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Research Report

An electroencephalogram biomarker of fentanyl drug effects

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Abstract

Opioid drugs influence multiple brain circuits in parallel to produce analgesia as well as side effects, including respiratory depression. At present, we do not have real-time clinical biomarkers of these brain effects. Here, we describe the results of an experiment to characterize the electroencephalographic signatures of fentanyl in humans. We find that increasing concentrations of fentanyl induce a frontal theta band (4 to 8 Hz) signature distinct from slow-delta oscillations related to sleep and sedation. We also report that respiratory depression, quantified by decline in an index of instantaneous minute ventilation, occurs at ≈1700-fold lower concentrations than those that produce sedation as measured by reaction time. The electroencephalogram biomarker we describe could facilitate real-time monitoring of opioid drug effects and enable more precise and personalized opioid administration.

Keywords: opioids, EEG monitoring, opioid anesthesia, OIRD

Significance Statement:

This past year more than 100,000 people lost their lives to opioid-related drug overdose. Fentanyl and its analogs are involved in approximately 20% of all overdose deaths. Opioid drugs influence multiple brain circuits in parallel to produce analgesia as well as side effects, including respiratory depression, the main cause of death in opioid overdose cases. Postsurgical pain is a significant driver of opioid dependence, but real-time clinical biomarkers to properly titrate opioid effects and avoid side effects are lacking. Here, we report a novel electroencephalogram signature highly correlated with fentanyl concentration and respiratory depression. Our studies could be used to facilitate real-time monitoring of opioid drug effects in medical settings and enable safer, more precise, and personalized opioid administration.

Introduction

Opioid drugs are essential tools for pain management, yet their side effects are profound, difficult to manage, and potentially deadly (1, 2). The challenge of balancing therapeutic opioid effects against side effects arises in part from the significant interindividual variability in these effects (3–5). At present, we have few tools to assess real-time opioid requirements in patients, relying instead on pharmacokinetic and pharmacodynamic models, indirect systemic physiologic indicators, and subjective reports of pain. The absence of such tools means that patients may be frequently underdosed, leading to poorly controlled pain, or overdosed, leading to oversedation and respiratory depression (RD). Although it is well known that opioid drugs influence multiple brain circuits to produce analgesia as well as its side effects (6, 7), at present it is not possible to directly measure how opioid drugs affect these circuits in a clinical setting (8).

Here, we report a novel electroencephalogram (EEG) biomarker for the opioid fentanyl, identified from a human study in which increasing doses of fentanyl were administered to patients prior to induction of general anesthesia. We find that this EEG biomarker is highly correlated with fentanyl concentration and RD and that it is readily visible in individual subjects. This novel biomarker could allow healthcare professionals to measure and titrate drug effects in real-time, mitigating the risk of over- or underdosing,

Competing Interests: P.L.P. is an inventor on patents assigned to MGH related to brain monitoring, an inventor on a patent licensed to Masimo by Massachusetts General Hospital and a Co-founder of PASCALL Systems, Inc., a company developing closed-loop physiological control systems for anesthesiology.
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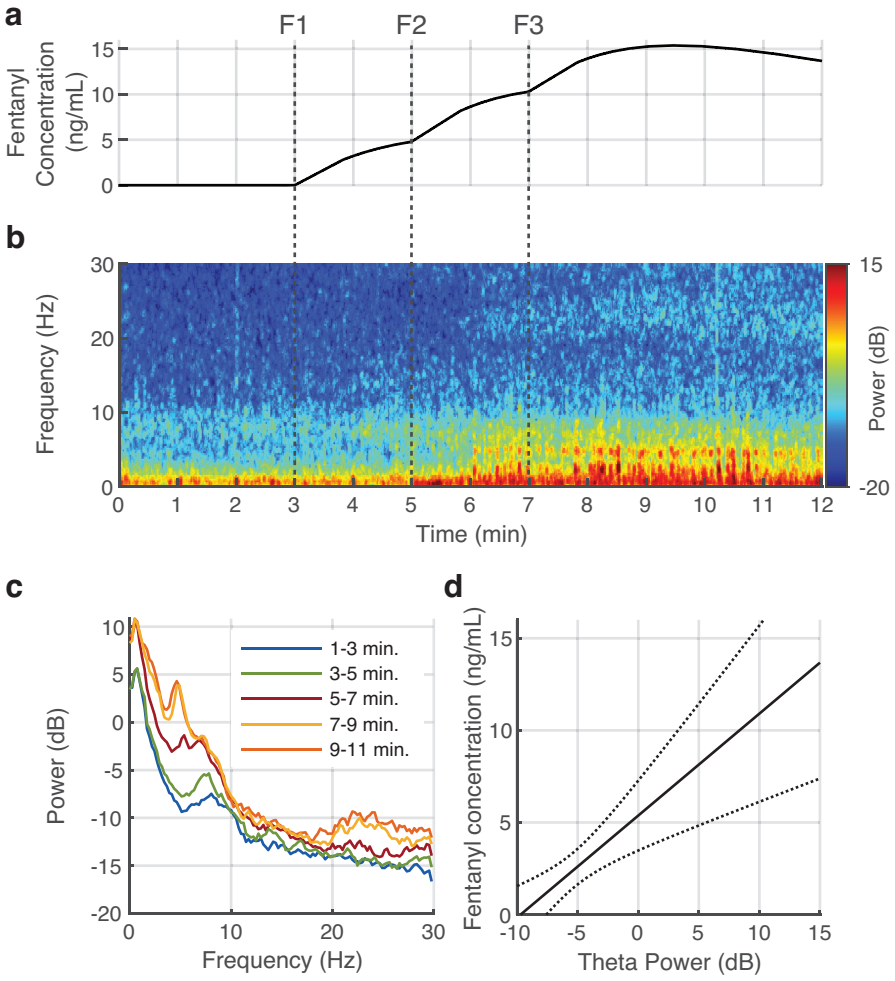

