Respiratory Effects of Chronic *in utero* Methadone or Morphine Exposure in the Neonatal Guinea Pig

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Abstract

This study uses a neonatal guinea pig model to compare the effects of *in utero* methadone or morphine exposure upon breathing control. We hypothesize that *in utero* methadone exposure will result in similar respiratory disturbances to those seen in morphine exposed neonates, but that the onset will be slower and the duration longer, due to methadone's longer elimination half-life. Pregnant Dunkin-Hartley guinea pigs received once-daily injections of methadone, morphine, or vehicle (saline) during the last half of gestation and pups were studied 3, 7, or 14 days after birth. *In utero* methadone or morphine exposure resulted in decreased birth weight compared to vehicle, and pups experienced a withdrawal syndrome which included increased locomotor activity and respiratory disturbances but no change in rectal temperature. Both opioid exposures increased inspiratory minute ventilation during CO_2 challenge at 3 days after birth, but only in morphine exposed pups was this withdrawal effect still present on day 7. Surprisingly, only morphine exposure increased inspiratory minute ventilation during room air breathing. We conclude that *in utero* methadone exposure is not equivalent to *in utero* morphine exposure. With respect to neonatal respiratory control, methadone-induced changes in respiration are only apparent during hypercapnia.

Keywords

In utero methadone and morphine; respiratory control; neonatal abstinence syndrome; development; opioids; substance abuse

1. Introduction

Methadone is the standard of care for opioid addicted pregnant women. The benefits of methadone treatment over continued heroin use include increased prenatal care, reduced fetal mortality, and increased birth weight [18]. However, infants born to women on methadone maintenance experience withdrawal symptoms of significantly greater severity than heroin exposed infants [46]. In human infants, the methadone-induced neonatal abstinence syndrome (NAS) is characterized by hyperexcitability [46], abnormal REM sleep [17], increased sleep

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apnea [44], respiratory alkalosis [9], decreased sensitivity to hypercapnia [43], diarrhea, fever and central nervous system irritability, which in severe cases progresses to seizures [13]. In addition, opioid exposed infants are smaller at birth and have an increased incidence of sudden infant death syndrome (SIDS), which may in part be due to abnormal respiratory control [20, 21].

The developmental and respiratory effects of *in utero* methadone exposure have been studied in infants of methadone maintenance patients [19,32]. However, these studies are often confounded by the use of alcohol, tobacco, cocaine, and/or illicit supplemental opioids; any one or combination of which can affect perinatal development [1,3,4,34]. In addition, poor maternal nutrition and disease state impact fetal development, and could account for low birth weight [11].

We use an animal model, free of confounding factors associated with drug abuse, to compare *in utero* methadone exposure with *in utero* morphine exposure, the main metabolite of heroin. The guinea pig was chosen because, like humans, guinea pigs have a hemomonochorial placenta and unlike rat or mouse pups, guinea pig pups metabolize morphine to both its major metabolites, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) in similar quantities to human infants [23,40]. Moreover, we have previously shown that acute methadone treatment is more potent, with regard to respiratory disturbances, than morphine in the newborn guinea pig [30] and that chronic *in utero* morphine exposure increases locomotor activity and causes hyperventilation during the first week of life [14]. Pregnant guinea pigs were treated once daily with methadone, morphine, or a saline control and a non-invasive plethysmographic method was used to measure respiratory parameters in their pups. We selected doses for both methadone and morphine that were the maximum daily dose tolerated without maternal or fetal mortality. We hypothesize that *in utero* methadone exposure will result in similar locomotor and respiratory disturbances. However, due to its long elimination half-life in pregnant guinea pigs [37], we expect methadone-induced changes to be of slower onset and longer duration than morphine whose elimination half-life is about one tenth as long, while both values are comparable to pregnant human subjects [16,36,40,41].

2. Materials and Methods

2.1. Animals

Dunkin-Hartley female guinea pigs (6 weeks old, 300 to 400 grams) were bred with male guinea pigs that were at least 5 months old at Oregon Health & Science University (OHSU). All animals were purchased from Charles River (Wilmington, MA). Dams and pups were housed together with 12 hr controlled light/dark cycles and *ad libitum* food and water. Studies were conducted in accordance with guidelines set forth by the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All experiments were approved by the OHSU Institutional Animal Care and Use Committee.

2.2. Treatment groups

A total of 30 pregnant female guinea pigs and 54 pups (27 males and 27 females) were included in the study. Pregnant guinea pigs were randomly assigned to one of three treatment groups: 1ml/kg vehicle (0.9% saline), 12 mg/kg of methadone in saline or 15 mg/kg of morphine in saline. The methadone dose was chosen based on pilot studies in which pregnant females received 10, 12, or 15 mg/kg methadone. In the pilot study, 10 or 12 mg/kg of methadone resulted in no drug associated maternal or neonatal mortality, however, 15 mg/kg methadone treatment resulted in an unacceptable mortality level among dams (67%) and their pups (100%). The morphine dose was chosen based on a previous study in our laboratory [14]. Even though this represents a higher molar dose of morphine (35%), these doses were used because they

represented the maximum daily dose tolerated by the maternal-fetal unit. The lower dose of methadone tolerated by the guinea pigs was most likely related to its ten times longer half-life compared to morphine and the propensity to accumulate upon repeated dosing. Pregnant females received once daily, subcutaneous injections from day 35 of pregnancy to parturition which was at about day 68. Day 35 was chosen because it precedes a critical period of neurological development in the fetal guinea pig and because at that time pregnancy could easily be confirmed by palpation. To reduce litter effects, only 1 female and 1 male pup per litter were included in the study. In the case of an all male or all female litter, only one pup was chosen and was later matched with a pup of opposite sex from a different litter. Each litter was randomly assigned to be studied on day 3, 7, or 14 post-partum with day 1 defined as the first day pups were observed in the tub.

