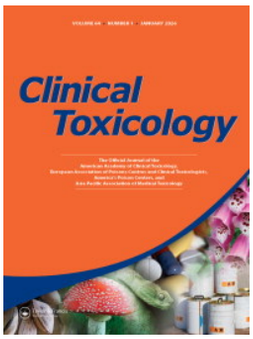

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BASIC RESEARCH

Respiratory and cardiac toxicity of xylazine and fentanyl overdose in rats

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ABSTRACT

Introduction: Xylazine, a veterinary sedative, is an adulterant increasingly found in illicit fentanyl supplies. Yet, the dose-dependent effects of xylazine on fentanyl overdose remain poorly characterized. We sought to determine how xylazine modifies fentanyl-induced mortality, respiratory depression, and cardiovascular dysfunction in rats.

Methods: Adult male and female Sprague-Dawley rats received non-lethal xylazine alone or combined with fentanyl (150 µg/kg) intravenously. For xylazine, three doses (0.1 mg/kg, 1 mg/kg, or 10 mg/kg) were chosen that produced a dose-dependent transition from a predominantly α_2-adrenergic receptor agonist to a non-specific α-adrenergic receptor agonist effect, and are consistent with reported human exposures. Ventilation and pulmonary gas exchange were measured using an open-flow plethysmograph. Cardiovascular function was assessed via telemetry and echocardiography.

Results: Bolus injection of xylazine alone at 0.1 mg/kg produced a typical profile of an α_2-adrenergic agonist transitioning to a pronounced and persistent hypertension at 10 mg/kg. Xylazine dose-dependently potentiated the toxicity of fentanyl. Xylazine at 0.1 mg/kg with fentanyl showed no mortality increase (12.5% versus 10.5% fentanyl alone) despite prolonging apnea. Of note, this dose reduced fentanyl-induced rigidity of respiratory muscles and oxygen consumption. Xylazine at 1 mg/kg dramatically increased fentanyl mortality (62.5%), with survivors showing progressive respiratory recovery while non-survivors developed cardiogenic shock secondary to respiratory failure. Xylazine at 10 mg/kg with fentanyl was universally fatal (100%), causing a unique clinical syndrome, consisting of rapid cardiogenic shock leading to a pulseless electrical activity within 2–3 min.

Discussion: Xylazine, when combined with a fentanyl overdose, has no increased mortality at 0.1 mg/kg, exacerbates hypoxemia-driven toxicity on breathing generation and cardiac function at 1 mg/kg, while producing an acute and very rapid lethal cardiocirculatory failure at 10 mg/kg.

Conclusion: Xylazine significantly potentiates fentanyl-induced respiratory depression and cardiovascular collapse in a dose-dependent manner in rats.

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Animal model; cardiac toxicity; drug overdose; fentanyl; respiratory toxicity; xylazine

Introduction

The toll of the opioid epidemic in the United States (US) has been devastating. Fueling this epidemic are vast amounts of illicitly manufactured opioids [1]. This source is inherently vulnerable to adulteration, which can dramatically alter fentanyl toxicity [2]. Xylazine, a veterinary sedative, has emerged as an adulterant of particular concern in the illicit fentanyl supply [3–5]. According to the US Drug Enforcement Administration, the presence of xylazine in seized fentanyl has increased rapidly from approximately 23% of fentanyl powder in 2022 to over 36% by 2024 [6]. The estimated number of drug-poisoning deaths involving xylazine grew from 102 in 2018 to over 3,000 in 2021 [7]. This rapid proliferation prompted the White House Office of National Drug Control Policy to declare fentanyl mixed with xylazine an emerging threat in 2023 and to release an updated implementation report in 2024 [8]. A major concern identified in this report is that xylazine has no US Food and Drug Administration-approved reversal agent. There is evidence in mice that xylazine increases the mortality associated with fentanyl [9,10]. However, there are also suggestions that xylazine is associated with decreased mortality [11], although this study had a clear

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survivorship bias by only including victims arriving alive in an emergency department. Characterizing the dose effects of xylazine on fentanyl toxicity is therefore the first crucial step before identifying effective therapeutic strategies.

Xylazine is an α_2-adrenergic receptor agonist commonly used in veterinary medicine as a sedative, analgesic, and muscle relaxant [12] but not approved for use in humans. α_2-Adrenergic receptors are found on pre- and postsynaptic neurons of the central and peripheral nervous systems [13]. Xylazine acts centrally to inhibit the release of norepinephrine and epinephrine, resulting in reduced sympathetic outflow [14]. As a result, low-dose xylazine predominantly produces sympatholytic effects [15], leading to vasodilation and decreased blood pressure [16–18]. In animals, sedative dosages of xylazine are approximately 0.5–1 mg/kg intravenously and 2–10 mg/kg intramuscularly, and doses in this range are often combined with dissociative agents (commonly ketamine) or central nervous system depressants for anesthesia [12,19].

Intoxication from xylazine alone is rare in humans [20], and there is a significant overlap between concentrations found in non-fatal and fatal (postmortem) blood samples following a xylazine overdose [20–22]. In humans, xylazine intoxication is known to produce sedation, respiratory depression, and a biphasic cardiovascular response characterized by initial vasoconstriction and hypertension, followed by central α_2-adrenergic-mediated sympatholytic effects and profound hypotension [23]. The pharmacokinetics of xylazine in humans can vary, with a reported elimination half-life of 12.0 h (range 5.9–20.8 h) [4,5]. It remains unclear how xylazine dose directly affects the respiratory and cardiovascular toxicity of fentanyl in an intravenous overdose setting.

Acute overdose of fentanyl, a potent μ-opioid receptor agonist, has three primary life-threatening effects that may be modulated by xylazine co-administration. First, the most recognized human toxicity of fentanyl is profound respiratory depression via activation of μ-opioid receptors in the medullary respiratory control centers, reducing respiratory drive [24]. In rats, rapid injection of high-dose fentanyl produces immediate central apnea lasting several minutes, followed by sustained respiratory depression that persists despite partial recovery of eupneic breathing [25], potentially leading to fatal hypoxemia [26]. Second, fentanyl overdose can induce a characteristic pattern of muscle rigidity, which dramatically reduces chest wall compliance and increases upper airway resistance through glottic closure, further compromising ventilation while simultaneously increasing oxygen (O_2) consumption due to widespread activation of skeletal muscle in humans [27,28] and rats [28,29]. Third, and perhaps most critically, our recent study in rats showed that acute fentanyl overdose causes severe cardiovascular symptoms [30]. This includes immediate and profound bradycardia, decreased cardiac output, and compromised cardiac contractility likely produced by fentanyl-induced hypoxemia, rather than by fentanyl per se. These cardiovascular effects substantially reduce O_2 delivery to vital organs that, when combined with respiratory depression and increased O_2 consumption due to muscle rigidity, can be rapidly fatal.

The present study seeks to determine how xylazine modifies known fentanyl toxicities, particularly focusing on its dose-dependent effects. We investigated the respiratory and cardiovascular responses to xylazine-fentanyl combinations, which have not been previously reported. The null hypothesis for this study was that non-fatal xylazine doses would not increase fentanyl mortality when combined. Understanding these interactions is crucial for developing targeted interventions for the growing population of individuals experiencing xylazine-fentanyl overdose, as traditional naloxone-based approaches may be insufficient to address the multi-receptor complexity of these combinations.

Materials and methods

Animal models, permissions, and ARRIVE statement

All studies were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, 8th edition, and the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines 2.0. The Institutional Animal Care and Use Committee approved the animal protocols at the Lerner Research Institute, Cleveland Clinic Foundation (Cleveland, OH). All male and female Sprague–Dawley rats (Crl: CD(SD), Strain code 001) were purchased from Charles River Laboratories (Raleigh, NC). Rats used for plethysmography alone were purchased with indwelling jugular vein catheters exteriorized from the back of the neck. A separate group of rats had implanted telemetry devices purchased from Data Sciences International (DSI, St. Paul, MN), which were implanted by Charles River Laboratories and were equipped with tail vein catheters on the day of the study. Rats used in echocardiography studies were a mix of rats with jugular vein catheters and telemetry rats with tail vein catheters. Rats were allowed at least seven days to recover from transport before studies.

Studies consisted of two distinct approaches in two groups of rats: i) ventilatory and gas exchange measurement (with and without telemetric blood pressure recording) and ii) echocardiography. A total of 86 experiments were conducted using 36 male and female rats, weighing 404 ± 92 g, across all experimental conditions. In accordance with the Three Rs principles of Replacement, Reduction, and Refinement [31] – particularly Reduction – some rats were used more than once, and no more than four times, to minimize the number of animals required for this study. All animals underwent a recovery period of at least seven days between treatments, during which they were closely monitored and assessed by a veterinarian to confirm full restoration of health and normal behavior before being considered for reuse. We performed 64 plethysmography experiments: 27 rats were studied once, 23 were studied twice, 13 were studied three times, and one rat was studied four times. Twenty-two echocardiogram experiments were performed: two rats were studied once, nine were studied twice, eight were studied three times, and three rats were studied four times. No rats were used for the same drug protocol more than once. It has been shown that tolerance does not develop for fentanyl-induced unconsciousness, muscle rigidity, or respiratory depression when given twice a week [32], which is consistent with our experience with this model. Rats were monitored daily after each experiment to ensure, within two to three days, that they had gained weight and demonstrated normal behavior. If normal behavior was observed, rats were randomly assigned to one of the remaining experimental conditions.

Drugs and dose justification

Drugs used in the study were fentanyl (fentanyl citrate injection [50 µg/mL], Hospira, Inc., USA) and xylazine (Rompun injection, 100 mg/mL, Dechra). Dilutions of xylazine were made with sterile saline. We have previously investigated the effects of fentanyl overdose (50–300 µg/kg) in rats and established that 150 µg/kg reliably induces respiratory depression and cardiovascular dysfunction while maintaining survivability in the majority of animals without intervention [29,33]. Therefore, fentanyl at 150 µg/kg was the dose used in this study, administered intravenously over 15 sec.

Xylazine doses of 0.1 mg/kg, 1 mg/kg, and 10 mg/kg fall within the established therapeutic range in veterinary practice [15]. These doses were selected based on pilot studies with xylazine alone that demonstrated effects on breathing and blood pressure consistent with a pharmacological transition from a selective α_2-adrenergic agonist to loss of receptor specificity (as described in the results). To provide context regarding whether these doses were representative of human xylazine exposure, we applied US FDA-recommended allometric scaling [34]. The rat xylazine doses of 0.1–10 mg/kg translate to human equivalent doses of 0.016–1.6 mg/kg using a conversion factor of 0.162 (human equivalent dose = rat dose × 0.162). Published human xylazine blood concentrations range from 0.003–2.8 mg/L [20–22], suggesting relevance, though direct dose comparison between species is difficult.

Plethysmography setup

On the day of the study, unrestrained rats were placed inside a custom-designed, leak-proof acrylic plethysmographic chamber (1.4 L volume) to measure ventilation [25,29,30]. Airflow was delivered via a precision rotameter to the inlet port of the chamber (flow of ~1.5 L/min) from a tank of compressed medical-grade air (Linde Gas, Danbury, CT). Both inlet and outlet ports were connected to independent bi-directional screen pneumotachs (Series 8421, Hans Rudolph, Shawnee, KS) via non-compliant tubing and linked to a pressure transducer (Pneumotach amplifier 1, Series 1110, Hans Rudolph) to measure gas flow. Each pneumotachograph was independently calibrated by passing a fixed volume of air via a syringe and integrating the flow signal to establish flow over time before each experiment. The sensors were then placed in a circuit with the empty chamber, and the rotameter was adjusted to 25 mL/sec using the two flow signals to ensure the system did not leak. Semi-quantitative measurements of tidal volume and minute ventilation were calculated using the outlet flow signal, and frequency was obtained from the raw data after filtering the signal, as described below.

Gas was sampled from the chamber outlet and analyzed using a Gemini Respiratory Analyzer (CWE, Inc.) to measure the fractions of carbon dioxide (CO_2) and O_2. These were used to calculate the rate of O_2 consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) using the outlet flow (mL/sec), the gas composition from the tank ($F_{in}O_2$, $F_{in}CO_2$), and the outflow gas ($F_{out}O_2$, $F_{out}CO_2$), which was determined at the beginning and verified at the end of the experiments to identify any drift in our analyzers. $\dot{V}O_2$ and $\dot{V}CO_2$ were computed as follows:

$$\dot{V}CO_2\,(\mathrm{mL/min}) = \text{outflow rate}\,(\mathrm{mL/sec}) * (F_{out}CO_2 - F_{in}CO_2) * (60/100),$$

$F_{in}CO_2$ is zero

$$\dot{V}O_2\,(mL/min) = \text{outflow rate}\,(mL/sec) * (F_{in}O_2 - F_{out}O_2) * (60/100)$$

For these calculations, the flow was expressed in standard temperature, pressure, and dry conditions. Analog signals were recorded using a digital data acquisition system (PowerLab/LabChart; AD Instruments, Colorado Springs, CO).

Rats were acclimated in the plethysmograph chamber for at least 30 min to establish baseline breathing recordings. The rats were then briefly removed to connect the venous catheter to a luer-lock stopcock valve embedded in the wall of the chamber via a polyethylene extension line. A syringe was connected to the portion of the stopcock external to the chamber. The rats were returned to the sealed chamber. Drugs were then pushed over 15 sec, and 1 mL of saline was flushed through the line over another 15 sec to ensure all the drug was delivered to the rats.

Telemetry setup

For continuous blood pressure monitoring, a subset of rats had implanted telemetry devices (HD-S10) purchased from DSI and implanted by Charles River Laboratories. These devices enabled the real-time measurement of blood pressure while the animals were in the plethysmography chamber, while simultaneously recording breathing. The signal was detected by the receiver (RPC-1), which was then acquired by the signal interface (MX2), along with an ambient pressure reference (APR-2), and fed into the PowerLab (ADInstruments) for recording and further analysis.

Echocardiography setup

To reduce the number of animals used, we incorporated data generated previously in our laboratory under identical experimental conditions (species, strain, sex, age, housing, diet, procedures, and endpoints). Six of the 10 rats in the baseline group and four of the six rats in the fentanyl group were previously published [30]. Echocardiography was performed by an echocardiographer with experience in rodent studies, as described in our previous publication [30]. Cardiac function was evaluated by transthoracic echocardiography in rats. Two-dimensional and M-mode studies were performed with a GE Logic-e ultrasound system, using an 8C-RS curved array ultrasound transducer with a frequency of 3.5–10.0 MHz, typically designed for pediatric studies. Left longitudinal parasternal views were obtained, and the settings were optimized to obtain the best image quality, adjusting the focus between 1 cm and 2 cm from the surface of the transducer. M-mode was used because of its excellent time resolution in determining changes in left ventricular dimension or contractility. Images were obtained at mid-left ventricle to allow a clear visualization of LV anterior and posterior wall endocardial borders, avoiding papillary muscles and mitral chordae. Simultaneous two-dimensional and M-mode imaging of the left ventricle was obtained throughout the experimentation. Ten-second clips were recorded and stored for subsequent analysis with GE software on the machine.