Fig. 1. Changes in fentanyl ESC and EEG. Subjects were administered up to three boluses in 2-minute intervals after a baseline period (F1, F2, and F3), and concentration levels were computed through PK/PD modeling. Panel (a) shows the predicted ESC of a representative subject. Exposure to fentanyl corresponded with changes in the spectrogram (b) and power spectrum changes in a representative subject (c), with notable increases in the theta (4 to 8 Hz) and slow/delta (0 to 4 Hz) bands. The increases in theta power correspond to increases in fentanyl concentration. A linear mixed-effects model was constructed across subjects [shown in (d) with 95% CI] to further describe the association between changes in theta power and Fentanyl concentration.

and could be used to enhance discovery and development of novel pain drugs or therapeutics (9). We also find that fentanyl causes clinically significant RD long before any noticeable sedation, making even the smallest amount of fentanyl potentially dangerous.

Anesthesiologists administer fentanyl during induction of general anesthesia to blunt the nociceptive response to intubation. We used this induction period to carry out an experiment in which we gradually administered fentanyl in 25 subjects undergoing general anesthesia for surgeries lasting 2 hours or more. Prior to entering the operating room, we placed respiratory inductance plethysmography (RIP) bands around each subject's abdomen and thorax to measure and track changes in respiration (10, 11) during the baseline period and after fentanyl administration. Patients were preoxygenated during this time via face mask (12), and pulseoximetry was carefully monitored by the anesthesiologist on our study team in accordance with clinical monitoring standards. We also recorded a four-channel frontal EEG throughout the study. We asked subjects to perform an auditory behavioral task with their eyes closed throughout the experiment to characterize their level of sedation (see the "Methods" section for details). After a 3 minute baseline period, we administered two or three doses of 2 μg/kg of ideal body weight (IBW) of fentanyl according to the subject's tolerance with 2 minutes of separation between each dose. After administration of the last bolus, we continued to record data for an additional 5 minutes.

Results

A distinct EEG theta oscillation develops with increasing fentanyl concentration

We estimated the fentanyl effect site concentration (ESC) using pharmacokinetic/pharmacodynamic (PK/PD) modeling implemented in StanpumpR (13), illustrated in Fig. 1a for a representative subject. We analyzed the EEG using multitaper spectral analysis (14). A spectrogram from a representative subject in Fig. 1b illustrates how EEG power in the theta (4 to 8 Hz) and slow/delta (0 to 4 Hz) bands increases as the fentanyl concentration increases. These same oscillations may also be appreciated in the spectrum, illustrated at different time points in Fig. 1c. We note that when the EEG waveforms are visualized in the time domain, the theta oscillations are difficult if not impossible to perceive (Supplementary Material Fig. S1). In contrast, the theta signal is readily apparent in the spectrum and spectrogram (Fig. 1a and b). To quan-

