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Assessing the Effects of Pharmacological Agents on Respiratory Dynamics Using Time-Series Modeling

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Abstract

Developing quantitative descriptions of how stimulant and depressant drugs affect the respiratory system is an important focus in medical research. Respiratory variables—respiratory rate, tidal volume, and end tidal carbon dioxide—have prominent temporal dynamics that make it inappropriate to use standard hypothesis-testing methods that assume independent observations to assess the effects of these pharmacological agents. We present a polynomial signal plus autoregressive noise model for analysis of continuously recorded respiratory variables. We use a cyclic descent algorithm to maximize the conditional log likelihood of the parameters and the corrected Akaike's information criterion to choose simultaneously the orders of the polynomial and the autoregressive models. In an analysis of respiratory rates recorded from anesthetized rats

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before and after administration of the respiratory stimulant methylphenidate, we use the model to construct within-animal z-tests of the drug effect that take account of the time-varying nature of the mean respiratory rate and the serial dependence in rate measurements. We correct for the effect of model lack-of-fit on our inferences by also computing bootstrap confidence intervals for the average difference in respiratory rate pre- and postmethylphenidate treatment. Our time-series modeling quantifies within each animal the substantial increase in mean respiratory rate and respiratory dynamics following methylphenidate administration. This paradigm can be readily adapted to analyze the dynamics of other respiratory variables before and after pharmacologic treatments.

Keywords

Bootstrap; general anesthesia; respiratory rate; serial correlation

I. INTRODUCTION

CHARACTERIZING the effects of stimulant and depressant drugs on respiration is an important question in medicine. For example, in patients requiring sedation in the intensive care unit as well as patients who are hospitalized following major surgery, a major side effect of most sedatives is respiratory depression [1], [2]. Following major surgery, the most commonly used medications for treatment of postoperative pain are the opioids. Like sedatives, the most significant side effect of the opioids is respiratory depression [3], [4]. Opioids administered by controlled infusions have been used to study respiratory dynamics [5], [6]. When respiratory depression leads to respiratory failure, it is not unusual to administer the opioid antagonist, naloxone [3], [4]. A significant drawback of this treatment is that naloxone also reverses the analgesic effects of the opioids [7], [8]. The respiratory depressive effects of opioids can be significantly reversed while sparing their analgesic effects by administering an ampakine [2], [9]. Other well-known respiratory stimulants include progesterone [10], phosphodiesterase inhibitors [11], dopaminergic agonists [12], [13], almitrine [14], and doxapram [15]. Methylphenidate (MPH), the dopaminergic agonist more widely known as Ritalin, can induce emergence from general anesthesia [16]. An almost immediate physiological change observed prior to emergence from general anesthesia is a dramatic increase in respiratory rate.

A typical experiment entails making extensive measurements on respiratory variables such as respiratory rate, tidal volume, and carbon dioxide production on each subject before and after administration of the pharmacological agent [1], [2], [9]–[13], [16]. Although the recording periods before and after drug administration usually last several minutes, these studies are only conducted on a few subjects. A standard analysis is to assess the effects of the drug by either performing a t-test on the average difference in the respiratory variables [11]–[13], [16], or a sign test on the signs of the average differences across subjects [1]. Repeated measures analysis of variance has been used when the objective is to determine an approximate dose-response relation [2], [9], [10].

This analysis approach has several shortcomings. First, using only a mean of a given respiratory variable to assess the state of the subject's respiratory system before and after treatment is inadequate because these variables often have strong temporal dynamics (see Fig. 1). Understanding these temporal dynamics, both time course and variability, can provide important information about the drug's effects on the respiratory system. Second, the measurements of the respiratory variables have strong serial dependence and the character of this dependence can be different pre- and postdrug administration. The effects of this serial dependence on the choice of the statistical analysis methods and the subsequent inference are not considered. Third, using either a t-test or a sign test reduces the amount of information in the study from several hundred observations per subject to a number of observations equal only to the small number of study subjects. Similarly, repeated measures analysis of variance loses information because the number of data points reduces to the number of subjects times the number of observation periods. Finally, because there can often be substantial between-subject differences in respiratory dynamics before and after drug administration, it is not clear that conducting an analysis that requires pooling across subjects will accurately provide a representative assessment of the respiratory physiology of the subjects before or after treatment.