For baseline echocardiography parameters, rats were briefly anesthetized with isoflurane, and baseline parameters were determined on a separate day. For drug administration studies, rats received the designated treatment via intravenous (IV) injection, and echocardiography was performed continuously to capture the dynamic changes in cardiac function.

Signal processing and data analysis

Respiratory Waveform Analysis: Raw plethysmographic signals underwent standardized signal processing to optimize signal-to-noise ratio. A band-pass filtration algorithm was applied with parameters optimized for respiratory signal isolation: low-frequency cutoff >0.1 Hz (eliminating direct current component) and high-frequency cutoff at 20 Hz (attenuating cardiac artifacts and ambient noise). The filtered signal provided the basis for quantification of respiratory rate and minute ventilation. To obtain minute ventilation from the filtered flow signal, positive deflections in the plethysmographic trace were integrated over 5 sec intervals. The result of this integration was then corrected, according to Boyle's law, for computation of the actual minute ventilation, as previously described [25,26,29,30]. This determination of minute ventilation should be considered as semiquantitative, represented in the same units as are appropriate for direct measurements of ventilation. Tidal volume was calculated as minute ventilation divided by frequency.

Traces underwent a further subjective analysis under blinded conditions. Apneic episodes were quantified using standardized criteria defined as the temporal interval required for reestablishment of regular respiratory rhythm [25,26,29,30]. Expiratory events were visually identified from the raw flow signal, and for each recording, were marked by any event that began with a negative inflection.

Telemetric pressure waveform analysis

The ambient pressure was subtracted from the raw pressure signal, leaving the measurement of blood pressure.

That signal was further analyzed in LabChart to determine the mean pressure, heart rate, and pulse pressure.

Echocardiographic image analysis

Echocardiographic data underwent blinded analysis by an echocardiographer with experience in rodent studies, as in our previous publication [30]. Left ventricular dimensions were quantified from M-mode tracings with measurement positions guided by corresponding two-dimensional images to ensure standardized anatomical localization at the mid-ventricular level. For each measurement interval, three to five consecutive cardiac cycles were analyzed with resultant values averaged to minimize beat-to-beat variability.

Derived cardiac functional parameters included:

- Ejection fraction (%), calculated via the Teichholz formula for ventricular volume estimation from linear dimensions.
- Cardiac output (mL/min/kg) derived from stroke volume and heart rate measurements and normalized to body weight.

Definition of relevant terms

Apnea

A period wherein eupneic breathing is absent, which we recently described in more detail [25]. During this period, two types of pathological respiratory events can still be observed following fentanyl: gasps and rhythmic expiratory activity, which are further defined below.

Gasps

Large inspiratory activity produced at a very slow rhythm with a short inspiratory time and high inspiratory flow [35,36]. They can be identified on the respiratory flow signal by their amplitude and very short duration. They typically occur during a period of apnea and are known to improve blood oxygenation and flow [37,38].

Expiratory activity

This pattern is easily identified on the respiratory flow signal and consists of biphasic signals starting with a negative (expiratory) deflection in flow. This activity is typically observed after a rapid IV injection of fentanyl, during the ensuing apnea [25,29,33,39].

Recovery from apnea

In contrast to gasps, eupneic breaths have a longer inspiratory time and a lower peak inspiratory flow [40]. However, fentanyl has multiple effects on breathing systems that complicate breathing patterns [41]. As such, the presence of rhythmic and regular breathing activity, typically with a low inspiratory and expiratory flow, sustained for at least 15 sec, was considered a recovery from apnea.

Statistical analysis

Data distributions were evaluated for normality and are presented as mean ± standard deviation (SD). Time-series data for ventilatory parameters are presented as mean ± standard error of the mean (SEM) with 15 sec or 1 min averaging intervals. Statistical comparisons between experimental conditions at defined time points employed two-way analysis of variance (ANOVA) with post-hoc multiple comparisons using Dunnett's multiple comparisons test. The threshold for statistical significance was $P<0.05$ for all analyses.

Results

Mortality due to intravenous xylazine, fentanyl, and a combination of xylazine and fentanyl

The primary outcome of this study was mortality, shown in Figure 1, which includes all 86 experiments from both the plethysmography/telemetry and echocardiography studies. Xylazine alone (0.1 mg/kg, 1 mg/kg, and 10 mg/kg) was tested in 33 individual experiments, with 100% survival. Fentanyl alone (150 µg/kg) was tested in 19 experiments, resulting in two deaths (10.5% mortality). When fentanyl was combined with xylazine at 0.1 mg/kg, one of eight rats died (12.5% mortality). Fentanyl with xylazine at 1 mg/kg was fatal in 10 of 16 experiments (62.5% mortality). Fentanyl with xylazine at 10 mg/kg was fatal in all 10 animals tested (100% mortality).

Effects of increasing doses of xylazine alone

Effects of xylazine on ventilation

Intravenous bolus injections of xylazine produced dose-dependent respiratory depression in freely moving, non-anesthetized rats, with representative examples shown in Figure 2. The effects on minute ventilation, frequency, and tidal volume over 30 min are shown in Figure 3. Xylazine at 0.1 mg/kg ($n=7$) induced a mild, transient reduction in minute ventilation, reaching a nadir of 93 ± 9.9 mL/min at 7 min post-injection from baseline values of 129 ± 24.5 mL/min. Minute ventilation gradually recovered by 15 min and remained stable through 30 min (Figure 4). This reduction was primarily due to a decreased breathing

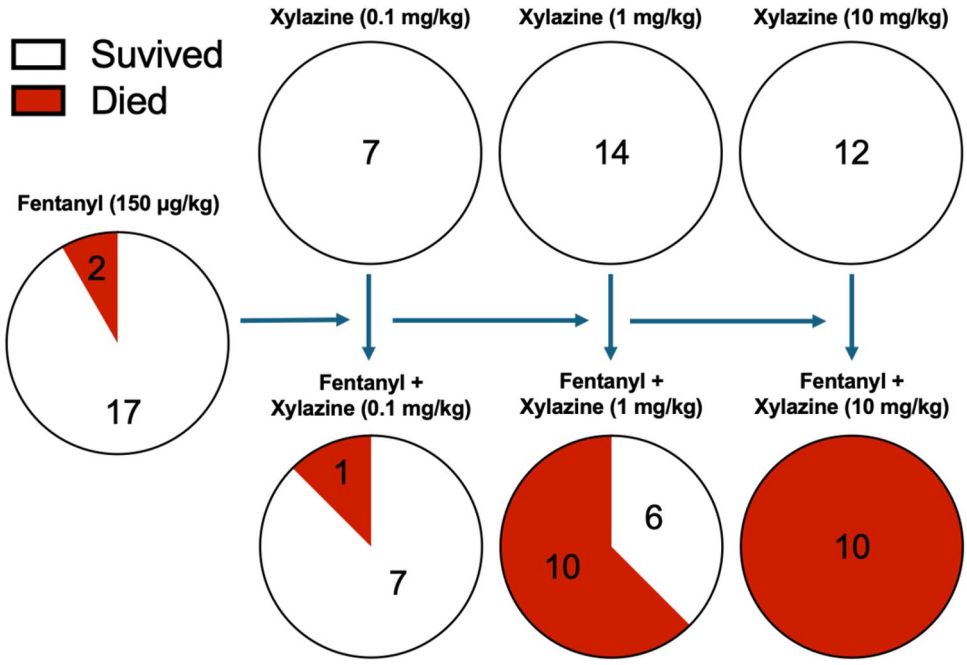

Figure 1. Xylazine dose-dependently increases the mortality associated with fentanyl. Fentanyl, xylazine, or a combination was intravenously administered to Sprague Dawley rats a total of 86 times. The cumulative mortality is reported here. Xylazine alone at 0.1 mg/kg ($n = 7$), 1 mg/kg ($n = 14$), and 10 mg/kg ($n = 12$) was not fatal in any individual experiment (0% mortality). Fentanyl alone at 150 µg/kg (n = 19) resulted in 2 deaths (10.5% mortality). When fentanyl was combined with xylazine at 0.1 mg/kg ($n = 8$), 1 rat died (12.5% mortality). Fentanyl with xylazine at 1 mg/kg ($n = 16$) was fatal in 10 experiments (62.5% mortality). Fentanyl with xylazine at 10 mg/kg ($n = 10$) was fatal in all 10 animals tested (100% mortality).

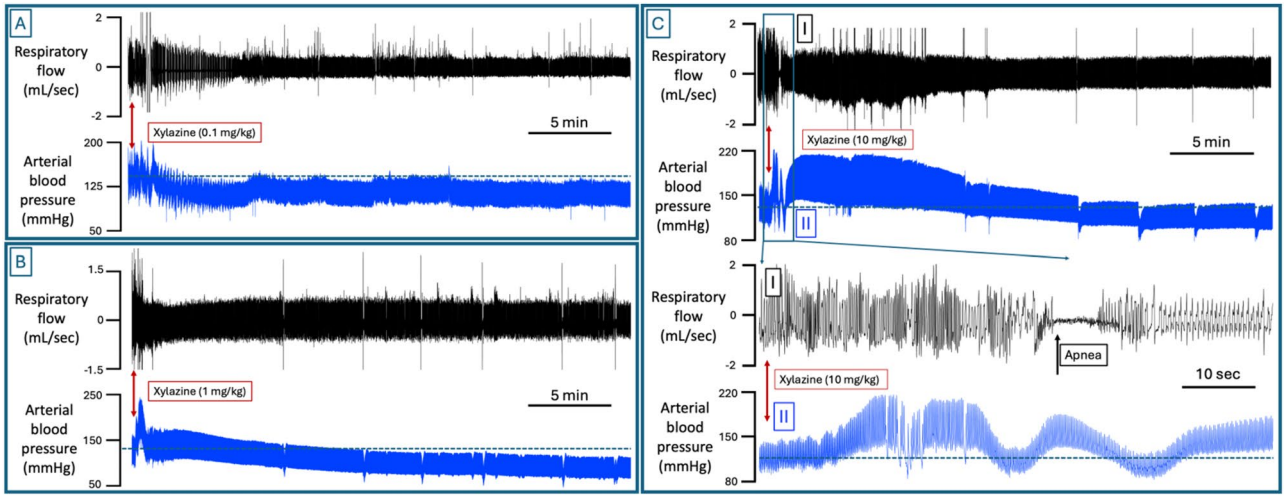

Figure 2. Representative examples of the effects of increasing intravenous xylazine doses on breathing and blood pressure in rats. Xylazine at 0.1 mg/kg (panel A) caused a mild reduction in respiratory flow and a brief, transient increase in arterial blood pressure, followed by a sustained hypotension. Xylazine at 1 mg/kg (panel B) produced more pronounced respiratory depression and a more pronounced hypertension that also resolved and was followed by a sustained hypotension. Xylazine at 10 mg/kg (panel C) also induced respiratory depression, now with brief apnea indicated with an arrow. This dose caused a pronounced hypertension, which took much longer to resolve and showed late or no subsequent hypotension. These traces are preprocessed plethysmography data.

frequency of 65 ± 6.1 breaths/min from baseline 120 ± 38.5 breaths/min, with minimal reduction in tidal volume. No apnea occurred at this dose. The increased variability over time likely reflects minimal sedation produced by this dose, as rats were observed to be conscious and sometimes moving during this time (not quantified). Xylazine at 1 mg/kg ($n = 9$) produced more pronounced respiratory depression without immediate apnea (representative example in Figure 2). The mean (±SEM) minute ventilation decreased rapidly

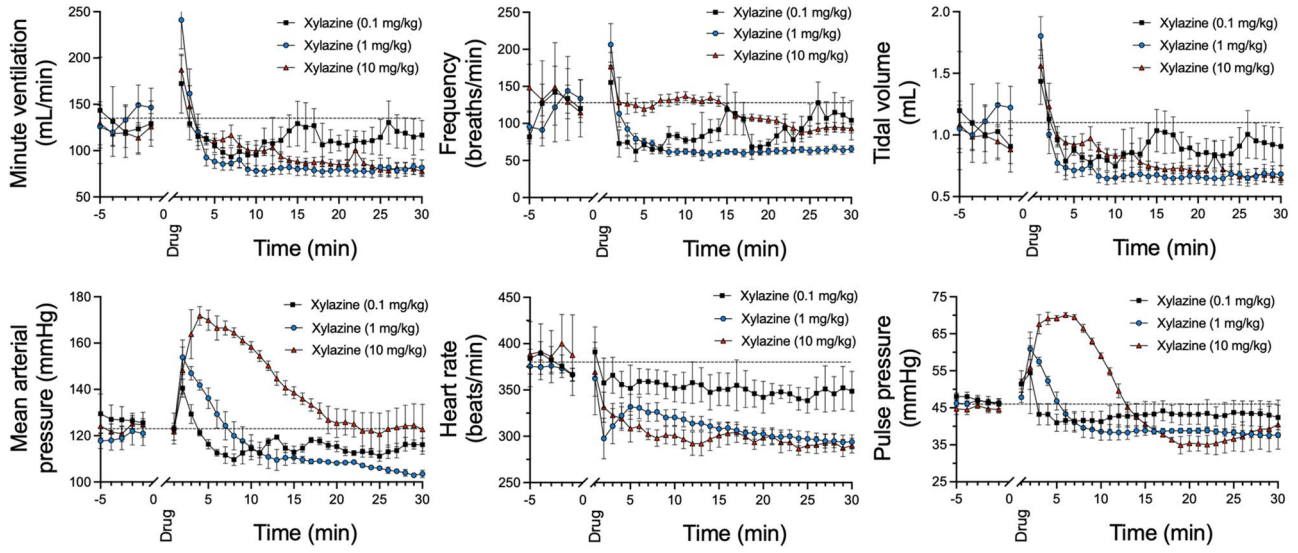

Figure 3. Averaged effects of increasing doses of intravenous xylazine on breathing and cardiovascular function in non-sedated rats. All xylazine doses cause respiratory depression, but a dose-dependent shift from brief hypertension followed by hypotension to sustained hypertension at higher doses in rats. Changes over time in minute ventilation (mL/min), frequency (breaths/min), tidal volume (mL), mean arterial pressure (mmHg), heart rate (beats/min), and pulse pressure (mmHg) in non-sedated, freely moving rats after injection of xylazine at 0.1 ($n=7$), 1 ($n=9$), or 10 ($n=9$) mg/kg. The dotted horizontal line indicates the 5 min average before the injection of xylazine. The break in the x-axis indicates where animals were removed from the chamber to attach the catheter for injections. Data are averaged every 1 min and are presented as means ± standard error of the mean.

to 78 ± 5.8 mL/min at 10 min post-injection, from a baseline of 146 ± 20.9 mL/min, then remained significantly below baseline throughout the 30 min observation period (Figures 3 and 4). This reduction resulted from a substantially reduced mean (±SEM) frequency of 62 ± 3.9 breaths/min at 10 min, from a baseline of 133 ± 25.7 breaths/min, which remained depressed for the remainder of the 30 min. Tidal volume was not reduced and exceeded that observed with 0.1 mg/kg by the end of the observation period.