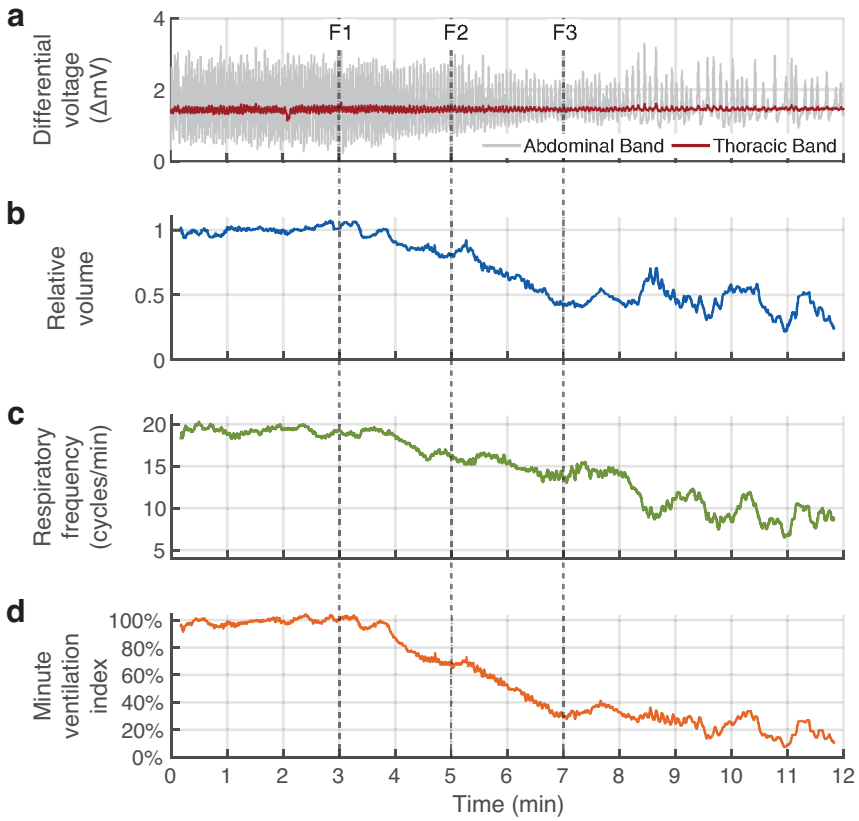

Fig. 2. Changes in respiratory dynamics drive RD. Subjects had thoracic and abdominal bands placed to measure expansion and compression, shown with an individual subject in (a). A state space oscillator model was used to characterize instantaneous changes in relative volume (b) and frequency (c), the moving average of which is displayed here. Declines in both relative volume and frequency contribute to the RD, shown by the decline in the MVI (d), the moving average of which is displayed here. Dashed lines correspond to the administration of the fentanyl boluses during the protocol.

tify the relationship between the EEG and fentanyl concentration, we constructed a linear mixed-effects model representing fentanyl concentration as a function of theta power, which revealed a strong association across all $n = 25$ subjects [slope: 0.55 (CI: 0.25 to 0.80), marginal $R^2 = 0.151$, conditional $R^2 = 0.744$; Fig. 1d].

Fentanyl-induced RD is driven by declines in both respiratory amplitude and frequency and can be quantified using an instantaneous minute ventilation index

The RIP waveforms showed obvious changes in respiratory amplitude and frequency on visual inspection, as illustrated in a representative subject in Fig. 2a. We noticed that in some subjects both respiratory amplitude and frequency decreased after fentanyl administration, while in others either amplitude or frequency decreased. In order to summarize the overall effect of changing amplitude and frequency on respiration, we developed an index related to minute ventilation that incorporated both aspects of respiration. Minute ventilation is defined as the volume of respiration per minute and is computed by multiplying the respiratory volume by the respiratory frequency (15). First, we used a state space oscillator model (16, 17) to estimate the instantaneous amplitude and frequency (Fig. 2b and c) from the raw waveform (Fig. 2a). Next, we derived analytical expressions to describe how changes in the chest and abdominal band measurements related to lung volume. We then used these expressions to calculate a minute ventilation index (MVI) calibrated for each subject to a value of 100% during the baseline period, corresponding to each subject's resting tidal volume (18), and to 0%, representing no change in volume (Fig. 2d). A full description of this derivation can be found in the Supplementary Material.

Minute ventilation decreases by almost two-thirds with increasing fentanyl concentration and is correlated with EEG theta power

We also found that MVI decreased by 30.1% (CI: 18.7 to 39.9) after the first bolus of fentanyl, by 54.6% (CI: 44.5 to 64.3) after the second, and by 59.9% (CI: 52.2 to 66.9) after the third bolus (Fig. S2). We noted that these effects occurred while the subjects maintained a blood oxygen saturation between 95% and 100% throughout the protocol as monitored by the study anesthesiologist and clinical care team. Even though the subjects remained stable clinically due to these precautions, the rapid decline in MV still occurred. To characterize the relationship between theta band power and respiratory changes we constructed a mixed-effects model [Fig. 3, slope: −1.62 (CI: −2.51 to 0.73), marginal $R^2 = 0.054$, conditional $R^2 = 0.248$]. Theta band power shows a clear association with the MVI changes across all $n = 25$ subjects, in which increasing theta power corresponds to decreasing MVI.

