inspiration (T_i) or expiration (T_e), with the sum of the two representing one breath (T_{tot}). The T_i/T_{tot} ratio is the active phase of breathing generated by brain stem respiratory neurons, also referred to as the “duty cycle”. The dose of fentanyl delivered into the medial PFC caused a

small decrease in respiratory rate, a significant increase in the volume of each breath (tidal volume (mL/breath)), and a significant increase in duty cycle (T_i/T_{tot}) in 19 mice.

The results summarized by Figs. 1 and 2 are significant in showing that microinjection of fentanyl into the PFC decreases breathing despite the fact that the PFC contains no primary respiratory neurons (Alheid & McCrimmon, 2008). To date, all primary respiratory neurons have been localized to an elaborate and complex neuronal network in the pontine and medullary brain stem (Janczewski & Feldman, 2006; Lindsey et al., 2000; Smith et al., 1991). Among humans, the endogenous respiratory rhythm begins in utero during the 34th week of gestation (Di Fiore et al., 2013). The endogenous rhythmicity of these neurons is modulated by cerebrospinal fluid pH, $PaCO_2$, and many other factors. The finding that PFC administration of fentanyl depressed breathing led to an additional set of experiments. As noted above, enhancing cholinergic transmission in the PFC has an excitatory effect on EEG and behavior.

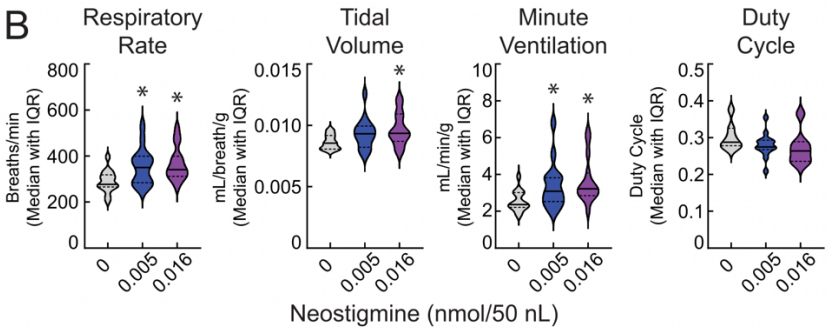


Fig. 3. From (Glovak et al., 2022). To examine the effects of enhancing cholinergic transmission on measures of breathing, Glovak et al, 2022 performed microinjection of acetylcholinesterase inhibitor neostigmine into the medial PFC at concentrations of 0, 0.005, and 0.016 nmol/50 nL. Fig. 3 shows that PFC neostigmine delivery significantly increased respiratory rate, tidal volume, and minute ventilation in 15 mice.

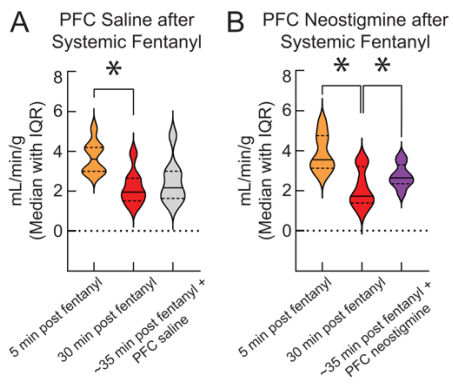


Fig. 4. From (Glovak et al., 2022). Fig. 4 shows the results of experiments designed to test the hypothesis that enhancing cholinergic transmission in the PFC would decrease respiratory depression caused by systemically administered fentanyl. Systemic administration of fentanyl (0.3 mg/kg in 8 mice) caused a decrease in minute ventilation. After 30 min, 50 nL of saline or neostigmine (0.016 nmol; 4.84 ng in 50 nL) was microinjected into the medial PFC. Fig. 4A shows that PFC microinjection of saline did not significantly affect the decrease in minute ventilation caused by systemic fentanyl. In contrast, 4B shows that PFC microinjection of neostigmine reduced the decrease in minute ventilation caused by systemic fentanyl administration. The Fig. 4 results in eight mice support the interpretation that the enhanced PFC cholinergic transmission can mitigate the respiratory depressive effects of systemic fentanyl.

