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Chronic *in utero* Buprenorphine Exposure Causes Prolonged Respiratory Effects in the Guinea Pig Neonate

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Abstract

Our laboratory studies the effects of *in utero* opioid exposure on the neonate. In this work we test the effects of chronic *in utero* exposure to buprenorphine on the neonate. Buprenorphine is a promising candidate for treatment of opioid addiction during pregnancy and it has been suggested to decrease the neonatal abstinence syndrome in human infants. In our guinea pig model, we focused not only on the respiratory effects of *in utero* exposure on the neonate, but also studied withdrawal signs in the neonate, a major concern of all opioid treatment during pregnancy. Pregnant guinea pigs were treated with daily subcutaneous injections of 0.1 mg/kg buprenorphine during the second half of gestation. We measured weight, locomotor activity and respiratory function in pups of ages 3 to 14 days. Respiratory response was recorded using a two-chamber plethysmograph, while pups were breathing either room air or 5% CO₂. Our results show that chronic *in utero* exposure to buprenorphine induces respiratory effects up to day 14 after birth, while earlier studies have shown that effects of either *in utero* methadone or morphine only persist in the first week after birth in the guinea pig model. These data provide important information for clinical trials of buprenorphine treatment suggesting that duration and severity of respiratory effects of *in utero* buprenorphine exposure should be monitored.

Keywords

In utero buprenorphine; respiratory control; neonatal abstinence syndrome; development; opioids; substance abuse

1. Introduction

Currently, the only approved drug treatment in the United States for opioid dependence in pregnant women is methadone maintenance [21,43]. Even though this therapy has proven beneficial for both maternal and fetal outcome, newborns often suffer from neonatal abstinence syndrome (NAS) as a result [27,39,44]. The NAS symptoms caused by *in utero* methadone exposure include dysfunctions of the respiratory system, seizures [15], and an increased risk

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Conflict of Interest Statement

The authors and author's institution have no financial or other relationships with other people or organizations that would constitute a conflict of interest.

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of sudden infant death syndrome [22,23]. Therefore alternate treatment options are sought that could improve maternal and neonatal outcome.

We have recently shown that in an animal model of chronic *in utero* methadone or morphine exposure, the respiratory effects of methadone on the neonate, though less severe than morphine, are still significant and potentially detrimental [33]. In addition, effects on neonatal weight and excitability, indicators for NAS, are more severe than effects of morphine. In humans these effects are offset somewhat by the socioeconomic factors associated with the treatment program, such as better medical care and nutrition [36].

Buprenorphine is one potential alternative treatment option for pregnant women. It is a long-lasting partial agonist at the mu opioid receptor that has recently been successfully introduced as an alternative treatment for opioid addiction in non-pregnant opioid addicts [4,24,37]. Preliminary studies indicate that pregnant female addicts can safely be transitioned to buprenorphine treatment and that NAS caused by *in utero* buprenorphine exposure may be less severe than that of methadone [11,12,17,20]. However, all these studies are hampered by confounding factors like multi-substance abuse, continued illicit drug use, insufficient medical care, or maternal neglect during and after the pregnancy. Studies using animal models are needed, as they are free of these confounding factors.

This study used the guinea pig model to evaluate the effects of chronic *in utero* buprenorphine exposure on the neonate. It was designed to test the hypothesis that the neonatal abstinence syndrome and respiratory depression effects of buprenorphine are less severe when compared with previously reported data on morphine and methadone *in utero* exposure [16,33].

Pregnant guinea pigs were treated with a once daily dose of buprenorphine during the second half of gestation. Dose selection was based on the survival of the maternal-fetal unit. Respiratory parameters of their litters have been studied in a longitudinal study during the first two weeks after birth using a non-invasive plethysmograph. We show that the respiratory effects of buprenorphine were equally as severe as those of methadone or morphine. Considering respiratory criteria alone, these data suggest that buprenorphine treatment of drug addiction during pregnancy is not more beneficial for the newborn child than methadone or morphine, as the respiratory effects persisted for two weeks after birth.