Because the experimental data consist of many measurements pre- and posttreatment made on only a few subjects, a more principled analysis approach would be to develop a detailed statistical model for the pretreatment and the posttreatment conditions within subject. We used a constant mean plus autoregressive (AR) noise model to analyze respiratory rate pre- and postadministration of MPH [16], [17]. The order of the AR model was chosen by corrected Akaike's Information Criterion (AICc) [18], [19]. The analyses in [17] offered analytic insights into the effect of serial dependence in the measurements on the effective sample size. Although these analyses made improvements over current approaches, the use of the constant mean in the models both pre- and postdrug administration does not allow an accurate assessment of respiratory dynamics.

To address this problem, we represent the respiratory variable in the predrug and the postdrug conditions with different signal plus correlated noise models. We model the signal as a polynomial function of order R and the serial dependence as an AR model of order P . We develop an efficient cyclic descent algorithm to fit the model by maximizing the conditional log likelihood of the data. We choose the orders of the polynomial and the AR models using the AICc [18], [19]. Standard errors for the parameters are computed both from an estimate of the inverse of the Fisher information matrix and by a bootstrap procedure to account for possible model lack-of-fit [19]. We derive a straight forward extension of the standard within subject z-test that takes account of both the time-varying nature of the mean and the serial dependence in the data. We apply the method in the analysis of data from a study in which anesthetized rats received MPH to assess its effects on the respiratory system [16].

II. METHODS

A. Experimental Procedures and Respiratory Measurements

This study was approved by the Massachusetts General Hospital Animal Care Committee. Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 351–565 g were anesthetized in a custom built plethysmography chamber with isoflurane at a constant concentration of 1.5% in oxygen. After equilibration in the chamber for 30 min, normal saline was administered through a previously inserted tail vein intravenous catheter, 5 min prior to administering intravenous MPH at a dose of 5 mg/kg. We used a differential pressure transducer and demodulator to convert chamber pressure into an analog signal. The signal was high-pass filtered at 15 s, acquired at 100 Hz, analyzed in 4-sec epochs using a USB-6009 data acquisition board and LabView Software and converted into breaths per minute at 15 samples per minute. We analyze here the 10–12 min of respiratory rate recordings (approximately 150–180 observations) made prior to the administration of MPH as the predrug condition and the 30–35 min of respiratory rate recordings (approximately 450–525 observations) made after the administration of MPH as the postdrug condition.

B. Polynomial and AR Process Model of Respiratory Dynamics

1) Model Definition, Parameter Estimation, and Model Order Selection: To describe the respiratory dynamics in our experiments, we assume a signal plus noise model in which the signal or time-dependent mean is represented as a polynomial of order R and the noise is represented as an AR process of order P . That is, let y_t be the respiratory rate observed at time t for $t = 1, 2, \dots, T$. We, then, have

$$y_t = \sum_{r=0}^R c_r (f + gt)^r + v_t \quad (1)$$

$$v_t = \sum_{p=1}^P a_p v_{t-p} + \epsilon_t \quad (2)$$

where the ϵ_t are independent zero mean Gaussian random variables with variance σ_ϵ^2 . We

choose f and g to be -1 and $\frac{2}{T+1}$ so that $f + gt$ lies between -1 and 1 for $t = 1, 2, \dots, T$.

Scaling $f + gt$ between $(-1, 1)$ helps insure the numerical stability of the polynomial evaluations during the estimation.

We let $c = (c_0, c_1, \dots, c_R)'$ and $a = (a_1, a_2, \dots, a_P)'$. Then, our objective is to estimate the $P + R + 2$ dimensional parameter $\theta = (a', c', \sigma_\epsilon^2)'$ from the data $y_1, y_2, \dots, y_T$. Because the dimension of θ needed to describe adequately the time series of respiratory rates may be large, we would like to develop an efficient estimation algorithm that can be used across a broad range of dimensions. Therefore, instead of maximizing the full likelihood of the parameter we simplify the calculation by conditioning on the first t_0 observations, i.e.