In the interest of expanding knowledge regarding the role of the prefrontal cortex in control of breathing, the present studies were designed to test the hypothesis that PFC-induced behavioral arousal can mitigate OIRD. The results provide additional information on the time course of neostigmine mitigation of respiratory depression caused by systemic fentanyl. Preliminary evidence from this honors thesis showed that in 7 mice, the PFC neostigmine mitigation of fentanyl-induced decrease in respiratory rate could be modeled using a gain modulation equation (Herzog, 2023; Woods et al., 2023). In our initial approach, preliminary data were fit to a gain modulation equation used previously to describe GABAergic modulation of breathing (Zuperku & McCrimmon, 2002). Gain was expressed as

$F_o = (1 + \alpha) F_i$, where F_i indicates pre- and F_o post-drug administration measures of respiratory rate, and α is a unitless modulation coefficient (Zuperku & McCrimmon, 2002). Following this evidence, similar data from prior studies in our lab were used as a further test of this hypothesis. Comparison of the time-course of respiratory rate following PFC-fentanyl versus PFC-saline administration (n=7) demonstrated that PFC fentanyl caused an inverse effect on breathing rate changes compared to PFC neostigmine (Herzog, 2023).

METHODS

Subjects: Before beginning, these studies were reviewed and approved by the University of Tennessee Institutional Animal Care and Use Committee and were conducted per the ARRIVE guidelines (Percie du Sert N, 2020). Adult male C57BL/6J (B6) mice (n=14). All mice were implanted with a microinjection guide tube aimed at the medial prefrontal cortex (1.94 mm anterior to bregma, 0.3 mm lateral (right) of midline, 2.5 mm ventral to skull surface) as well as an RFID chip for unique identification of each mouse.

Surgical procedure for medial PFC microinjection guide cannula implantation:

Prior to surgery, mice were weighed and then deeply anesthetized with 2 to 3% isoflurane delivered by 100% oxygen at a flow rate of 1.0 L/min. Upon loss of righting reflex, loss of consciousness was confirmed via paw withdrawal test. The head was shaven at the implantation site, and ketoprofen (5 mg/kg) and saline were delivered subcutaneously to prevent dehydration and induce a deeper anesthetic state. Mice were transferred to a stereotaxic frame, scrubbed with 7.5% povidone-iodine and 10% beta iodine, and administered

ophthalmic ointment. Mice rested on a warm water heating pad, and isoflurane concentration was maintained at 1.3% through the surgery. Isoflurane delivery via mouse adaptor (model 921) and anesthesia mask (model 907) was monitored by spectrophotometry (CardioCap/5; Datex-Ohmeda Inc., Madison, WI). Body temperature and breathing rate were monitored during surgery to maintain core body temperature (36-37°C) and adequate respiratory function (>100 breaths/min). Breathing rate, isoflurane delivery concentration, and body temperature were recorded every 10 minutes.

A dorsal skin incision was made to expose the skull, and stereotaxic zero was confirmed using bregma and lambda measurements. A craniotomy was formed with a drill (Microkopf) at the aim site for medial PFC implantation, and the cortex depth was measured. A 26-gauge stainless steel guide cannula was then implanted. The stereotaxic coordinates of the aim site for the medial PFC were 1.9 mm anterior to bregma, 0.3 mm lateral (right) of the midline, and 3.3 mm below the surface of the skull (Paxinos, G., Franklin, K.B., 2007. The Mouse Brain in Stereotaxic Coordinates, Third ed. Academic Press, San Diego). This aim site for microinjection was chosen based on previous data from our lab that demonstrated an autonomic response following injection in this site (Glovak et al., 2022). The guide cannula was secured to the surface of the skull with dental acrylic (Jet Acrylic Self Curing Resin and Liquid; Lang Dental Manufacturing Co. Inc., Wheeling, IN) and four stainless steel anchor screws (Antrin Miniature Specialties, Fallbrook, CA). Each mouse received a unique subcutaneous RFID implant for identification purposes.

Confirmation of Microinjection Site:

In n=7 mice, mice were euthanized, and histology of post-mortem brains was performed by NeuroScience Associates (2023). Iron staining was used to localize the site of injection in the prefrontal cortex in tissue using the mouse brain atlas published by Paxinos and Franklin. The average injection site for n=7 mice was 1.49 ± 0.66mm anterior from bregma, 0.60 ± 0.37 lateral of midline, and 2.42 ± 0.43mm below the skull surface

Quantification of Breathing:

The breathing quantification system used for both studies was breathing measured by whole body plethysmography (WBP) and analyzed using "FinePointe" software (Data Sciences International, St. Paul, MN). This system was used to measure breathing in freely moving, unanesthetized mice. Each WBP chamber is fitted with a pneumotach screen and a sensitive pressure transducer that measures changes in air pressure. These changes in air pressure are then used to calculate box flow. The first contributor to box flow is the expansion of air when it is warmed during inhalation and the contraction of air when it is cooled after exhalation. The other contributor to the box flow is the expansion of air due to a negative pressure within the lungs during a forced inspiration. The WBP system was calibrated via a reference water column prior to each experiment.