2. Materials and methods

2.1. Animals and treatment groups

All experiments were approved by the Oregon Health & Science University Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health “*Guide for the Care and Use of Laboratory Animals*” guidelines. Female Dunkin-Hartley guinea pigs (6 weeks old, 300 to 400 grams) from Charles River Laboratories (Wilmington, MA) were bred at Oregon Health & Science University. Pregnant guinea pigs were randomly assigned to receive a daily injection of either 1 ml/kg of 0.9% saline solution (vehicle control) or 0.1 mg/kg buprenorphine in saline. Buprenorphine injection volumes were standardized to 1 ml/kg. Subcutaneous injections in the shaved scruff of the neck started at day 35 of pregnancy prior to critical periods of *in utero* nervous system development in the guinea pig [10,35] and were given once daily in the morning until the day of parturition [33]. Six pups (2 litters, 3 males and 3 females) from two saline treated dams, and eight pups from three buprenorphine treated dams (3 litters, 6 males and 2 females) were included in the study. All pups from a litter were studied, but in order to avoid litter effects, data analysis was performed using the litter as the basic unit.

After parturition, dams and pups were housed together with 12 hr controlled light/dark cycles and *ad libitum* food and water. The first day pups were observed in the tub was defined as day 1. A longitudinal study of all pups of a litter was carried out on days 3, 7, 10, and 14.

2.2. Locomotor activity

Locomotor activity of pups was measured on study days immediately prior to respiratory measurements. A pup was separated from its mother, weighed, and placed immediately in an isolated Opto-Varimax mini activity monitor (Columbus Instruments, Columbus, OH) for a period of 15 min. The activity monitor counted breaks of the light beams and separately recorded total movements including grooming, digging, and head movements (every beam break is recorded) and ambulatory movements (two consecutive beam breaks are recorded).

2.3. Ventilatory and metabolic measurements

Ventilatory and metabolic data were collected using the same protocol as in previous studies in our laboratory [16,33]. Pups were placed in a non-invasive, head-out, two-chamber plethysmograph [35] (Buxco Electronics, Sharon, CT) for a 10 min acclimation period. Interchangeable head chambers allowed separate inspiration of the two study gases; “Room Air”, (RA; 21% O₂, balance N₂) and “5% CO₂” (5% CO₂, 30% O₂, balance N₂). Therefore both room air and hypercapnic respiratory response could be measured in unanesthetized, unintubated and only slightly restrained pups [3]. First, room air data were collected with a steady flow of RA (1 L·min⁻¹) for 10 min, after which the head chamber was quickly changed and the 5% CO₂ gas mixture (1.7 L·min⁻¹) was administered for 5 min to measure the hypercapnic response. Metabolic parameters were only measured during RA flow by sampling the head chamber over 30 sec periods. Both oxygen consumption (VO₂; ml·min⁻¹·100g⁻¹) and CO₂ production (VCO₂; ml·min⁻¹·100g⁻¹) were measured and V_I/VO₂ and V_I/VCO₂ were calculated (Oxymax software version 2.4.2, Columbus Instruments, Columbus, OH). From the separate body chamber animal breathing movements were recorded and averaged over 1 min periods. Directly 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), which were used to calculate 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) using BioSystem XA software version 2.7.9 (Buxco Electronics, Sharon, CT).

2.4. Data analysis and statistics

Gestational length was defined as the number of days from the first day of vaginal opening to the day pups were observed in the tub. Maternal weight gain was monitored daily and weight recorded on the day before pups were born was reported as maternal weight at parturition. All pups of a litter were studied and averaged to avoid litter effects. Previous studies in the guinea pig in our group as well as clinical observations have shown that respiratory effects of opioids are not sex dependent [5,30,32], therefore both male and female pups were studied and data was collapsed for gender.

Respiratory data of all pups were collected on days 3, 7, 10, and 14 after birth as previously described [33]. In brief, during the 10 min RA breathing period, metabolic parameters were measured for the first 6 min and breathing movements for the remaining 4 min. During the 5 min CO₂ challenge, only breathing movements were measured. In order to ensure a steady-state had been reached, the data reported and analyzed are the averages of the last two minutes of each measurement period. All values are reported as the mean ± standard error.

Significant statistical differences for gestational length, maternal weight, and maternal weight gain were determined using the Student's t-test with treatment as factor (SigmaStat version 3.11, SysStat Software Inc., Richmond, CA). All neonate data were averaged for each litter

and were analyzed by two-way repeated-measures analysis of variance (ANOVA) with factors of age and treatment. An overall P-value of less than 0.05 was considered significant and all post-hoc comparisons were performed using the Holm-Sidak method. Weight data for all litters was analyzed using litter size as a covariant (SPSS, SPSS Inc, Chicago, IL).