2.3. Drug preparation

Methadone·HCl or morphine·SO_4 (Research Triangle Institute, Research Triangle Park, NC) was diluted in sterile 0.9% saline (Abbott Laboratories, Chicago, IL). Injection volumes were standardized to 12 mg of methadone base per ml or 15 mg of morphine base per ml to get equal injections volumes (1ml/kg) for each treatment group. Dilutions were sterile filtered and stored at −80 °C in 1 ml aliquots until needed.

2.4. Activity measurement

Locomotor activity was measured for a 15 minute period prior to respiratory measurements. On day 3, 7 or 14 after birth, each individual pup was placed in a Plexiglas cage located between the optical sensors of the Opto-Varimax mini activity monitor (Columbus Instruments, Columbus, OH). The monitor measured both total and ambulatory movements. Total activity recorded one count for every broken beam, and measured all animal movements including scratching, grooming, digging, and any stereotypical non-horizontal movements. Ambulatory activity (horizontal movements) was recorded only when two beams were broken consecutively.

2.5. Temperature measurement

Rectal body temperature was measured for each pup following respiratory measurements in order to document withdrawal associated hyper- or hypothermia, which could increase or decrease V_{O_2} and V_I, respectively. A TM-200D Bi-Temp Monitor (Respiratory Support Products, Irvine, CA) with a flexible small animal thermistor (Model 402; Yellow Springs Instrument, OH) was used.

2.6. Ventilatory measurements

Ventilatory data were collected using a non-invasive two-chamber plethysmograph (Buxco Electronics, Sharon, CT). Detailed methods have been previously described [14,29,30,35]. Two interchangeable head chambers allow separate inspiration of each study gas; "Room Air", (RA; 21% O_2, balance N_2) or "5% CO_2" (5% CO_2, 30% O_2, balance N_2). Using BioSystem XA software version 2.7.9 (Buxco Electronics, Sharon, CT), animal breathing movements from the body chamber were averaged over 1 min periods. Measured parameters were: breathing frequency (f_R; breaths·min^{-1}), inspiratory time (T_I; sec), expiratory time (T_E; sec), and respiratory air flow (ml·sec^{-1}) from which were derived, tidal volume (V_T; ml·100g^{-1}), inspiratory minute ventilation (V_I; ml·min^{-1}·100g^{-1}), and inspiratory effort (V_T/T_I; ml·sec^{-1}·100g^{-1}).

2.7. Metabolic measurements

During RA flow, the head chamber was sampled over 30 sec periods. Oxygen consumption (V_{O_2}; ml·min^{-1}·100g^{-1}) and CO_2 production (V_{CO_2}; ml·min^{-1}·100g^{-1}) were measured and

the respiratory exchange ratio (RER, V_{CO_2}/V_{O_2}) was calculated using Oxymax software version 2.4.2 (Columbus Instruments, Columbus, OH).

2.8. Experimental setup

Hypercapnic ventilatory response was measured by exposing the pups to increased CO_2. In addition, depression of the ventilatory response to CO_2 is characteristic of opioids and thus served as a control to rule out residual effects of opioids on breathing. The plethysmograph chamber was used to study unanesthesized, unintubated and only slightly restrained animals. Therefore we only minimally interfere with the physiological state of the animal and don't affect the respiratory system [2].

2.9. Experimental procedure

A pup was weighed then placed in the activity monitor for a period of 15 min during which both total and ambulatory movements were measured. Next the pup was placed in the plethysmograph with RA flow (1 L·min^{-1}) for a 10 min acclimation period prior to the start of measurement. Ventilatory data were collected with a steady flow of RA for an additional 10 min, after which the head chamber was changed and 5% CO_2 was administered for 5 min. The pup was then removed from the chamber and returned to housing. When ventilatory measurements of all pups were complete, rectal temperatures were measured.

2.10. Data analysis

Gestational length was defined as the number of days from the date of vaginal opening to the date pups were observed in the tub, during which pregnant dams were weighed daily. The weight recorded on the day before pups were born is reported as maternal weight at parturition.

Neonatal respiratory data were collected for 10 min while breathing RA and for 5 min during 5% CO_2 challenge. During RA breathing, metabolic parameters were measured for the first 6 min and breathing movements for the remaining 4 min. During CO_2 challenge, only breathing movements were measured. In order to ensure a steady-state had been reached, the data reported are the average of minutes 5 and 6 for metabolic parameters and the average of minutes 9 and 10 for breathing movements while breathing RA. During 5% CO_2 challenge the average of minutes 4 and 5 was reported, since steady-state had been reached by this time. On occasion a preceding minute may have been substituted due to disruptive animal movements within the chamber. All values are reported as the mean ± standard error (SE) unless otherwise specified.