Experimental Design:

Dual Injection Study

Following microinjector guide tube implantation, mice recovered for a minimum of one week before starting at least one week of handling and injection conditioning. Conditioning was achieved when the behavior of each mouse revealed the absence of stress or escape behavior in response to being picked up and gently handled. Mice underwent baseline data collection before receiving any injections. For baseline recordings, mice were weighed and placed in DSI whole-body plethysmography (WBP) chambers. Mice were habituated to chambers for 15 minutes and then received mock injections (systemic and PFC). After mock injections, mice were placed back into WBP chambers, and breathing measures were recorded for an hour. After preliminary conditioning and baseline recordings, experiments occurred weekly, and conditioning persisted on days that experiments did not occur.

Mice received an intraperitoneal (ip) injection (0.3 mL) of either saline or fentanyl (0.1 mg/kg). After the systemic injection is administered, mice received a PFC microinjection (50 nL) of saline or neostigmine (0.016 nmol). Following injections, mice were immediately placed in WBP containers, and breathing was recorded for one hour. During recording, mice were consistently monitored to ensure wakefulness by researchers. All mice received the treatment conditions in the following order: ip saline, PFC saline; ip fentanyl, PFC saline; ip fentanyl, PFC neostigmine. Depending on opioid dose, respiratory depression is normally variable between individual mice, so this study utilized a within-subjects design to determine the effects of PFC neostigmine on OIRD. Tidal volume, frequency, and minute ventilation were collected from WBP using FinePointe software and analyzed statistically using Excel and Prism.

PFC Microinjection Study

After implantation of a microinjector guide tube, mice recovered for a minimum of one week before starting one week of handling and injection conditioning. Conditioning was achieved when each mouse could be handled safely. Mice underwent baseline data collection before receiving any injections. For baseline recordings, mice were weighed and placed in DSI whole-body plethysmography chambers. Mice were habituated to chambers for 15 minutes and then received mock PFC injections. After mock injections, mice were placed back into WBP chambers, and breathing measures were recorded for an hour. After preliminary conditioning and baseline recordings, experiments occurred weekly, and conditioning persisted on days that experiments did not occur.

Mice received a PFC microinjection (50 nL) of saline or fentanyl (1 nmol). All mice received the PFC saline administration, then the PFC fentanyl administration. Following PFC administration, mice were immediately placed in WBP, and breathing was recorded for one hour. During recording, mice were consistently monitored to ensure wakefulness by researchers.

Table 1

Week	Treatment
Week 1 Experiment	Saline (ip), Saline (PFC)
Week 2 Experiment	Fentanyl (ip), Saline (PFC)
Week 3 Experiment	Fentanyl (ip), Neostigmine (PFC)

Table 2

Week	Treatment
Week 1 Experiment	Saline (PFC)
Week 2 Experiment	Fentanyl (PFC)

Data Analysis: The primary dependent variables for breathing were tidal volume, frequency, minute ventilation, and duty cycle. Tidal volume (mL/breath/gram) is the average amount of air that is inspired and expired in each breath, while frequency (breaths/minute) is the average number of breaths per minute. Minute ventilation (mL/minute/gram) is the product of tidal volume and frequency and is the average total volume of air that a mouse inspires per minute. Duty cycle is the ratio of time spent inspiring to the total amount of time breathing (T_i/T_{tot}) and is a measure of the active phase of breathing and reflects respiratory drive. Tidal volume, minute ventilation, and duty cycle were standardized between mice by expressing measures per mass of each individual mouse. Data were analyzed by descriptive (mean ± standard deviation (SD), median, and interquartile range (IQR)), inferential statistics, and percent change. Data from both studies were analyzed using a one-tailed, paired t-test. Various regression models were also applied to breathing data to identify trends in the time course effects of PFC neostigmine and fentanyl.