$\{y_1, y_2, \dots, y_{t_0}\}$ for some $t_0 \leq P$. It follows under the Gaussian assumption for the ϵ_t that the conditional log likelihood of θ is

$$\ell(\theta|y) = -\frac{T-t_0}{2} \log(2\pi\sigma_\epsilon^2) - \frac{\epsilon' \epsilon}{2\sigma_\epsilon^2} \quad (3)$$

where we define $y = (y_{t_0+1}, \dots, y_T)'$, $\epsilon = (\epsilon_{t_0+1}, \dots, \epsilon_T)'$

We can maximize (3) with respect to both a_i for $i = 1, 2, \dots, P$ and c_j for $j = 0, 1, \dots, R$ to obtain a maximum likelihood estimates of each of the parameters $\{a_i, c_j\}$ in terms of the others. This structure in the problems suggests that an efficient approach to maximizing the conditional log likelihood in (3) is to use a cyclic descent algorithm [20]. Efficient implementations of cyclic descent procedures for signal plus noise models have been reported in [21]–[23].

At iteration h a cyclic descent algorithm for our model is

$$\hat{c}_j^{(h)} = (\hat{u}_j^{(h)'} \hat{u}_j^{(h)})^{-1} \hat{u}_j^{(h)'} (\hat{\epsilon}^{(h,j)} + \hat{c}_j^{(h-1)} \hat{u}_j^{(h)}) \quad (4)$$

$$\hat{a}_i^{(h)} = (\hat{v}_i^{(h)'} \hat{v}_i^{(h)})^{-1} \hat{v}_i^{(h)'} (\hat{\epsilon}^{(h,R+i)} + \hat{a}_i^{(h-1)} \hat{v}_i^{(h)}) \quad (5)$$

where $\hat{v}_i^{(h)} = (\hat{v}_{t_0+1-i}^{(h)}, \dots, \hat{v}_{T-i}^{(h)})'$, as $\hat{v}_i^{(h)} = y_t - \sum_{r=0}^R \hat{c}_i^{(h)} (f + gt)^r$;

$\hat{u}_j^{(h)} = (\hat{u}_{t_0+1,j}^{(h)}, \dots, \hat{u}_{T,j}^{(h)})'$, where $\hat{u}_{t,j}^{(h)}$ is the AR filtering of $(f + gt)^j$ defined by

$\hat{u}_{t,j}^{(h)} = (f + gt)^j - \sum_{p=1}^P \hat{a}_p^{(h-1)} [f + g(t-p)]^j$; and $\hat{\epsilon}^{(h,j)} = (\hat{\epsilon}_{t_0+1}^{(h,j)}, \dots, \hat{\epsilon}_T^{(h,j)})'$ which has to

be updated when any a_i or c_j is updated, i.e.,

$\hat{\epsilon}_t^{(h,j)} = y_t - \sum_{p=1}^P \hat{a}_p^{(h-1)} y_{t-p} - \sum_{r < j} \hat{c}_r^{(h)} \hat{u}_{t,r}^{(h)} - \sum_{r \geq j} \hat{c}_r^{(h-1)} \hat{u}_{t,r}^{(h)}$ for $j = 0, 1, \dots, R$ and

$\hat{\epsilon}_t^{(h,R+i)} = \hat{v}_t^{(h)} - \sum_{p < i} \hat{a}_p^{(h)} \hat{v}_{t-p}^{(h)} - \sum_{p \geq i} \hat{a}_p^{(h-1)} \hat{v}_{t-p}^{(h)}$ for $i = 1, 2, \dots, P$.

Since each parameter depends on the others, it is essential to use the best approximation of input parameters to obtain the best output parameters. The initial estimate $\hat{c}_j^{(0)}$ can be easily computed by least squares assuming a zero order AR, whereas $\hat{c}_j^{(0)}$ the initial estimate given $\hat{a}_i^{(0)}$ can be computed by least squares or the Burg algorithm [24]

$$\hat{c}^{(0)} = (X' X)^{-1} X' y \quad (6)$$

$$\hat{a}^{(0)} = (V' V)^{(-1)} V' (y - X \hat{c}^{(0)}) \quad (7)$$

where $\hat{a}^{(0)} = (\hat{a}_1^{(0)}, \dots, \hat{a}_P^{(0)})'$, $\hat{c}^{(0)} = (\hat{c}_0^{(0)}, \dots, \hat{c}_R^{(0)})'$; X is the $(T - t_0) \times (R + 1)$ design matrix for the $(f + gt)^r$ for $t = t_0 + 1, \dots, T$ and $r = 0, \dots, R$, i.e., $X_{ij} = [f + g(t_0 + i)]^{j-1}$; and V is the $(T - t_0) \times P$ matrix of lagged values for the $\hat{v}_t^{(0)}$, i.e., $V_{ij} = \hat{v}_{t_0+i-j}^{(0)}$.

