Current Research in Toxicology 3 (2022) 100078

Contents lists available at ScienceDirect

Current Research in Toxicology

journal homepage: www.journals.elsevier.com/current-research-in-toxicology

Current research in pathophysiology of opioid-induced respiratory depression, neonatal opioid withdrawal syndrome, and neonatal antidepressant exposure syndrome

Brian A. Baldo

Kolling Institute of Medical Research, Royal North Shore Hospital of Sydney and Department of Medicine, University of Sydney, Sydney, NSW, Australia

ARTICLE INFO

Handling Editor: Thomas B Knudsen

Keywords:
Pathophysiology of opioid-induced respiratory depression
Pre-Bötzinger complex and opioid-induced respiratory depression
Kölliker-Fuse nucleus and opioid-induced respiratory depression
Neural mediation of opioid-induced respiratory depression
Neonatal opioid withdrawal syndrome

ABSTRACT

Respiratory depression (RD) is the primary cause of death due to opioids. Opioids bind to mu (μ)-opioid receptors (MORs) encoded by the MOR gene *Oprm1,* widely expressed in the central and peripheral nervous systems including centers that modulate breathing. Respiratory centers are located throughout the brainstem. Experiments with *Oprm1*-deleted knockout (KO) mice undertaken to determine which sites are necessary for the induction of opioid-induced respiratory depression (OIRD) showed that the pre-Bötzinger complex (preBötC) and the pontine Kölliker-Fuse nucleus (KF) contribute equally to OIRD but RD was not totally eliminated. Morphine showed a differential influence on preBötC and KF neurons – low doses attenuated RD following deletion of MORs from preBötC neurons and an increase in apneas after high doses whereas deletion of MORs from KF neurons but not the preBötC attenuated RD at both high and low doses. In other KO mice studies, morphine administration after deletion of *Oprm1* from both the preBötC and the KF/PBN neurons, led to the conclusion that both respiratory centres contribute to OIRD but the preBötC predominates. MOR-mediated post-synaptic activation of GIRK potassium channels has been implicated as a cause of OIRD. A complementary mechanism in the preBötC involving KCNQ potassium channels independent of MOR signaling has been described. Recent experiments in rats showing that morphine depresses normal, but not gasping breathing, cast doubt on the belief that eupnea, sighs, and gasps, are under the control of preBötC neurons. Methadone, administered to alleviate symptoms of neonatal opioid withdrawal syndrome (NOWES), desensitized rats to OIRD. Protection lost between postnatal days 1 and 2 coincides with the preBötC becoming the dominant generator of respiratory rhythm. Neonatal antidepressant exposure syndrome (NADES) and serotonin toxicity (ST) show similarities including RD. Enzyme CYP2D6 involved in opioid detoxification is polymorphic. Individuals of different CYP2D6 genotype may show increased, decreased, or no enzyme activity, contributing to the variability of patient responses to different opioids and OIRD.

Introduction

While what has been called the 'opioid epidemic' continues, a further disturbing trend in recent years has been the three-times faster increase in deaths than the increase in the number of addicted individuals. Figures released by the US National Institutes of Health National Institute on Drug Abuse for 1999–2020 showed that national overdose deaths involving any opioid were, for example, 21,088 in 2010, 46,802 in 2018, and 68,630 in 2020 (National Institute on Drug Abuse, 2022). In November 2021, data released by the US Centres for Disease Control and Prevention (CDC) revealed that the total number of drug overdose deaths in the US reached a record; in the period May 2020–April 2021

Abbreviations: AAV, adeno-associated virus; CDC, Centers for Disease control and prevention; CTAP, MOR agonist (*D*-Phe-Cys-Tyr-*D*-Trp-Arg-Thr-Pen-Thr-NH_2); DAMGO, synthetic specific MOR agonist [D-Ala^2, *N*-MePhe^4, Gly-ol]-enkephalin; DRG, dorsal respiratory group; FDA, Food and Drug Administration; GIRK, G protein-gated inwardly-rectifying potassium (K^+); GPCR, G protein-coupled receptor; KCNQ, voltage-gated potassium (Kv) channels in the KCNQ (Kv7) family; KF, Kölliker-Fuse nucleus; MOR, mu opioid receptor; NAS, neonatal abstinence syndrome; NIH, National Institutes of Health; NK-1R, neurokinin-1 receptor; OAD, opioid analgesic drug; OIRD, opioid-induced respiratory depression; PBL, lateral parabrachial; PBN, parabrachial nucleus; preBötC, pre-Bötzinger complex; PRG, pontine respiratory group; RD, respiratory depression; TACR1, tachykinin receptor 1; VRG, ventral respiratory group; NADES, neonatal antidepressant exposure syndrome; NOWES, neonatal opioid withdrawal syndrome.

E-mail address: babaldo@iinet.net.au.

https://doi.org/10.1016/j.crtox.2022.100078
Received 17 March 2022; Received in revised form 20 May 2022; Accepted 1 June 2022
Available online 6 June 2022

there were over 100,000 deaths, over 75,000 due to opioids and, of these, over 64,000 were due to synthetic opioids, particularly fentanyl (CDC Overdose Death Counts, 2022).

The most serious of the many adverse effects of the administration of opioid analgesic drugs (Baldo, 2021) is respiratory depression (RD) which includes depression of respiratory rate, minute ventilation, alveolar-arterial gas exchange and respiratory responsiveness to hypoxia and hypercapnia. Opioid-induced respiratory depression (OIRD) occurs with an incidence of 0.5–2% (Dahan et al., 2010) and is the primary cause of death due to opioid analgesic drugs (OADs) in both drug addicts and overdose cases (Pattinson, 2008). Partly as a result of increased research funding to counter the opioid epidemic, for example, the NIH 'Helping to end addiction over the long-term program', there has been an increase in grants to study the opioid overdose problem including a focus on OIRD. Although there remains much to learn about the development of safer opioid pain therapies with reduced respiratory side effects (Montandon and Slutsky, 2019), considerable progress in adding to the understanding of the pathophysiology of OIRD has been made in the last few years. This progress is examined here.

Respiratory centers are located throughout the brainstem. OADs exert their pharmacological actions via opioid G protein-coupled receptors (GPCRs). In mammals such as humans and rodents, the mu (μ)-opioid receptor (MOR), encoded by the MOR gene, *Oprm1,* is widely expressed in the central and peripheral nervous systems including centres that modulate breathing (Kirby and McQueen, 1986; Lonergan et al., 2003; Mansour et al., 1994). OADs bind to MORs triggering G protein signaling and modulation of ion channels to produce analgesia. Studies with knockout mice lacking the MOR gene *Oprm1*, demonstrated the absence of morphine antinociception at normal analgesic doses and the abolition of morphine-induced RD, (Kieffer, 1999; Matthes et al., 1996; Sora et al., 1997) findings that implicated the MOR as the mediator of the opioid-induced analgesia and RD.

Mechanisms of opioid-induced apnea and respiratory depression

Brainstem sites contributing to OIRD

In experiments designed to study the neurological progression to opioid-induced apnea, in particular the involvement of the KF/PBN in opioid-induced apnea, Saunders and Levitt (2020) examined 3-phase respiratory motor output in a rat arterially perfused working heart-brainstem (half-rat) preparation. Systemic low doses of fentanyl slowed the respiratory rate to a 2-phase breathing pattern whereas high doses rapidly provoked apnea that could be reversed by naloxone. The selective MOR antagonist CTAP (*D*-Phe-Cys-Tyr-*D*-Trp-Arg-Thr-Pen-Thr-NH_2) when used before (as in prevention) and after (as in reversal) direct bilateral microinjection of fentanyl into the KF/PBN, restored a phasic respiratory pattern at a rate similar to that recorded prior to fentanyl exposure.

Over a period of many years, studies have demonstrated that multiple sites can independently exert a depressive effect on breathing (Cinelli et al., 2020; Kirby and McQueen, 1986; Montandon et al., 2011; Mustapic et al., 2010; Palkovic et al., 2020; Prkic et al., 2012) but doubts have remained about which site makes the more significant contribution for the induction of OIRD. Following early disagreements on the relative importance of the preBötC and KF/PBN in the induction of OIRD (Montandon and Horner, 2014; Lalley et al., 2014), recent experiments with remifentanil infusion in an in vivo decerebrate, hyperoxic rabbit model, showed that injection of naloxone into the KF/PBN increased the respiratory rate and prevented apnoea in most of the animals while subsequent injection of naloxone into the preBötC prevented apnoea in all of the animals. However, naloxone injection did not completely reverse RD and injection into the preBötC increased the respiratory rate in rabbits exposed to analgesic doses of remifentanil but not in animals given apneic doses (Palkovic et al., 2021). The authors interpreted the failure to fully reverse RD as a consequence of reduced respiratory drive sensitive to high doses of opioid resulting in reduced activity of the KF/PBN and preBötC and a decrease in tidal volume. Relevant to these findings are recent experimental results of Varga et al. (2020) employing homozygous mice with selectively deleted MORs from either preBötC or KF neurones and challenged with morphine. Both site-specific and morphine dosage effects on respiration control were seen. At low dose (10 mg), morphine-induced RD was significantly attenuated after deletion of MORS from either the preBötC or KF neurons, demonstrating an equal contribution to RD in a cumulative network effect. However, depression of respiratory rate was not eliminated by deletion of MORs from preBötC or KF neurons, a finding consistent with the observation that both the preBötC and KF each partially contribute to RD induced by clinical doses of remifentanil (Miller et al., 2017; Stucke et al., 2015). When the dose of morphine was increased to 30 mg/kg, deletion of MORs from the preBötC showed no effect on rate depression but ataxic breathing with apneas occurred. In contrast, deletion of MORs from the KF but not the preBötC, significantly attenuated respiratory rate depression and resulted in less apneic events in the breathing patterns at both low and high doses of morphine. The findings indicate that at low therapeutic doses of morphine, both the preBötC and KF contribute to respiratory rate depression and suggest that at higher doses, *further* depression of the respiratory rate occurs via inhibition of KF neurones as well as other brainstem respiratory nuclei (Varga et al., 2020).

Other recent studies to elucidate mechanisms of OIRD with *Oprm1* genetically deleted knockout mice have had the aim of clarifying the question of which sites are necessary for the induction of OIRD. In first seeking a suitable model for OIRD, Bachmutsky et al. (2020) set out to measure quantitative breathing changes in awake behaving mice before and after administration of morphine. Morphine induced both a slower breathing rate and decrease in inspiratory airflow (Fig. 1a). In hypercapnic air, a trace of a single breath after saline injection (black) showed two phases, inspiration (Ti) and expiration (Te), each of about 50 msec. After injection of morphine (red), time Ti was significantly increased while following Te, a pause occurred (Fig. 1b, c) showing two changes to breathing, a decreased inspiratory airflow prolonging Ti, and Te, and a prolonged period with little or no airflow delaying the start of the next breath (Fig. 1d). These results demonstrated preservation of tidal volume by an increase in inspiration time during a decrease in opioid-induced inspiratory airflow.