RESULTS

Fig. 5A-C. From (Woods et al., 2023)

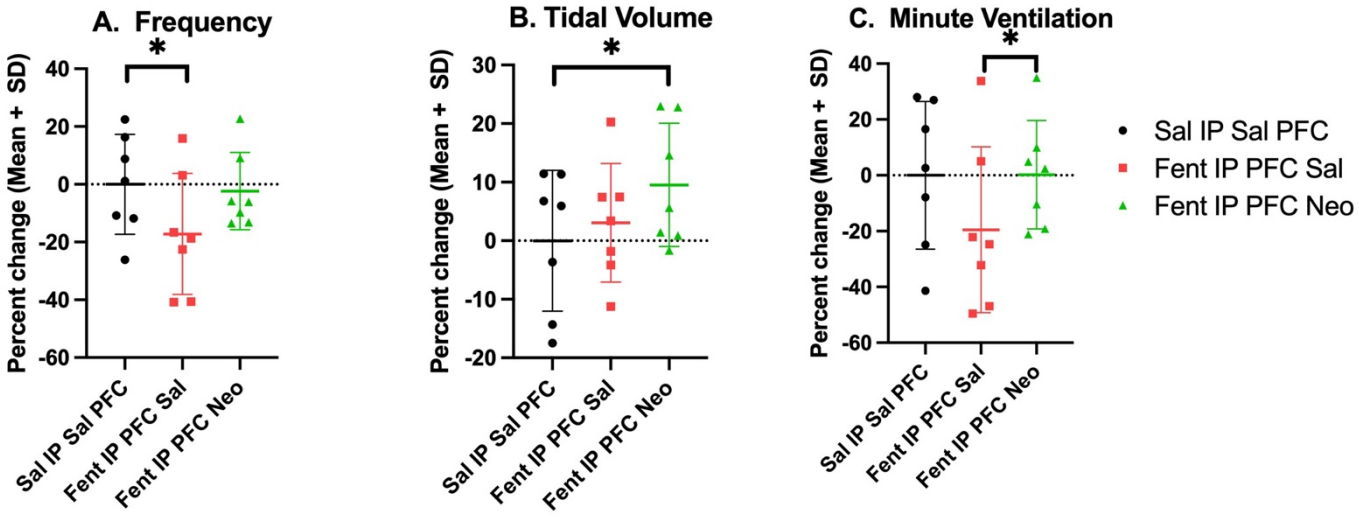


Fig. 5A-C. quantifies the percent change in frequency, tidal volume, and minute ventilation relative to the control condition over the 15-minute recording period. Fig. 5A shows that systemic fentanyl significantly decreased breathing frequency and that this significant decrease was mitigated by PFC neostigmine. Fig. 5B shows that PFC neostigmine also caused a further increase in tidal volume relative to both control and systemic fentanyl conditions. Fig. 5C shows that neostigmine mitigated the substantial decrease in minute ventilation induced by systemic fentanyl.

Fig. 6D-E. From (Woods et al., 2023).

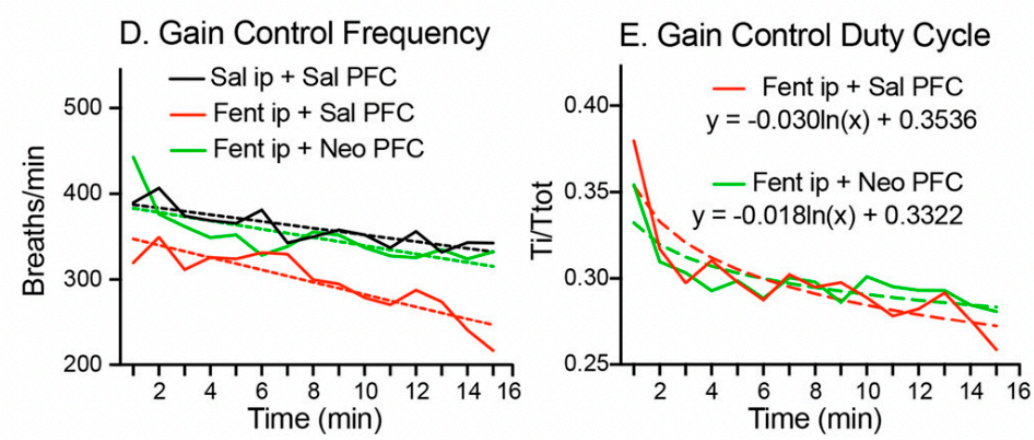
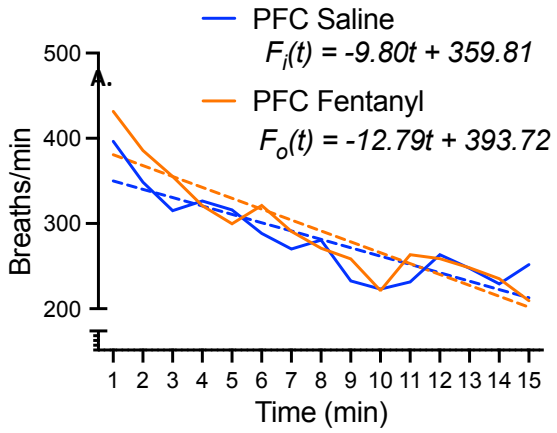