2.11. Statistical analysis

Statistical significance for gestational length, maternal weights, and maternal weight gain was determined by one-way analysis of variance (ANOVA) with drug treatment as the group factor (SigmaStat version 3.11, SysStat Software Inc., Richmond, CA). Neonate data were analyzed initially by three-way ANOVA with factors of sex, drug, and age. However, since there were no sex effects within any parameter, all data were collapsed with respect to sex and analyzed by two-way ANOVA with factors of age and drug. An overall "p" value less than 0.05 was considered significant and all multiple comparisons were performed using the Holm-Sidak method.

3. Results

3.1. Gestational length, litter size

The length of gestation was not different among methadone, morphine or vehicle treated dams. The average length of gestation was 68.4±0.3 days. Likewise, average litter size was similar

among the treatment groups with an average of 3.1 ± 0.1 pups per litter and a range of 2 to 5 pups per litter.

3.2. Maternal weight, neonatal weight

Maternal weight was not different among groups at the start of treatment (686 ± 10 g). However, there were significant differences between groups for weight at parturition ($p < 0.01$). Methadone and morphine treated females gained a significantly lower percentage of weight over the treatment period compared to weight at the start of treatment ($p < 0.01$ and $p < 0.05$, respectively, Table 1). Therefore, opioid treated females had a significantly lower weight at parturition compared to vehicle ($p < 0.01$, Table 1). Weights of guinea pig pups are shown in Table 2. There were main effects of drug ($p < 0.001$) and age ($p < 0.001$) for pup weight. *In utero* methadone or morphine exposed pups were significantly smaller than vehicle pups from birth to day 7. However, by day 14 morphine exposed pups caught up to vehicle pups, while methadone pups remained significantly smaller than vehicle and morphine exposed pups (Table 2).

3.3. Pup activity and rectal temperature

Total locomotor activity, a measure of both ambulatory activity and grooming movements, is shown in Fig. 1. There were main effects of age ($p < 0.05$) and drug ($p < 0.05$), and an age-drug interaction for total activity ($p < 0.01$). On day 3, methadone or morphine exposed pups were more active than control pups and methadone pups were more active than morphine pups. On day 7 and 14 there were no differences between the treatment groups. Methadone exposed pups were significantly more active on day 3 than on day 7 or 14.

There was a main effect of drug ($p < 0.01$) when the ratio of ambulatory to total activity was analyzed. Ambulatory activity was a greater portion of total activity for methadone or morphine exposed pups compared to control pups ($p < 0.01$, both). For methadone or morphine exposed pups the ratio was 65 ± 3 % and 64 ± 3 %, respectively. For vehicle exposed pups ambulatory activity was 53 ± 3 % of total activity.

Rectal body temperature was not different among pups at any age or in any treatment group.

3.4. Respiratory measurements

3.4.1. Inspiratory and tidal volume—For V_I there were main effects of drug ($p < 0.01$) and age ($p < 0.001$), and a drug-age interaction ($p < 0.01$) during RA breathing. V_I of vehicle and methadone exposed pups did not change with age and V_I of methadone pups was not different from control at any age (Fig. 2A). However, V_I for morphine exposed neonates on day 3 was significantly greater than V_I of control or methadone pups at this age as well as morphine exposed pups on day 7 and 14. The increased V_I on day 3 for morphine exposed pups was due to an increased V_T.

There were main effects for V_T (Fig. 2D) of drug ($p < 0.05$) and age ($p < 0.01$), and a drug-age interaction ($p < 0.05$). On day 3, V_T of morphine exposed pups was greater than V_T of control and methadone pups, as well as V_T of older morphine pups (days 7 and 14). V_T of vehicle and methadone treated pups did not change with age.

During 5% CO_2 challenge, there were main effects of age ($p < 0.001$) and drug-age interactions ($p < 0.01$) for both V_I (Fig. 2B) and V_T (Fig. 2E). V_I and V_T of vehicle exposed pups did not change with age, however there was a change with age for morphine and methadone exposed pups. For methadone and morphine exposed pups, V_I and V_T were greater than control only on day 3. On day 7, V_I of morphine exposed pups was significantly greater than methadone.

An increase in inspired CO_2 is a powerful stimulus to breathing. For the relative increase (delta response) in V_I and V_T from RA breathing during 5% CO_2 challenge there were main effects of drug ($p \leq 0.01$) and drug-age interactions ($p<0.01$) (Fig. 2C,F). On day 3 and 14, ΔV_I and ΔV_T for morphine pups was lower than for control (ΔV_I only on day 14) or methadone pups. Morphine pups also showed an age dependent change in the delta response for both V_I and V_T.

3.4.2. Breathing frequency—For f_R there was a main effect of age ($p<0.001$) (Fig. 2G), but no main drug effect during RA breathing. Control pups had a greater f_R on day 3 than on day 14, while f_R of methadone exposed pups did not change with age. For morphine f_R was elevated on day 3 compared to day 7 and day 14 pups of the same treatment.

There was only a main effect of age ($p<0.001$) during 5% CO_2 challenge for f_R (Fig. 2H). Compared to 3 day old pups f_R of vehicle treated pups decreased on day 7 and 14 compared and for methadone or morphine exposed pups f_R decreased on day 14. Only on day 7, f_R of methadone and morphine exposed pups was significantly greater than control.