The highest dose of xylazine, 10 mg/kg ($n=9$), also induced respiratory depression. In addition, a brief apnea occurred at a mean (±SD) time after injection of 36 ± 6.9 sec, lasting 14 ± 10 sec (a representative example is shown in Figure 2(C and I)). The mean (±SEM) minute ventilation gradually decreased to 88 ± 5.0 mL/min at 15 min from a baseline of 125 ± 14.2 mL/min. Unlike lower doses, this effect was primarily due to mean (±SEM) reduced tidal volume (0.8 ± 0.09 mL at 12 min, from baseline 1.3 ± 0.10 mL) (Figures 3 and 4). In stark contrast to lower doses, frequency remained at baseline through 15 min, then gradually declined to to a mean (±SEM) 87.7 ± 8.3 breaths/min at 24 min from a baseline of 115 ± 13.4 breaths/min.

Effects of xylazine on cardiovascular function

Representative examples are shown in Figure 2. Low-dose xylazine (0.1 mg/kg, $n=3$) induced a transient increase in blood pressure, with mean (±SD) arterial pressure peaking at 140 ± 3.9 mmHg at 2 min post-injection, from a baseline of 125 ± 1.7 mmHg (Figure 3). Mean (±SD) arterial pressure then fell below baseline to 109 ± 2.5 mmHg at 8 min, remaining depressed thereafter, though only significantly so during the 20–30 min period (Figure 4). Heart rate and pulse pressure were not significantly affected.

Xylazine at 1 mg/kg ($n=3$) produced a more pronounced initial increase in blood pressure, reaching a mean (±SD) of 154 ± 7.5 mmHg within 2 min of injection, from a baseline of 126 ± 4.3 mmHg. This hypertension took longer to resolve, remaining significantly elevated for 10 min before dipping below baseline, remaining depressed for the rest of the observation period (Figure 4). Heart rate was depressed throughout the 30 min, and pulse pressure showed gradual depression. Echocardiography ($n=5$) revealed a significant reduction in cardiac output (Figure 5(A)) and ejection fraction (Figure 5(B)) during the 30 min observation period compared to baseline.

Xylazine at 10 mg/kg ($n=3$) produced a profound and sustained hypertension, with mean (±SD) arterial pressure reaching 172 ± 4.0 mmHg and remaining elevated throughout the observation period (Figure 4). Unlike lower doses, this hypertension did not resolve, and no subsequent hypotension was observed. Heart rate remained significantly reduced after the injection. However, there was a large, immediate increase in pulse pressure that corresponded with the increase in

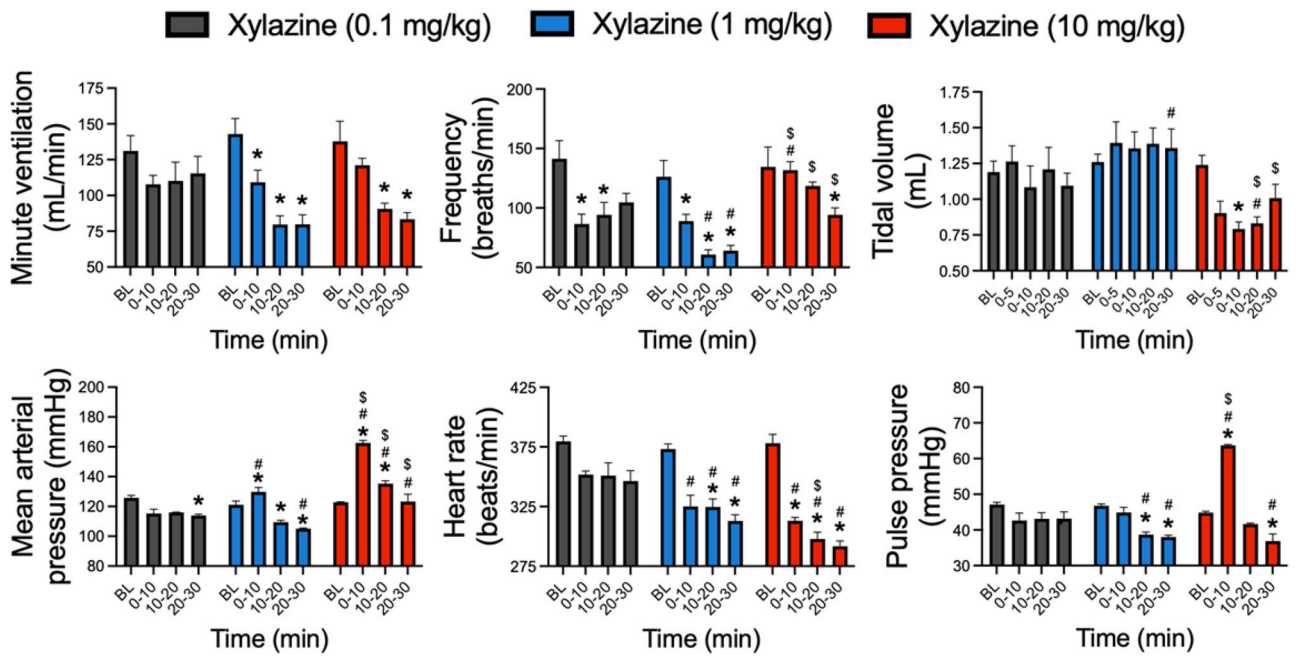

Figure 4. Statistical comparisons of the breathing and cardiovascular effects of bolus injections of xylazine in non-anesthetized rats. Changes over time in minute ventilation (mL/min), frequency (breaths/min), tidal volume (mL), mean arterial pressure (mmHg), heart rate (beats/min), and pulse pressure (mmHg) in non-sedated, freely moving rats after injection of xylazine at 0.1 ($n=7$), 1 ($n=9$), or 10 ($n=9$) mg/kg. The baseline (BL) was averaged over 5 min before xylazine administration. Subsequent intervals are the responses averaged over 0–10, 10–20, and 20–30 min after injection. The data are shown as means ± standard deviation. *$P<0.05$, a significant change from baseline (BL) values within the group. #$P<0.05$, treatment versus 0.1 mg/kg at the corresponding time point, and \$$P<0.05$, 10 mg/kg versus 1 mg/kg at the corresponding time point.

mean (±SD) arterial pressure, reaching 70 ± 0.7 mmHg from a baseline of 45 ± 0.9 mmHg. This resolved and dipped below baseline after 20 min. Echocardiography ($n=3$) revealed similar depression in cardiac output (Figure 5(A)) and ejection fraction (Figure 5(B)) compared to 1 mg/kg.

Effects of fentanyl alone

We have published extensive reports on the effects of high-dose fentanyl on both breathing [25,26,29,33] and cardiovascular function [30]. While the ventilatory and blood responses were obtained in separate groups of animals, the echocardiography studies on baseline values and fentanyl alone have been re-analyzed using the recording obtained for that study to reduce the number of animals used [30].

Effects of fentanyl on breathing and gas exchange rate

Fentanyl bolus (150 µg/kg intravenous) produced apnea in all rats ($n=14$), consistent with previous findings [25,26,29]. Apnea onset occurred at a mean (±SD) time of 14 ± 2.7 sec post-injection. Spontaneous eupneic breathing resumed in 13 of 14 experiments (93%) after 217 ± 55 sec. One rat failed to recover and was excluded from further combined analysis. Expiratory activity interrupted apnea in all 13 experiments in which the rats recovered, characterized by a mean (±SEM) of 22 ± 14 expiratory events beginning at a mean (±SD) time of 68 ± 18 sec post-fentanyl, lasting 95 ± 45 sec (representative example in Figure 6(A and I)). Also consistent with previous reports, fentanyl depressed minute ventilation and frequency throughout the observation period and caused transient depression of tidal volume (Figures 6(B) and 7). Both $\dot{V}CO_2$ and $\dot{V}O_2$ were sustained or increased after the period of apnea compared to baseline (Figure 8). As a result, the ventilatory equivalent for O_2 ($\dot{V}E/\dot{V}O_2$) and for CO_2 ($\dot{V}E/\dot{V}CO_2$) were depressed throughout the recovery period (Figure 8).

Effects of fentanyl on cardiovascular function

Though fentanyl ($n=5$) did not cause a statistically significant difference in mean arterial pressure in these animals (Figure 6(B)), mean (±SD) heart rate was transiently depressed to 126 ± 14.2 beats per min from baseline 340 ± 20.5 beats per min, returning to baseline after 15 min. Mean (±SD) pulse pressure substantially increased from baseline 40 ± 3.8 mmHg to 78 ± 5.0 mmHg at 5 min, remaining significantly elevated for 20 min (Figure 7). Fentanyl ($n=6$) transiently

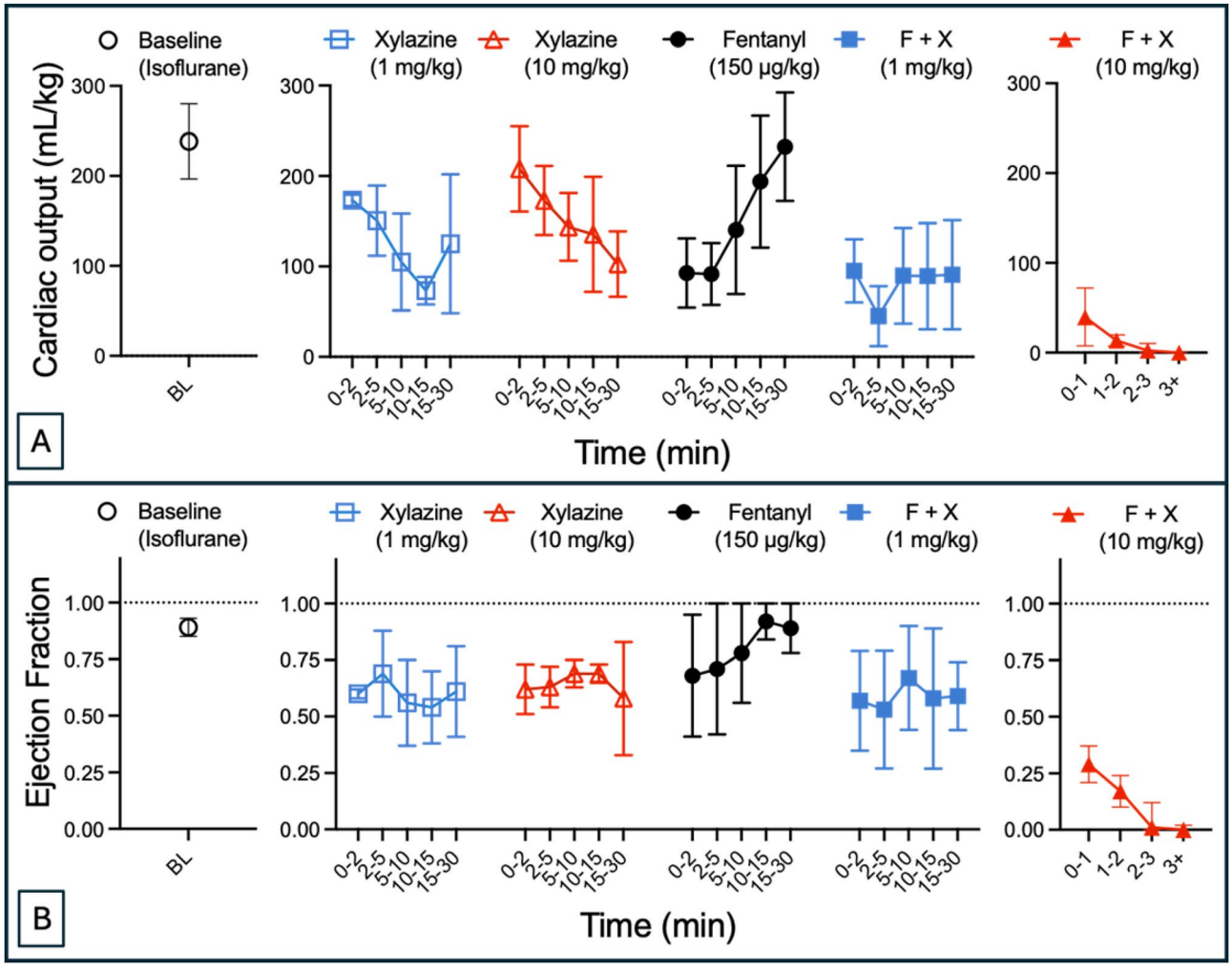

Figure 5. Effects of xylazine (X), fentanyl (F), and the combination on cardiac parameters computed from echocardiographic data. Baseline measurements were taken with animals under brief isoflurane anesthesia ($n=10$). In a separate group of animals, bolus of xylazine at 1 mg/kg ($n=3$) or 10 mg/kg ($n=3$), fentanyl at 150 µg/kg ($n=6$), fentanyl mixed with xylazine at 1 mg/kg ($n=5$), or 10 mg/kg ($n=3$) was administered intravenously, and echocardiography recorded. All but fentanyl and xylazine (10 mg/kg) were observed for 30 min. One animal receiving fentanyl, two receiving fentanyl and xylazine (1 mg/kg), and all three receiving fentanyl and xylazine (10 mg/kg) died. The data are shown as means ± standard deviation. *$P<0.05$, a significant change from the corresponding baseline (BL) value.

depressed cardiac output to a mean (±SD) of 92 ± 34.2 mL/min from baseline 238 ± 41.7 mL/min ($n=10$) at 5 min and created large variability in ejection fraction for the first 10 min, which resolved to baseline by 15 min (Figure 5).