RD begins several minutes before noticeable changes in reaction time and at more than 1000-fold lower fentanyl concentrations

The subjects were also asked to perform an auditory behavioral task throughout the protocol to track sedation and loss of consciousness (unresponsiveness). Briefly, subjects heard sounds

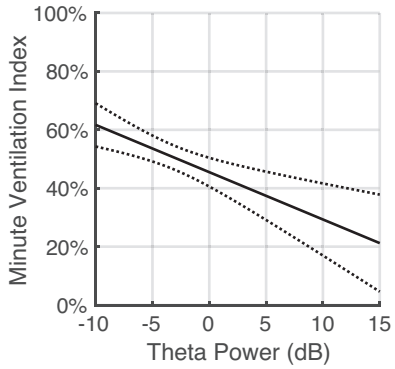

Fig. 3. A linear mixed-effects model across all subjects indicates an inverse association between frontal theta power and MVI. Most subjects showed a clear decline in MV, characteristic of fentanyl-induced RD. Theta power, shown to have a positive association with fentanyl ESC (Fig. 1d), also has an inverse relationship with this decline, indicating the ability to track the respiratory effects of the opioid using the EEG.

played from a headphone and were asked to press a button in response (see the "Methods" section). We analyzed changes in reaction time (RT) in relation to the onset of RD in each subject (Fig. 4a). We observed that while RD presented quickly after the initial bolus of fentanyl, most subjects did not show a noticeable change in RT until many minutes later, after multiple boluses of fentanyl had already been administered. In Fig. 4(a), we show an example of this dramatic time lag between initial RD and RT in a typical subject. We estimated this time lag in each subject using a cross-correlation analysis between the MVI and RT time series. We estimated a mean lag of 277 seconds (CI: 219.5 to 332.5) between changes in MVI and changes in RT using a bootstrap analysis of 10,000 samples. We also observed that the MVI would decline by 51.4% (CI: 30.9 to 80.4) before these increases in RT occurred (Fig. 4c). Finally, we estimated that the predicted ESC of fentanyl needed to induce a 10% drop in MVI is roughly 1750-fold lower than the concentration present upon RT changes (CI: 839 to 2854) (Fig. 4d). Although sedation due to opioid exposure is well-documented (19, 20), our results show that the respiratory decline induced by fentanyl far outpaces any behavioral change.

Fentanyl-induced theta power correlates with slowing RT, while slow/delta oscillations indicate loss of consciousness

We used a linear mixed-effects models to characterize RT as a function of MVI (Fig. S3a), theta power (Fig. S3b), and slow/delta power (Fig. S3c). MVI is poorly correlated with changes in RT (slope: −1.94, conditional $R^2 = 0.180$), as expected given the significant lag between RD and RT. However, the theta band EEG power proved to be highly correlated to RT (slope: 30.11, conditional $R^2 = 0.313$), with 1 dB increases in power corresponding to roughly 30 ms increases in RT. The development of slow/delta power also correlates well with the slowing RT of subjects after fentanyl administration (slope: 17.30, conditional $R^2 = 0.261$).

Beyond a slowing RT, a significant result of opioid administration is loss of consciousness (LOC). We defined LOC as a period of at least three consecutive failures to respond to auditory stimuli (approximately 12 seconds). We used a logistic mixed-effects model to characterize LOC. Toward the end of the protocol, when fentanyl concentrations were highest, subjects were less likely to respond to the auditory stimuli. By this time, most subjects already experienced significant declines in their minute ventilation, and thus, MVI shows no association with LOC (Fig. S3d; odds ratio: 0.99). While theta power is associated with LOC (Fig. S3e; odds ratio 0.86), slow/delta band power appears to be even more informative (Fig. S3f; odds ratio 0.85), as the increased range in slow/delta band power available corresponds with a larger range in the probability of consciousness. At some point, 16 out of 25 subjects reached LOC, all of whom were arousable to either verbal or physical stimulation, with four requiring a jaw thrust to support their airway.

Discussion

The EEG has been used previously to study opioids (19, 21–25), but the specific signatures we describe here and their unique associations with respiration, sedation, and LOC have not, to our knowledge, been previously reported. The sample size of our study is comparable to or exceeds those of previous studies of fentanyl EEG effects (21–25). A major strength of our study was our ability to use the operating room environment to safely study fentanyl in humans. A key innovation in our approach was that we were able to administer the drug gradually over a relatively long period of time, making it possible to discern the theta oscillation signature and its association with fentanyl concentration and RD that had not been previously reported. Finally, a major strength of our work was our ability to develop and apply novel methods to precisely characterize instantaneous changes in respiratory dynamics using noninvasive methods. This allowed us to characterize fentanyl-induced RD with greater sensitivity, and in turn to analyze fentanyl's marked differential effects on respiration compared to sedation. More work will be needed to study the extent to which these effects can be observed in a larger patient population, but the strong and novel associations we found in this study are nonetheless compelling. In particular, our findings suggest that it may be possible to predict RD in patients using processed EEG.