In view of the known differences of opinion concerning the involvement and/or relative importance of different brainstem sites in OIRD (Montandon and Horner, 2014; Lalley et al., 2014), Bachmutsky et al. (2020) next undertook experiments in which *Oprm1* was genetically deleted from the preBötC and KF/PBN. In hypercapnia and after morphine administration, breathing in mice with *Oprm1* deletion in the preBötC was less depressed compared to control but faster, peak inspiratory flow was larger, and pauses were almost eliminated (Fig. 2a, b). This showed an improvement in OIRD, but rescue was not total. In comparison to mice with *Oprm1* deletion from the preBötC, genetic deletion from the KF/PBN, induced a more moderate effect after morphine, seen as only slight increases in the respiratory rate and inspiratory airflow. In mice with *Oprm1* deleted from both sites, breathing after morphine administration appeared to be almost identical to that observed in the saline control animals with breathing rate and inspiratory airflow depressed by about 20% compared to saline injected mice (Fig. 2c, d). The authors concluded that *both* respiratory centres contribute to OIRD *but the preBötC predominates*. Even with supersaturating doses of opioid, for example fentanyl 150 mg/ml, breathing remained resilient in mice with *Oprm1* deletion from both the preBötC and the KF/PBN. Using *Oprm1*$^{fl/fl}$ floxed mice which possess *loxP* sites flanking exons 2–3 of the MOR (*Oprm1*) gene, RD resulted in *Oprm1*-positive floxed mice (Fig. 2e) but a normal breathing pattern was maintained in mice after *Oprm1* deletion (Fig. 2f). In identifying the cells involved in depressing breathing, single cell transcriptome profiling of preBötC cells from postnatal day 0 mice showed that only 21 cells of 267 (8%) presumed preBötC neurones expressed *Oprm1* mRNA. About 50%

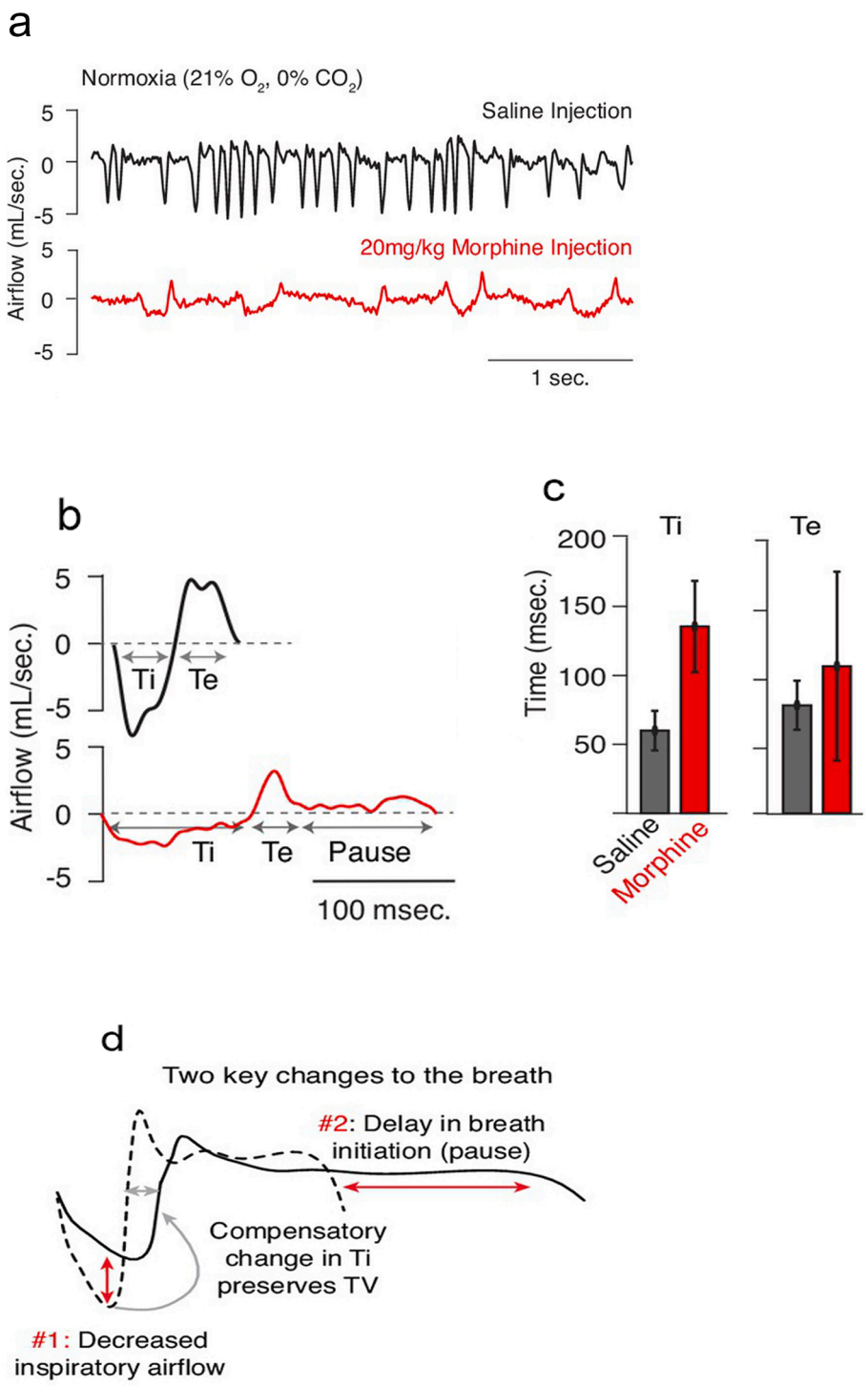

Fig. 1. Changes to breathing in mice during OIRD induced by injection of morphine. (a) Breathing airflow (mL/sec) in normoxia (21% O_2, 0% CO_2) 15 min after injection of saline (black) or morphine (red). (b) Trace of the airflow from a single breath in hypercapnia after saline (black) and morphine (red) injection. Two phases, inspiration Ti and expiration Te, are seen after saline (black) whereas after morphine (red), airflow is markedly reduced and a pause, or third phase, is seen. (c) Shows the inspiration Ti, and expiration Te, time lengths. (d) Diagrammatic representation of morphine-induced change to a breath. The resultant decrease in respiratory rate is due to an extended inspiration time Ti and a pause phase which is extended to preserve tidal volume, TV. This results in breaths after morphine showing an approximately similar TV as seen with control breaths. Adapted from Bachmutsky I, Wei XP, Kish E et al (2020) Opioids depress breathing through two small brainstem sites. eLife 9:e52694 10.7554/eLife.52694, an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0).

of the preBötC neurones expressing *Oprm1* were glutamatergic neurones and approximately 140 of these were involved in depressing breathing. In turn, only about half, ~ 70, appeared to be necessary to rescue opioid-induced depression of the preBötC rhythm (Bachmutsky et al., 2020).

With the aim of dissecting the cellular and network mechanisms of OIRD, Baertsch et al., (2021) developed an in silico model of OIRD employing transgenic mice and the application of optogenetics to *Oprm1*-expressing neurons together with electrophysiology and computational modelling approaches. Although the preBötC has both excitatory and inhibitory neurons that interact in the regulation of breathing frequency (Winter et al., 2009; Baertsch et al., 2018), the excitatory glutamatergic neurons are critical for rhythmogenesis and OIRD. From their results, the authors propose that MORs inhibit a subset of preBötC neurons expressing *Oprm1*, inhibition of the respiratory network is due to reduced pre-inspiratory spiking, and inhibition of excitatory synaptic transmission leads to disruption of the rhythmogenic network.

Since OIRD can only be partially antagonized in the preBötC and KF/PBN complex, the possible involvement of other sites has been suggested and anticipated. To investigate whether the caudal medullary raphe might contribute to OIRD, Palkovic et al. (2022) administered remifentanil to ventilated, vagotomized, decerebrate rabbits at what was

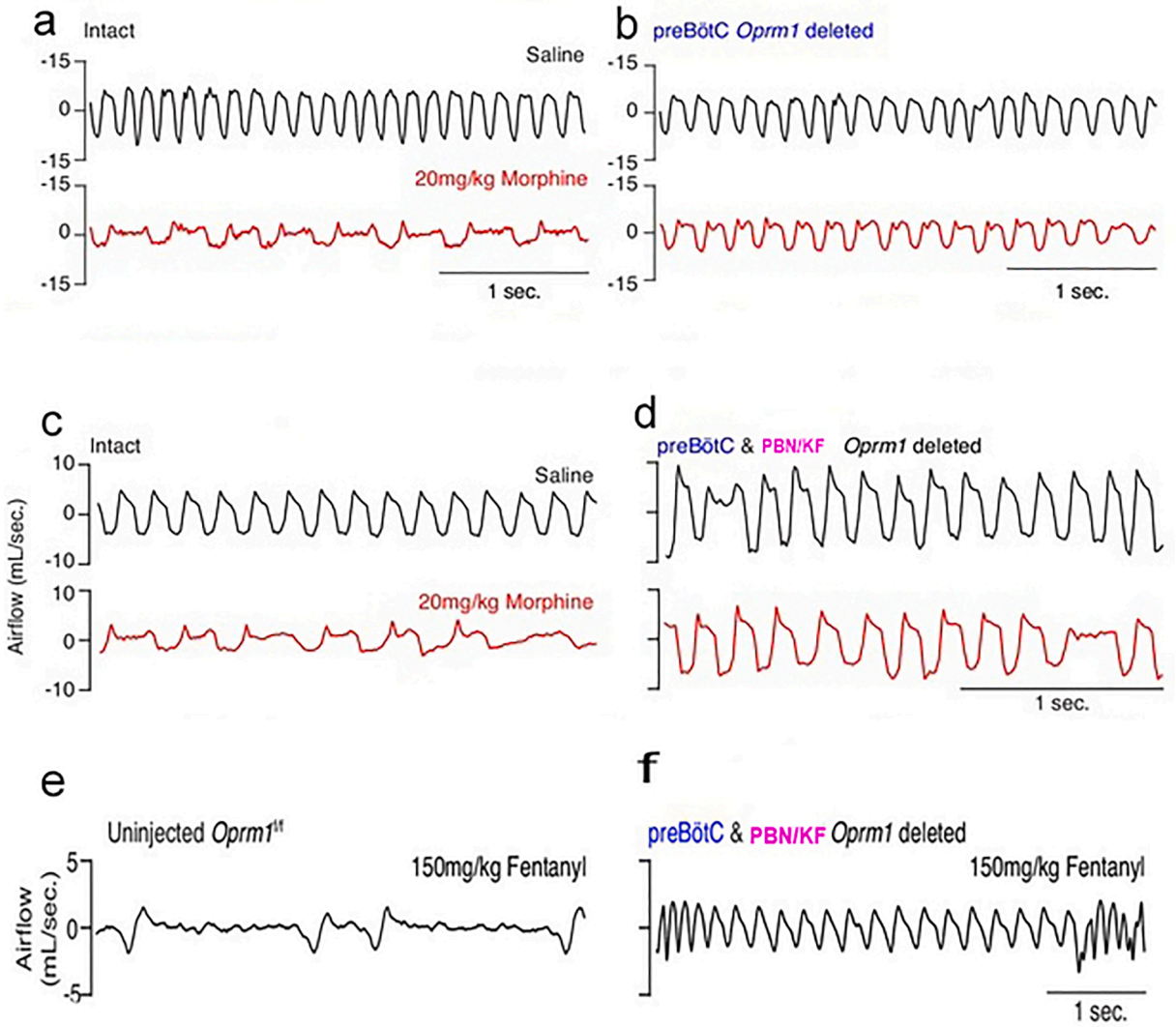

Fig. 2. Plethysmography traces from breathing in mice (airflow in mL/sec) in hypercapnia after injection of saline or opioid into the preBötC before and after *Oprm1* deletion. (a) and (b). Unlike saline, the effect on breathing by morphine was clearly depressive. (c) and (d). After *Oprm1* deletion from the preBötC and Kölliker-Fuse/parabrachial nucleus (KF/PBN), breathing after morphine in mice subjected to double deletion appeared almost identical to breathing seen in control mice. (e) and (f). Traces from breathing of $Oprm1^{fl/fl}$ floxed mice in normoxia after injection of a super-saturating dose of fentanyl (150 mg/kg) showing RD in *Oprm1*-positive floxed mice but a normal breathing pattern in mice after *Oprm1* deletion from the preBötC and KF/PBN. Adapted from Bachmutsky I, Wei XP, Kish E et al (2020) Opioids depress breathing through two small brainstem sites. eLife 9:e52694 https://doi.org/10.7554/eLife.52694, an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0).

called "analgesic", "apneic", and "very high" doses followed by naloxone microinjections into the preBötC, KF/PBN complex, and caudal medullary raphe. Complete reversal of RD was seen after analgesic and apneic, but not after very high, doses of remifentanil while, in separate animals, injection of naloxone without remifentanil into the raphe, increased the respiratory rate. The authors concluded that OIRD results from a combined effect on the respiratory rhythm generator and respiratory drive (Palkovic et al., 2022).

Neural mediation of OIRD

Understanding and differentiating mechanisms involved in RD and the regulation of nociception will be necessary before opioid pain therapy can be made safe from OIRD. Montandon and Slutsky (2019) have pointed out that the preBötC and medullary raphe are not part of nociceptive descending pathways and may therefore offer a way to prevent OIRD without, at the same time, affecting analgesia.

In the rat, preBötC glutamatergic neurones expressing the neurokinin-1 receptor (NK-1R; also known as tachykinin receptor 1, TACR1 or substance P receptor) are necessary for breathing; their deletion or depletion results in ataxic and sleep-disordered breathing and/or apnea (Gray et al., 2001; McKay et al., 2005; Tan et al., 2008). A subset of preBötC neurons coexpress NK-1R and somatostatin (Gray et al., 2010; Llona and Eugenín, 2005; Stornetta et al., 2003) while another subset expresses somatostatin (Stornetta et al., 2003). Inhibition of preBötC cells expressing somatostatin abolishes respiratory activity (Tan et al., 2008). With this background, recent research has focussed on preBötC neurons and their respiratory modulatory signal pathways.