The conditional maximum likelihood estimates are the parameter estimates $\hat{c} = \hat{c}^{(H)}$ and $\hat{a} = \hat{a}^{(H)}$ computed at the final iteration H of the algorithm where we use as a stopping criterion $|\ell(\theta^{(H)} | y) - \ell(\theta^{(H-1)} | y)| < \delta$. At the final iteration we use the conditional maximum likelihood estimate of σ_e^2

$$\hat{\sigma}_e^2 = \frac{\hat{\epsilon}^{(H)'} \hat{\epsilon}^{(H)}}{T - t_0}. \quad (8)$$

The cyclic descent algorithm is predicated on knowing the order of the polynomial R and the order of the AR process P . Because the model orders are unknown, we use the AICc defined as

$$\begin{aligned} & \text{AICc}(R + P + 2) \\ &= -2\ell(\hat{\theta} | y) + \frac{2(T - t_0)(R + P + 2)}{T - t_0 - R - P - 3} \end{aligned} \quad (9)$$

where $\hat{\theta} = (\hat{a}', \hat{c}', \hat{\sigma}_e^2)'$ is the conditional maximum likelihood estimate of θ for polynomial model order R and AR model order P .

2) Model-Based Inferences: The model in (1) and (2) has a time-varying mean. Thus, in order to estimate the mean respiratory rate for the period before (after) injection of the MPH, we integrate the polynomial terms in our model in (1) between the initiation and the termination times of the before (after) periods. That is, we have

$$\hat{\mu}_t = \sum_{r=0}^R \hat{c}_r (f + gt)^r \quad (10)$$

and thus

$$\begin{aligned}
\hat{\mu} &= \frac{1}{T-t_0} \int_{t_0}^T \hat{\mu}_t dt \\
&= \frac{1}{T-t_0} \int_{t_0}^T \sum_{r=0}^R \hat{c}_r (f+gt)^r dt \\
&= \frac{1}{T-t_0} \left[\sum_{r=0}^R \hat{c}_r \frac{(f+gt)^{r+1}}{g(r+1)} \right]_{t_0}^T \\
&= \sum_{r=0}^R \frac{[(f+gT)^{r+1} - (f+gt_0)^{r+1}]}{(r+1)g(T-t_0)} \hat{c}_r \\
&= w' \hat{\theta}
\end{aligned} \tag{11}$$

where $w = (0, \dots, 0, w_{p+1}, \dots, w_{p+1+R}, 0)'$ and $w_{p+1+r} = \frac{[(f+gT)^{r+1} - (f+gt_0)^{r+1}]}{(r+1)g(T-t_0)}$ for $r = 0, \dots, R$.

The variance of the estimated parameters is given by the inverse Fisher Information matrix which we estimate as the negative of the Hessian (observed Fisher Information) matrix evaluated at $\hat{\theta}$. Therefore, the approximate variance of $\hat{\theta}$ is $I(\hat{\theta})^{-1}$ [19]. It follows that the variance of the mean respiratory rate is given by

$$\text{Var}(\hat{\mu}) = w' I(\hat{\theta})^{-1} w. \tag{12}$$

We can now test a hypothesis about the difference between the pre-MPH and the post-MPH mean respiratory rates. This z-statistic is

$$z = \frac{\hat{\mu}_{\text{after}} - \hat{\mu}_{\text{before}}}{\left[\text{Var}(\hat{\mu}_{\text{before}}) + \text{Var}(\hat{\mu}_{\text{after}}) \right]^{\frac{1}{2}}} \tag{13}$$

where $\hat{\mu}$ is defined in (11) and $\text{Var}(\hat{\mu})$ is given in (12).

3) Bootstrap Analysis: In cases in which the chosen model has lack-of-fit, i.e., the polynomial plus AR model fails to capture all of the salient structure in the data, the observed Fisher Information matrix may not provide an accurate assessment of the parameter variances. To analyze the data in the setting of possible lack-of-fit due predominantly to the AR noise model, we use a bootstrap procedure [25]. Using the

estimated errors, i.e., the residuals $\hat{\epsilon}$, and the conditional maximum likelihood parameter estimates, we simulate multiple bootstrap samples and find the conditional maximum likelihood estimates of the parameters for each sample.