Effects of fentanyl-xylazine on ventilation and gas exchange rate

Effects of fentanyl and xylazine at 0.1 mg/kg on ventilation and gas exchange rate

Like fentanyl alone, fentanyl mixed with xylazine at 0.1 mg/kg ($n=8$) caused prolonged apnea starting at a mean (±SD) time of 15 ± 3.9 sec post-injection. Eupneic breathing resumed after a mean (±SD) time of 449 ± 127 sec, representing a 107% increase from fentanyl alone. Unlike fentanyl alone, no expiratory events occurred during apnea (Figure 9(A)), except in the one animal that died (Figure 9(B)), which never resumed normal eupneic breathing. After 5 min, minute ventilation was more depressed than with fentanyl alone, while frequency and tidal volume were similar, except for tidal volume at 20–30 min. Both $\dot{V}O_2$ and $\dot{V}CO_2$ were significantly reduced compared to both baseline and fentanyl alone, while $\dot{V}E/\dot{V}O_2$ was depressed immediately post-injection and at 20–30 min and $\dot{V}E/\dot{V}CO_2$ was depressed compared to baseline.

Effects of fentanyl and xylazine at 1 mg/kg on ventilation and gas exchange rate

Fentanyl with xylazine at 1 mg/kg ($n=11$) caused immediate apnea in all animals starting at a mean (±SD) time

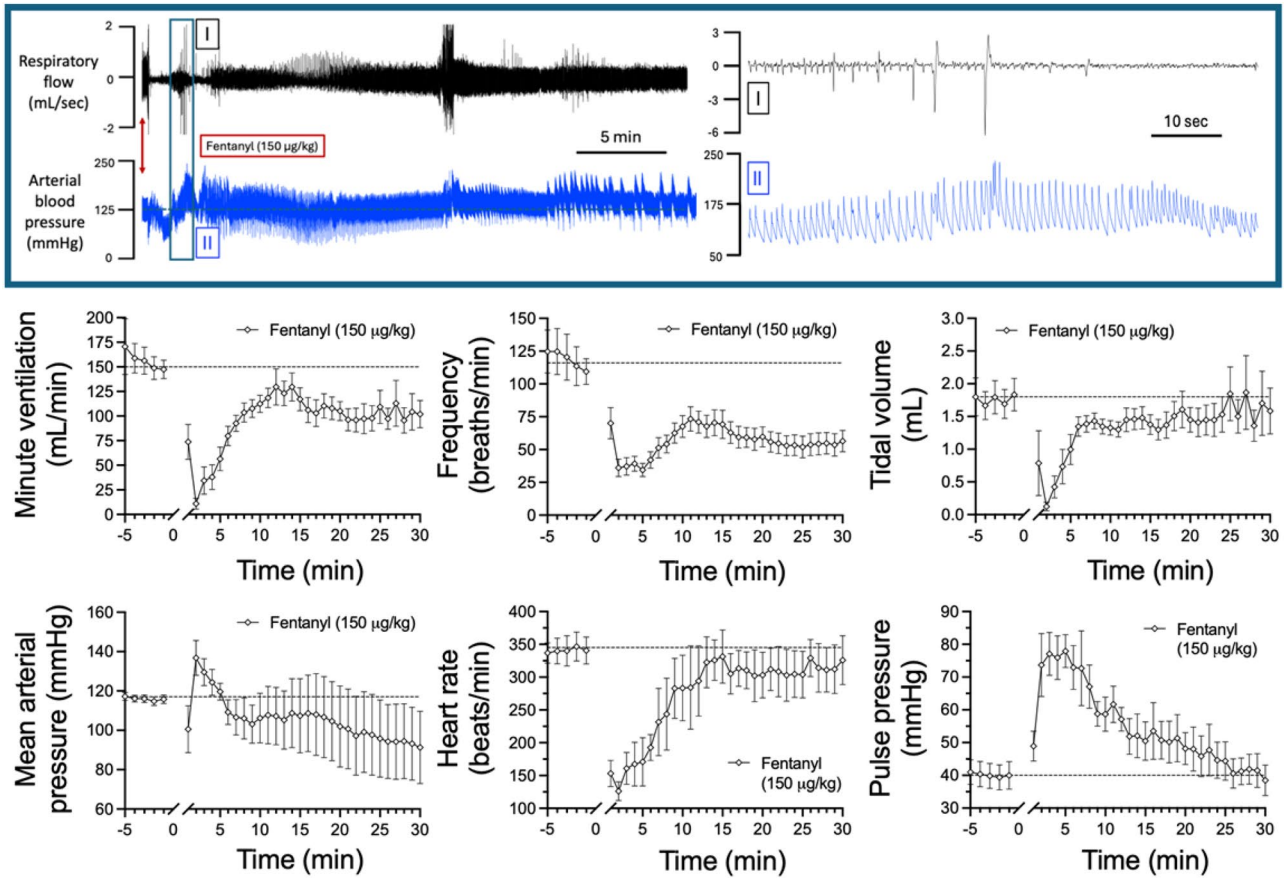

Figure 6. Representative examples and averaged effects of fentanyl overdose on breathing and cardiovascular function in non-sedated rats. Fentanyl (150 µg/kg) caused immediate apnea, expiratory activity (expanded on the right side of the panel), a sustained reduction in respiratory flow (mL/sec), and a brief, transient increase in arterial blood pressure (mmHg), followed by sustained hypotension. These traces in the top panel are preprocessed plethysmography data The bottom panel shows changes over time in minute ventilation (mL/min), frequency (breaths/min), tidal volume (mL), mean arterial pressure (mmHg), heart rate (beats/min), and pulse pressure (mmHg) in non-sedated, freely moving rats after injection of fentanyl ($n = 13$). One animal did not survive and was excluded from the combined analysis. The dotted horizontal line indicates the 5 min average before the injection of xylazine. The break in the x-axis indicates where animals were removed from the chamber to attach the catheter before fentanyl injection. Data are averaged every 1 min and are presented as means ± standard error of the mean.

of 14 ± 4.3 sec post-injection, but produced two distinct breathing patterns. In surviving rats ($n = 4$), it took a mean (±SD) time of 167 ± 79.3 sec for eupneic breathing to resume (Figure 10(A)). Three of four survivors showed expiratory activity beginning at a mean (±SD) time of 57 ± 32 sec post-injection, with a mean (±SD) of 19 ± 3.6 events lasting a mean (±SD) time of 112 ± 60 sec. In non-surviving rats ($n = 7$), breathing activity (gasps and augmented breaths) occurred, but eupneic breathing never resumed (Figure 10(B)). These rats showed similar expiratory activity (beginning at a mean (±SD) time of 55 ± 22 sec post-injection, a mean (±SD) number of 20 ± 4.4 events lasting a mean time (±SD) 92 ± 76 sec). Last detectable breathing occurred at a mean (±SD) time of 536 ± 262 sec post-injection.

During the initial 10 min, minute ventilation, frequency, and tidal volume showed little difference between the fentanyl with xylazine at 0.1 mg/kg or 1 mg/kg (Figure 11). Overall, minute ventilation was more depressed than fentanyl alone at all timepoints after 5 min, but similar to the 0.1 mg/kg group (Figure 7). A clear divergence emerged when survivors and non-survivors were analyzed separately (Figure 12). At 8 min post-injection, both groups had similar minute ventilation: mean (±SEM) of 79 ± 6.5 mL/min (survived) versus 78 ± 25 mL/min (died). By 10 min, minute ventilation in survivors increased to a mean (±SEM) of 138 ± 18 mL/min, then stabilized at 81 ± 7.7 mL/min (20 min) and 71 ± 14 mL/min (30 min). In non-survivors, minute ventilation dropped to a mean (±SEM) of 27 ± 12 mL/min at 10 min and continued declining, with no detectable breathing in any animal by 17 min. Survivors showed lower $\dot{V}O_2$ and $\dot{V}CO_2$ than fentanyl alone beyond 5 min but higher $\dot{V}E/\dot{V}CO_2$ than both fentanyl and fentanyl + xylazine (0.1 mg/kg) groups (Figure 8). An increase in $\dot{V}E/\dot{V}O2$ was also observed, but only during the 0–5 min period, compared to both groups.

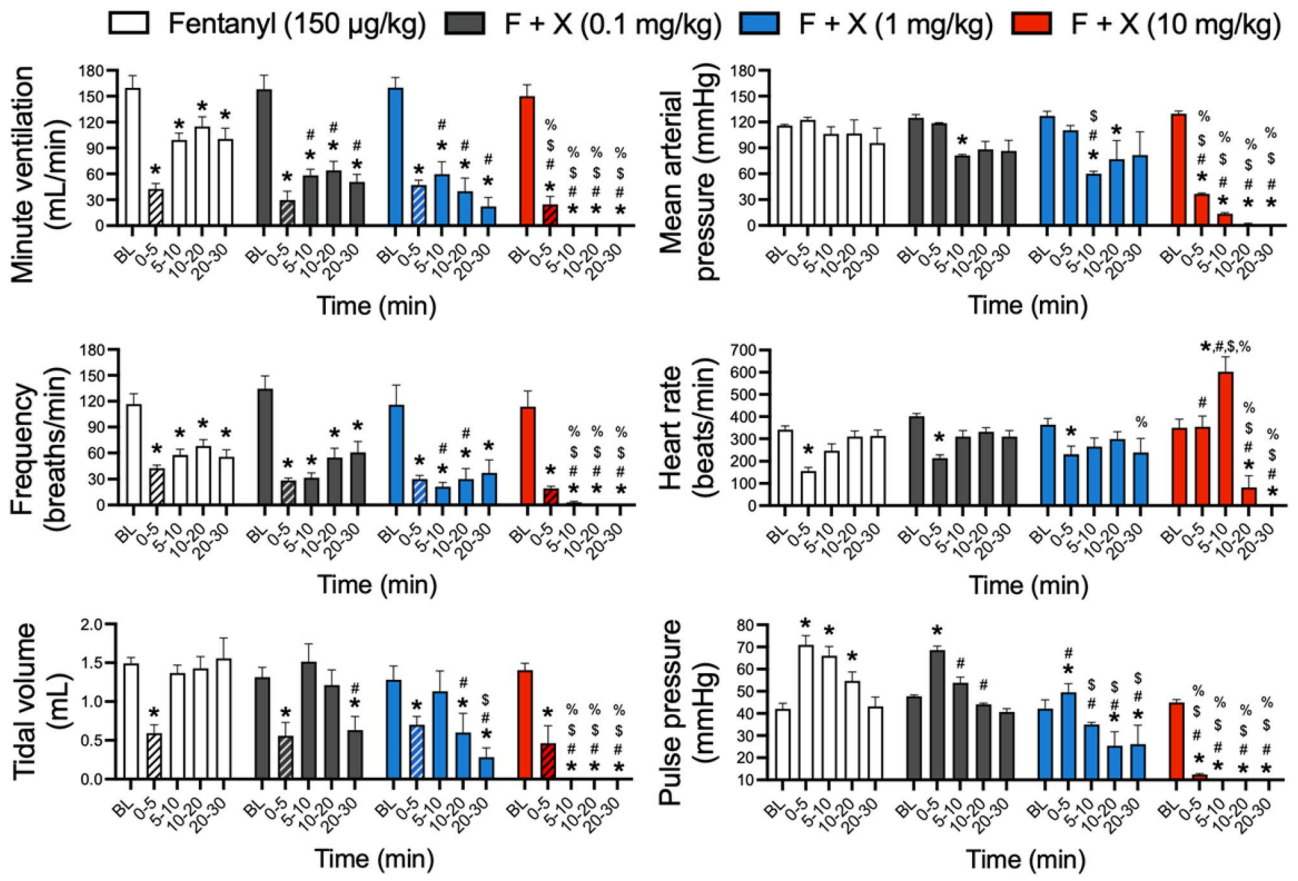

Figure 7. Statistical comparisons of the breathing and cardiovascular effects of bolus injections of fentanyl alone and fentanyl with xylazine at 0.1 mg/kg, 1 mg/kg, or 10 mg/kg in non-anesthetized rats. Changes over time in minute ventilation (mL/min), frequency (breaths/min), and tidal volume (mL), mean arterial pressure (mmHg), heart rate (beats/min), and pulse pressure (mmHg) in non-sedated, freely moving rats after injection of fentanyl (F, 150 µg/kg, $n = 13$), and fentanyl mixed with xylazine (X) at 0.1 ($n = 8$), 1 ($n = 11$), or 10 ($n = 7$) mg/kg. The baseline (BL) was averaged over 5 min before xylazine administration. Subsequent intervals are the responses averaged over 0–5 10, 10–20, and 20–30 min after injection. Note that the 0–5 min interval (hatched bars) indicates that the period includes apnea, recovery, as well as non-eupneic breaths (expiratory activity and gasps). The data are shown as means ± standard deviation. *$P < 0.05$, a significant change from baseline (BL) values within the group. #$P < 0.05$ treatment versus fentanyl, $$P < 0.05$ treatment versus 0.1 mg/kg, %$P < 0.05$ 10 mg/kg versus 1 mg/kg, each at the corresponding time point.

Effects of fentanyl and xylazine at 10 mg/kg on ventilation and gas exchange rate

Fentanyl with xylazine at 10 mg/kg ($n = 7$) was rapidly lethal in all animals. A typical example of this lethality is shown in Figure 13. Consistent with other doses, apnea began at a mean (±SD) time of 15 ± 3.5 sec post-injection, but no animal regained eupneic breathing. Six of seven animals showed brief expiratory activity beginning at a mean (±SD) time of 40 ± 11 sec post-injection, lasting only 12 ± 8.5 sec. Last discernible breathing (including gasps or augmented breaths) occurred at a mean (±SD) time of 65 ± 14 sec post-injection. Consequently, minute ventilation, frequency, and tidal volume had no measurable values beyond this point (Figures 7 and 11), and pulmonary gas exchange could not be calculated and is therefore not presented.

Effects of xylazine-fentanyl on cardiovascular function

Effects of fentanyl and xylazine at 0.1 mg/kg on cardiovascular function

Fentanyl with 0.1 mg/kg xylazine ($n = 3$) induced transient hypotension, with mean (±SD) arterial pressure reaching 62 ± 9.5 mmHg at 7 min from baseline 131 ± 5.4 mmHg (Figure 11) before recovering. Only the 5–10 min period showed significant depression from baseline, with no differences from fentanyl alone (Figure 7). Heart rate briefly dropped to a mean (±SD) of 115 ± 16 beats per min within 1 min from baseline 395 ± 34 beats per min, but quickly recovered. Pulse pressure spiked to a mean (±SD) of 88 ± 1.6 mmHg within 1.5 min from baseline 48 ± 1.0 mmHg, also recovering rapidly. Only the 0–5 min period showed significant changes from baseline for both parameters.