Fentanyl and its analogs are the primary drivers of the opioid overdose crisis in the United States (26–28), which has led to more than 100,000 deaths in 2020 and 2021 (29–31). Our results help to explain why fentanyl is so deadly and has practical implications that may be useful in the fight to reduce opioid-related overdoses and deaths. First, our study quantifies, to our knowledge for the first time, that fentanyl is more than 1700-fold more potent for RD than for sedative effects. Our results suggest that when used outside a medical setting, fentanyl would induce apnea well in advance of sedation or behavioral effects. Opioids or other drugs taken during substance abuse may contain fentanyl in unknown yet significant proportions; the respiratory effects we describe here make clear that no amount of fentanyl would be safe in this context. As fentanyl exposure is likely to remain a persistent risk during illicit use (28, 32), the rapid and significant RD we observed supports the need for increased availability of medical observation or supervision units, naloxone, medication-assisted therapy (33, 34), and other harm-reduction tools (35, 36) to reduce the risk of death among substance-use-disorder patients. Our results could also enable the development of novel monitoring and delivery technologies designed to optimize opioid administration for pain management that could reduce the risk of persistent opioid use and dependence.

Methods

Study design and oversight

This study was approved by the Institutional Review Board (IRB) at the Massachusetts General Hospital (MGH), study ID number: 2018P000383. It was conducted in accordance with the provisions of the Declaration of Helsinki. All subjects enrolled in the study provided written informed consent. The study was registered on ClinicalTrials.gov (Identifier: NCT03866278).

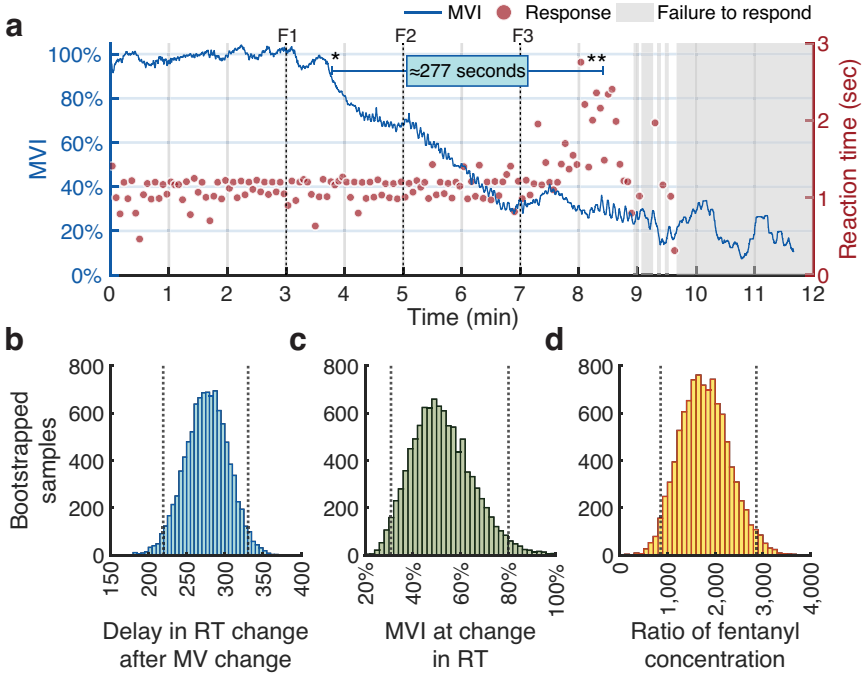

Fig. 4. Significant behavioral changes after fentanyl administration occur with a delay and significantly increased fentanyl concentration. While changes in MV (moving average shown) for the subject in (a) occur shortly after the initial fentanyl exposure at 3 minutes, noticeable increases in RT to stimuli occur closer to 7 minutes in the displayed individual subject. (b) Simulating 10,000 bootstrapped samples shows an average delay of 277 seconds (95% CI: 219.5 to 332.5 seconds) in RT changes from MV changes (c). The MVI drops to 51% (95% CI: 31% to 79%) upon the onset of an increase in RT (d). Comparing the fentanyl concentration upon initial decline of MV against the concentration upon changes to RT shows a 1754-fold (95% CI: 839 to 2854 fold) increase in fentanyl concentration underlying these behavioral changes (d). ∗10% decrease in MV; ∗∗noticeable increase in RT.