The bootstrap algorithm is

1. Draw ϵ_t^* from $\hat{\epsilon}$ with replacement, for $t = -(b - t_0), \dots, T$, where b is a positive number which is reasonably chosen as the burn-in period.
2. Compute $v_t^* = \sum_{p=1}^P \hat{a}_p v_{t-p}^* + \epsilon_t^*$ for $t = -(b - t_0), \dots, T$ assuming $\left\{v_{-(b-t_0)-P}, \dots, v_{-(b-t_0)-1}\right\}$ are all zeros. Discard the burn-in period $\{v_{-t_0}^*, \dots, v_0^*\}$.
3. Compute $y_t^* = \sum_{r=0}^R \hat{c}_r (f + gt)^r + v_t^*$ for $t = t_0 + 1, \dots, T$.
4. Given $\mathbf{y}^* = (y_{t_0+1}^*, y_{t_0+2}^*, \dots, y_T^*)'$, compute $\hat{\theta}^*$ by maximizing $\ell(\theta | \mathbf{y}^*)$.
5. Carry out A through D B^* times.

Using this procedure, we obtained B^* sets of the parameter estimates θ^* . For each set of parameter estimates, the value of $\hat{\mu}$ can be estimated as in (11). A 95% confidence interval for $\hat{\mu}$ is given by the 2.5th and the 97.5th percentiles of the bootstrap sample. Similarly, subtracting each $\hat{\mu}_{\text{before}}$ from each $\hat{\mu}_{\text{after}}$ we obtain B^* mean differences, a 95% confidence interval for $\hat{\mu}_{\text{after}} - \hat{\mu}_{\text{before}}$ is given by 2.5th and the 97.5th percentiles of the mean differences. Drawing the errors from the empirical distribution defined by the residuals, allows us to evaluate the effect of lack-of-fit due to the AR model because this allows for possibility of larger errors (outliers), and as a result, larger parameter standard errors than would be predicted by the Gaussian white noise component of the AR model.

To conduct a hypothesis test of the difference in means using this bootstrap method, we examine if the 95% confidence interval of $\hat{\mu}_{\text{after}} - \hat{\mu}_{\text{before}}$ contains zero. We reject H_0 at significance level 0.05 if the interval does not contain zero and conclude that there is a significant effect of MPH on respiration.

III. ANALYSIS OF RESPIRATORY RATE TIME SERIES

A. Model Selection and Goodness-of-Fit Analysis

Fig. 1 shows the respiratory rate time series of the eight rats that we studied. While all eight animals had stable breathing patterns with very little excursion in their rates prior to injection of MPH, all showed a time-varying base line respiratory rate and much wider excursions following injection. The instantaneous respiratory rates range from 70 to 110 breaths per minute before injection and from 90 to 200 breaths per minute after injection. The changes in breathing patterns suggest a substantial change in the animals' physiologic states following injection of MPH.

To choose the orders for the polynomial and AR components of the model for each animal, we evaluated the AICc for 160 models by considering P ranging from 0 to 15 and R from 0 to 9 (see Fig. 2). We identified for each animal a single pair of P and R (see Fig. 2, Table I). There was not a consistent pattern in the model orders before injection compared with after injection within or between animals. The only exception is that the orders for the AR components tended to be higher after injection. This agrees with our observation of increased variability in the respiratory rate following injection. Although we chose a single order for both components pre- and postinjection (see Fig. 2, dark blue cell) the AICc surface in the neighborhood of the optimal pair tended to be flat (see Fig. 2, light blue region) suggesting that models near the one we chose most likely had similar descriptive power.

The order of the AR component P was greater than or equal to 1 for all animals before and after MPH injection, suggesting that a simple analysis using only the mean respiratory rate to analyze the effect of this stimulant on breathing would not give an accurate assessment (see Fig. 3, Table I). Similarly, because only animals 1 and 6 had optimal AR orders of 0 (see Table I) the white noise Gaussian assumption required for standard hypothesis testing is also not satisfied by the data in this experiment. In both the pre- and postinjection conditions, the polynomial components (see Fig. 3, green curve) capture well for each animal the time-varying nature of the mean respiratory rate whereas the AR model describes well the variability about that time-varying mean. Together the polynomial and AR components (see Fig. 3, red curve) capture the majority of the structure in the respiratory rate time series.