3. Results

3.1. Effects of chronic in utero buprenorphine treatment on the dam and pup weight

The buprenorphine dose of 0.1 mg/kg used in these experiments was determined in pilot studies as the maximum tolerable dose for the maternal-fetal unit with no fatal outcome seen for either dams or pups. In the pilot study, four dams had been tested with buprenorphine doses of 0.2 (2 dams), 0.5, and 1.0 mg/kg. The treatment with the two highest doses resulted in unacceptable weight loss of the dams and had to be terminated. The 0.2 mg/ml dose resulted in premature still birth on day 49 and 57, respectively. Table 1 shows the maternal weight data recorded during gestation. Treatment groups were not different in the first half of gestation. Buprenorphine treatment during the second half of gestation caused significantly lower weight gain and resulted in lower maternal weight at parturition. All three dams treated with 0.1 mg/kg of buprenorphine gave birth to live, full term litters.

Pup weight was recorded on the day pups were first observed in the tub (day 1) and on all days respiratory measurements were taken. Weight of *in utero* buprenorphine exposed pups was significantly lower on all days than the weight of the saline exposed control group. The relative weight gain of pups compared to their birth weight was not different between treatment groups (Table 1).

3.2. Effects of chronic in utero buprenorphine treatment on pup activity

Immediately before respiratory measurements were recorded, pups were removed from their moms and their activity was monitored in an isolated locomotor chamber (Table 2). Buprenorphine exposed pups showed withdrawal related hyperactivity only on day 3 compared to control animals, while older pups had no statistically significant hyperactivity.

3.3. Respiratory measurements

3.3.1. Inspiratory minute ventilation and tidal volume—For V_I , there was a main effect for age ($F(3,9) = 4.03, p < 0.05$), and an age-drug interaction ($F(3,9) = 3.97, p < 0.05$) during RA breathing (Fig. 1A). There were no main effects for V_T (Fig 1E). While V_I and V_T of saline exposed pups did not change with age, both were drastically elevated in 3 day old buprenorphine pups, indicating withdrawal related hyperventilation even under rest conditions. This increase was not seen in older pups. During 5% CO_2 challenge, there was a main effect for age ($F(3,9) = 22.46, p < 0.001$ and $F(3,9) = 6.53, p < 0.05$, respectively) for both V_I and V_T (Fig. 1B, F). In contrast to RA breathing V_I decreased between week 1 (days 3 and 7) and week 2 (days 10 and 14) for the buprenorphine treatment group. For saline pups V_I of the youngest pups (day 3) was greater than V_I of the oldest pups (day 14). Similar trends were seen for V_T , but these decreases were not statistically significant. In addition, buprenorphine exposed pups had a significantly increased V_I up to day 7, while it was slightly increased during week 2. V_T of the buprenorphine treated pups was slightly increased on all days and significantly increased on day 7 compared to the control group. This indicates that respiratory related withdrawal symptoms are present longer under hypercapnic conditions.

3.3.2. Breathing frequency—For f_R there was a main effect for drug ($F(1,9) = 66.84, p < 0.01$) and an age-drug interaction ($F(3,9) = 4.96, p < 0.05$) during RA breathing (Fig. 1C). There were no changes in f_R for control pups with age. On day 3, buprenorphine exposed pups had a greater f_R than control pups of the same age and 7 and 14 day old buprenorphine pups.

Breathing frequency was also elevated on day 10 compared to older buprenorphine pups and control pups of the same age. During 5% CO₂ challenge, there was a main effect for age ($F(3,9) = 19.59, p < 0.001$) and an age-drug interaction ($F(3,9) = 4.20, p < 0.05$) (Fig. 1D). No age effect was seen for control pups. In contrast, 3 day old buprenorphine pups had significantly elevated f_R compared to control pups and older buprenorphine pups. In older pups, f_R was not different from control pups.

3.3.3. Inspiratory effort—During RA breathing (Fig. 1G), three day old buprenorphine treated pups had a drastically increased inspiratory effort compared to control pups as well as compared to older pups of the same treatment. There were no age effects for saline treated pups. During 5% CO₂ challenge, there was a main effect for age ($F(3,9) = 14.65, p < 0.001$). The inspiratory effort of buprenorphine exposed pups was significantly increased on day 7, but elevated compared to control on all four test days (Fig 1H). For both treatment groups, the inspiratory effort was higher in the first week (days 3 and 7) than in the second week (days 10 and 14) for buprenorphine treated pups.