G protein-gated inwardly-rectifying potassium (K^+) (GIRK) channels, found throughout the CNS, have been implicated in the modulation of thermal nociception and as a contributor to morphine analgesia (Blednov et al., 2003; Ikeda et al., 2000; Marker et al., 2004). In an extension of the conclusion of Montandon et al. (2011) that that neurokinin-1 receptor-expressing PreBötC neurons are selectively inhibited by opioids, presumably via potassium channels (Gray et al., 1999), subsequent investigations by this group (Montandon et al., 2016b) with GIRK2

knockout mice showed that OIRD induced by systemic administration of fentanyl substantially resulted from the opening of GIRK channels. Further investigations (Montandon et al., 2016a, 2016b) indicated that two separate preBötC neuronal circuits mediate respiratory rhythm and motor output – stimulation of respiratory rhythm and motor output by NK-1R-expressing neurones involves GIRK channels while inhibition of rhythm and motor output involves neurons expressing two somatostatin receptor subtypes. Knowledge of the somatostatin receptors remains limited (Tupal et al., 2014). Fig. 3 shows a speculative interpretation in schematic form of the molecular pathways regulating neuronal inhibition by MORs showing activation of GIRK channels and inhibition of voltage-gated $Ca^{++}$and cAMP pathways (Fig. 3a) and opioid-induced inhibition of respiratory circuits producing RD by opening of GIRK channels (Fig. 3b) (Montandon and Slutsky, 2019). A strategy of specific pharmacological targeting of GIRK channel pathways to prevent or reverse drug-induced RD but not analgesia has been suggested (Lujan et al., 2014; Marker et al., 2004).

The explanation implicating MOR-mediated post-synaptic activation

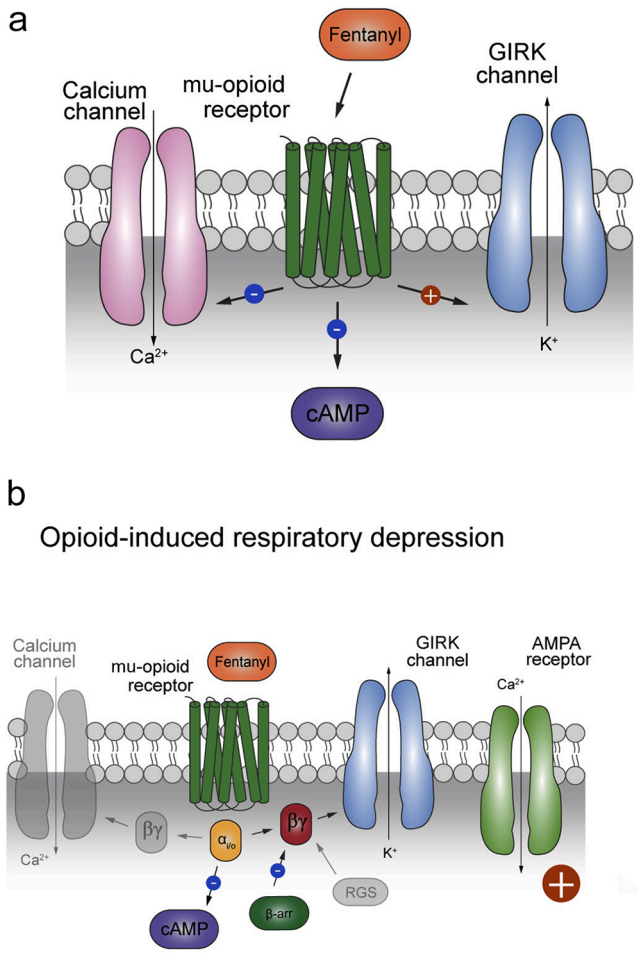

Fig. 3. Proposed speculative pathways in opioid-induced activation of the G-protein-coupled MOR triggering of (a) activation of GIRK channels and inhibition of voltage-gated $Ca^{++}$and cAMP pathways and (b) opioid-induced inhibition of respiratory circuits by opening of GIRK channels, inhibition of adenyl cyclase and recruitment of β-arrestin2. Shaded gray areas indicate pathways not known to be involved in opioid inhibition of the corresponding neuronal function. AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; βarr2, beta-arrestin2; $G\alpha_i$, G-protein α_i; $G\beta\gamma$, G-protein βγ; GIRK, G-protein-gated inwardly rectifying potassium; RGS, regulators of G-protein signaling. Adapted from Montandon G, Slutsky AS. Solving the opioid crisis: Respiratory depression by opioids as critical endpoint. Chest 2019; 156: 653–8. Reproduced with permission from Elsevier.

of GIRK potassium channels should not be seen as the only cause of OIRD. From their studies of opioid action on medullary slices containing the preBötC, and in awake mice, Wei and Ramirez (2019) concluded that MOR-mediated GIRK activation "contributed only modestly to OIRD" but KCNQ potassium channels (Abbott, 2020) independent of MOR signaling are implicated as a modulator of OIRD. Evidence advanced to support these conclusion were results showing OIRD was mimicked by KCNQ-specific activators ICA69673 and retigabine (Fig. 4a, b) while the KCNQ-specific blockers chromanol 293B and XE991 reversed the rhythmic inspiratory bursts (Fig. 4c, d). In contrast, the GIRK-specific channel blocker tertiapin-Q failed to reverse MOR agonist DAMGO ([*D*-Ala2, *N*-Me-Phe4, Gly-ol]-enkephalin)-induced OIRD and the GIRK activator ML297 failed to mimic OIRD (Fig. 4e, f).

These conclusions were supported by results obtained using an in vitro OIRD model, namely, rhythmic inspiratory bursts recorded from mouse preBötC tissue slices. Opioids activate MORs at many synapses (Hjelmstad et al., 2013; Tao and Auerbach, 2005; Zhu and Pan, 2005). To investigate whether OIRD and KCNQ channels act at a pre- or post-synaptic site, mice expressing the homeobox gene *Dbx1* were utilized. *Dbx1* is necessary for breathing during embryonic development and *Dbx1*-derived neurons are regarded as the core inspiratory rhythm generator. Injection of DAMGO significantly suppressed excitatory post-synaptic current frequency in Dbx1-expressing inspiratory interneurons but the KCNQ-specific blocker XE991 which is known to reverse morphine-induced OIRD, restored the frequency in about 70% of the neurons. Considered against the findings that the KCNQ-specific activator retigabine depressed respiratory frequency and the failure of the GIRK-specific activator ML297 to suppress morphine-induced OIRD, the results were interpreted as supporting a presynaptic mode of action by KCNQ potassium channels (Wei and Ramirez, 2019).

Studies identifying the preBötC as the primary rhythm generator (Cui et al., 2016; Del Negro et al., 2018; Smith et al., 1991) and the parabrachial complex (made up of the PBL, medial parabrachial, and KF nuclei) as a modulator of breathing (Navarrete-Opazo et al., 2020) in OIRD pathogenesis have been criticized for their use of pharmacological approaches that lack precise and localized targeting due to diffusion in situ and genetic methods that may lead to incomplete genetic deletion. With these potential difficulties in mind, Liu et al. (2021) employed contemporary cell type-specific tools with breathing monitoring in awake behaving mice to investigate the neural basis of opioid-induced respiratory depression and its rescue with a focus on the relationship between OIRD and intact PBL^{Oprm1} neurons.

While breathing rate and PBL^{Oprm1} neuronal activity were found to be tightly correlated, administration of morphine depressed the respiratory rate by about 50% and eliminated spontaneous fluctuations in breathing. Results indicated that PBL^{Oprm1} neurons modulate respiratory rate and morphine suppresses both PBL^{Oprm1} neuronal activity and the coupling of this activity with the respiratory rate. In relation to OIRD, the results were interpreted as showing that the genetically defined PBL^{Oprm1} neurons are an important regulator of RD and opioid-induced inhibition of these neurons leads to RD while their activation via endogenous or GPCR signaling rescues OIRD in mice (Liu et al., 2021).

For a more detailed discussion of cellular, physiological and neural aspects of OIRD, see the recent extensive review by Ramirez et al., (2021).

Eupnea, sighs, and gasps

The control of breathing can be seen as two mechanisms that are not always separate and distinct, namely the generation of rhythm and pattern. In the former, rhythm generating mechanisms control breathing frequency in a one-, two-, or three-phase rhythm while regulation of tidal volume and activation of upper airway and respiratory muscles are considered pattern generation (Fogarty et al., 2018; Ramirez and Baertsch, 2018). The three phases of breathing, inspiration,

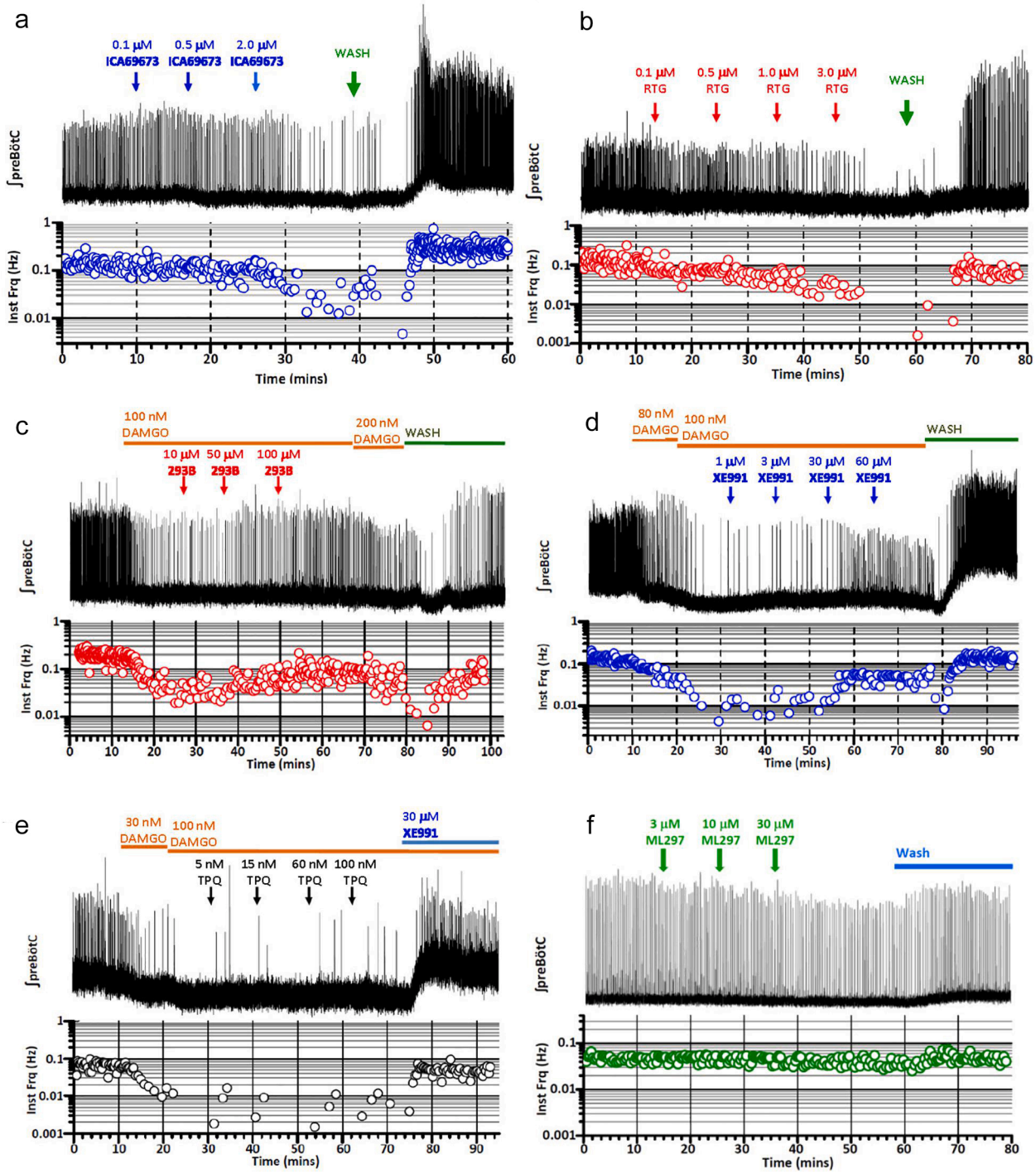

Fig. 4. Inspiratory rhythmic bursts recorded in an in vitro model of DAMGO-induced OIRD in mouse preBötC slices in the presence or absence of GIRK and KCNQ potassium channel activators and blockers. Inspiratory bursts mimicking OIRD following increasing doses of the KCNQ-specific activators (a) ICA69673 and (b) retigabine. Rescue from DAMGO-induced OIRD with increasing doses of the KCNQ-specific blockers (c) chromanol 293B and (d) XE991. Failure to rescue DAMGO-induced OIRD with increasing doses of the GIRK-specific blocker tertiapin-Q (e) and failure to mimic OIRD with increasing doses of the GIRK-specific activator ML297 (f). RTG, retigabine; TPQ, tertiapin-Q. From Wei AD, Ramirez J-M. Presynaptic mechanisms and KCNQ potassium channels modulate opioid depression of respiratory drive. Front. Physiol. 2019; 10: 1407. https://doi.org/10.3389/fphys.2019.01407, an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0).

postinspiration, and expiration, can be switched to a different breathing rhythm, for example, to gasping during hypoxia, a one-phase inspiratory rhythm (Ramirez and Baertsch, 2018; Wang et al., 1996). The processes of rhythm and pattern generation can be connected, for example, changing breathing rhythm can change its pattern and altering the pattern can change the breathing rhythm (Baertsch et al., 2018).