For the relative increase in f_R from RA to 5% CO_2 breathing there were main effects of drug ($p<0.05$) and drug-age interaction ($p=0.001$), but no effect of age (Fig. 2I). On day 3, the percent increase in frequency (Δf_R) for morphine pups was lower compared to both vehicle or methadone exposed pups as well as compared to older morphine exposed pups due to the increased f_R of morphine pups during RA breathing. Both methadone and morphine pups had an increased delta response compared to vehicle on day 7.

3.4.3. Inspiratory and expiratory time—There was only a main age effect for both T_I and T_E ($p<0.01$) (Table 3). There were no age related changes in T_I or T_E for vehicle or methadone exposed pups, and no difference between any treatment groups on any particular day. However, T_I and T_E were lower on day 3 than on day 14 for morphine exposed pups.

In contrast, there was a main effect of age for T_I and T_E ($p<0.001$) and a main effect of drug for T_I ($p<0.001$) with a drug-age interaction ($p<0.05$) during 5% CO_2 challenge (Table 3). For control pups, T_I increased between day 3 and day 7, while T_I of methadone or morphine exposed pups did not and was significantly lower than control on day 7. On day 14, T_I of all treatment groups was not different from each other, but increased compared to day 3. T_E of methadone or morphine treated pups was not different from control at any age. The age effect was present because T_E increased on day 7 and remained at this level on day 14 for all treatment groups.

3.4.4. Inspiratory effort—For inspiratory effort (V_T/T_I), there were main effects of age ($p<0.001$) and drug ($p<0.01$), with a drug-age interaction ($p<0.01$) (Table 3). V_T/T_I of methadone exposed pups was not different from control pups at any age. Morphine exposed pups had a significantly greater V_T/T_I on day 3 than on day 7 or 14 and a significantly greater V_T/T_I than vehicle or methadone exposed pups on day 3.

There were main effects of age ($p<0.001$) and drug ($p<0.01$) and a drug-age interaction ($p<0.001$) during 5% CO_2 challenge (Table 3). On day 3, V_T/T_I for methadone and morphine exposed pups was greater than control. For vehicle treated pups V_T/T_I did not change with age. On day 7, V_T/T_I of morphine exposed pups remained above control levels but pups exposed to methadone did not. On day 14 there was no difference between treatment groups.

3.5. Oxygen consumption and CO_2 production

3.5.1. Oxygen consumption V_{O_2}—There was a main effect of age ($p<0.001$) for V_{O_2}. For vehicle exposed pups, V_{O_2} was equivalent on day 3 and 7 and decreased on day 14 (Table 4). V_{O_2} of both methadone and morphine exposed pups decreased with age. Methadone and

morphine exposed pups had a greater V_{O_2} on day 3 than control but were not different from control on day 7 and 14.

3.5.2. Carbon dioxide production V_{CO_2}—For V_{CO_2}, there was a main effect of drug ($p<0.05$) and age ($p<0.001$), but no drug-age interaction. V_{CO_2} for methadone was not different from control for all ages (Table 4). Morphine exposed pups had a greater V_{CO_2} on day 3, but showed a decrease in V_{CO_2} back to control levels between day 3 and day 7. V_{CO_2} of all pups decreased on day 14.

3.5.3. V_I/VO_2 and V_I/VCO_2—There were main effects of age ($p<0.05$) and drug ($p<0.01$) for V_I/V_{O_2} and V_I/V_{CO_2}, two parameters commonly used to indicate hypo- or hyperventilation (Table 4). Both did not change with age for control or methadone pups, but were drastically elevated on day 3 in morphine exposed pups, indicating withdrawal induced hyperventilation.

3.5.4. Respiratory exchange rate—There was a main effect of age ($p<0.001$), but no drug effect for the respiratory exchange ratio. RER did not change with age for vehicle treated pups and was not different among treatment groups at any particular age (Table 4). However, RER of methadone treated pups was greater on day 14 than on day 3. RER of morphine treated pups was equivalent on day 3 and 7 but increased on day 14.

4. Discussion

Methadone, a long acting synthetic opioid, is the standard of care for opioid addiction during pregnancy. Human studies of the effects of *in utero* methadone exposure are often confounded by maternal alcohol, tobacco, and/or supplemental opioid use [1,3,4,34]. The present study compares the effects of *in utero* methadone to *in utero* morphine (the predominant active metabolite of heroin) exposure in an animal model free of the confounding factors related to maternal polypharmacy, nutritional deficiencies, or disease states.

The neonatal guinea pig was chosen for several important reasons. Guinea pigs [12] and humans [25,38] hyperventilate during pregnancy, which is suppressed by opioids, and both species have a hemomonochorial placenta with similar permeability characteristics [6,31]. Importantly, the elimination half-life of methadone is 15 hrs in the pregnant guinea pig [37] which is comparable to that in humans [16,36,41]. In contrast, the elimination half-life of morphine in the pregnant guinea pig is 1.3 hr [40]. Consequently, daily administration of methadone would be more prone to drug accumulation than morphine requiring different doses for the two drugs in a model of once daily drug administration over a long period of time. Finally, human infants and guinea pig pups, unlike rat or mouse pups, metabolize morphine to both pharmacologically active metabolites, M3G and M6G [23,40], making the guinea pig an excellent model for the present study.