Subjects

Subjects were recruited from patients aged 18 to 65 scheduled to undergo surgery lasting 2 hours or more, and with American Society of Anesthesiologists (ASA) physical status classification I to III. Subjects had their medical history reviewed by the study staff anesthesiologist (E.T.P.) to rule out active and chronic medical problems. Once a potential subject was identified by the research, the study staff anesthesiologist approached the potential subject to obtain consent as early as possible prior to surgery and anesthesia. A total of 31 subjects provided informed consent. There was no randomization or treatment assignment. The onset of the COVID-19 pandemic led to a cessation of nearly all clinical research at our institution and a severely reduced number of surgical procedures, making it difficult if not impossible to continue subject recruitment. We therefore stopped recruiting subjects and proceeded to analyze the data and report the results herein. We completed the protocol in 28 out of the 31 consented subjects. Two subjects were not studied because of scheduling conflicts, and one further was not studied due to withdrawal of consent. There were technical problems in three additional subjects that led to data loss: In one subject, we were unable to record the respiratory signals; in another, we were unable to obtain the behavioral responses; and in yet another, the EEG signal was too noisy to analyze. In the end, we analyzed complete data sets from 25 out of 31 subjects. From these participants, 13 were female (52%), with a mean age of 54.4 years (range: 31 to 64 years) and a mean weight of 78.76 kg (SD = 22.6).

Exclusion criteria

Our exclusion criteria included: craniofacial abnormalities, allergies to fentanyl, bisulfite, eggs or egg products, latex, soybeans, soybean oil, BMI $\geq$ 30 (kg/m^2), known or suspected difficult intubation, known or suspected need, for rapid sequence induction and intubation, history of obstructive sleep apnea requiring CPAP, history of uncontrolled gastroesophageal reflux disease (GERD), opiate use within 24 hours, and history of opiate abuse within 3 years.

Study procedure

The protocol consisted of recording EEG, RIP, and sedation state during administration of fentanyl prior to induction of general anesthesia. Fentanyl is generally administered before intubation to blunt the circulatory response to laryngoscopy (37, 38). We designed our protocol to administer fentanyl gradually over a 4-minute period in doses of 2 μg/kg of IBW every 2 minutes with a maximum dose of 6 μg/kg IBW according to patient tolerance and performance on the behavioral task. To ensure the subject's safety, vital signs and potential side effects were closely monitored by the research anesthesiologist staff. Interventions up to and including abandonment of the protocol were considered depending on the subject's tolerance.

Data recording

A four-channel Masimo SEDLine (Masimo Corporation, Irvine, CA, USA) frontal EEG sensor was placed with electrode positions corresponding to Fp1, Fp2, F7, and F8 in the international 10-20 system. We made sure to keep the electrode impedances $\leq$5 kΩ. RIP was measured using a SleepSense 9003-L90 Semi-Reusable inductive elastic bands placed across each subject's chest and abdomen. The data from the RIP sensor were captured using a Neuroelectrics NIC2 device at a sampling frequency of 500 Hz. The auditory behavioral task was administered using a computer-driven script written in Python. The subjects were asked to listen to a series of sounds played every 4 seconds, and to respond via button press to identify the sound as either a train of clicks,

or verbal stimuli. Auditory stimuli were delivered using Etymotic ER-3C earphones. Button presses were recorded using a computer mouse strapped to the subject's hand.

Data analysis

EEG processing

EEG information acquired through the 4-channel frontal EEG monitor was analyzed using custom in-house scripts written in MATLAB 2021a (MathWorks, Inc,., Natick, MA, USA) employing the Chronux v.2.12 toolbox (http://chronux.org) (14). The EEG was sampled at 250 Hz and was detrended and bandpass filtered between 0.1 and 40 Hz. Multitaper spectral analysis was used to compute spectra and spectrograms, with a 4-second window sampled every second (i.e. 3-second overlap) with $K = 3$ tapers, time-bandwidth product of $TW = 2$, and a spectral resolution of 1 Hz (39). The median power across channels was computed and analyzed across the slow/delta (0.1 to 4 Hz), theta (4 to 8 Hz), alpha (8 to 12 Hz), beta (12 to 20 Hz), and gamma (>20 Hz) frequency bands.

RIP processing

The RIP signals from the chest and abdominal bands were downsampled to 25 Hz. A state space oscillator model was used to directly characterize the instantaneous amplitude and frequency of the respiratory signals (16). We used a multivariable version of the model in which the abdominal and chest signals shared the same oscillatory frequency but were otherwise independent. The instantaneous amplitude for both the chest and abdominal bands was extracted using this model, along with the shared instantaneous frequency, as described in the Supplementary Material. We then estimated a minute ventilation index (MVI) using expressions described in the Supplementary Material ("Derivation of an Expression to Estimate Instantaneous Minute Ventilation"). The MVI was calibrated in each subject to a value of 100 at baseline, corresponding to each subject's resting tidal volume, and zero when both the abdominal and chest respiratory amplitudes were zero. Values above 100%, which correspond to physiological variations of tidal volume or forced inspiration, were excluded from analysis.

Statistical analysis

We used linear and logistic mixed-effects models to describe the associations between EEG theta power, fentanyl ESC, MVI, and behavioral changes, implemented using the *lme4* R package (40). EEG power was calculated as described above. MVI was calculated as described above and in further detail in the Supplementary Material. The predicted fentanyl ESC through stanpumpR (13). Behavioral changes were represented in terms of RT and a binary variable representing the presence or absence of a response.