Transformation of normal breathing into sighs or gasps, originally thought to be due to a eupneic center in the pons and a gasping center in the medulla, raises the question of which brainstem neural networks are involved (Del Negro et al., 2018; Feldman and Gray, 2000; Lieske et al., 2000; St-John, 1998). Early studies using brainstem slices containing the preBötC, demonstrated breathing patterns similar to eupnea, sighs, and

gasps (Lieske et al., 2000); normal oxygenation generated rhythms similar to normal breathing and sighs, and anoxia produced gasping, facilitating autoresuscitation (Guntheroth and Kawabori, 1975). These findings led to the conclusion that the three patterns of breathing were each controlled by rhythm-generating neurons of the preBötC. In recent experiments on spontaneously breathing rats, Shoemaker et al. (2020) demonstrated that a clinically-relevant dose of morphine (3 mg/kg), significantly depressed normal breathing but did not affect gasping breathing (Fig. 5) suggesting to the authors two possible explanations. Firstly, neurons involved in opioid-induced depression of eupnea are distinct from those involved in gasping rhythm; since preBötC neurons are heterogeneous, gasping may be controlled by a subpopulation of neurons not sensitive to opioids. Secondly, the threshold for opioid-induced depression of respiration is higher for gasping breathing. Relevant to this explanation is the possibility that eupnea and gasping may be modulated by different ion channels or synaptic mechanisms (Viemari et al., 2011). In another possible mechanism, the inhibited preBötC neurons might receive upstream drive that is insensitive to opioids but able to promote the inspiratory activity needed for gasping. Whatever the mechanism, the results are difficult to reconcile with an explanation implicating a single population of preBötC neurons in the generation of both eupnea and gasping.

The finding of a 20% increased mortality in the morphine-treated hypoxic rats and cardiovascular changes including decreased blood pressure (Fig. 5) led the authors to speculate that mortality in OIRD is a failure in breathing rhythm rather than RD and changes in vasomotor tone, hypotension, and possibly cardiac arrhythmia, may prevent respiratory autoresuscitation and the return of eupneic breathing (Shoemaker et al., 2020).

Neonatal opioid withdrawal syndrome

Opioid use during pregnancy is increasing (Epstein et al., 2013; Stover and Davis, 2015) and this, in turn, has led to increasing numbers of infants with neonatal opioid withdrawal syndrome (NOWS), also called neonatal abstinence syndrome (NAS), a dysfunction of the CNS and autonomic nervous and gastrointestinal systems (McQueen and Murphy-Oikonen 2016). The number of cases of NOWS has increased many-fold in the last decade with an incidence up to ~14 per 1000 in some groups (Winkelman et al., 2018). In 2019, the use of prescription opioids was self-reported by 6.5% of pregnant women in the US (Ko et al., 2020).

Opioids cross the placenta and may suppress neonatal rhythmic respiratory activity (Hauser and Knapp, 2018) modulated by the preBötC in the medulla but the effects of opioids on maternal and neonatal respiratory circuits are inadequately investigated and the pathophysiology of NOWS remains poorly understood. Methadone, a standard treatment for maternal opioid dependence and administered to alleviate symptoms of NOWS (Bhavsar et al., 2018), was recently used by Hocker et al. (2021) to investigate early onset respiratory rhythmogenesis in the rat where NK1R-positive neurons in the area of the preBötC appear at gestational day(s) 12.5–13.5 (Pagliardini et al., 2003) and generation of respiratory rhythm and breathing begin on gestational day 17. From day 17, pregnant dams were injected daily with methadone or saline until postnatal (P) day 5 (P5) before neonatal assessment of baseline breathing and ventilatory responses by whole body plethysmography (Fig. 6). Over a 5-day postnatal period, neonates exposed to methadone showed an increase in apneas and destabilized breathing. Also, and surprisingly, desensitization to RD occurred – at P0 and P1, frequency of methadone-induced RD and tidal volume were blunted, although protection was rapidly lost or normalized between P1 and P2. (Fig. 6). The authors suggested that the loss of protection against OIRD was a result of its coincidence with the preBötC becoming the dominant generator of respiratory rhythm. The relevance of these results to humans remains to be determined but the findings may be important for the further understanding of NOWS and treatments for the alleviation of

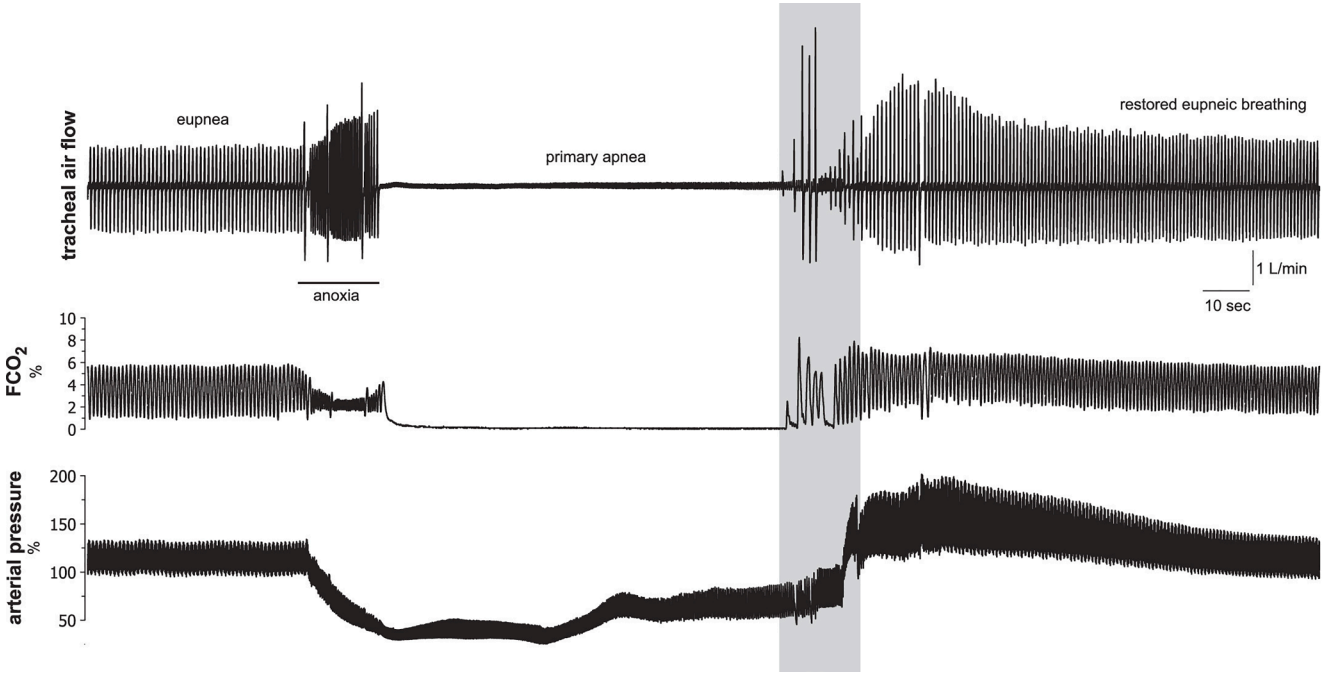

Fig. 5. Responses to transient anoxia during autoresuscitation in a pentobarbital anesthetized rat preinjected with a clinically-relevant dose of morphine (3 mg/kg) showing respiratory flow, FCO_2 fraction, and arterial blood pressure waveforms. Left to right shows resting breathing in normal air (marked 'eupnea'), followed by breathing anoxic air (100% nitrogen) during phase marked 'anoxia'. Access to room air was restored at the onset of respiratory arrest. Eupnea, restored by gasping, was followed by a brief secondary apnea before the return of full eupneic breathing. During autoresuscitation, a profound cardiovascular response in the form of a marked fall in blood pressure occurred. Adapted and reproduced from Shoemaker, A., Steelman, K., Srbu, R., Bell, H.J., 2020. Disparity in the effect of morphine on eupnea and gasping in anesthetized spontaneous breathing adult rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 319, R526-540, with permission from the American Physiological Society.

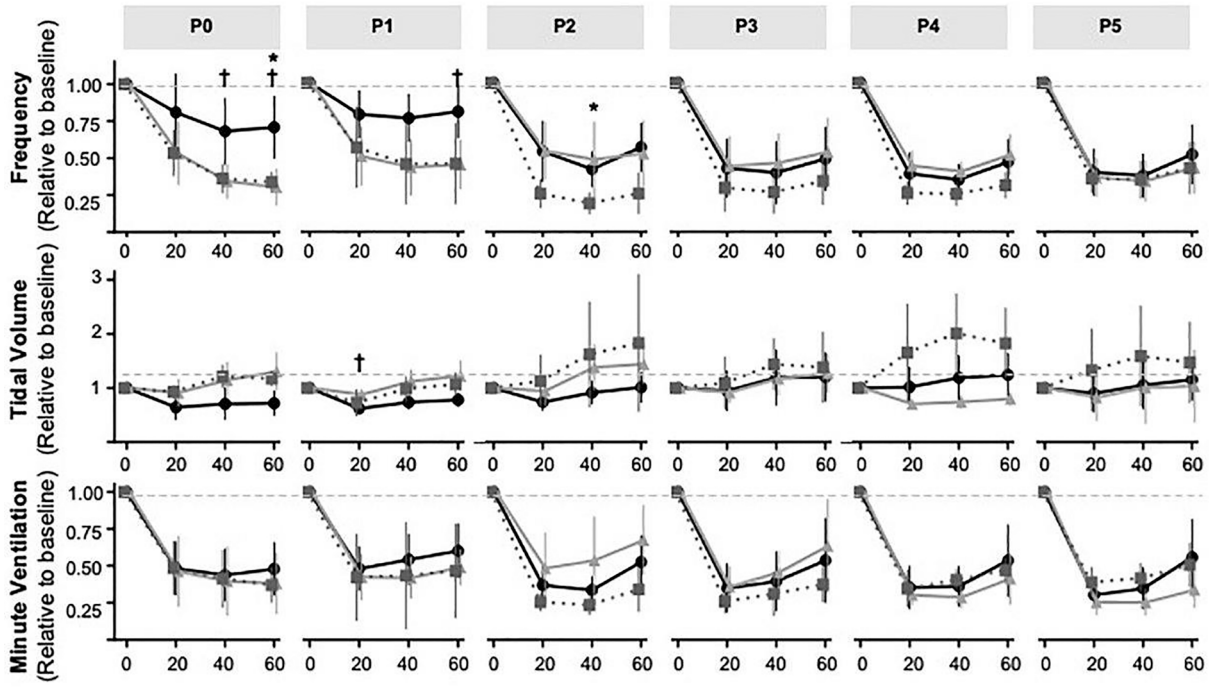

Fig. 6. Maternal methadone (MM) desensitizes neonatal rats at postnatal (P) days P0 and P1 to methadone-induced respiratory depression. Ventilation was measured from P0 to P5 using whole body plethysmography. Hypoxic ventilatory responses were measured in neonates 1 h after injection of methadone 1 mg/kg i.p. At P0 and P1, frequency of methadone-induced RD and tidal volume were blunted compared to MN (maternal no injection) and MS (maternal saline injection) and MM neonates at P2 also showed blunted RD frequency compared to MN at 40 min. Maternal methadone dosage MM, black circles; maternal no treatment MN, black squares; maternal saline dosage MS, gray triangles. P, postnatal day number. *MM $p < 0.05$ from MN; †MM $p < 0.05$ from MS. Adapted and reproduced from Hocker AD, Morrison NR, Selby ML et al (2021) Maternal methadone destabilizes neonatal breathing and desensitizes neonates to opioid-induced respiratory frequency depression. Front Physiol 12:604593. https://doi.org/10.3389/fphys.2021.604593, an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY).

its symptoms.