Birth weights of pups exposed *in utero* to methadone or morphine were lower than control pups. However, by day 14, morphine exposed pups had achieved weights equivalent to control pups, while methadone pups remained below expected weights.

This is contrary to human studies where methadone infants were reported to have higher birth weights than heroin and presumably morphine exposed infants. This discrepancy may be the result of social factors as opposed to a true drug effect because women in methadone maintenance programs are more likely to receive adequate prenatal care and have improved nutrition, thus their infants are likely to have higher birth weights than the infants of heroin abusing women [18]. In addition, methadone treatment in humans often reduces the abuse of other drugs and reduces the risk of I.V. drug related diseases (*i.e.* HIV and hepatitis) [7]. The explanation for the difference in the weight gain between methadone and morphine exposed

pups on day 14 is unclear. This effect could be due to lingering methadone in the body, but this has not been documented in the literature.

In addition to the difference in weight gain, there was also a difference in total locomotor activity between treatment groups. On day 3, *in utero* methadone exposed pups had a greater total activity count than morphine or control pups. The greater total activity of methadone exposed pups may reflect a change in N-methyl-D-aspartate (NMDA) receptors, since methadone acts as a non-competitive NMDA antagonist [10]. In infant rats, co-treatment with the non-competitive NMDA antagonist MK-801 and the mu opioid receptor agonist morphine increased the incidence of certain withdrawal behaviors [45] including walking behaviors. The developmental maturity of the NMDA receptor is likely more advanced in the 3 day old guinea pig compared to the young rat; nonetheless, the study of Zhu and Barr [45] does demonstrate the involvement of the NMDA receptor in opioid withdrawal and emphasizes an important difference between methadone and morphine. Increased locomotor activity may indicate *in utero* alterations or adaptation of the NMDA receptor system due to the presence of methadone, which has both NMDA antagonist activity [10] and mu opioid agonist activity [42].

In regard to breathing control, the results were contrary to our hypothesis. Methadone did not have a slower onset of withdrawal symptoms nor did symptoms last longer than in morphine induced withdrawal. Most surprising, methadone exposed pups only showed signs of respiratory disturbances during the stress of CO_2 challenge while morphine induced changes were seen during both RA and CO_2 breathing.

During RA breathing, methadone exposed pups showed no withdrawal-related respiratory changes, while morphine exposed pups had an elevated V_I. Although this could be a result of central nervous system stimulation by M3G, a morphine metabolite that reportedly has an excitatory effect on the central nervous system [22,33], in a previous study, M3G did not affect breathing when administered postnatally to neonatal guinea pigs [28]. Besides, it is unlikely that M3G would remain in the blood by day 3. Instead the respiratory changes may indicate a down-regulation of the opioid system due to chronic *in utero* morphine exposure or a change in receptor efficiency. The literature remains controversial with regard to chronic opioid induced up- or downregulation of opioid receptors, but previous studies from our laboratory support a change in receptor efficiency. For example, after chronic *in utero* morphine exposure guinea pig pups showed decreased DAMGO-stimulated ^{35}S-GTPγS binding in respiratory control areas of the brainstem [26] with no difference in brainstem mu opioid receptor mRNA expression or protein levels [39], which suggests a change in receptor efficiency rather than a decrease in density. Unfortunately similar studies have not been done in the guinea pig with methadone.

In addition, the inspiratory effort of morphine exposed pups was increased while breathing RA during the withdrawal period, which corresponded to a period of hyperventilation. This is consistent with previous work from our laboratory in which *in utero* morphine exposed pups hyperventilated during withdrawal [14] and studies in human infants withdrawing from heroin [9]. This hyperventilation would increase oxygen needs for the newborn and in that sense would be deleterious. It should be noted that Glass and colleagues [9] excluded acid-base disturbances as the cause of hyperventilation. In contrast, the methadone exposed guinea pig pups did not exhibit increased inspiratory effort and hyperventilation, similar to a previous study of human neonates of methadone maintenance patients [32]. The mechanism for this difference is not apparent but may be due in part to differences in pharmacokinetics [15] or different agonist properties of methadone and morphine at the kappa and delta opioid receptors [24]. However, the latter seems unlikely, as mu opioid receptor knock-out studies have shown that morphine had no effect on the hypercapnic response in mu knock-out mice [5].

During 5% CO_2 challenge both methadone and morphine caused a withdrawal related increase in V_I on day 3. However, this effect remained in morphine pups through day 7. This is interesting because we expected the respiratory effects of methadone to be of longer duration and slower onset than that of morphine. One possible explanation is the effect of lipid solubility which is two orders of magnitude greater for methadone than morphine [27]. It is possible that the long elimination half-life of methadone, combined with its high lipid solubility, results in a constant release of drug from fat stores to the bloodstream that prevents or reduces respiratory withdrawal effects.

Abnormal respiratory control caused by chronic drug use has long been presumed to be the leading cause of an increased risk of sudden infant death syndrome [20,21]. Recent evidence shows that SIDS may result from a combination of prenatal vulnerability, postnatal stress and a critical developmental stage and may be caused by defiencies in breathing, autonomic and sleep-wake control [8]. Our data clearly show an altered response to hypercapnic challenge in the very young caused by in utero drug exposure. Therefore this could significantly increase the likelihood of all three causes to occur simultaneously and result in an increased SIDS incidence in infants of substance abusing mothers [20,21].