Fig. 6D plots the time course for min 1 - 14 of breathing frequency (solid lines) for each of the three treatment conditions. Dashed lines illustrate negative slope values of -3.9 (black),-7.2 (red), and -4.8 (green). Fig. 5E plots the time course for min 1 - 14 of the respiratory drive. The logarithmic regression analysis yielded multiplicative k values of -.03 and -.018, respectively, indicating a .6 scalar increase in duty cycle between these two conditions.

Fig. 7A&B. From (Herzog, 2023).



B. Gain Factor of PFC Fentanyl:

$$\alpha = 1 - \left(\frac{Fo(t)}{Fi(t)}\right)$$

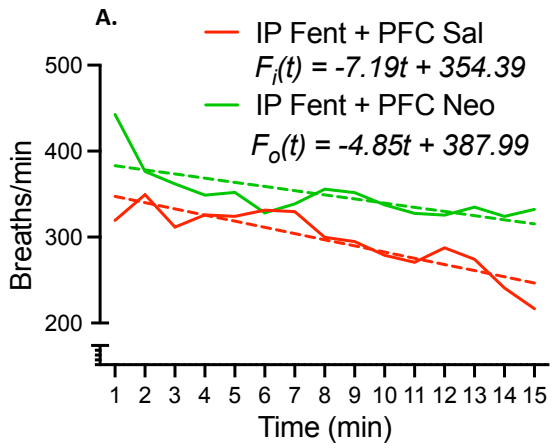
$$\alpha = 1 - \left(\frac{Fo(t) = -12.791t + 393.72}{Fi(t) = -9.7984t + 359.81}\right)$$

$$\alpha = 1 - \left(\frac{-12.791t}{-9.7984t}\right)$$

$$\alpha = -.3054$$

Fig. 7A shows that fentanyl microinjected into the prefrontal cortex caused a time-dependent decrease in respiratory rate. Fig. 7B shows the gain modulation equation, which provides alpha (α) as a quantitative expression of the decreased gain in respiratory drive caused by fentanyl.

Fig. 8A&B. From (Herzog, 2023).



B. Gain Factor of PFC Neostigmine:

$$\alpha = 1 - \left(\frac{Fo(t)}{Fi(t)}\right)$$

$$\alpha = 1 - \left(\frac{Fo(t) = -4.8458t + 387.99}{Fi(t) = -7.1897t + 354.49}\right)$$

$$\alpha = 1 - \left(\frac{-4.8458t}{-7.1897t}\right)$$

$$\alpha = +.3260$$

Fig. 8A shows that neostigmine (green lines) microinjected into the prefrontal cortex mitigated the time-dependent decrease in respiratory rate caused by systemic fentanyl (red lines). Fig. 8B quantifies the alpha (α) value (+0.3260), which shows that the gain modulation equation also quantifies a positive gain modulation of respiratory drive caused by enhancing cholinergic neurotransmission in the prefrontal cortex.

By examining pre- and post-PFC administration of either fentanyl or neostigmine, a bi-directional, time-dependent change in breathing rate was revealed (Herzog, 2023; Woods et al., 2023). The bidirectional, time-dependent changes in breathing rate caused by PFC neostigmine versus PFC fentanyl support the hypothesis that the PFC alters breathing via gain

modulation of brainstem respiratory rhythm generation.

Discussion:

The first phase of this project indicated that the administration of neostigmine into the PFC mitigated fentanyl-induced respiratory depression by increasing both breathing frequency and tidal volume. Not only did PFC neostigmine increase respiratory measures averaged over a 15-minute period, but these effects were also time-dependent. The finding that PFC neostigmine time-dependently altered breathing supports the hypothesis that the PFC is a region that provides gain modulation of breathing. This means that the PFC provides input to the respiratory control centers in the brainstem, potentially enhancing sensitivity to respiratory sensory input.