Neonatal antidepressant exposure syndrome

Anxiety, depression, and other psychiatric comorbidities occur in an estimated 25–45% of pregnant women with opioid use disorder who are most commonly treated with serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors (SRIs) (Kaltenbach et al., 2012). In neonates, so-called neonatal antidepressant exposure syndrome (NADES) (Nordeng et al., 2001; Moses-Kolko et al., 2005), may occur following in utero exposure to selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs) during the last trimester of pregnancy. Exposed children display changes in motor development and movement control (Casper et al., 2003; Masiag et al., 2006) while in rats, signs and symptoms of neonates with NADES include CNS, motor, respiratory, and gastrointestinal effects (Moses-Kolko et al., 2005). In experiments on postnatal rats (days 8–21), Maciag et al. (2006) found that exposure to SSRIs led to a reduction in both tryptophan hydroxylase-2 (TPH2) (Matthes and Bader, 2018) in dorsal raphe and serotonin reuptake transporter protein (SERT) in cortex. Along with this, changes occurred in behavior including increased locomotor activity. It was concluded that SSRIs at an early age disrupt the normal maturation of the serotonin system causing lasting effects on behavior and serotonin-dependent neuronal processes. Importantly, up to 30% of human neonates with prenatal exposure to SRIs show an increased risk of RD. Adverse outcomes, including RD, were shown to be associated with SERT genotype SLC6A4 with reduced Apgar scores in neonates primarily due to reduced respiratory effort (Oberlander et al., 2008). In 2004 the FDA issued a warning recommending that SSRIs administration be tapered off 7–10 days prior to delivery. An assessment of 700,000 infants with antenatal exposure to SSRIs revealed neonates were more likely to experience RD and CNS-related symptoms (Einarson et al., 2010). A recent case of RD in a neonate born to a mother on maximum dose sertraline provides a good example of the increased respiratory risk and consequences of the perinatal complications (Marchand et al., 2021).

In rodents, the presence of serotonin has been shown to be critical for eupnea and the generation of gasping and autoresuscitation in response to hypoxia (Cummings et al., 2011; Hodges et al., 2009). Early results obtained in vitro and in situ demonstrated that fictive gasping in mice was eliminated by 5-HT_{2A} antagonists (Tryba et al., 2006) and endogenous activation of the 5-HT_{2A} receptor is required for eupneic ventilation (Hodges et al., 2009; Peña and Ramirez, 2002; Ptak et al., 2009). Recently, in vivo results with tryptophan hydroxylase 2 KO ($TPH2^{-/-}$) and wild-type mice, 5-HT_{2A} and 5-HT_{1A} agonists, and a 5-HT_{2A} antagonist, also showed that both eupnea and gasping proceed via 5-HT_{2A} receptors (Cummings, 2021).

Similarities of neonatal antidepressant exposure syndrome and opioid-induced serotonin toxicity

The central pool of serotonin is formed by TPH2-catalysed decarboxylation and hydroxylation of tryptophan (Gutknecht et al., 2008; Walther and Bader, 2003), an isoform expressed in raphe neurons in the brainstem (Saper, 2000). Serotonergic neurons in this region form a neurotransmitter system that affects most parts of the CNS. Depolarization of the presynaptic axon releases 5-HT from storage in presynaptic vesicles into the synaptic cleft where it can bind to postsynaptic 5-HT receptors effecting neurotransmission. Control of 5-HT levels proceeds by a number of mechanisms including feedback loops, metabolizing

enzymes (e.g., monoamine oxidase A) and, in particular for the discussion here, reuptake via the SERT (Fig. 7). SERT is a monoamine transporter, recycling serotonin from the synaptic cleft to the presynaptic terminal (Murphy et al., 2008). Inhibition of SERT results in raised intrasynaptic and postsynaptic concentrations of serotonin that activate postsynaptic 5-HT receptors effecting neurotransmission and multiple downstream cognitive, motor, and autonomic functions. Postsynaptic receptors 5-HT_{1A} and 5-HT_{2A} have been implicated in serotonin toxicity/syndrome (ST) where they may act in concert (Arnt and Hyttel, 1989; Scotton et al., 2019). In addition to the effects of opioids Baldo, 2018, 2021; Baldo and Rose, 2020) commonly used drugs that inhibit serotonin reuptake include tricyclic antidepressants, cocaine, 3,4-methylenedioxymethamphetamine (MDMA, ecstasy), and importantly, SSRIs and SNRIs (Gillman, 2006; Gillman and Whyte, 2004; Scotton et al., 2019). From the mechanistic perspective, given the increase in intrasynaptic serotonin concentrations, postsynaptic 5-HT receptor stimulation, and resultant receptor-mediated adverse effects in both ST and NADES, the latter might be seen as a condition analogous to ST e (see also Oberlander et al., 2008) except for some difference in the overall spectra of resulting adverse events: clonus, agitation, irritability, diaphoresis, tremor, hyperreflexia, diarrhea, nausea, RD (in severe cases) and vomiting for ST and RD, hypoxia, tachypnea, jitteriness, irritability, tremors, diarrhea, nausea, vomiting and increased muscular and neuromotor tone for NADES. However, some of these signs in the two conditions are the same or similar, in particular, RD may occur in both conditions. Relevant to this hypothesis, and of particular interest here, are results of the recent prospective study of Bakhireva et al. (2022) showing the association in pregnant women of more severe NOWS with the use of SRIs. Thus, both mechanistically and clinically, NADES, and ST show some close similarities.

The CYP2D6 phenotype, opioids, and opioid-induced respiratory depression

The possible importance of genetic polymorphisms, in particular, the highly polymorphic cytochrome drug-metabolizing enzyme P450 phenotype CYP2D6, needs to be recognized as a contributor to the individual variability of patient responses to opioids and the occurrence of OIRD since this polymorphism can be a major factor affecting drug detoxification, activation, and plasma concentration of some opioids. Of the more than 100 designated CYP2D6 allelic variants and subvariants, patients of different CYP2D6 genotype may show normal, increased, decreased, or no CYP2D6 enzyme activity. With, for example, tramadol, CYP2D6 converts this prodrug to the active metabolite *O*-desmethyltramadol (M1) which has higher μ-opioid receptor affinity and is up to six times more potent as an analgesic. Examples of allelic variants producing different enzyme functions are: normal function, CYP2D6*1 and CYP2D6*2; decreased function, CYP2D6*9 and CYP2D6*10; function absent, CYP2D6*3 and CYP2D6*4; and increased function, a duplication of alleles such as CYP2D6*1, CYP2D6*2, and CYP2D6*35. Patients showing reduced CYP2D6 activity are designated 'intermediate metabolizers', those showing no CYP2D6 activity are known as 'poor metabolizers', and the label 'ultrarapid metabolizers' is used for patients with increased enzyme activity. It follows that for patients with no, or decreased CYP2D6 activity, a standard dose of tramadol will not be fully converted to M1 and hence pain relief may be lessened. For ultrarapid

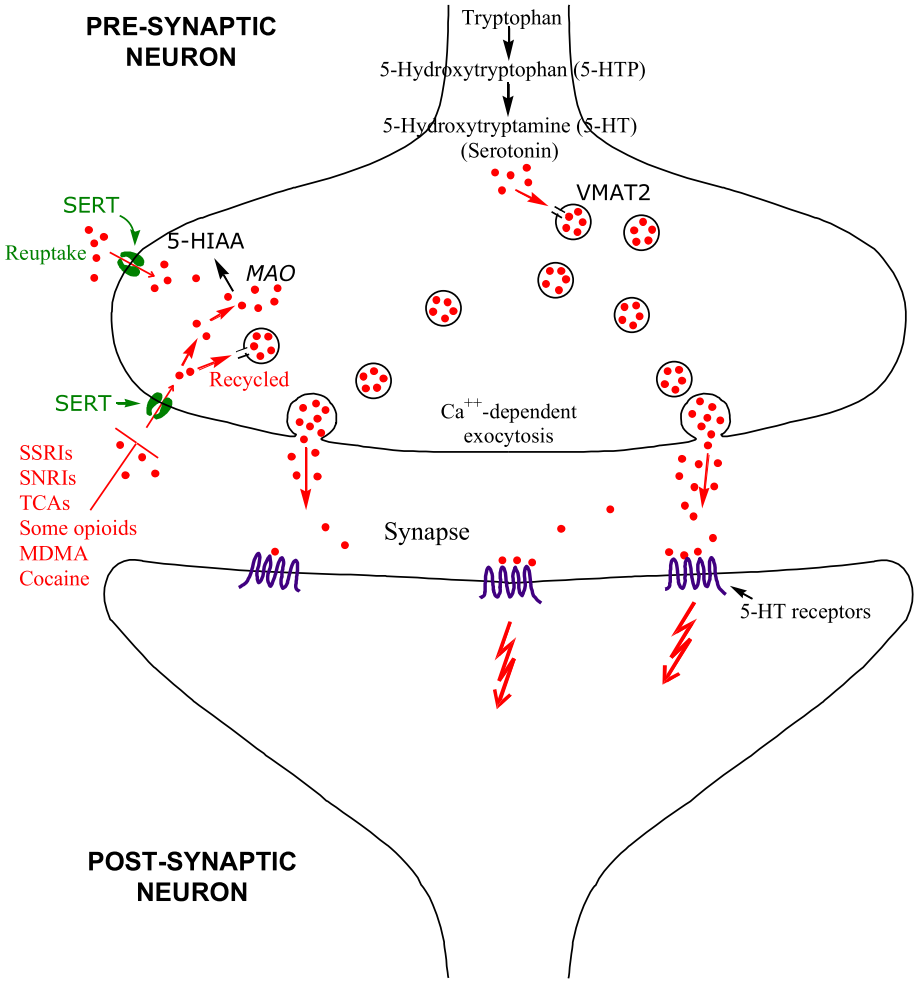

Fig. 7. Diagrammatic representation of raised intrasynaptic concentrations of serotonin (5-hydroxytryptamine, 5-HT) and inhibition of reuptake of 5-HT by different serotonergic drugs. Serotonin, packaged into vesicles, is released by Ca++-dependent exocytosis into the synapse where it diffuses to its receptors on the post-synaptic neuron. Reuptake of the neurotransmitter into pre-synaptic nerve terminals is effected by serotonin transporter (SERT) which ensures and maintains low 5-HT plasma levels and is important for the rapid reuptake of the neurotransmitter into pre-synaptic nerve terminals. Opioids, in particular, tramadol, dextromethorphan, pethidine, methadone, and tapentadol (but generally not morphine and other phenanthrenes), SRIs, and other serotonergic agents inhibit reuptake of serotonin by inhibiting SERT, thus increasing plasma and synaptic cleft serotonin concentrations available to bind and activate the post-synaptic 5-HT receptors. In the pre-synaptic neuron, serotonin is metabolized by monoamine oxidase (MAO) to 5-hydroxyindoleacetic acid (5-HIAA). Reproduced from Baldo, BA., Rose, MA., 2020. The anaesthetist, opioid analgesic drugs, and serotonin toxicity: a mechanistic and clinical review. Br. J. Anaesth. 124, 44–62, with permission from Elsevier,

metabolizers, life-threatening effects after a standard dose can occur (Elkalioubie et al., 2011 30). A standard dose has been reported to induce RD due to an increased exposure to M1 in a patient with CYP2D6 gene duplication (Stamer et al. 2008). Note that different ethnic groups show differences in incidences of enzyme function. In 2021, the FDA boxed warning for tramadol included interactions with drugs affecting cytochrome P450 isoenzymes with the specific requirement for "careful consideration of the effects of the parent drug, tramadol, and the active metabolite, M1". The warning also covers the risk of life-threatening RD in infants of nursing mothers, children under 12 and, in some cases, those under 18. In addition, under the Warnings and Precautions for tramadol, the FDA states that individuals who are ultra-rapid metabolizers should not use the drug (Deane and Kane, 2021; Tramadol. Highlights of prescribing information, 2021).