There is no doubt that illicit drug abuse during pregnancy has fetal and neonatal consequences. This study has demonstrated that *in utero* methadone or morphine exposure is not equivalent with respect to neonatal withdrawal. In fact, overall *in utero* methadone exposure had fewer detrimental effects than *in utero* morphine and presumably heroin exposure when ventilatory effects are taken into account. This could be related to the lower dose of methadone used, but more likely reflects the difference in elimination half-lives and the different effects of the two drugs on other receptors, such as the NMDA receptor. With the exception of birth weight, where both methadone and morphine exposure reduced pup weight at birth, morphine had fewer disadvantageous outcomes for postnatal weight gain and central nervous system stimulation (as measured by locomotor activity during withdrawal), however, methadone clearly had fewer ventilatory effects, and does not alter hypercapnic ventilatory response.

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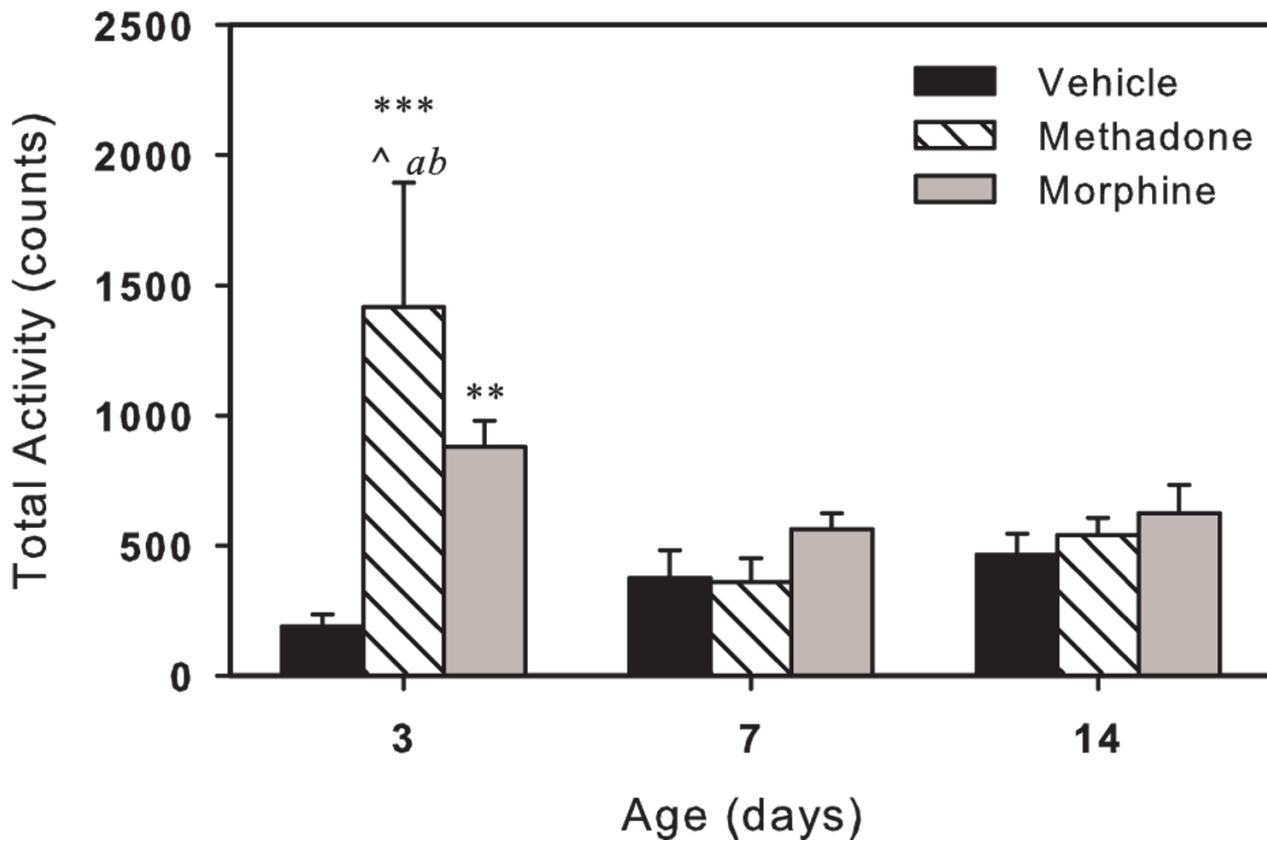
Fig. 1.