3.3.4. Inspiratory and expiratory time—There was a main effect for drug for both T_I and T_E ($F(1,9) = 63.62, p < 0.01$, and $F(1,9) = 12.41, p < 0.05$, respectively) during RA breathing (Table 3). Both parameters were lower in 3 and 10 day old buprenorphine treated pups compared to vehicle. In addition, T_E of 3 day old buprenorphine pups was lower compared to 14 day old pups in the same treatment group. During 5% CO₂ challenge, there was a main effect- for age ($F(3,9) = 116.18, p < 0.001$), and an age- drug interaction for T_E ($F(3,9) = 29.61, p < 0.001$). T_I of buprenorphine pups was not different from control pups and showed no changes with age. In contrast, T_E of buprenorphine exposed pups increased during the first 10 days, while control pups had no age related effects. Comparison of both treatment groups showed that T_E of buprenorphine pups is smaller in 3 day old, identical in 7 day old, and greater than control in 10 and 14 day old pups.

The ratio of expiratory to inspiratory time (T_E/T_I) was different between treatment groups only under 5% CO₂ challenge (Table 3). There were main effects for age ($F(3,9) = 30.53, p < 0.001$) and drug ($F(1,9) = 15.18, p < 0.05$), and an age-drug interaction ($F(3,9) = 21.13, p < 0.001$). Like the T_E of buprenorphine pups during 5% CO₂ challenge, the ratio of T_E/T_I was smaller for 3 day old pups and identical on day 7, while it was higher in these pups compared to control in the second week of testing.

3.3.5. Comparison of room air breathing and CO₂ challenge—Fig. 2 shows the delta response from room air breathing to 5% CO₂ challenge. For ΔV_I , ΔV_T and $\Delta V_T/T_I$ (Fig 2A, C, D), there was an age-drug interaction ($F(3,9) = 19.88, p < 0.001$, $F(3,9) = 9.88, p < 0.01$, and $F(3,9) = 27.19, p < 0.001$, respectively). For Δf_R (Fig 2B), there was a main effect of age ($F(3,9) = 6.85, p < 0.05$). The delta response for all four parameters was drastically decreased for buprenorphine exposed animals on day 3, due to the increase of all parameters during normal RA breathing (see above). In contrast, ΔV_T had a tendency to be increased on day 7 ($p = 0.08$) and was increased on day 10; plus $\Delta V_T/T_I$ was increased on day 10 and 14 compared to control animals. In addition, ΔV_I showed a tendency to be increased on day 7 ($p = 0.1$) and 14 ($p = 0.06$). Also, Δf_R on day 10 was different from control due to a negative Δf_R response on this day for all test animals, while Δf_R was not different from control on days 7 and 14.

3.4. Metabolic measurements

3.4.1. Oxygen consumption (VO₂) and carbon dioxide production (VCO₂)—There were main effects of age ($F(3,9) = 14.83, p < 0.001$ and $F(3,9) = 4.25, p < 0.05$, respectively) and drug ($F(1,9) = 29.29, p < 0.05$ and $F(1,9) = 12.05, p < 0.05$) for both VO₂ and VCO₂ (Fig 3). VO₂ for control animals decreased during the two weeks of testing and was different

between day 3 and day 14, while for buprenorphine treated pups VO_2 was strongly increased on day 3 and lowest on day 14 (Fig 3A). Buprenorphine treatment significantly increased oxygen consumption on day 3 compared to control. Similarly, on day 3, buprenorphine pups had a strongly increased CO_2 production compared to control pups as well as older buprenorphine exposed pups (Fig 3B). In contrast to VO_2 though, there were no age related effects for control pups for VCO_2 .

3.4.2. V_I/VO_2 and V_I/VCO_2 —For both parameters, V_I/VO_2 and V_I/VCO_2 there was an age-drug interaction ($F(3,9) = 7.38, p < 0.01$ and $F(3,9) = 11.64, p < 0.01$, respectively). In addition, there was a main effect for age for V_I/VCO_2 ($F(3,9) = 7.24, p < 0.01$). Both parameters indicate a strong hyperventilation of buprenorphine treated pups on day 3 (Fig 3C, D) compared to control pups. This was not seen in 7 day or older pups of both treatment groups.