Like tramadol, codeine is a prodrug that binds weakly to the μ-opioid receptor and exerts its analgesic action mainly by ~5–10% of the drug undergoing CYP2D6-catalysis to morphine. Consequently, the regulatory warnings and precautions issued for tramadol, also apply to codeine. Also, like tramadol, variant alleles of CYP2D6 in some individuals who are ultrarapid metabolizers, or poor metabolizers, lead to higher or lower percentage conversions, respectively to morphine (Eckhardt et al., 1998). Ultrarapid metabolizers may have toxic concentrations of morphine even after low codeine doses (Gasche et al., 2004; Kirchheiner et al., 2007).

Hydrocodone and oxycodone are also metabolized to more potent metabolites by CYP2D6 but the resultant effects on analgesia and toxicity remain to be fully determined (Crews et al., 2021; St Sauver et al., 2017). Methadone is metabolized to a small extent by CYP2D6 but individuals of CYP2D6 genotype appear to show little or no effect in relation to opioid dose, analgesia, or adverse reactions (Crews et al., 2021).

In CYP2D6 normal metabolizers, only about 5% of a given dose of hydrocodone is *O*-demethylated by the enzyme to hydromorphone (Cone et al., 1978), an opioid with a MOR affinity 100-fold higher than the parent (Volpe et al., 2011). However, with regard to analgesia, the relative contributions of parent and metabolite are not clear. In CYP2D6 poor metabolizers, the mean plasma concentration of hydromorphone is only one-fifth that of normal metabolizers. It is not yet known whether this coincides with lesser analgesia and any adverse events (Otton et al., 1993).

Approximately 11% of a dose of oxycodone is demethylated by CPY2D6 to oxymorphone which has an affinity for the MOR 60-times that of oxycodone (Volpe et al., 2011). However, it appears that the parent drug is predominately responsible for pain relief (Klimas et al., 2013). Data is conflicting on the relationship between CYP2D6 metabolizer phenotype and analgesic and toxic effects of oxycodone and more studies are needed to determine the effects and risks, if any.

Concluding remarks

Despite RD remaining the main cause of death due to opioid overdose, the underlying mechanisms leading to fatalities are still being worked out with significant knowledge gaps outstanding. The number and variety of animal models used in the study of OIRD, namely mice, rats, rabbits, cats, dogs, and goats has led to some confusing interpretations and doubtful extrapolations to humans all of which has not helped in achieving consensus on the causes and prevention of OIRD. Even so, and as outlined here, considerable progress has been made principally by the application of combined pharmacological and genetic approaches together with electrophysiological recordings on tissue slices in vitro and whole-body plethysmography in vivo. Although multiple sites are involved in OIRD, the preBötC and KF/PBN are major contributors to it with the former of prime importance if only because its inhibition leads to respiratory failure. Inhibition of KF and/or PBN neurons leads to significant RD and can lead to apnea. Note, however, removal of MORs from the preBötC only partially prevents OIRD. Ongoing OIRD research needs to be focussed on understanding neuromodulation involving opioids acting on rhythm-generating neurons at respiratory centers in the brainstem, particularly the preBötC where reduced synaptic glutamatergic transmission and rhythmogenic capacities result in opioid-induced apnea. A consequence of the present gaps in our understanding of OIRD is that, with the exception of naloxone (which brings its own difficulties including its short elimination half-life and reversal of analgesia sometimes leading to acute withdrawal symptoms), there are currently limited treatment options for it and opioid addiction (Algera et al., 2019). Clearly, alternative targeted therapies for rescuing RD, preventing opioid overdose, and alleviating addiction are sorely needed.

Although protection against OIRD after maternal administration of methadone is quickly lost in rats soon after birth when the preBötC becomes the primary rhythm generator (Hocker et al., 2021), it remains to be determined if the same is true in humans. Given the increasing number of neonates exposed to gestational opioids, further investigation of the effects of opioids on neurorespiratory disruption and developing respiratory circuits is warranted. In rats, symptoms of neonates with NADES include CNS and respiratory effects while in humans, NADES may result after in utero exposure to SRIs during the last trimester of pregnancy. Up to 30% of human neonates with prenatal exposure to SRIs show an increased risk of RD. Considering the increase in intrasynaptic serotonin concentrations, postsynaptic 5-HT receptor stimulation, resultant receptor-mediated adverse effects in both ST and NADES, and a similarity of some of the symptoms, the two conditions have some obvious similar mechanistic features. This hypothesis is reinforced by results showing an association in pregnant women of more severe NOWS with the use of SRIs.

The demonstration by Shoemaker et al. (2020) that morphine depresses normal, but not gasping breathing in rats may indicate that an individual population of preBötC neurons is not involved in both eupnea and gasping and the underlying mechanisms of these two forms of breathing are therefore distinct. Further investigation should follow the finding of increased morphine-induced mortality due to hypoxemia even in the absence of gasping rhythm, since this suggests that opioid- related cardiovascular rather than opioid-related depression of eupnea, may be involved in cases of opioid overdose (Shoemaker et al., 2020).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Brian A. Baldo: Conceptualization, Data curation, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

Brian A. Baldo has no support to declare and there are no other relationships or activities of any kind to declare.

References

Abbott, G.W., 2020. KCNQs: Ligand- and voltage-gated potassium channels. Front. Physiol. 11, 583. https://doi.org/10.3389/fphys.2020.00583.

Algera, M.H., Kamp, J., van der Schrier, R., van Velzen, M., Niesters, M., Aarts, L., Dahan, A., Olofsen, E., 2019. Opioid-induced respiratory depression in humans: a review of pharmacokinetic-pharmacodynamic modelling of reversal. Br. J. Anaesth. 122 (6), e168–e179. https://doi.org/10.1016/j.bja.2018.12.023.

Arnt, J., Hyttel, J., 1989. Facilitation of 8-OHDPAT-induced forepaw treading of rats by the 5-HT2 agonist DOI. Eur. J. Pharmacol. 161, 45–51. https://doi.org/10.1016/0014-2999(89)90178-7.

Bachmutsky, I., Wei, X.P., Kish, E., Yackle, K., 2020. Opioids depress breathing through two small brainstem sites. eLife 9, e52694. https://doi.org/10.7554/eLife.52694.

Baertsch, NA., Baertsch, HC., Ramirez, J-M., 2018. The interdependence of excitation and inhibition for the control of dynamic breathing rhythms. Nature Commun. 9, 843. https://doi.org/10.1038/s41467-018-03223-x.

Baertsch, NA., Bush, NE., Burgraff, NJ., Ramirez, J-M., 2021. Dual mechanisms of opioid-induced respiratory depression in the inspiratory rhythm-generating network. eLife 10:e67523, https://doi.org/10.7554.eLife.67523.

Bakhireva, L.N., Sparks, A., Herman, M., Hund, L., Ashley, M., Salisbury, A., 2022. Severity of neonatal opioid withdrawal syndrome with prenatal exposure to serotonin reuptake inhibitors. Pediatr. Res. 91 (4), 867–873. https://doi.org/10.1038/s41390-021-01756-4.

Baldo, B.A., 2018. Opioid analgesic drugs and serotonin toxicity (syndrome): mechanisms, animal models, and links to clinical effects. Arch. Toxicol. 92, 2457–2473. https://doi.org/10.1007/s00204-018-2244-6.

Baldo, B.A., 2021. Toxicities of opioid analgesics: respiratory depression, histamine release, hemodynamic changes, hypersensitivity, serotonin toxicity. Arch. Toxicol. 95, 2627–2642. https://doi.org/10.1007/s00204-021-03068-2.

Baldo, B.A., Rose, M.A., 2020. The anaesthetist, opioid analgesic drugs, and serotonin toxicity: a mechanistic and clinical review. Br. J. Anaesth. 124, 44–62. https://doi.org/10.1016/j.bja.2019.08.010.

Bhavsar, R., Kushnir, A., Kemble, N., 2018. Incidence and severity of neonatal abstinence syndrome in infants with prenatal exposure to methadone versus buprenorphine. Pediatrics 142, 145. https://doi.org/10.1542/PEDS.142.1_MEETINGABSTRACT.145.

Blednov, Y.A., Stoffel, M., Alva, H., Harris, R.A., 2003. A pervasive mechanism for analgesia: activation of GIRK2 channels. Proc. Natl. Acad. Sci. USA 100 (1), 277–282. https://doi.org/10.1073/pnas.012682399.

Casper, R.C., Fleisher, B.E., Lee-Ancajas, J.C., Gilles, A., Gaylor, E., DeBattista, A., Hoyme, H.E., 2003. Follow-up of children of depressed mothers exposed or not exposed to antidepressant drugs during pregnancy. J. Pediatr. 142 (4), 402–408. https://doi.org/10.1067/mpd.2003.139.

CDC for Disease Control and Prevention. NVSS Vital Statistics Rapid Release Overdose Death Counts. Update 2/16/2022. https://www.cdc.gov/nchs/nvss/vsrr/drug-overdose-data.htm. Accessed March 1, 2022.

Cinelli, E., Bongianni, F., Pantaleo, T., Mutolo, D., 2020. Activation of μ-opioid receptors differentially affects the preBötzinger Complex and neighbouring regions of the respiratory network in the adult rabbit. Resp. Physiol. Neurobiol. 280, 103482. https://doi.org/10.1016/j.resp.2020.103482.

Cone, E.J., Darwin, W.D., Gorodetzky, C.W., Tan, T., 1978. Comparative metabolism of hydrocodone in man, rat, guinea pig, rabbit, and dog. Drug Metab. Dispos. 6, 488–493. https://doi.org/10.1111/j.2042-7158.1979.tb13507.x.

Crews, K.R., Monte, A.A., Huddart, R., Caudle, K.E., Kharasch, E.D., Gaedigk, A., Dunnenberger, H.M., Leeder, J.S., Callaghan, J.T., Samer, C.F., Klein, T.E., Haidar, C. E., Van Driest, S.L., Ruano, G., Sangkuhl, K., Cavallari, L.H., Müller, D.J., Prows, C. A., Nagy, M., Somogyi, A.A., Skaar, T.C., 2021. Implementation Consortium Guideline for *CYP2D6, OPRM1*, and *COMT* genotypes and select opioid therapy. Clin. Pharmacol. Ther. 110 (4), 888–896. https://doi.org/10.1002/cpt.2149.

Cui, Y., Kam, K., Sherman, D., Janczewski, W.A., Zheng, Y., Feldman, J.L., 2016. Defining preBötzinger complex rhythm- and pattern-generating neural microcircuits in vivo. Neuron 91, 602–614. https://doi.org/10.1016/j.neuron.2016.07.003.

Cummings, K.J., 2021. Eupnea and gasping in vivo are facilitated by activation of 5-HT2A receptors. J. Neurophysiol. 125, 1543–1551. https://doi.org/10.1152/jn.00088.2021.

Cummings, K.J., Hewitt, J.C., Li, A., Daubenspeck, J.A., Nattie, E.E., 2011. Postnatal loss of brainstem serotonin neurones compromises the ability of neonatal rats to survive episodic severe hypoxia. J. Physiol. 589, 5247–5256. https://doi.org/10.1113/jphysiol.2011.214445.

Dahan, A., Aarts, L., Smith, T.W., 2010. Incidence, reversal, and prevention of opioid-induced respiratory depression. Anesthesiology 112, 226–238. https://doi.org/10.1097/ALN.0b013e3181c38c25.

Deane, L., Kane, M., 2021. Tramadol therapy and CYP2D6 genotype. 2015 Sep 10 [Updated 2021 July 20]. In: Pratt, V.M., Scott, S.A., editors. Medical Genetic Summaries. Bethesda MD: National Center for Biotechnology Information (US); 2012. p. 1-20. Available from https://www.ncbi.nlm.nih.gov/books/NBK315950/ (accessed March 1, 2022).

Del Negro, C.A., Funk, G.D., Feldman, J.L., 2018. Breathing matters. Nat. Rev. Neurosci. 19, 351–367. https://doi.org/10.1038/s41583-018-0003-6.

Eckhardt, K., Li, S., Ammon, S., Schanzle, G., Mikus, G., Eichelbaum, M., 1998. Same incidence of adverse drug events after codeine administration irrespective of the genetically determined differences in morphine formation. Pain 76, 27–33. https://doi.org/10.1016/S0304-3959(98)00021-9.