Total activity of *in utero* drug exposed neonatal guinea pigs. Activity is the total count of photo-beam breaks recorded in a 15 minute period. Methadone (striped bars) or morphine (gray bars) exposed pups showed signs of withdrawal related hyperactivity on day 3; by day 7 and 14 counts were not different from control (black bars). Values are average of 6 pups per treatment. Vertical bars represent standard error (S.E). ** $p<0.01$, *** $p<0.001$ vs. vehicle; ^ $p<0.05$ vs. morphine; [a] $p<0.05$ vs. 7-day same treatment; [b] $p<0.05$ vs. 14-day same treatment.
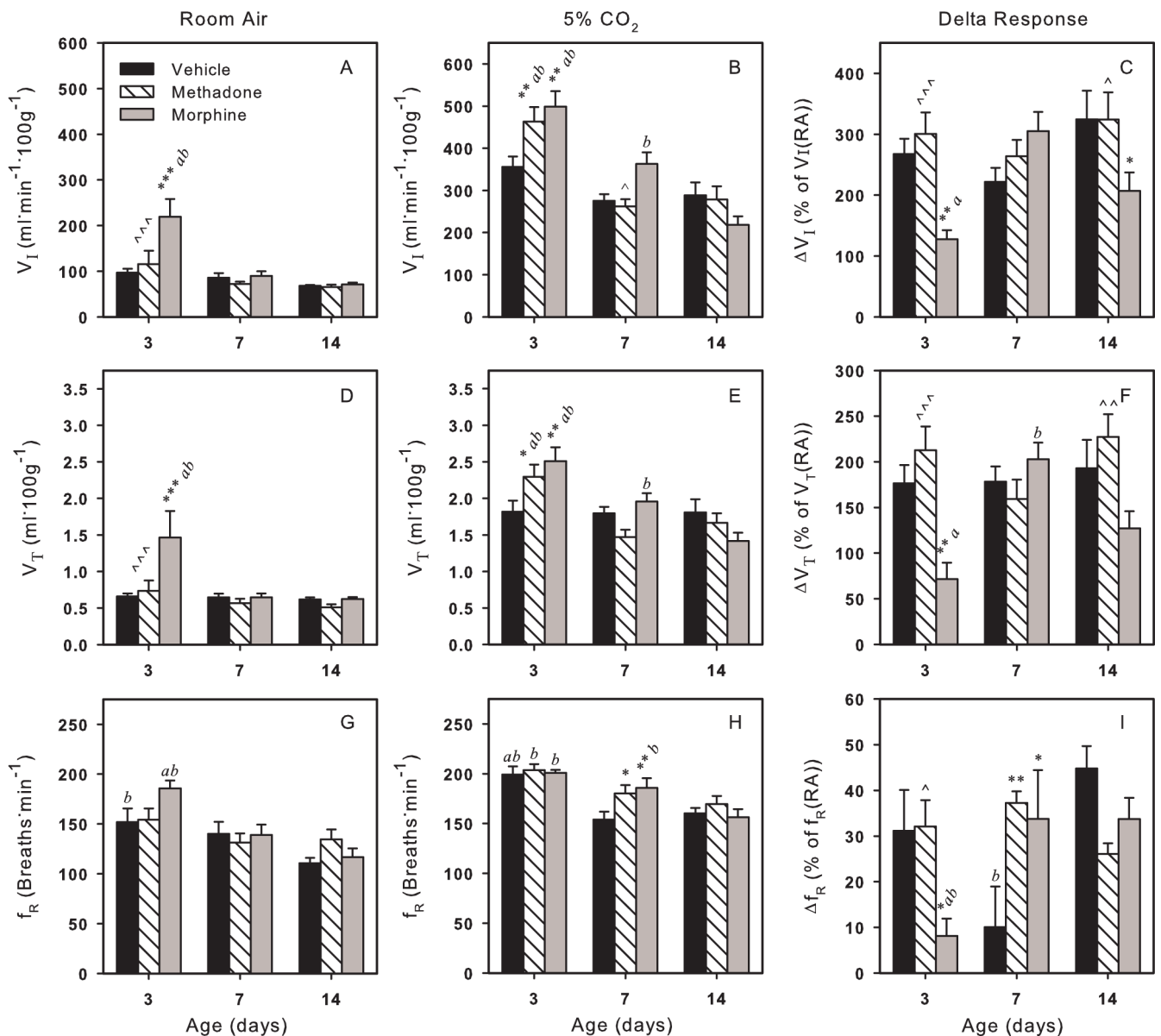
Fig. 2.
Respiratory effects of *in utero* methadone (striped bars) or morphine (gray bars) exposures compared to vehicle (black bars) during room air (RA) breathing (left column) and during 5% CO_2 challenge (middle column). CO_2 challenge induced delta response is shown as percent of baseline (RA breathing) (right column). Three major respiratory parameters, inspiratory volume (V_I) (A–C), tidal volume (V_T) (D–F) and breathing frequency (f_R) (G–I) are shown. Several differences exist between methadone and morphine exposed pups. Vertical bars represent standard error (S.E). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. vehicle; ^^^ $p<0.001$ vs. morphine; [a] $p<0.05$ vs. 7-day same treatment; [b] $p<0.05$ vs. 14-day same treatment.