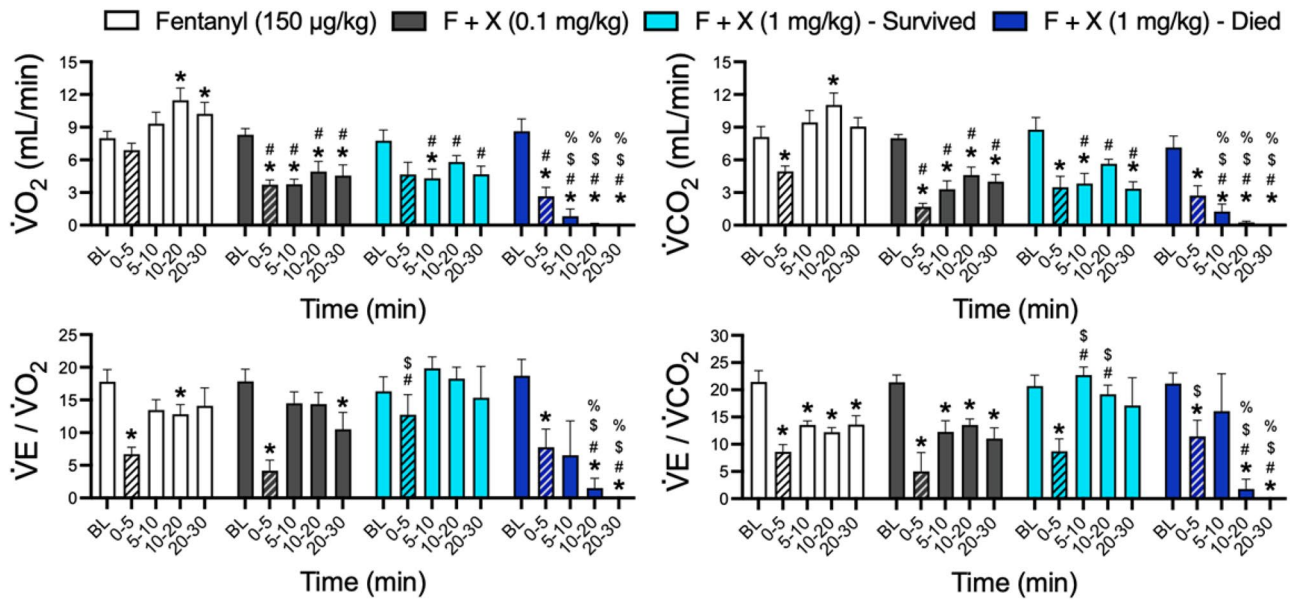

Figure 8. Statistical comparisons of oxygen consumption and carbon dioxide production, and the ventilatory equivalent for O_2 and CO_2. Changes over time in O_2 consumption ($\dot{V}O_2$, mL/min), CO_2 production ($\dot{V}CO_2$, mL/min), and the ratio of minute ventilation ($\dot{V}E$) to $\dot{V}O_2$ and $\dot{V}CO_2$ as an assessment of ventilatory efficiency in non-sedated, freely moving rats after injection of fentanyl (150 µg/kg, $n=13$), fentanyl (F) mixed with xylazine (X) at 0.1 mg/kg ($n=8$), or 1 mg/kg xylazine in rats that survived ($n=4$), or died ($n=7$). The baseline (BL) was averaged over 5 min before xylazine administration. Subsequent intervals are the responses averaged over 0–5 min, 10 min, 10–20 min, and 20–30 min after injection. Note that, like in Figure 7, the 0–5 min interval (hatched bars) indicates that the period includes apnea, recovery, as well as non-eupneic breaths (expiratory activity and gasps). The data are shown as means ± standard deviation. *$P<0.05$, a significant change from baseline (BL) values within the group. #$P<0.05$ treatment versus fentanyl, $$P<0.05$ treatment versus 0.1 mg/kg, %$P<0.05$ 1 mg/kg (died) versus 1 mg/kg (survived), each at the corresponding time point.

Effects of 0.1 mg/kg xylazine and fentanyl on cardiovascular function

Fentanyl with 1 mg/kg xylazine ($n=5$) decreased mean arterial pressure gradually over 10 min, reaching a mean (±SD) of 46 ± 6.1 mmHg at 8 min from a baseline of 132 ± 5.6 mmHg. Heart rate was briefly depressed but remained mostly stable in survivors. As with breathing, cardiovascular responses diverged between survivors ($n=3$) and non-survivors ($n=2$). At 9 min post-injection (approximately 1 min after the breathing divergence), mean arterial pressure in survivors gradually increased, returning to baseline by 15 min and remaining stable. Mean arterial pressure continued to decline in non-survivors (Figure 12). Echocardiography ($n=5$) revealed a dramatic reduction in cardiac output throughout the observation period, dropping to a mean (±SD) of 45 ± 33 mL/kg within 5 min from a baseline of 238.45 ± 41.74 mL/kg (Figure 5(A)). Ejection fraction was depressed initially to a mean (±SD) of 0.57 ± 0.22% and again after 15 min to 0.59 ± 0.15% compared to a baseline of 0.89 ± 0.04% (Figure 5(B)).

Effects of xylazine at 10 mg/kg and fentanyl on cardiovascular function

The combination of fentanyl and xylazine at 10 mg/kg was fatal in all animals ($n=3$), producing cardiovascular collapse within min, as seen in a representative example in Figure 13. Mean (±SD) arterial pressure dropped to 16 ± 2.6 mmHg within 3 min from a baseline of 132 ± 6.7 mmHg and never recovered in any animal (Figure 11). Similarly, pulse pressure collapsed to a mean (±SD) of 1.5 ± 0.13 mmHg from baseline 49 ± 2.1 mmHg within 3 min, and also never recovered, resulting in pulseless electrical activity and death. This cardiogenic origin of the hemodynamic failure was confirmed by echocardiogram ($n=3$). The ejection fraction was a mean (±SD) of 0.29 ± 0.08% after 1 min, down from 0.89 ± 0.04% at baseline (Figure 5(B)). There was no measurable ejection fraction or identifiable cardiac contractions after 3 min. Similarly, cardiac output was down to a mean (±SD) of 40 ± 32 mL/kg, from a baseline of 238 ± 42 mL/kg (Figure 5(A)). By 3 min, there was no detectable output.

Discussion

The present study demonstrates a dose-dependent toxicological profile of xylazine when co-administered with fentanyl. We recently showed in rats that high-dose fentanyl produces immediate bradycardia, reduced cardiac output, and transient decreases in contractility [30], creating a vulnerable cardiovascular state that high-dose xylazine exacerbates. This

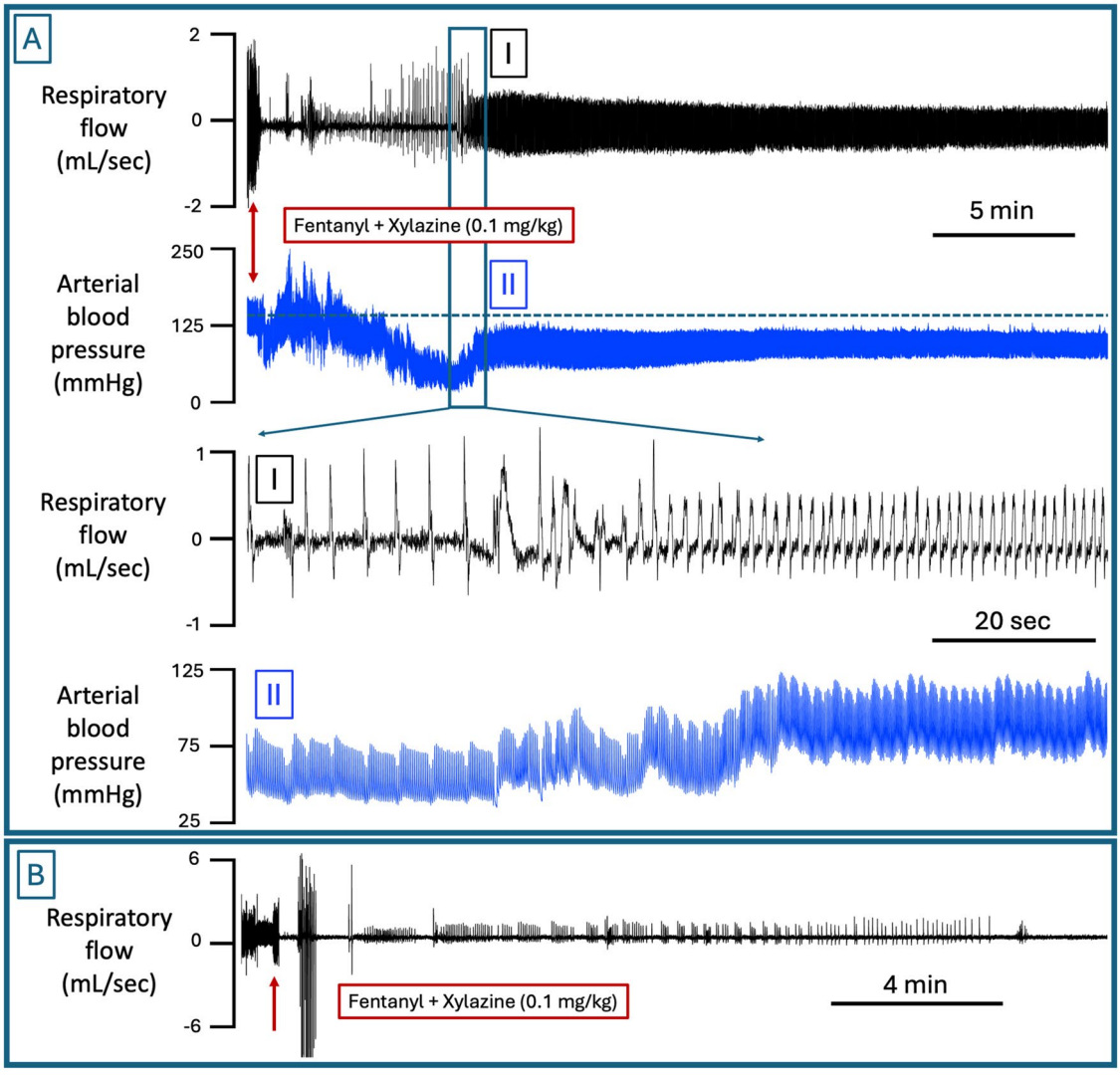

Figure 9. Representative examples of the effects of fentanyl and xylazine at 0.1 mg/kg on breathing and blood pressure in non-anesthetized rats. A combination of fentanyl (150 µg/kg) and xylazine (0.1 mg/kg) intravenous bolus injections was fatal in one of eight total rats tested (panel A). These animals did not show the large expiratory activity during apnea typical of fentanyl alone. Despite a delayed restoration of eupneic breathing, animals resumed a depressed pattern seen in respiratory flow (mL/sec). This was preceded by the reversal of an initial hypotension seen in the arterial blood pressure (mmHg). Subsequently, blood pressure stabilized, although it remained depressed. The one rat that did not survive (panel B) had large expiratory events and never reestablished eupneic breathing. These traces are preprocessed plethysmography data.

dose-dependent increase in toxicity can be explained by a shift from the expected α_2-adrenergic receptor agonist effect to that of a non-specific α receptor agonist as the dose of xylazine increases.

Effects of xylazine administered alone

At the lowest dose tested, xylazine (0.1 mg/kg) produced a response characteristic of selective α_2-adrenergic receptor agonists: a brief initial increase in blood pressure followed by sustained mild hypotension. This biphasic response reflects initial peripheral α_2B adrenergic receptor activation on vascular smooth muscle, causing transient vasoconstriction, followed by central α_2-adrenergic receptor-mediated sympatholytic effects, reducing sympathetic outflow [13,14]. At this dose, xylazine is likely to maintain its α_2/α_1 selectivity [42]. The respiratory depression observed at 0.1 mg/kg was mild and transient, likely reflecting only light sedation via partial activation of α_2A-adrenergic receptor activation in the locus coeruleus and medullary respiratory centers seen with other α_2 agonists [43,44].

Xylazine at 1 mg/kg produced a more pronounced initial hypertension, followed by sustained hypotension, suggesting a beginning loss of α_2 selectivity with increasing α_1-adrenergic receptor activation. We suspect that the enhanced initial vasopressor response likely reflects greater peripheral vasoconstriction from