Einarson, A., Choi, J., Einarson, T.R., Koren, G., 2010. Adverse effects of antidepressant use in pregnancy: an evaluation of fetal growth and preterm birth. Depression Anxiety 27, 35–38. https://doi.org/10.1002/da.20598.

Elkalioubie, A., Allorge, D., Robriquet, L., Wiart, F.-W., Garat, A., Broly, F., et al., 2011. Near-fatal tramadol cardiotoxicity in a CYP2D6 ultrarapid metabolizer. Eur J. Clin. Pharmacol. 67, 855–858. https://doi.org/10.1007/s00228-011-1080-x.

Epstein, R.A., Bobo, W.V., Martin, P.R., Morrow, J.A., Wang, W., Chandrasekhar, R., et al., 2013. Increasing pregnancy-related use of prescribed opioid analgesics. Ann. Epidemiol. 23, 498–503. https://doi.org/10.1016/j.annepidem.2013.05.017.

Feldman, J.L., Gray, P.A., 2000. Sighs and gasps in a dish. Nat. Neurosci. 3, 531–532. https://doi.org/10.1038/75695.

Fogarty, M.J., Mantilla, C.B., Sieck, G.C., 2018. Breathing: Motor control of diaphragm muscle. Physiology (Bethesda) 33, 113–126. https://doi.org/10.1152/physiol.00002.2018.

Gasche, Y., Daali, Y., Fathi, M., Chiappe, A., Cottini, S., Dayer, P., et al., 2004. Codeine intoxication associated with ultrarapid CYP2D6 metabolism. N. Engl. J. Med. 351, 2827–2831. N. Engl. J. Med. 351, 2827–2831. https://doi.org/10.1056/NEJMoa041888.

Gillman, P.K., 2006. A review of serotonin toxicity data: implications for the mechanisms of antidepressant drug action. Biol Psychiatry 59, 1046–1051. https://doi.org/10.1016/j.biopsych.2005.11.016.

Gillman, P.K., Whyte, I.M., 2004. Serotonin syndrome. In: Haddad, P., Dursun, S., Deakin, B. (Eds.), Adverse Syndromes and Pschiatric Drugs. Oxford University Press, Oxford, pp. 37–49.

Gray, P.A., Hayes, J.A., Ling, G.Y., Llona, I., Tupal, D., Picardo, M.C.D., et al., 2010. Developmental origin of preBötzinger complex respiratory neurons. J. Neurosci. 2010 (30), 14883–14895. https://doi.org/10.1523/JNEUROSCI.4031-10.2010.

Gray, P.A., Janczewski, W.A., Mellen, N., McCrimmon, D.R., Feldman, J.L., 2001. Normal breathing requires pre-Bötzinger complex neurokinin-1 receptor expressing neurons. Nat. Neurosci 4, 927–930. https://doi.org/10.1038/nn0901-927.

Gray, P.A., Rekling, J.C., Bocchiaro, C.M., Feldman, J.L., 1999. Modulation of respiratory frequency by peptidergic input to rhythmogenic neurons in the preBötzinger complex. Science 286, 1566–1568. https://doi.org/10.1126/science.286.5444.1566.

Guntheroth, W.G., Kawabori, I., 1975. Hypoxic apnea and gasping. J. Clin. Invest. 56, 1371–1377. https://doi.org/10.1172/JCI108217.

Gutknecht, L., Waider, J., Kraft, S., Kriegebaum, C., Holtmann, B., Reif, A., et al., 2008. Deficiency of brain 5-HT synthesis but serotonergic neuron formation in Tph2 knockout mice. J. Neural. Transm. (Vienna) 115, 1127–1132. https://doi.org/10.1007/s00702-008-0096-6.

Hauser, K.F., Knapp, P.E., 2018. Opiate drugs with abuse liability hijack the endogenous opioid system to disrupt neuronal and glial maturation in the central nervous system. Front. Pediatr. 5, 294. https://doi.org/10.3389/fped.2017.00294.

Hjelmstad, G.O., Xia, Y., Margolis, E.B., Fields, H.L., 2013. Opioid modulation of ventral pallidal afferents to ventral tegmental area neurons. J. Neurosci. 33, 6454–6459. https://doi.org/10.1523/JNEUROSCI.0178-13.2013.

Hocker, A.D., Morrison, N.R., Selby, M.L., Huxtable, A.G., 2021. Maternal methadone destabilizes neonatal breathing and desensitizes neonates to opioid-induced respiratory frequency depression. Front. Physiol. 12, 604593. https://doi.org/10.3389/fphys.2021.604593.

Hodges, M.R., Wehner, M., Aungst, J., Smith, J.C., Richerson, G.B., 2009. Transgenic mice lacking serotonin neurons have severe apnea and high mortality during development. J. Neurosci. 29, 10341–10349. https://doi.org/10.1523/JNEUROSCI.1963-09.2009.

Ikeda, K., Kobayashi, T., Kumanishi, T., Niki, H., Yano, R., 2000. Involvement of G-protein-activated inwardly rectifying K+ (GIRK) channels in opioid-induced analgesia. Neurosci. Res. 38, 113–116. https://doi.org/10.1016/s0168-0102(00)00144-9.

Kaltenbach, K., Holbrook, A.M., Coyle, M.G., Heikl, S.H., Salisbury, A.L., Stine, S.M., et al., 2012. Predicting treatment for neonatal abstinence syndrome in infants born to women maintained on opioid agonist medication. Addiction 107, 45–52. https://doi.org/10.1111/j.1360-0443.2012.04038.x.

Kieffer, B.L., 1999. Opioids: first lessons from knockout mice. Trends Pharmacol. Sci. 20, 19–26. https://doi.org/10.1016/s0165-6147(98)01279-6.

Kirby, G.C., McQueen, D.S., 1986. Characterization of opioids receptors in the cat carotid body involved in chemosensory depression in vivo. Br. J. Pharmacol. 88, 889–898. https://doi.org/10.1111/j.1476-5381.1986.tb16263.x.

Kirchheiner, J., Schmidt, H., Tzvetkov, M., Keulen, J-Tha., Lötsch, J., Roots, I., et al. 2007. Pharmacokinetics of codeine and its metabolite morphine in ultra-rapid metabolizers due to CYP2D6 duplication. Pharmacogenomics J., 257–265. https://doi.org/10.1038/sj.tpj.6500406.

Klimas, R., Witticke, D., El Fallah, S., Mikus, G., 2013. Contribution of oxycodone and its metabolites to the overall analgesic effect after oxycodone administration. Expert Opin. Drug Metab. Toxicol. 9, 517–528. https://doi.org/10.1517/17425255.2013.779669.

Ko, J.Y., D'Angelo, D.V., Haight, S.C., Morrow, B., Cox, S., Salvesen von Essen, B., et al., 2020. Vital signs: Prescription opioid pain reliever use during pregnancy – 34 U.S. jurisdictions. Morb. Mortal. Wkly Rep. 69, 897–903.

Lalley, P.M., Pilowsky, P.M., Forster, H.V., Zuperku, E.J., 2014. CrossTalk opposing view: The pre-Bötzinger complex is not essential for respiratory depression following systemic administration of opioid analgesics. J. Physiol. 592, 1163–1166. https://doi.org/10.1113/jphysiol.2013.258830.

Lieske, S.P., Thoby-Brisson, M., Telgkamp, P., Ramirez, J.M., 2000. Reconfiguration of the neural network controlling multiple breathing patterns: eupnea, sighs and gasps. Nat. Neurosci. 3, 600–607. https://doi.org/10.1038/75776.

Liu, S., Kim, D.-I., Oh, T.G., Han, S., 2021. Neural basis of opioid-induced respiratory depression and its rescue. Proc. Natl. Acad. Sci. USA 118: e2022134118. doi.org/10.1073/pnas.2022134118.

Llona, I., Eugenín, J., 2005. Central actions of somatostatin in the generation and control of breathing. Biol. Res. 38, 347–352. https://doi.org/10.4067/s0716-97602005000400006.

Lonergan, T., Goodchild, A.K., Christie, M.J., Pilowsky, P.M., 2003. Mu opioid receptors in rat ventral medulla: effects of endomorphin-1 on phrenic nerve activity. Respir. Physiol. Neurobiol. 138, 165–178. https://doi.org/10.1016/s1569-9048(03)00173-3.

Lujan, R., Fernandez, M., de Velasco, E., Aguado, C., Wickman, K., 2014. New insights into the therapeutic potential of Girk channels. Trends Neurosci. 37, 20–29. https://doi.org/10.1016/j.tins.2013.10.006.

Marchand, G.J., Meassick, K., Wolf, H., Hopewell, S.K., Sainz, K., Anderson, S.M., 2021. Respiratory depression in a neonate born to mother on maximum dose setraline: a case report. J. Med. Case Rep. 15, 88. https://doi.org/10.1186/s13256-020-02640-1.

Masiag, D., Simpson, K.L., Coppinger, D., Lu, Y., Wang, Y., Lin, R.C.S., et al., 2006. Neonatal antidepressant exposure has lasting effects on behavior and serotonin circuitry. Neuropsychopharmacology 31, 47–57. https://doi.org/10.1038/sj.npp.1300823.

Mansour, A., Fox, C.A., Burke, S., Meng, F., Thompson, R.C., Akil, H., Watson, S.J., 1994. Mu, delta, and kappa opioid receptor mRNA expression in the rat CNS: an in situ hybridization study. J. Comp. Neurol. 350, 412–438. https://doi.org/10.1002/cne.903500307.

Marker, C.L., Stoffel, M., Wickman, K., 2004. Spinal G-protein-gated K^+ channels formed by GIRK1 and GIRK2 subunits modulate thermal nociception and contribute to morphine analgesia. J. Neurosci. 24, 2806–2812. https://doi.org/10.1523/JNEUROSCI.5251-03.2004.

Matthes, S., Bader, M., 2018. Peripheral serotonin synthesis as a new drug target. Trends Pharmacol. Sci. 39, 560–572. https://doi.org/10.1016/j.tips.2018.03.004.

Matthes, H.W.D., Maldonado, R., Simonin, F., Valverde, O., Slowe, S., Kitchen, I., et al., 1996. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the μ-opioid-receptor gene. Nature 383, 819–823. https://doi.org/10.1038/383819a0.

McKay, L.C., Janczewski, W.A., Feldman, J., 2005. Sleep-disordered breathing after targeted ablation of preBotzinger complex neurons. Nat. Neurosci. 8, 1142–1144. https://doi.org/10.1038/nn1517.

McQueen, K., Murphy-Oikonen, J., 2016. Neonatal abstinence syndrome. N. Engl. J. Med. 375, 2468–2479. https://doi.org/10.1056/NEJMra1600879.

Montandon, G., Horner, R., 2014. Cross talk proposal: The preBötzinger complex is essential for the respiratory depression following systemic administration of opioid analgesics. J. Physiol. 592, 1159–1162. https://doi.org/10.1113/jphysiol.2013.261974.

Montandon, G., Liu, H., Horner, R.L., 2016a. Contribution of the respiratory network to rhythm and motor output revealed by modulation of GIRK channels, somatostatin and neurokinin-1 receptors. Sci. Rep. 6, 32707. https://doi.org/10.1038/srep32707.

Montandon, G., Qin, W., Liu, H., Ren, J., Greer, J.J., Horner, R.L., 2011. PreBötzinger complex neurokinin-1 receptor-expressing neurons mediate opioid-induced respiratory depression. J. Neurosci. 31, 292–1301. https://doi.org/10.1523/JNEUROSCI.4611-10.2011.

Montandon, G., Ren, J., Victoria, N.C., Liu, H., Wikman, K., Greer, J.J., et al., 2016b. G-protein-gated inwardly rectifying potassium channels modulate respiratory depression by opioids. Anesthesiology 124, 641–650. https://doi.org/10.1097/ALN.0000000000000984.

Montandon, G., Slutsky, A.S., 2019. Solving the opioid crisis. Respiratory depression by opioids as critical end point. Chest 156, 653–658. https://doi.org/10.1016/j.chest.2019.05.015.

Moses-Kolko, E.L., Bogan, D., Perel, J., Bregar, A., Uhl, K., Levin, B., et al., 2005. Neonatal signs after late in utero exposure to serotonin reuptake inhibitors: literature review and implications for clinical application. JAMA 293, 2372–2383. https://doi.org/10.1001/jama.293.19.2372.