Table 1

Weight of pregnant guinea pigs treated once daily with vehicle (saline, 1 ml/kg), methadone (12 mg/kg), or morphine (15 mg/kg) starting at day 35 of gestation

	Vehicle	Methadone	Morphine
Maternal wt. at parturition (g)	1040 ± 39	869 ± 21 [***]	913 ± 28 [**]
Wt. gain (% of treatment start wt)	50 ± 6	30 ± 2 [**]	34 ± 2 [*]
N (dams/treatment)	9	11	10

[*] $p<0.05$ vs. vehicle

[**] $p<0.01$ vs. vehicle

[***] $p<0.001$ vs. vehicle; all values reported as mean $\pm$ S.E.

Table 2

Weights (g) of *in utero* opioid exposed neonatal guinea pigs compared to vehicle

Age	Vehicle	Methadone	Morphine
Birth	102 ± 4	81 ± 4[**]	82 ± 3[**]
3 days	102 ± 4	82 ± 5[**]	84 ± 3[**]
7 days	132 ± 6	116 ± 8[*]	112 ± 5[**]
14 days	189 ± 12	147 ± 10[*^^]	178 ± 9

[*]
 p<0.05 vs. vehicle

[**]
 p<0.01 vs. vehicle

[***]
 p<0.001 vs. vehicle

[^^]
 p<0.01 vs. morphine, all values reported as mean ± S.E. of all pups in each treatment group, n (per treatment): Birth and day 3, 18 pups; day 7, 12 pups; day 14, 6 pups.

Table 3

Respiratory measurements of *in utero* opioid exposed neonatal guinea pigs

	Age	Room Air			5% CO_2		
		Vehicle	Methadone	Morphine	Vehicle	Methadone	Morphine
T_I [sec]	3 days	0.18 ± 0.02	0.16 ± 0.01	0.14 ± 0.01^b	0.14 ± 0.01^{ab}	0.13 ± 0.00^b	0.13 ± 0.00^b
	7 days	0.19 ± 0.02	0.20 ± 0.02	0.18 ± 0.01	0.17 ± 0.01	$0.13 \pm 0.01^{***}$	$0.13 \pm 0.00^{***b}$
	14 days	0.23 ± 0.01	0.20 ± 0.02	0.23 ± 0.02	0.16 ± 0.00	0.15 ± 0.01	0.16 ± 0.01
T_E [sec]	3 days	0.25 ± 0.03	0.25 ± 0.03	0.21 ± 0.01^b	0.17 ± 0.01^{ab}	0.17 ± 0.01^{ab}	0.17 ± 0.01^{ab}
	7 days	0.27 ± 0.03	0.27 ± 0.02	0.28 ± 0.02	0.23 ± 0.01	0.21 ± 0.01	0.21 ± 0.01
	14 days	0.32 ± 0.02	0.27 ± 0.02	0.33 ± 0.03	0.22 ± 0.01	0.22 ± 0.01	0.22 ± 0.01
V_T/T_I	3 days	3.8 ± 0.3	$4.7 \pm 1.1^{\wedge\wedge\wedge}$	$10.4 \pm 2.3^{***ab}$	13.2 ± 0.9	$18.1 \pm 1.2^{**ab}$	$18.9 \pm 1.2^{***ab}$
[ml·sec^{-1}·100g^{-1}]	7 days	3.7 ± 0.6	2.8 ± 0.2	3.7 ± 0.4	10.8 ± 0.5	$11.2 \pm 0.6^{\wedge\wedge}$	$15.3 \pm 0.6^{**b}$
	14 days	2.7 ± 0.1	2.6 ± 0.1	2.8 ± 0.2	11.2 ± 1.1	11.4 ± 1.1	8.8 ± 0.7

**
  p<0.01 vs. vehicle

***
  p<0.001 vs. vehicle

^
  p<0.01 vs. morphine

^^^
  p<0.001 vs. morphine

a
  p<0.05 vs. 7 day same treatment

b
  p<0.05 vs. 14 day same treatment, all values reported as mean ± S.E.

Table 4

Ventilatory measurements of *in utero* opioid exposed neonatal guinea pigs during RA breathing

	Age	V_{O_2} [ml·min^{-1}·100g^{-1}]	V_{CO_2} [ml·min^{-1}·100g^{-1}]	V_I/V_{O_2}	V_I/V_{CO_2}	RER
Vehicle	3 days	3.5 ± 0.5^b	2.7 ± 0.2^b	30 ± 4	37 ± 3	0.82 ± 0.06
	7 days	3.3 ± 0.1^b	2.5 ± 0.1	25 ± 3	34 ± 4	0.75 ± 0.01
	14 days	2.5 ± 0.1	2.1 ± 0.1	27 ± 1	32 ± 1	0.86 ± 0.03
Methadone	3 days	$4.2 \pm 0.1^{*ab}$	3.0 ± 0.1^b	$27 \pm 6^{\wedge\wedge}$	$38 \pm 9^{\wedge\wedge}$	0.75 ± 0.02^b
	7 days	3.3 ± 0.2^b	2.7 ± 0.1	22 ± 2	27 ± 2	0.81 ± 0.02
	14 days	2.7 ± 0.3	2.4 ± 0.1	25 ± 2	28 ± 2	0.92 ± 0.06
Morphine	3 days	$4.4 \pm 0.2^{**ab}$	$3.3 \pm 0.1^{**ab}$	$51 \pm 9^{**ab}$	$68 \pm 12^{***ab}$	0.74 ± 0.02^b
	7 days	3.3 ± 0.2^b	2.6 ± 0.1	28 ± 4	36 ± 5	0.78 ± 0.04^b
	14 days	2.3 ± 0.1	2.1 ± 0.1	32 ± 2	34 ± 2	0.94 ± 0.06

*
$p<0.05$ *vs.* vehicle

**
$p<0.01$ *vs.* vehicle

***
$p<0.001$ *vs.* vehicle

^
$p<0.01$ *vs.* morphine

^^^
$p<0.001$ *vs.* morphine

a
$p<0.05$ *vs.* 7 day same treatment

b
$p<0.05$ *vs.* 14 day same treatment, all values reported as mean ± S.E.