Acknowledgments

We would like to thank nurses, surgeons, and anesthesiologists at Massachusetts General Hospital (MGH) who helped facilitate these studies. Without you, this study would not have been possible. We also extend our thanks to all the subjects who contributed their time and trust for this investigation.

Supplementary Material

Supplementary material is available at *PNAS Nexus* online.

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Authors' Contributions

P.L.P.: conceptualization, methodology, data collection, data analysis, data dnalysis methods development, funding acquisition, supervision, writing—original draft, and writing—review and editing.

G.A.B.: data collection, data preprocessing, data analysis, data visualization, project administration, writing—original draft, and writing—review and editing.

K.M.B.: data collection, data preprocessing, data analysis, data visualization, data analysis methods development, writing—original draft, writing—review and editing, and statistical modeling.

A.C.M.: conceptualization, methodology, data collection, data preprocessing, data analysis, funding acquisition, and project administration.

A.M.B.: data analysis methods development.

E.C.W.: data collection, funding acquisition, and project administration.

F.J.M.: data collection.

E.T.P.: conceptualization, methodology, data collection, and supervision.

T.T.H.: data analysis, and statistical modeling.

Previous Presentation

These results were previously presented as an abstract at the International Anesthesia Research Society, Association of University Anesthesiologists, and Society of Critical Care Anesthesiologists Annual meeting in March 2022.

Data Availability

The processed data used to characterize the relationships among drug concentration, EEG power, RT, and respiration are currently stored in Harvard Dataverse. The data are not publicly available as they are the subject of ongoing analyses but can be requested from the corresponding author and could be shared after establishing a data use agreement with the corresponding author's institution.

Code Availability

Custom MATLAB analysis and code was created to generate the data set, statistical analysis was done in R with the lme4 package from https://cran.r-project.org/web/packages/lme4/, and signal processing of EEG data was done with done with the chronux library from http://chronux.org. The state space modeling code is not publicly available at present but can be requested from the corresponding author and could be shared after establishing a data use agreement with the corresponding author's institution.

References

1. Long DR, *et al.* 2018. Association between intraoperative opioid administration and 30-day readmission: a pre-specified analysis of registry data from a healthcare network in New England. Brit J Anaesth. 120(5):1090–1102.