Murphy, D.L., Fox, M.A., Timpano, K.R., Moya, P., Ren-Patterson, R., Andrews, A.M., 2008. How the serotonin story is being rewritten by new gene-based discoveries principally related to SLC6A4, the serotonin transporter gene, which functions to influence all cellular serotonin systems. Neuropharmacology 55, 932–960. https://doi.org/10.1016/j.neuropharm.2008.08.034.

Mustapic, S., Radocaj, T., Sanchez, A., Dogas, Z., Stucke, A.G., Hopp, F.A., et al., 2010. Clinically relevant infusion rates of μ-opioid agonist remifentanil cause bradypnea in decerebrate dogs but not via direct effects in the pre-Bötzinger complex region. J. Neurophysiol. 103, 409–418. https://doi.org/10.1152/jn.00188.2009.

National Institute on Drug Abuse. Overdose Death Rates. January 20, 2022. https://nida.nih.gov/drug-topics/trends-statistics/overdose-death-rates. Accessed 1 March, 2022.

Navarrete-Opazo, A.A., Cook-Snyder, D.R., Miller, J.R., Callison, J.J., McCarthy, N., Palkovic, B., et al., 2020. Endogenous glutamatergic inputs to the Parabrachial Nucleus/Kölliker-Fuse Complex determine respiratory rate. Resp. Physiol. Neurobiol. 277, 103401. https://doi.org/10.1016/j.resp.2020.103401.

Nordeng, H., Lindemann, R., Perminov, K.V., Reikvam, A., 2001. Neonatal withdrawal syndrome after in utero exposure to selective serotonin reuptake inhibitors. Acta. Paediatr. 90, 288–291. https://doi.org/10.1111/j.1651-2227.2001.tb00306.x.

Oberlander, T.F., Bonaguro, R.J., Misri, S., Papsdorf, M., Ross, C.J.D., Simpson, E.M., 2008. Infant serotonin transporter (SLC6A4) promotor genotype is associated with adverse neonatal outcomes after prenatal exposure to serotonin uptake inhibitor medications. Mol. Psychiatry 13, 65–67. https://doi.org/10.1038/sj.mp.4002007.

Otton, S.V., Schadel, M., Cheung, S.W., Kaplan, H.L., Busto, U.E., Sellers, E.M., 1993. CYP2D6 phenotype determines the metabolic conversion of hydrocodone to hydromorphone. Clin. Pharmacol. Ther. 54, 463–472. https://doi.org/10.1038/clpt.1993.177.

Pagliardini, S., Ren, J., Greer, J.J., et al., 2003. Ontogeny of the pre-Bötzinger complex in perinatal rats. J. Neurosci. 23, 9575–9584. https://doi.org/10.1523/jneurosci.23-29-09575.2003.

Palkovic, B., Callison, J.J., Marchenko, V., Stuth, E.A.E., Zuperku, E.J., Stucke, A.G., 2021. Dose-dependent respiratory depression by remifentanil in the rabbit parabrachial nucleus/ Kölliker-Fuse complex and preBötzinger complex. Anesthesiology 135, 649–672. https://doi.org/10.1097/ALN.0000000000003886.

Palkovic, B., Cook-Snyder, D., Callison, J.J., Langer, T.M., Nugent, R., Stuth, E.A.E., et al., 2022. Contribution of the caudal medullary raphe to opioid induced respiratory depression. Respir. Physiol. Neurobiol. 299, 103855. https://doi.org/10.1016/j.resp.2022.103855.

Palkovic, B., Marchenko, V., Zuperku, E.J., Stuth, E.A.E., Stucke, A.G., 2020. Multi-level regulation of opioid-induced respiratory depression. Physiology (Bethesda) 35, 391–404. https://doi.org/10.1152/physiol.00015.2020.

Pattinson, K.T.S., 2008. Opioids and the control of respiration. Br. J. Anaesth. 100, 747–758. https://doi.org/10.1093/bja/aen094.

Peña, F., Ramirez, J.-M., 2002. Endogenous activation of serotonin-2A receptors is required for respiratory rhythm generation in vitro. J. Neurosci. 22, 11055–11064. https://doi.org/10.1523/jneurosci.22-24-11055.2002.

Prkic, I., Mustapic, S., Radocaj, T., Stucke, A.G., Stuth, E.A.E., Hopp, F.A., et al., 2012. Pontine μ-opioid receptors mediate bradypnea caused by intravenous remifentanil infusions at clinically relevant concentrations in dogs. J. Neurophysiol. 108, 2430–2441. https://doi.org/10.1152/jn.00185.2012.

Ptak, K., Yamanishi, T., Aungst, J., Milescu, L.S., Zhang, R., Richerson, G.B., Smith, J.C., 2009. Raphe neurons stimulate respiratory circuit activity by multiple mechanisms via endogenously released serotonin and substance P. J. Neurosci. 29, 3720–3737. https://doi.org/10.1523/JNEUROSCI.5271-08.2009.

Ramirez, J.-M., Baertsch, N.A., 2018. Defining the rhythmogenic elements of mammalian breathing. Physiology 33, 302–316. https://doi.org/10.1152/physiol.00025.2018.

Ramirez, J.-M., Burgraff, N.J., Wie, A.D., Baertsch, N.A., Varga, A.G., Baghdoyan, H.A., et al., 2021. Neural mechanisms underlying opioid-induced respiratory depression: our current understanding. J. Neurophysiol. 125, 1899–1919. https://doi.org/10.1152/jn.00017.2021.

Saper, C.B., 2000. Brainstem modulation of sensation, movement, and consciousness. In: Kandel, E.R., Schwartz, J.H., Jessell, T.M. (Eds.), Principles in Neural Science., 4th ed. McGraw-Hill, New York, p. 896.

Saunders, S.E., Levitt, E.S., 2020. Kölliker-Fuse/parabrachial complex mu opioid receptors contribute to fentanyl-induced apnea and respiratory rate depression. Respir. Physiol. Neurobiol. 275, 103388. https://doi.org/10.1016/j.resp.2020.103388.

Scotton, W.J., Hill, L.J., Williams, A.C., Barnes, N.M., 2019. Serotonin syndrome: pathophysiology, clinical features, management, and potential future directions. Int. Tryptophan. Res. 12, 1178646919873925. 10.1177/1178646919873925.

Shoemaker, A., Steelman, K., Srbu, R., Bell, H.J., 2020. Disparity in the effect of morphine on eupnea and gasping in anesthetized spontaneous breathing adult rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 319, R526-540. doi: 10.1152/ajpregu.00031.2020.

Smith, J.C., Ellenberger, H.H., Ballanyi, K., Richter, D.W., Feldman, J.L., 1991. Pre-Bötzinger complex: a brainstem region that may generate respiratory rhythm in mammals. Science 254, 726–729. https://doi.org/10.1126/science.1683005.

Sora, I., Takahashi, N., Funada, M., Uhl, G.R., 1997. Opiate receptor knockout mice define μ receptor roles in endogenous nociceptive responses and morphine-induced analgesia. Proc. Natl. Acad. Sci. USA 94, 1544–1549. https://doi.org/10.1073/pnas.94.4.1544.

Stamer, U.M., Stüber, F., Muders, T., Musshoff, F., 2008. Respiratory depression with tramadol in a patient with renal impairment and CYP2D6 gene duplication. Anesth. Analg. 107, 926–929. https://doi.org/10.1213/ane.0b013e31817b796e.

St-John, W.M., 1998. Neurogenesis of patterns of autonomic ventilatory activity. Prog. Neurobiol. 56, 97–117. https://doi.org/10.1016/s0301-0082(98)00031-8.

St Sauver, J.L., Olson, J.E., Roger, V.L., Nicholson, W.T., Black, J.L., Takahashi, P.Y., et al., 2017. CYP2D6 phenotypes are associated with adverse outcomes related to opioid medications. Pharmacogenomics Pers. Med. 10, 217–227. https://doi.org/10.2147/PGPM.S136341.

Stornetta, R.L., Rosin, D.L., Wang, H., Sevigny, C.P., Weston, M.C., Guyenet, P.G., 2003. A group of glutamatergic interneurons expressing high levels of both neurokinin-1 receptors and somatostatin identifies the region of the pre-Bötzinger complex. J. Comp. Neurol. 455, 499–512. https://doi.org/10.1002/cne.10504.

Stover, M.W., Davis, J.M., 2015. Opioids in pregnancy and neonatal abstinence syndrome. Semin. Perinatol. 39, 561–565. https://doi.org/10.1053/j.semperi.2015.08.013.

Tan, W., Janczewski, W.A., Yang, P., Shao, X.M., Callaway, E.M., Feldman, J.L., 2008. Silencing preBotzinger complex somatostatin-expressing neurons induces persistent apnea in awake rat. Nat. Neurosci. 11, 538–540. https://doi.org/10.1038/nn.2104.

Tao, R., Auerbach, S.B., 2005. mu-Opioids disinhibit and kappa-opioids inhibit serotonin efflux in the dorsal raphe nucleus. Brain Res. 1049, 70–79. https://doi.org/10.1016/j.brainres.2005.04.076.

Tramadol. Highlights of prescribing information. Available from https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/020281s048lbl.pdf (accessed 3 March 2022).

Tryba, A.K., Peña, F., Ramirez, J.-M., 2006. Gasping activity in vitro: a rhythm dependent on 5-HT2A receptors. J. Neurosci. 26, 2623–2634. https://doi.org/10.1523/JNEUROSCI.4186-05.2006.

Tupal, S., Rieger, M.A., Ling, G.-Y., Park, T.J., Dougherty, J.D., Goodchild, A.K., et al., 2014. Testing the role of preBötzinger complex somatostatin neurons in respiratory and vocal behaviours. Eur. J. Neurosci. 40, 3067–3077. https://doi.org/10.1111/ejn.12669.

Varga, A.G., Reid, B.T., Kieffer, B.L., Levitt, E.S., 2020. Differential impact of two critical respiratory centres in opioid-induced respiratory depression in awake mice. J. Physiol. 598, 189–205. https://doi.org/10.1113/JP278612.

Viemari, J.-C., Garcia 3rd, A.J., Atsushi, D., Ramirez, J.-M., 2011. Activation of alpha-2-noradrenergic receptors is critical for the generation of fictive eupnea and fictive gasping inspiratory activities in mammals in vitro. Eur. J. Neurosci. 33, 2228–2237. https://doi.org/10.1111/j.1460-9568.2011.07706.x.

Volpe, D.A., McMahon Tobin, G.A., Mellon, R.D., Katki, A.G., Parker, R.J., Colatsky, T., et al., 2011. Uniform assessment and ranking of opioid Mu receptor binding constants for selected opioid drugs. Regul. Toxicol. Pharmacol. 59, 385–390. https://doi.org/10.1016/j.yrtph.2010.12.007.

Walther, D.J., Bader, M., 2003. A unique central tryptophan hydroxylase isoform. Biochem. Pharmacol. 66, 1673–1680. https://doi.org/10.1016/s0006-2952(03)00556-2.

Wang, W., Fung, M.-L., Darnall, R.A., St John, W.M., 1996. Characterizations and comparisons of eupnoea and gasping in neonatal rats. J. Physiol. 490, 277–292. https://doi.org/10.1113/jphysiol.1996.sp021143.

Wei, A.D., Ramirez, J.-M., 2019. Presynaptic mechanisms and KCNQ potassium channels modulate opioid depression of respiratory drive. Front. Physiol. 2019 (10), 1407. https://doi.org/10.3389/fphys.2019.01407.

Winter, S.M., Fresemann, J., Schnell, C., Oku, Y., Hirrlinger, J., Hülsmann, S., 2009. Glycinergic interneurons are functionally integrated into the inspiratory network of mouse medullary slices. Pflügers Arch. Eur. J. Physiol. 458, 459–469. https://doi.org/10.1007/s00424-009-0647-1.

Winkelman, T.N.A., Villapiano, N., Kozhimannil, K.B., Davis, M.M., Patrick, S.W., 2018. Incidence and costs of neonatal abstinence syndrome among infants with Medicaid: 2004–2014. Pediatrics 141, e20173520. https://doi.org/10.1542/peds.2017-3520.

Zhu, W., Pan, Z.Z., 2005. Mu-opioid-mediated inhibition of glutamate synaptic transmission in rat central amygdala neurons. Neuroscience 133, 97–103. https://doi.org/10.1016/j.neuroscience.2005.02.004.