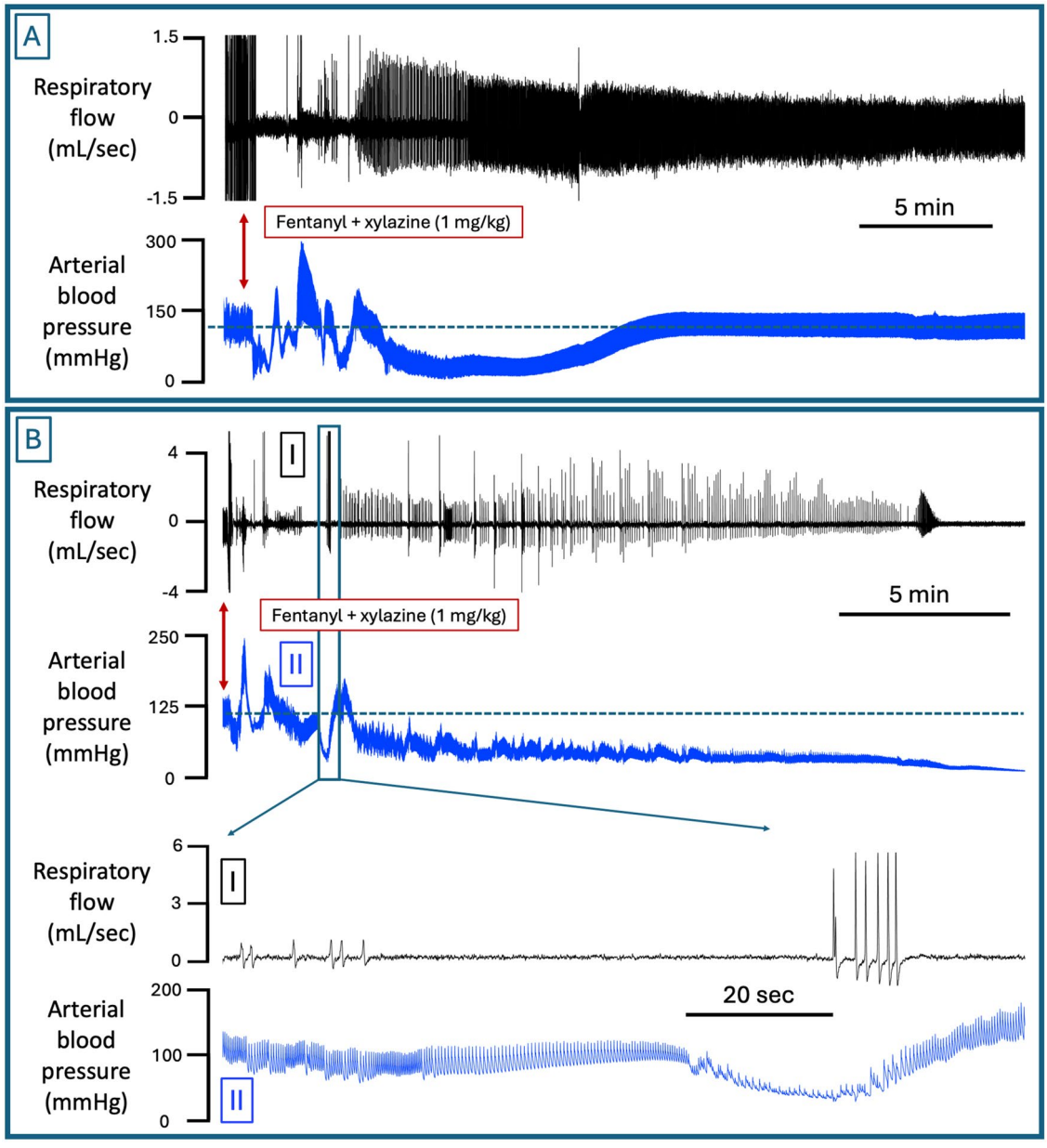

Figure 10. Representative examples of the effects of fentanyl and xylazine at 1 mg/kg combination on breathing and blood pressure in non-anesthetized rats. Of the 11 total tests of fentanyl and xylazine at 1 mg/kg, $n = 4$ rats survived (example in panel A), and $n = 7$ rats died (example in panel B). The animals that survived had immediate apnea and some expiratory activity, but resumed eupneic, though depressed breathing (respiratory flow, mL/sec), which was followed by blood pressure stabilizing to baseline levels when still conscious (arterial blood pressure, mmHg). Animals that died never re-established eupneic breathing, and blood pressure never stabilized, but instead continued to deteriorate over time until both breathing and heart function stopped. These traces are preprocessed plethysmography data.

α_1-adrenergic receptors on vascular smooth muscle, while the subsequent sustained hypotension suggests persistent central α_2 adrenergic effects [15,45]. The respiratory depression at 1 mg/kg was pronounced and sustained, which can potentially be explained by increased sedation reducing ventilation to match arousal [46].

The most striking finding occurred with xylazine at 10 mg/kg, which produced a large, sustained hypertension lasting over 20 min, indicating α_1-adrenergic receptor activation overwhelming central α_2-adrenergic effects and causing peripheral vasoconstriction, dramatically increasing afterload. At this dose, xylazine resembles a mixed α_1/α_2-adrenergic agonist like norepinephrine, including increased mean arterial pressure, decreased heart rate, and increased peripheral resistance [47]. The respiratory pattern also differed from lower doses, with brief apnea followed by

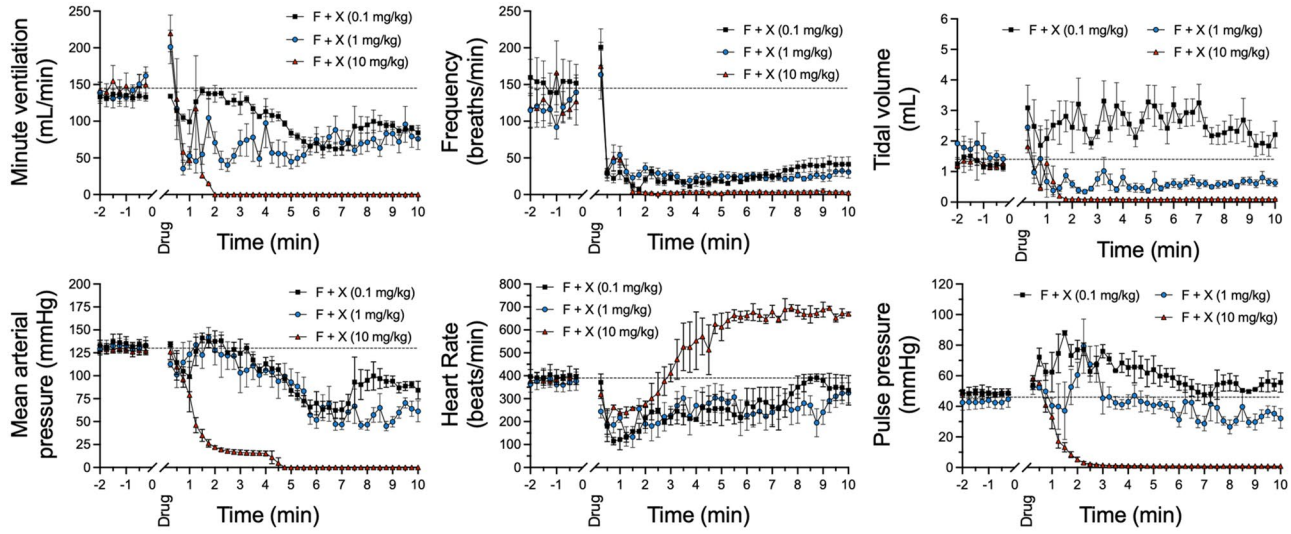

Figure 11. Averaged effects of increasing doses of xylazine mixed with fentanyl on breathing and cardiovascular function in non-sedated rats. Xylazine increases fentanyl toxicity in a dose-dependent manner when administered intravenously. Changes over time in minute ventilation (mL/min), frequency (breaths/min), and tidal volume (mL), mean arterial pressure (mmHg), heart rate (beats/min), and pulse pressure (mmHg) in non-sedated, freely moving rats after a combined injection of fentanyl (F, 150 µg/kg) and xylazine (X) at 0.1 ($n=8$), 1 ($n=11$), or 10 ($n=7$) mg/kg. The dotted horizontal line indicates the 5 min average before the injection of xylazine. The break in the x-axis indicates where animals were removed from the chamber to attach the catheter before fentanyl injection. The data are averaged every 15 sec and presented as means ± standard error of the mean.

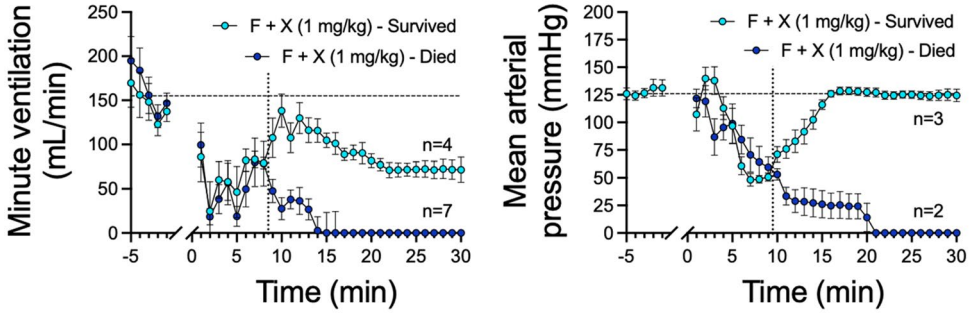

Figure 12. Averaged effects of fentanyl and xylazine at 1 mg/kg on breathing and cardiovascular function in non-sedated rats, analyzed according to outcome (survived or died). Changes over time in minute ventilation (mL/min) and mean arterial pressure (mmHg) in non-sedated, freely moving rats after injection of fentanyl (F) and xylazine (X) at 1 mg/kg in rats that survived ($n=7$) and those that died ($n=4$). Telemetric blood pressure was recorded in five of these ($n=3$ survived, $n=2$ died). The dotted vertical lines indicate an inflection point in breathing (8.5 min, left) and blood pressure (9.5 min, right) between the 'survived' and the 'died' groups. The dotted horizontal line indicates the 5-min average before the injection of xylazine. The break in the x-axis indicates where animals were removed from the chamber to attach the catheter before fentanyl injection. The data are averaged every 1 min and presented as means ± standard error of the mean.

a gradual decline in minute ventilation primarily due to reduced tidal volume rather than frequency at 1 mg/kg. α_1-Adrenergic receptor activation in the retrotrapezoid nucleus can stimulate respiratory drive, potentially explaining why respiratory frequency remained at baseline initially [48].

Fentanyl and low-dose xylazine (0.1 mg/kg): α_2-selective effects and potential protection

When combined with fentanyl, xylazine at 0.1 mg/kg displayed both potentially protective and harmful mechanisms operating simultaneously. Time to eupneic breathing resuming was doubled (a mean (±SD) time of 449 ± 127 sec versus 217 ± 55 sec for fentanyl alone), yet without a corresponding increase in mortality. This paradoxical observation suggests that apnea duration alone is not the critical determinant of survival outcomes, but rather the balance between ventilation and O_2 consumption.

Intravenous fentanyl is known to generate tetanic contractions of a skeletal muscle in humans [49] and rats [29,33,50], increasing metabolic O_2 demand [33]. This includes inspiratory and expiratory muscles,

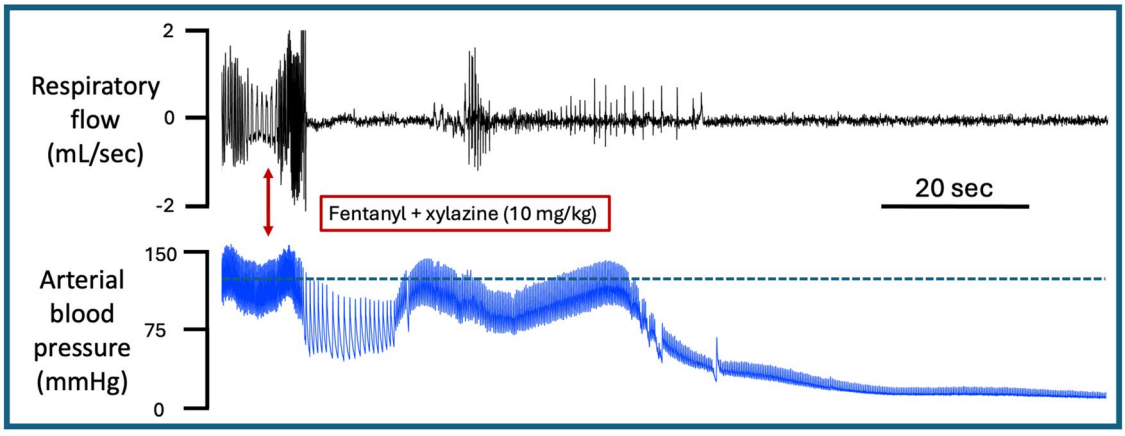

Figure 13. A representative example of the effects of fentanyl and 10 mg/kg xylazine on breathing and blood pressure in non-anesthetized rats. The combination of fentanyl and xylazine at 10 mg/kg ($n = 7$) was rapidly lethal in all animals. Consistent with other doses, there was immediate apnea, but no animal regained eupneic breathing despite some brief expiratory activity and gasping. Blood pressure rapidly collapsed within 1–2 min, often before any breathing activity had ceased. There was no detectable blood or pulse pressure beyond 3 min in any animal. These traces are preprocessed plethysmography data.

inhibiting normal breathing [39,49–51]. Unlike fentanyl alone, which consistently produced expiratory activity (a marker for the onset of rigidity) during apnea in all surviving animals, the combination with xylazine 0.1 mg/kg abolished these expiratory events in survivors. The inhibition of α_1-adrenergic receptors [52] and agonism of α_2-adrenergic receptors [53,54] have both been shown to restore normal function of the coerulospinal pathway in opioid intoxicated models, thereby inhibiting muscle rigidity. The elimination of expiratory activity during apnea likely reflects α_2-adrenergic mediated suppression of noradrenergic drive from the locus coeruleus, as seen with other α_2-adrenergic agonists, including dexmedetomidine [27,33,53]. Dexmedetomidine has also been shown to attenuate alfentanil-induced increased expiratory muscle activity measured via diaphragmatic electromyogram in rats [55]. The absence of these potentially inefficient breathing attempts, combined with reduced activation of skeletal muscle, may conserve O_2 and reduce the metabolic burden during the critical apneic period. Evidence for this is seen in the significant reduction in both CO_2 production ($\dot{V}CO_2$) and the rate of O_2 consumption ($\dot{V}O_2$) compared to fentanyl alone (Figure 8), indicating xylazine at 0.1 mg/kg reduces the O_2 demand-supply mismatch that characterizes fentanyl overdose. So, despite a significantly reduced minute ventilation ($\dot{V}E$) compared to fentanyl alone, decreased O_2 demand due to reduced rigidity can explain why ventilatory efficiency, $\dot{V}E/\dot{V}CO_2$ and $\dot{V}E/\dot{V}O_2$, were not different between the groups.

Unlike higher doses that produced significant cardiovascular failure, xylazine at 0.1 mg/kg combined with fentanyl showed cardiovascular responses similar to fentanyl alone, and sped up the restoration of pulse pressure after the initial spike (Figure 7). This suggests that selective α_2-agonist administration may not compromise cardiovascular adaptations necessary for survival during prolonged apnea. Xylazine at 0.1 mg/kg appears to occupy a narrow therapeutic window where α_2-adrenergic selective effects predominate, as xylazine has been shown to have a 100-fold lower affinity for α_2-adrenergic receptors compared to more selective agonists like medetomidine, making it more prone to off-target effects as doses increase [56].

These results could potentially help explain a rather surprising finding by Love et al. [11], who conducted a multicenter study of emergency departments and found cardiac arrest and coma due to opioid overdose were significantly less severe in those also testing positive for xylazine. As the authors point out, this study did not account for overdose victims who died before being brought to the hospital. So, while higher doses of xylazine may increase mortality in humans, lower doses may confer some reduction in muscle rigidity and O_2 demand. The reality of such a protective mechanism certainly requires confirmation. Additionally, in humans taking these combinations, the practicality of increased xylazine doses may mean lower doses of fentanyl being administered, and therefore reducing toxicity. Further studies with a range of fentanyl concentrations could also better reflect human drug use.