4. Discussion

Over the years we have reported on the effects of *in utero* exposure to different drugs of abuse or drugs used to treat drug abuse during gestation [16,28,33,35]. The purpose of this study was to determine the effects of chronic *in utero* buprenorphine exposure on the neonatal guinea pig. Buprenorphine was studied in an animal model free of confounding factors because it is a promising candidate drug for substitution of the current standard methadone maintenance treatment program during pregnancy [11,12,17,20]. The present study focuses on respiratory effects associated with buprenorphine, but it also presents data on parameters associated with neonatal abstinence syndrome. To our knowledge, this is the first *in utero* buprenorphine study in the guinea pig.

In order to determine the dose for *in utero* buprenorphine exposure, we preformed preliminary studies based on previous literature on buprenorphine in the adult guinea pig model [6,14]. Similar to previous *in utero* studies for different opioids in both the rat and the guinea pig [33,42], we chose to study the highest tolerable dose for the maternal-fetal unit. The treatment schedule was based on our previous studies with methadone [33], as well as on clinical treatment programs for buprenorphine where buprenorphine is given once a day or every other day [4]. Data presented here show that *in utero* buprenorphine exposure affects both maternal weight gain and pup weight at birth and during the first two weeks of life. These results are similar to a study of *in utero* buprenorphine exposure in rats that showed reduction in pup weight that was comparable to that of methadone treated pups [38]. In addition, we used withdrawal induced hyperactivity in pups as a measure of NAS. We found extreme hyperactivity in 3 day old pups when removed from the moms, but not in older animals, suggesting that immediate withdrawal symptoms are only present in the youngest animals. This increase is comparable to the one we have seen in a previous study with methadone, while both are greater than for morphine [33]. In human infants, the score for NAS for infants born to mothers in buprenorphine treatment is better [20] or equal [26] in most cases compared to methadone maintained infants, while both are less severe than for morphine exposed infants [29].

Respiratory effects of *in utero* buprenorphine are seen during the immediate withdrawal period on day 3 and continue for the first two weeks of life. In the youngest pups, we have seen a substantial increase in inspiratory effort for buprenorphine treated pups, both under normcapnic and hypercapnic conditions. This is accompanied by extreme hyperventilation in these animals, due to increased inspiratory minute ventilation that is above and beyond any increase in oxygen consumption or carbon dioxide production. On the following study days, there is a continued increase in the delta effect of the inspiratory effort in response to CO_2 inhalation. Furthermore, expiratory time and the ratio of expiratory to inspiratory time is altered in buprenorphine pups up to the last study day. This suggests an abnormal neuro-control of breathing during the acute

withdrawal period in buprenorphine exposed pups. Furthermore, buprenorphine causes a prolonged and exaggerated response to hypercapnia beyond this initial withdrawal period, which implies an abnormal control of breathing in the central nervous system [25,40].

Previous studies have shown that there is an increased risk of sudden infant death syndrome (SIDS) associated with the respiratory effects caused by *in utero* methadone [22,23], morphine [34], or buprenorphine [19]. The data reported here suggest that the risk of SIDS for the neonate exposed to buprenorphine treatment during pregnancy may be as likely or frequent as for methadone maintained or morphine abusing moms. Furthermore, the increased risk factors might be manifested for a long time after birth. Reasons for these prolonged effects are unclear. Moms were not given any drug after parturition in this study. We did not measure drug levels in the mom or the pup during our studies. Previous studies though have shown that the placental transfer of buprenorphine to the fetal circuit is low [31], but have also indicated that due to the high lipophilicity of buprenorphine [13] it may be retained longer [38]. Furthermore, buprenorphine exhibits slower pharmacokinetics and metabolism in infants compared to adults [1]. Prenatal buprenorphine exposure in the rat also down-regulates mu opioid binding in the brain [2] and potentially affects respiratory control this way. Independent of the mechanism through which buprenorphine causes these prolonged effects in the neonate, our results indicate a need for longer and careful supervision of offspring of buprenorphine treated moms.