The finding that PFC fentanyl caused time-dependent breathing depression relative to PFC saline similarly supports the hypothesis that the PFC is a region that provides gain modulation of breathing. The fact that local administration of fentanyl to the mPFC caused respiratory depression similar to depressed breathing caused by systemic administration of fentanyl supports the interpretation that the PFC is a brain region contributing to OIRD. The quantification of the 'gain factor' of PFC fentanyl and neostigmine provides a quantitative index of the degree of modulation of breathing via the PFC. As shown by Fig. 5C, the fentanyl-induced decrease in minute ventilation was overcome by PFC administration of neostigmine, consistent with a cholinergically mediated wakefulness stimulus for breathing arising from the PFC.

Considered together, these results from mice demonstrate that the PFC contributes a

wakefulness stimulus for breathing that can attenuate respiratory depression caused by systemic administration of fentanyl. Depending on opioid dose and body mass, opioid-induced respiratory depression can cause intervals of periodic breathing between episodes of apnea. Periodic breathing due to decreased alertness has been implicated in exacerbating congestive heart failure and cerebrovascular disease (Cherniack & Longobardo, 2006). Clinical studies have systemically co-administered physostigmine, another acetylcholinesterase inhibitor, with morphine to preoperative patients to prevent OIRD while preserving analgesia (Bourke et al., 1984).

The results of these preliminary studies encourage future experiments exploring PFC administration of donepezil, an acetylcholinesterase inhibitor used clinically for palliative treatment of dementia (Kumar A, 2023). Experiments delivering PFC donepezil following systemic fentanyl administration may provide further support for the hypothesis that enhanced cholinergic activity in the prefrontal cortex mitigates OIRD by increasing behavioral arousal.

Connections to Future Endeavors:

Upon graduation, I will matriculate into the Vanderbilt University School of Medicine, where I plan to bridge my past experiences with my future goals as a clinical care provider and my interests in achieving a better understanding of opioid addiction. Growing up in Northeast Tennessee, I was surrounded by many community members who had participated in some form of opioid use. One neighborhood adjacent to our high school was notorious for drug abuse. It was difficult to understand the circumstances that led to this situation. Why did all these

people suffer in this way, and how did they find themselves in that situation? The question I wondered about most of all was where these opioids were coming from. I was ignorant of the scale of the addictive potential of opioids and the ease with which they could be attained.

As a volunteer at a local reduced-cost medical clinic in Knoxville, I continued to interact closely with people whose lives have been altered by opioid dependence. These interactions have provided insight into what it means to face opioid dependence and the extent to which opioid dependence can wreak havoc on relationships, potential income sources, and general well-being. The more I have learned about the impact of opioid use on a person, the more complex my understanding of opioid dependence becomes. Acknowledging these complexities will be critical for providing optimal care to people from different backgrounds and situations. One relevant way that this issue presents itself in clinical care is the increased likelihood (twice as likely) of uninsured patients to demonstrate prescription opioid misuse (Lasser, 2017). Experts hypothesize this is because buying another's prescription opioids is significantly cheaper than a clinic visit. However, the pain management of opioids is a surface-level solution to an oftentimes deep problem. Tooth decay, other chronic pains, or psychological ailments will linger and can be resolved through medical care. However, this care is not accessible to those vulnerable to opioid misuse.

In medicine, a treatment alleviates pain while limiting unnecessary adverse effects. In the field of intensive care, any imbalances between treatment and side effects can lead to long-term effects on mental status. In Dr. Wes Ely's book, *Every Deep-Drawn Breath*, he reveals the delirium and dementia that result from ICU visits (Ely, 2021). After long periods of immobility and heavy sedation, patients were unable to return to the lives they had lived. By revealing this

effect, changes could be made in the ICU to limit sedation and increase mobility. Obtaining knowledge about the effect of commonplace sedative drugs such as fentanyl or morphine on the brain, respiration, and sleep will undoubtedly aid future medical treatment.

Making associations between my endeavors in neuroscience research, clinical experience, and coursework has enhanced my understanding of what it means to suffer from drug dependence and addiction. Serving disadvantaged populations through volunteer work, I have met many people actively experiencing opioid addiction or undergoing substance abuse recovery. Making connections between basic science and clinical endeavors allows me to remain motivated and productive in my research while also incorporating my learning into better patient care. As I progress into medical school and a career as a physician, I will continue to learn through seeking understanding and connection with my patients and my community.

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