Fentanyl and xylazine at 1 mg/kg: Loss of receptor selectivity and transition to toxicity

The combination of fentanyl with xylazine at 1 mg/kg increased mortality, potentially due to excessive α_2-adrenergic receptor activation, and/or activation of

α_1-adrenergic receptors. All animals receiving fentanyl with xylazine at 1 mg/kg experienced immediate apnea, but not all resumed eupneic breathing. Critically, a clear divergence emerged between survivors and non-survivors post-injection. An average of 8 min after injection, both groups had similar minute ventilation, but by 10 min, survivors had increased ventilation, unlike non-survivors. This delayed mortality pattern reflects the complex interplay between respiratory depression and progressive hypoxemia. Classic studies by Guntheroth and Kawabori [57] demonstrated that severe hypoxia causes apnea when the partial pressure of O_2 in arterial blood falls below 10 mmHg, regardless of arterial CO_2 concentration and pH levels. Once the partial pressure of O_2 in arterial blood drops below this threshold, hypoxia directly depresses the metabolic activity of respiratory neurons in the medulla, creating a vicious cycle where decreased ventilation leads to worsening hypoxemia, which further suppresses respiratory drive [58,59]. This hypoxemia-mediated central respiratory inhibition counteracts peripheral chemoreceptor stimulation and renders breathing depression naloxone-resistant during an opioid overdose [26]. Further, the incomplete reversal of muscle rigidity at this dose (evidenced by the expiratory activity present in all but 1 animal) means that O_2 consumption remains elevated while O_2 delivery is severely compromised.

The cardiovascular data support this hypoxemia-driven mechanism. Mean arterial pressure gradually declined over 10 min in all animals, but critically, mean arterial pressure divergence between survivors and non-survivors occurred approximately 1 min after the respiratory divergence (Figure 12). This suggests that cardiovascular failure follows, rather than precedes, the respiratory failure. The dramatic reduction in cardiac output throughout the observation period indicates severe compromise of O_2 delivery to tissues that, combined with respiratory depression and increased O_2 demand from muscle rigidity, creates life-threatening hypoxemia. We have shown that fentanyl overdose induces decreased cardiac contractility mediated by fentanyl induced hypoxemia, rather than by fentanyl per se [30]. Further, xylazine has been shown to potentiate brain hypoxia due to fentanyl in rats, a specific example of decreased O_2 delivery [60].

The loss of xylazine α_2-adrenergic selectivity at this dose likely contributes to a reduced O_2 delivery through multiple mechanisms. First, α_1-adrenergic receptor activation produces peripheral vasoconstriction that increases cardiac afterload [61], at a time when cardiac output is already severely compromised by fentanyl and reduced peripheral perfusion. Second, the mixed α_1/α_2-adrenergic effects may disrupt the normal compensatory responses to hypoxemia [62,63]. Under conditions of severe hypoxia, peripheral chemoreceptors normally trigger both increased ventilation and sympathetic activation to maintain O_2 to vital organs (see [64] for review). However, xylazine at 1 mg/kg may exacerbate fentanyl disruption of these protective reflexes, preventing the coordinated cardiovascular and respiratory responses necessary for survival.

Fentanyl and xylazine at 10 mg/kg

The universal mortality observed when xylazine at 10 mg/kg was combined with fentanyl reveals a catastrophic interaction leading to pulseless electrical activity within minutes. This cardiovascular collapse (>180 sec) happened faster than eupneic breathing resumes in animals administered fentanyl alone (mean (±SD) time of 217 ± 55 sec), suggesting that this rapid death must also involve direct cardiac toxicity. However, we do not yet know what causes such a severe response and can only speculate.

Several converging mechanisms may explain this rapid progression. Xylazine at 10 mg/kg appears to act as an agonist of both α_1- and α_2-adrenergic receptors, causing peripheral vasoconstriction and acute hypertension [65,66], thereby significantly increasing the pulse pressure required to maintain effective perfusion. Higher doses of xylazine may produce sympathomimetic effects via activation of α_1-adrenergic receptors, leading to peripheral vasoconstriction, hypertension, and cerebrovascular and myocardial ischemia [15]. Xylazine has been shown to constrict isolated small canine coronary arteries in an α_2-dependent manner [67], which would rapidly reduce myocardial perfusion and therefore O_2 available to maintain myocardial contractility. Fentanyl causes an acute bradycardia, a rapid decrease in cardiac output and ejection fraction (Figure 6), and we have previously demonstrated that fentanyl overdose induces a rapid decrease in O_2 delivery [30]. Fentanyl is known to have acute cardiac toxicity, including inducing ischemic stroke, myocardial infarction, acute heart failure, and dysrhythmias [68,69]. This combination of xylazine and fentanyl effects would greatly decrease cardiac contractility, leading to a fatal cardiogenic shock.

Clinical implications

These findings have critical implications for managing xylazine-adulterated opioid overdose. The heterogeneous responses observed clinically could well reflect dose-dependent exposure levels similar to our findings. Recognition of the temporal patterns of toxicity – rapid

cardiogenic shock versus delayed hypoxemic death – is essential for tailoring therapeutic interventions.

Xylazine at 1 mg/kg, which represents a typical veterinary sedative dose, transforms a typically non-fatal fentanyl overdose into a lethal one in the majority of cases in our model. This occurs not through direct toxicity, like at 10 mg/kg. Rather, a cascade of events is initiated by prolonged apnea, exacerbated by cardiovascular compromise, and ultimately in hypoxemia-induced failure of respiratory control mechanisms. Understanding this mechanistic sequence is crucial for developing targeted interventions to bolster traditional naloxone therapy that only addresses the opioid component of overdose. If hypoxemia has reached a critical level, additional intervention may be required to restore normal breathing and cardiovascular function.

Studies investigating a combination of naloxone and atipamezole (α_2-adrenergic receptor antagonist only approved for veterinary use) for reversal of fentanyl with xylazine at non-fatal doses are encouraging [70]. However, our results suggest that for suspected high-dose exposures, ventilatory and circulatory support may also be necessary, given the narrow window for effective intervention. Understanding the dose-dependent transition from α_2-to-α_1-adrenergic effects is crucial for developing targeted therapeutic strategies to resolve a combination overdose.

Study limitations

There is currently no universally agreed preclinical model for acute opioid overdose. Species-specific adaptive physiology may limit relevance to humans and must be considered, as we previously discussed [71]. Mice possess a mass-specific metabolic rate 15–20 times higher than humans, largely due to nonshivering thermogenesis [72], which is absent in adult humans. This allows mice to rapidly reduce their O_2 metabolism by up to 90% in response to hypoxic stress without affecting adenosine triphosphate production, a protective adaptation that has no equivalent in larger mammals [73]. Rats demonstrate some intermediate physiological responses that more closely approximate human respiratory control mechanisms, though still with differences that must be considered [74]. Further, rats demonstrate sensitivity and symptomatic responses to fentanyl that more closely reflect human outcomes when compared to mice. Mice require much higher doses than rats or humans (typically 10–100 times higher doses than clinically relevant in humans) to elicit respiratory depression [75–77], and doses in the mg/kg range are known to cause hyperlocomotion in mice, rather than sedation or coma [75,78]. We contend that rat models provide improved translational relevance, particularly when ventilatory responses are normalized by making ventilation a function of metabolism ($\dot{V}E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$) to account for baseline metabolic disparities.

A potential limitation of this study is that our mechanistic interpretation relies on existing pharmacological literature, rather than direct receptor-specific evidence. We hypothesize that the dose-dependent increase in xylazine toxicity is due to a transition from α_2-adrenergic selective to mixed α_1/α_2-adrenergic agonism. However, we do not yet have direct evidence of receptor occupancy or activation for the doses we studied. The α_2/α_1-adrenergic selectivity ratio and the loss of this selectivity at higher doses are extrapolated from *in vitro* binding studies and *ex vivo* tissue preparations. While we do not question the integrity of this work, these assays may not fully reflect the complex *in vivo* pharmacology during overdose. Furthermore, the concurrent activation of pre- and postsynaptic α_2-adrenergic receptors, α_2-adrenergic receptor subtypes (α_2A, α_2B, α_2C), and the potential for both fentanyl and xylazine metabolites to have different receptor profiles all complicate our mechanistic conclusions.

Definitively proving these receptor-specific mechanisms *in vivo* presents significant experimental challenges. While we are currently testing whether atipamezole, a selective α_2-adrenergic antagonist and common xylazine antidote, can prevent or reverse toxicity at intermediate and high doses, administering adrenergic antagonists in the setting of severe cardiovascular compromise also has risks. Antagonism of α_2-adrenergic receptors could exacerbate the muscle rigidity observed during fentanyl overdose. Antagonism of α_1-adrenergic receptors could theoretically prevent hypertension and increased afterload at high xylazine doses, but would likely cause profound hypotension in animals already experiencing cardiovascular failure due to fentanyl overdose, potentially accelerating death rather than preventing it. Similarly, non-selective α-adrenergic antagonists like phentolamine could address both α_1-adrenergic and α_2-adrenergic effects but would eliminate any protective α_2-adrenergic mediated reduction in O_2 consumption while simultaneously causing severe vasodilation and cardiovascular decompensation. These pharmacological constraints highlight why developing effective treatments for xylazine-fentanyl overdoses remains so challenging – interventions targeting one mechanism may exacerbate others, and the narrow therapeutic window leaves little room for error in an already critically compromised physiological state. Even if atipamezole proves an effective treatment for combination overdose, it is

not approved for use in humans, nor is any parenteral α_2-adrenergic receptor antagonist, so translation to the clinic could be some distance away.

A further potential limitation of this study is that intravenous delivery of fentanyl and xylazine may not precisely replicate how humans administer these drugs. This method has proved to be a reliable model for consistently creating fentanyl overdose [25,29,30]. However, studies that examine the rapidity of administration may reveal important insights. In addition, our previous studies have demonstrated that the dose of fentanyl (150 µg/kg) selected for this study is reliable for causing an acute, but typically survivable overdose in rats [25,30]. In human drug use, the addition of xylazine may reduce the total fentanyl concentration administered, so future studies with other fentanyl doses are also important.

Conclusions

This study reveals that xylazine may fundamentally transform fentanyl toxicity in a dose-dependent manner, shifting from α_2-adrenergic selective effects with no increased mortality at low doses to catastrophic cardiogenic shock and rapid pulseless electrical activity at high doses in a rat model of overdose.

Disclosure statement

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References

[1] Palamar JJ, Fitzgerald ND, Carr TH. An analysis of the size of law enforcement seizures of illicit fentanyl in the United States, 2018–2023. Drug Alcohol Rev. 2025;44(3):793–798. doi: 10.1111/dar.14022.

[2] Singh VM, Browne T, Montgomery J. The emerging role of toxic adulterants in street drugs in the US illicit opioid crisis. Public Health Rep. 2020;135(1):6–10. doi: 10.1177/0033354919887741.

[3] Reyes JC, Negrón JL, Colón HM, et al. The emerging of xylazine as a new drug of abuse and its health consequences among drug users in Puerto Rico. J Urban Health. 2012;89(3):519–526. doi: 10.1007/s11524-011-9662-6.

[4] Nunez J, DeJoseph ME, Gill JR. Xylazine, a veterinary tranquilizer, detected in 42 accidental fentanyl intoxication deaths. Am J Forensic Med Pathol. 2021;42(1):9–11. doi: 10.1097/PAF.0000000000000622.

[5] Johnson J, Pizzicato L, Johnson C, et al. Increasing presence of xylazine in heroin and/or fentanyl deaths, Philadelphia, Pennsylvania, 2010–2019. Inj Prev. 2021;27(4):395–398. doi: 10.1136/injuryprev-2020-043968.

[6] Administration DE. DEA Reports widespread threat of fentanyl mixed with xylazine. DEA.gov; 2023. Available from https://www.dea.gov/alert/dea-reports-widespread-threat-fentanyl-mixed-xylazine

[7] Kariisa M, O'Donnell J, Kumar S, et al. Illicitly manufactured fentanyl-involved overdose deaths with detected xylazine – United States, January 2019–June 2022. MMWR Morb Mortal Wkly Rep. 2023;72(26):721–727. doi: 10.15585/mmwr.mm7226a4.

[8] Fentanyl adulterated or associated with xylazine implementation report; 2024. Available from: https://www.whitehouse.gov/wp-content/uploads/2025/03/ONDCP-2024-FAAX-Implementation-Report.pdf

[9] Smith MA, Biancorosso SL, Camp JD, et al. "Tranq-dope" overdose and mortality: lethality induced by fentanyl and xylazine. Front Pharmacol. 2023;14:1280289. doi: 10.3389/fphar.2023.1280289.

[10] Acosta-Mares P, Violante-Soria V, Browne TJr., et al. Xylazine potentiates the lethal but not the rewarding effects of fentanyl in mice. Drug Alcohol Depend. 2023;253:110993. doi: 10.1016/j.drugalcdep.2023.110993.

[11] Love JS, Levine M, Aldy K, et al. Opioid overdoses involving xylazine in emergency department patients: a multicenter study. Clin Toxicol (Phila). 2023;61(3):173–180. doi: 10.1080/15563650.2022.2159427.

[12] Gupta R, Holtgrave DR, Ashburn MA. Medical and public health imperatives of xylazine. Reply. N Engl J Med. 2023;389(8):771–772.

[13] Giovannitti JAJr., Thoms SM, Crawford JJ. Alpha-2 adrenergic receptor agonists: a review of current clinical applications. Anesth Prog. 2015;62(1):31–39. doi: 10.2344/0003-3006-62.1.31.

[14] Khan ZP, Ferguson CN, Jones RM. Alpha-2 and imidazoline receptor agonists. Their pharmacology and therapeutic role. Anaesthesia. 1999;54(2):146–165. doi: 10.1046/j.1365-2044.1999.00659.x.

[15] Greene SA, Thurmon JC. Xylazine – a review of its pharmacology and use in veterinary medicine. J Vet Pharmacol Ther. 1988;11(4):295–313. doi: 10.1111/j.1365-2885.1988.tb00189.x.

[16] Ball NS, Knable BM, Relich TA, et al. Xylazine poisoning: a systematic review. Clin Toxicol (Phila). 2022;60(8):892–901. doi: 10.1080/15563650.2022.2063135.

[17] Hoffman GR, Giduturi C, Cordaro NJ, et al. Classics in chemical neuroscience: xylazine. ACS Chem Neurosci. 2024;15(11):2091–2098. doi: 10.1021/acschemneuro.4c00172.

[18] Edinoff AN, Sall S, Upshaw WC, et al. Xylazine: a drug adulterant of clinical concern. Curr Pain Headache Rep. 2024;28(5):417–426. doi: 10.1007/s11916-024-01211-z.