We saw no reduction in the level of respiratory effects for buprenorphine in the neonate when comparing our studies side by side with our recent study on methadone and morphine [33]. In contrast, the comparison shows that buprenorphine induced respiratory effects tend to be more severe in the immediate withdrawal phase as they are more apparent under normcapnia and are still apparent and measurable for several parameters during a hypercapnic challenge during the second week of life. Both of which was not seen for methadone in our earlier study, and only seen for morphine during normcapnia in the youngest animals [33]. Several studies have shown that, because buprenorphine is a partial agonist, respiratory effects of increasing doses exhibit a ceiling effect; while analgesic effects do not [8,9]. Therefore, we expected to see less respiratory effects with buprenorphine treatment, but this has not been seen when we compare equally effective *in utero* opioid drug doses. This could be due to the relative low dose of buprenorphine used; however, the dose of buprenorphine used had similar effects on the pregnant dam as the doses of methadone and morphine that we are comparing it to, and was the maximal dose tolerated by the maternal-fetal unit.

Currently, buprenorphine is only approved for treatment of opioid addiction in non-pregnant populations [7,41]. Nevertheless, it is increasingly prescribed to pregnant patients, even though studies examining the safety and risks of prenatal exposure to buprenorphine are currently in progress and still incomplete [18,20]. Therefore, it is more and more important to gather data on the effects of *in utero* buprenorphine on the newborn infant. The importance of the data reported here is that there may be considerable risks involved with transferring pregnant females from methadone to buprenorphine maintenance treatment, especially with regard to the consequences for the respiratory control of the newborn. Overall the switch from methadone to buprenorphine might be beneficial for both the mom and the child due to the socioeconomic factors of the treatment change [36], but our data clearly demonstrate that the risks involved with buprenorphine maintenance treatment are significant and need to be monitored carefully.

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Figure 1.

Respiratory effects of *in utero* buprenorphine treatment (gray bars) were compared to vehicle (black bars) during room air (RA) breathing (left) and during 5% CO₂ challenge (right). Major respiratory parameters, inspiratory minute ventilation (V_I) (A, B), breathing frequency (f_R) (C, D), tidal volume (V_T) (E, F), and inspiratory effort (V_T/T_I) (G, H) are shown. Several differences exist between buprenorphine and saline exposed pups. Differences are greatest in the youngest animals and during CO₂ challenge. Vertical bars represent standard error (S.E.). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle; ^a $p < 0.05$ vs. 7-day same treatment; ^b $p < 0.05$ vs. 10-day same treatment; ^c $p < 0.05$ vs. 14-day same treatment.

Figure 2.

Delta response for buprenorphine (gray bars) and vehicle (black bars) treated pups was calculated as the percent change from RA breathing to CO₂ challenge compared to the mean for all litters of the same age and treatment group during RA breathing. ΔV_I (A), Δf_R (B), ΔV_T (C), and $\Delta V_T/T_I$ (D) are graphed. The delta response is vastly decreased for all parameters in the youngest animals, while it is increased in older animals (ΔV_T , and $\Delta V_T/T_I$). Vertical bars represent standard error (S.E.). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle; ^a $p < 0.05$ vs. 7-day same treatment; ^b $p < 0.05$ vs. 10-day same treatment; ^c $p < 0.05$ vs. 14-day same treatment.

Figure 3.

Effects on oxygen metabolism of *in utero* buprenorphine treatment (gray bars) were compared to vehicle (black bars). Oxygen consumption VO₂ (A), CO₂ production VCO₂ (B), V_I/VO₂ (C), and V_I/VCO₂ (D) are graphed for all test days. Differences between treatment groups are greatest in 3 day old pups, indicating withdrawal related hyperventilation in buprenorphine exposed pups. Vertical bars represent standard error (S.E.). ** $p < 0.01$, *** $p < 0.001$ vs. vehicle; ^a $p < 0.05$ vs. 7-day same treatment; ^b $p < 0.05$ vs. 10-day same treatment; ^c $p < 0.05$ vs. 14-day same treatment.

Table 1

Weight data of dams and pups: Pregnant guinea pigs were treated once daily with vehicle (saline), or buprenorphine (0.1 mg/kg) starting at day 35 of gestation. Maternal weight during gestation and pup weight at days of respiratory testing was recorded. Buprenorphine treatment affected both maternal weight gain during the injection period and pup weight during the first two weeks of life, but did not affect the relative weight gain of the pups. Data from vehicle treated dams and pups were not different from previous studies. All values are mean $\pm$ SE.