2. Lee LA, *et al.* 2015. Postoperative opioid-induced respiratory depression. Anesthesiology. 122(3):659–665.
3. Angst M, *et al.* 2012. Aversive and reinforcing opioid effects. Anesthesiology. 117(1):22–37.
4. Angst MS, *et al.* 2012. Pain sensitivity and opioid analgesia: a pharmacogenomic twin study. Pain. 153(7):1397–1409.
5. Kumar S, Kundra P, Ramsamy K, Surendiran A. 2019. Pharmacogenetics of opioids: a narrative review. Anaesthesia. 74(11):1456–1470.
6. Manning BH, Morgan MJ, Franklin KBJ. 1994. Morphine analgesia in the formalin test: evidence for forebrain and midbrain sites of action. Neuroscience. 63(1):289–294.
7. Matthies BK, Franklin KBJ. 1992. Formalin pain is expressed in decerebrate rats but not attenuated by morphine. Pain. 51(2):199–206.
8. Belzeaux R, Lalanne L, Kieffer BL, Lutz PE. 2018. Focusing on the opioid system for addiction biomarker discovery. Trends Mol Med. 24(2):206–220.
9. Darcq E, Kieffer BL. 2018. Opioid receptors: drivers to addiction? Nat Rev Neurosci. 19(8):499–514.
10. Grossman P, Wilhelm FH, Brutsche M. 2010. Accuracy of ventilatory measurement employing ambulatory inductive plethysmography during tasks of everyday life. Biol Psychol. 84(1):121–128.
11. Clarenbach CF, Senn O, Brack T, Kohler M, Bloch KE. 2005. Monitoring of ventilation during exercise by a portable respiratory inductive plethysmograph. Chest. 128(3):1282–1290.
12. Benumof J. 1999. Preoxygenation. Anesthesiology. 91(3):603–603.
13. Shafer SL. 2019. stanpumpR. [accessed in 11/29/2021]. https://github.com/StevenLShafer/stanpumpR
14. Bokil H, Andrews P, Kulkarni JE, Mehta S, Mitra PP. 2010. Chronux: a platform for analyzing neural signals. J Neurosc Meth. 192(1):146–151.
15. Kavanagh BP, Hedenstierna G. 2019. Respiratory physiology and pathophysiology. In: Gropper Michael A Miller Ronald D Cohen Neal H. Miller's anesthesia. 9thed. Philadelphia, PA: Elsevier. p. 354–383.
16. Matsuda T, Komaki F. 2017. Time series decomposition into oscillation components and phase estimation. Neural Comput. 29(2):332–367.
17. Beck AM, Stephen E, Purdon P. 2018. State space oscillator models for neural data analysis. Paper presented at: 2018 40th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC); Honolulu (HI), USA.
18. Quanjer PH, *et al.* 1993. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Supp. 16:5–40.
19. Streisand J, *et al.* 1993. Fentanyl-induced rigidity and unconsciousness in human volunteers incidence, duration, and plasma concentrations. Anesthesiology. 78(4):629–634.
20. Katoh T, *et al.* 1999. The effect of fentanyl on sevoflurane requirements for somatic and sympathetic responses to surgical incision. Anesthesiology. 90(2):398–405.
21. Scott J, Ponganis K, Stanski D. 1985. EEG quantitation of narcotic effect: the comparative pharmacodynamics of fentanyl and alfentanil. Anesthesiology. 62(3):234–241.
22. Gambus PL, Gregg KM, Shafer SL. 1995. Validation of the alfentanil canonical univariate parameter as a measure of opioid effect on the electroencephalogram. Anesthesiology. 83(4):747–756.
23. Montandon G, Horner RL. 2019. Electrocortical changes associating sedation and respiratory depression by the opioid analgesic fentanyl. Sci Rep. 9(1):14122.
24. Smith NT, *et al.* 1989. Seizures during opioid anesthetic induction—are they opioid-induced rigidity? Anesthesiology. 71(6):852–862.
25. Egan TD, *et al.* 1996. Remifentanil versus alfentanil: comparative pharmacokinetics and pharmacodynamics in healthy adult male volunteers. Anesthesiology. 84(4):821–833.
26. Scholl L, Seth P, Kariisa M, Wilson N, Baldwin G. 2018. Drug and opioid-involved overdose deaths—United States, 2013–2017. MMWR Morbid Mortal Week Rep. 67(5152):1419–1427.
27. Gladden RM, O'Donnell J, Mattson CL, Seth P. 2019. Changes in opioid-involved overdose deaths by opioid type and presence of benzodiazepines, cocaine, and methamphetamine—25 states, July–December 2017 to January–June 2018. MMWR Morbid Mortal Week Rep. 68(34):737–744.
28. Shover CL, *et al.* 2020. Steep increases in fentanyl-related mortality west of the Mississippi River: recent evidence from county and state surveillance. Drug Alcohol Depen. 216:108314.
29. CDC 2021. Drug overdose deaths in the U.S. top 100,000 annually. [accessed 11/22/2021]. http://www.cdc.gov/nchs/pressroom/nchs_press_releases/2021/20211117.htm
30. Ahmad FB, Rossen LM, Spencer MR, Warner M, Sutton P. 2018. Provisional drug overdose death counts. National Center for Health Statistics.
31. Friedman J, Akre S. 2021. COVID-19 and the drug overdose crisis: uncovering the deadliest months in the United States, January–July 2020. Am J Pub Health. 111(7):1284–1291.
32. Kandel DB, Hu MC, Griesler P, Wall M. 2017. Increases from 2002 to 2015 in prescription opioid overdose deaths in combination with other substances. Drug Alcohol Depen. 178:501–511.
33. National Academies of Sciences, Engineering, and Medicine; Health and Medicine Division; Board on Health Sciences Policy; Committee on Medication-Assisted Treatment for Opioid Use Disorder. 2019. Medications for opioid use disorder save lives. The National Academies Collection: Reports funded by National Institutes of Health. Washington (DC): National Academies Press.
34. Substance Abuse and Mental Helth Services Administration. 2021. MAT Medications, Counseling, and Related Conditions. [Accessed 11/22/2021]. http://www.samhsa.gov/medication-assisted-treatment/medications-counseling-related-conditions
35. Marotta PL, *et al.* 2021. Assessing the relationship between syringe exchange, pharmacy, and street sources of accessing syringes and injection drug use behavior in a pooled nationally representative sample of people who inject drugs in the United States from 2002 to 2019. Harm Reduct J. 18:115.
36. Des Jarlais DC, McKnight C, Goldblatt C, Purchase D. 2009. Doing harm reduction better: syringe exchange in the United States. Addiction. 104(9):1441–1446.
37. Cork RC, Weiss JL, Hameroff SR, Bentley J. 1984. Fentanyl preloading for rapid-sequence induction of anesthesia. Anesth Analg. 63(1):60–64.
38. Dahlgren N, Messeter K. 1981. Treatment of stress response to laryngoscopy and intubation with fentanyl. Anaesthesia. 36(11):1022–1026.
39. Prerau MJ, Brown RE, Bianchi MT, Ellenbogen JM, Purdon PL. 2017. Sleep neurophysiological dynamics through the lens of multitaper spectral analysis. Physiology. 32(1):60–92.
40. Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. J Stat Software. 67(1):1–48