[19] Valverde A, Skelding AM. Alternatives to opioid analgesia in small animal anesthesia: alpha-2 agonists. Vet Clin North Am Small Anim Pract. 2019;49(6):1013–1027. doi: 10.1016/j.cvsm.2019.07.010.

[20] Ruiz-Colón K, Chavez-Arias C, Díaz-Alcalá JE, et al. Xylazine intoxication in humans and its importance as an emerging adulterant in abused drugs: a comprehen-

sive review of the literature. Forensic Sci Int. 2014;240:1–8. doi: 10.1016/j.forsciint.2014.03.015.
[21] Hoffmann U, Meister CM, Golle K, et al. Severe intoxication with the veterinary tranquilizer xylazine in humans. J Anal Toxicol. 2001;25(4):245–249. doi: 10.1093/jat/25.4.245.
[22] Choon LK, Khiruddin AI, Annuar W, et al. A case series of accidental xylazine intoxication in humans; is there a role of naloxone as an antidote? Turk J Emerg Med. 2023;23(2):119–122. doi: 10.4103/tjem.tjem_198_22.
[23] Gallanosa AG, Spyker DA, Shipe JR, et al. Human xylazine overdose: a comparative review with clonidine, phenothiazines, and tricyclic antidepressants. Clin Toxicol. 1981;18(6):663–678. doi: 10.3109/15563658108990293.
[24] Pattinson KT. Opioids and the control of respiration. Br J Anaesth. 2008;100(6):747–758. doi: 10.1093/bja/aen094.
[25] Haouzi P, Mellen N, McCann M, et al. Evidence for the emergence of an opioid-resistant respiratory rhythm following fentanyl overdose. Respir Physiol Neurobiol. 2020;277:103428. doi: 10.1016/j.resp.2020.103428.
[26] Haouzi P, Guck D, McCann M, et al. Severe hypoxemia prevents spontaneous and naloxone-induced breathing recovery after fentanyl overdose in awake and sedated rats. Anesthesiology. 2020;132(5):1138–1150. doi: 10.1097/ALN.0000000000003156.
[27] Torralva R, Janowsky A. Noradrenergic mechanisms in fentanyl-mediated rapid death explain failure of naloxone in the opioid crisis. J Pharmacol Exp Ther. 2019;371(2):453–475. doi: 10.1124/jpet.119.258566.
[28] Kinshella MW, Gauthier T, Lysyshyn M. Rigidity, dyskinesia and other atypical overdose presentations observed at a supervised injection site, Vancouver, Canada. Harm Reduct J. 2018;15(1):64. doi: 10.1186/s12954-018-0271-5.
[29] Lewis T, Haouzi P. Nalfurafine reverses fentanyl-induced muscle rigidity and respiratory depression without affecting sedation in rats: decoupling respiration from sedation. J Appl Physiol (1985). 2025;138(5):1161–1172. doi: 10.1152/japplphysiol.00952.2024.
[30] Judenherc-Haouzi A, Lewis T, Reinhardt A, et al. Life-threatening fentanyl overdose beyond medullary depression in breathing. Am J Physiol Regul Integr Comp Physiol. 2025;328(3):R408–R421. doi: 10.1152/ajpregu.00238.2024.
[31] Hubrecht RC, Carter E. The 3Rs and humane experimental technique: implementing change. Animals (Basel). 2019;9(10):754. doi: 10.3390/ani9100754.
[32] Obert DP, Park GH, Strong K, et al. Repeated high-dose fentanyl administration in rats reveals minimal tolerance to unconsciousness, bradycardia, muscle rigidity, and respiratory depression. Anesthesiology. 2025;142(3):465–475. doi: 10.1097/ALN.0000000000005324.
[33] Haouzi P, Tubbs N. Effects of fentanyl overdose-induced muscle rigidity and dexmedetomidine on respiratory mechanics and pulmonary gas exchange in sedated rats. J Appl Physiol (1985). 2022;132(6):1407–1422. doi: 10.1152/japplphysiol.00819.2021.
[34] U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research. Guidance for industry: estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers; 2005. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/estimating-maximum-safe-starting-dose-initial-clinical-trials-therapeutics-adult-healthy-volunteers.
[35] St John WM, Bartlett DJr. Comparison of phrenic motoneuron activity during eupnea and gasping. J Appl Physiol Respir Environ Exerc Physiol. 1981;50(5):994–998. doi: 10.1152/jappl.1981.50.5.994.
[36] St John WM, Knuth KV. A characterization of the respiratory pattern of gasping. J Appl Physiol Respir Environ Exerc Physiol. 1981;50(5):984–993. doi: 10.1152/jappl.1981.50.5.984.
[37] Ristagno G, Tang W, Sun S, et al. Spontaneous gasping produces carotid blood flow during untreated cardiac arrest. Resuscitation. 2007;75(2):366–371. doi: 10.1016/j.resuscitation.2007.04.020.
[38] Suzuki M, Funabiki T, Hori S, et al. Spontaneous gasping increases cerebral blood flow during untreated fatal hemorrhagic shock. Resuscitation. 2009;80(1):109–112. doi: 10.1016/j.resuscitation.2008.08.013.
[39] Burns G, DeRienz RT, Baker DD, et al. Could chest wall rigidity be a factor in rapid death from illicit fentanyl abuse? Clin Toxicol (Phila). 2016;54(5):420–423. doi: 10.3109/15563650.2016.1157722.
[40] St-John WM, Paton JF. Defining eupnea. Respir Physiol Neurobiol. 2003;139(1):97–103. doi: 10.1016/s1569-9048(03)00193-9.
[41] Baby SM, May WJ, Getsy PM, et al. Fentanyl activates opposing opioid and non-opioid receptor systems that control breathing. Front Pharmacol. 2024;15:1381073. doi: 10.3389/fphar.2024.1381073.
[42] Lemke KA. Perioperative use of selective alpha-2 agonists and antagonists in small animals. Can Vet J. 2004;45(6):475–480.
[43] Sinclair MD. A review of the physiological effects of alpha2-agonists related to the clinical use of medetomidine in small animal practice. Can Vet J. 2003;44(11):885–897.
[44] Murrell JC, Hellebrekers LJ. Medetomidine and dexmedetomidine: a review of cardiovascular effects and antinociceptive properties in the dog. Vet Anaesth Analg. 2005;32(3):117–127. doi: 10.1111/j.1467-2995.2005.00233.x.
[45] Paddleford RR, Harvey RC. Alpha 2 agonists and antagonists. Vet Clin North Am Small Anim Pract. 1999;29(3):737–745. doi: 10.1016/s0195-5616(99)50058-2.
[46] Brown EN, Purdon PL, Van Dort CJ. General anesthesia and altered states of arousal: a systems neuroscience analysis. Annu Rev Neurosci. 2011;34(1):601–628. doi: 10.1146/annurev-neuro-060909-153200.
[47] Allwood MJ, Cobbold AF, Ginsburg J. Peripheral vascular effects of noradrenaline, isopropylnoradrenaline and dopamine. Br Med Bull. 1963;19(2):132–136. doi: 10.1093/oxfordjournals.bmb.a070031.
[48] Oliveira LM, Moreira TS, Kuo FS, et al. Alpha1- and alpha2-adrenergic receptors in the retrotrapezoid nucleus differentially regulate breathing in anesthetized adult rats. J Neurophysiol. 2016;116(3):1036–1048. doi: 10.1152/jn.00023.2016.
[49] Streisand JB, Bailey PL, LeMaire L, et al. Fentanyl-induced rigidity and unconsciousness in human volunteers. Incidence, duration, and plasma concentrations. Anesthesiology. 1993;78(4):629–634. doi: 10.1097/00000542-199304000-00003.

[50] Lui PW, Lee TY, Chan SH. Fentanyl-induced muscle rigidity in unanesthetized and ketamine- or thiopental-anesthetized rats. Anesthesiology. 1989;70(6):984–990. doi: 10.1097/00000542-198906000-00017.
[51] Drummond GB, Duncan MK. Abdominal pressure during laparoscopy: effects of fentanyl. Br J Anaesth. 2002;88(3):384–388. doi: 10.1093/bja/88.3.384.
[52] Lui PW, Lee TY, Chan SH. Involvement of coerulospinal noradrenergic pathway in fentanyl-induced muscular rigidity in rats. Neurosci Lett. 1990;108(1–2):183–188. doi: 10.1016/0304-3940(90)90728-r.
[53] Weinger MB, Segal IS, Maze M. Dexmedetomidine, acting through central alpha-2 adrenoceptors, prevents opiate-induced muscle rigidity in the rat. Anesthesiology. 1989;71(2):242–249. doi: 10.1097/00000542-198908000-00013.
[54] Jerussi TP, Capacchione JF, Benvenga MJ. Reversal of opioid-induced muscular rigidity in rats: evidence for alpha-2 adrenergic involvement. Pharmacol Biochem Behav. 1987;28(2):283–289. doi: 10.1016/0091-3057(87)90226-7.
[55] Campbell C, Weinger MB, Quinn M. Alterations in diaphragm EMG activity during opiate-induced respiratory depression. Respir Physiol. 1995;100(2):107–117. doi: 10.1016/0034-5687(94)00119-k.
[56] Schwartz DD, Clark TP. Affinity of detomidine, medetomidine and xylazine for alpha-2 adrenergic receptor subtypes. J Vet Pharmacol Ther. 1998;21(2):107–111. doi: 10.1046/j.1365-2885.1998.00113.x.
[57] Guntheroth WG, Kawabori I. Hypoxic apnea and gasping. J Clin Invest. 1975;56(6):1371–1377. doi: 10.1172/JCI108217.
[58] Jacobi MS, Gershan WM, Thach BT. Mechanism of failure of recovery from hypoxic apnea by gasping in 17- to 23-day-old mice. J Appl Physiol (1985). 1991;71(3):1098–1105. doi: 10.1152/jappl.1991.71.3.1098.
[59] Jacobi MS, Thach BT. Effect of maturation on spontaneous recovery from hypoxic apnea by gasping. J Appl Physiol (1985). 1989;66(5):2384–2390. doi: 10.1152/jappl.1989.66.5.2384.
[60] Choi S, Irwin MR, Kiyatkin EA. Xylazine effects on opioid-induced brain hypoxia. Psychopharmacology (Berl). 2023;240(7):1561–1571. doi: 10.1007/s00213-023-06390-y.
[61] Kayki-Mutlu G, Papazisi O, Palmen M, et al. Cardiac and vascular alpha(1)-adrenoceptors in congestive heart failure: a systematic review. Cells. 2020;9(11):2412.
[62] Bölter C, Gabriel P, Appelt P, et al. Effects of adrenergic agonists and antagonists on cardiopulmonary function during normobaric hypoxia in rat. Front Physiol. 2019;10:860. doi: 10.3389/fphys.2019.00860.
[63] Weisbrod CJ, Minson CT, Joyner MJ, et al. Effects of regional phentolamine on hypoxic vasodilatation in healthy humans. J Physiol. 2001;537(Pt 2):613–621. doi: 10.1111/j.1469-7793.2001.00613.x.
[64] López-Barneo J, Ortega-Sáenz P, González-Rodríguez P, et al. Oxygen-sensing by arterial chemoreceptors: mechanisms and medical translation. Mol Aspects Med. 2016;47-48:90–108. doi: 10.1016/j.mam.2015.12.002.
[65] Talke P, Lobo E, Brown R. Systemically administered alpha2-agonist-induced peripheral vasoconstriction in humans. Anesthesiology. 2003;99(1):65–70. doi: 10.1097/00000542-200307000-00014.
[66] Muszkat M, Kurnik D, Sofowora GG, et al. Independent regulation of alpha1 and alpha2 adrenergic receptor-mediated vasoconstriction in vivo. J Hypertens. 2011;29(2):251–256. doi: 10.1097/HJH.0b013e3283407ffd.
[67] Teng B, Muir WW.3rd. Effects of xylazine on canine coronary artery vascular rings. Am J Vet Res. 2004;65(4):431–435. doi: 10.2460/ajvr.2004.65.431.
[68] Doshi R, Majmundar M, Kansara T, et al. Frequency of cardiovascular events and in-hospital mortality with opioid overdose hospitalizations. Am J Cardiol. 2019;124(10):1528–1533. doi: 10.1016/j.amjcard.2019.07.068.
[69] Krantz MJ, Palmer RB, Haigney MCP. Cardiovascular complications of opioid use: JACC state-of-the-art review. J Am Coll Cardiol. 2021;77(2):205–223. doi: 10.1016/j.jacc.2020.11.002.
[70] Tackett WR, Yalakala J, Hambuchen MD. Corrigendum to "Co-administration of naloxone and dexmedetomidine to simultaneously reverse acute effects of fentanyl and methamphetamine in rats". Drug Alcohol Depend. 2024;261:111380. doi: 10.1016/j.drugalcdep.2024.111380.
[71] Haouzi P. Murine models in critical care research. Crit Care Med. 2011;39(10):2290–2293. doi: 10.1097/CCM.0b013e3182227550.
[72] Singer D. Metabolic adaptation to hypoxia: cost and benefit of being small. Respir Physiol Neurobiol. 2004;141(3):215–228. doi: 10.1016/j.resp.2004.02.009.
[73] Mortola JP. Implications of hypoxic hypometabolism during mammalian ontogenesis. Respir Physiol Neurobiol. 2004;141(3):345–356. doi: 10.1016/j.resp.2004.01.011.
[74] Gonzalez NC, Kuwahira I. Systemic oxygen transport with rest, exercise, and hypoxia: a comparison of humans, rats, and mice. Compr Physiol. 2018;8(4):1537–1573. doi: 10.1002/j.2040-4603.2018.tb00050.x.
[75] Haouzi P, McCann M, Tubbs N. Respiratory effects of low and high doses of fentanyl in control and beta-arrestin 2-deficient mice. J Neurophysiol. 2021;125(4):1396–1407. doi: 10.1152/jn.00711.2020.
[76] Hill R, Santhakumar R, Dewey W, et al. Fentanyl depression of respiration: comparison with heroin and morphine. Br J Pharmacol. 2020;177(2):254–266. doi: 10.1111/bph.14860.
[77] Varshneya NB, Hassanien SH, Holt MC, et al. Respiratory depressant effects of fentanyl analogs are opioid receptor-mediated. Biochem Pharmacol. 2022;195:114805. doi: 10.1016/j.bcp.2021.114805.
[78] Varshneya NB, Walentiny DM, Moisa LT, et al. Fentanyl-related substances elicit antinociception and hyperlocomotion in mice via opioid receptors. Pharmacol Biochem Behav. 2021;208:173242. doi: 10.1016/j.pbb.2021.173242.