		Vehicle	Buprenorphine
Maternal weight [g]	Gestation start	463 $\pm$ 59	420 $\pm$ 16
	Parturition	1046 $\pm$ 75	795 $\pm$ 18 *
Weight gain [%]	Day 1 – day 35	54 $\pm$ 16	56 $\pm$ 6
	Day 35 - parturition	49 $\pm$ 7	22 $\pm$ 2 *
N (dams)		2	3
Pup weight [g]	Birth (day 1)	108 $\pm$ 15	87 $\pm$ 5 *
	Day 3	103 $\pm$ 12	83 $\pm$ 8 *
	Day 7	130 $\pm$ 20	102 $\pm$ 12 *
	Day 10	155 $\pm$ 26	125 $\pm$ 15 *
	Day 14	193 $\pm$ 31	157 $\pm$ 17 *
Weight gain [% of birth weight]	Day 3	-4 $\pm$ 12	-4 $\pm$ 9
	Day 7	21 $\pm$ 18	18 $\pm$ 15
	Day 10	35 $\pm$ 6	43 $\pm$ 17
	Day 14	80 $\pm$ 29	80 $\pm$ 19
N (litter)		2	3

*
p<0.05 vs. vehicle

Activity data of pups: Pups were removed from their moms on days of respiratory testing for a period of 15 min and their ambulatory as well as grooming and digging movements were monitored. Buprenorphine treated pups show withdrawal related hyperactivity only on day 3. All values are mean $\pm$ SE.

Table 2

		3 day	7 day	10 day	14 day
Total activity [counts]	Vehicle	313 $\pm$ 9	311 $\pm$ 3	236 $\pm$ 20	420 $\pm$ 113
	Buprenorphine	1696 $\pm$ 354 *** <i>abc</i>	601 $\pm$ 33	422 $\pm$ 89	479 $\pm$ 98
Ambulatory activity [counts]	Vehicle	146 $\pm$ 24	168 $\pm$ 14	81 $\pm$ 8	214 $\pm$ 100
	Buprenorphine	1376 $\pm$ 308 *** <i>abc</i>	358 $\pm$ 30	228 $\pm$ 93	253 $\pm$ 68
Activity ratio [% ambulatory/total]	Vehicle	43 $\pm$ 5	50 $\pm$ 1	35 $\pm$ 6	48 $\pm$ 11
	Buprenorphine	79 $\pm$ 2 *** <i>abc</i>	59 $\pm$ 2	47 $\pm$ 11	51 $\pm$ 4

p<0.001 vs. vehicle,
a,b,c
p<0.05 vs. 7, 10, and 14 day same treatment, (n = 6 for vehicle, n = 8 for buprenorphine)

Table 3Respiratory measurements of inspiratory and expiratory time of *in utero* buprenorphine exposed neonatal guinea pigs.

Age	Room Air		5% CO ₂	
	Vehicle	Buprenorphine	Vehicle	Buprenorphine
T _I [sec]	3 days	0 0.00 0 0.01 0 0.0 0 0.01 0 0.01		
		.20 .13**	.14 0	.13
7 days	0 0.01 0 0.01 0 0.0 0 0.00 0 0.00			
	.17 .16	.14 0	.14	
10 days	0 0.01 0 0.00 0 0.0 0 0.01 0 0.01			
	.17 .13*	.15 0	.14	
14 days	0 0.02 0 0.01 0 0.0 0 0.00 0 0.00			
	.16 .16	.16 1	.14	
T _E [sec]	3 days	0 0.00 0 0.01 0 0.0 0 0.01 0 0.01*		
	.26 .18**c	.17 0 ^{abc}	.13* ^{abc}	
7 days	0 0.01 0 0.01 0 0.0 0 0.00 0 0.00 ^b			
	.25 .23	.19 0	.19 ^c	
10 days	0 0.00 0 0.00 0 0.0 0 0.01 0 0.01*			
	.25 .20*	.20 1	.23	
14 days	0 0.01 0 0.03 0 0.0 0 0.00 0 0.00*			
	.25 .26	.21 0	.23	
T _E /T _I	3 days	1 0.0 1 0.1 1 0.0 1 0.0**		
	.3 .4	.2 0 ^{abc}		
7 days	1 0.0 1 0.1 1 0.0 1 0.0 ^{bc}			
	.5 .5	.3 .4		
10 days	1 0.1 1 0.1 1 0.1 1 0.1**			
	.5 .5	.3 .7*		
14 days	1 0.1 1 0.1 1 0.0 1 0.0**			
	.5 .6	.3 .6*		

* p<0.05,

**
p<0.01,

p<0.001 vs. vehicle;

a,b,c p<0.05 vs. 7,10, or 14 day same treatment.

All values are reported as mean $\pm$ S.E.