We computed 95% confidence intervals for the residuals based on the empirical distribution of the residuals (see Fig. 4, dashed horizontal bands, 2.5th and 97.5th percentiles) and based on $\hat{\sigma}_\epsilon^2$ (see Fig. 4 shaded horizontal bands, $\pm 1.96\hat{\sigma}_\epsilon$). Before administering MPH, the residuals for each animal were well within the 95% limits for both types of confidence intervals (see Fig. 4, dashed lines). In contrast, the residuals from the model fits after MPH had much wider variability and wider 95% confidence intervals of both types despite the fact that respiratory rates were recorded for longer periods after injection. In addition to being larger, a greater fraction of the postinjection residuals (6%) fell outside of both sets of 95% confidence intervals. Nevertheless, the autocorrelation function analyses showed that these residuals were uncorrelated for each animal (analysis not shown). Our Q-Q plot analyses (not shown) showed that the empirical distributions of the residuals were symmetric. However, for several of the animals post-MPH, these distributions had heavier tails—due to the outliers in Fig. 4—than Gaussian distributions. We conclude that there is some lack-of-fit of the AR component of our model.

Nevertheless, the residuals clearly illustrate the change in respiratory dynamics and physiologic state in each animal following injection of MPH. The variability in animals 1 and 6 resembles their variability preinjection. Animals 2, 3, and 7 show substantially increased variability postinjection, whereas animals 4, 5, and 8 show little to no change in variability immediately following the MPH injection and develop more variability several minutes later.

B. Effect of MPH on Respiratory Rate

The goal of the model fitting was to analyze for each of the eight animals the effect of MPH on respiratory rate (see Tables I and II). The increases in the mean respiratory rates ranged from 11 to 51 breaths per minute. All of these increases were statistically significant ($p < 10^{-5}$) based on the z-test de-rived in (11) to (13). To assess the effect of model lack-of-fit on this inference, we also performed bootstrap analyses of the mean increases in respiratory rates by drawing for each animal $B^* = 200$ bootstrap samples. The widths of the 95% bootstrap confidence intervals for the mean increases ranged from 1.94 to 9.37 breaths per minute (see Table II). The distance between the lower bound of these intervals and 0—the point of no effect—ranged from 7 to 50 breaths per minute confirming that MPH induces a substantial increase respiratory rate in anesthetized rats.

In nearly all of the animals the increase in minute ventilation following MPH administration were due almost entirely to the increases in respiratory rates as their tidal volumes were unchanged. Therefore, we did not analyze minute ventilation or tidal volume.

C. Standard Errors of the Model Parameters

We transformed the time axis to the interval from $(-1,1)$ to ensure numerical stability of the polynomial evaluations during the estimation. As an indicator of the algorithm's performance, we report the model parameters and associated standard errors post-MPH administration for Animal 5 (see Table III) as a representative example. The AR parameters are on the order of 10^{-1} whereas the polynomial coefficients are on the order 10^2 . With the exception of c_4 , c_5 , and c_7 , the ratios of the coefficients to the standard errors suggest that all estimates are statistically significant. This is consistent with our ability to compute standard errors for $\hat{\mu}$ (see Table I) for all animals pre- and post-MPH administration. Our ability to compute reasonable standard errors for the parameter estimates suggests that the final Hessian matrix is negative definite and at least consistent with the parameter estimates being from a local maximum of the conditional log likelihood.

D. Convergence of the Cyclic Descent Algorithm

In a preliminary analysis of these data fit with a mean plus AR(1) noise model, the cyclic descent algorithm converged in 20 or fewer iterations [17]. To give a sense of the algorithm's convergence behavior for our larger models, we analyzed the convergence of the mean estimates (11) before and after injection (see Fig. 5). These analyses show that in each case, the initial mean estimate was within 0.2 of the final estimate and the algorithm was close to the final estimate within 100 iterations.

IV. DISCUSSION

We have introduced a polynomial plus AR noise model to show how more informative studies of the effects of pharmacologic agents on respiratory variables can be accomplished by using detailed statistical modeling. To illustrate our approach, we analyzed the stimulant effects of MPH on the respiratory rates of anesthetized rodents. We used a cyclic descent algorithm to fit the model and the AICc criterion to choose the polynomial and AR orders. Our time-series models provided accurate descriptions of the time-varying mean and the

serial dependence in these respiratory rate measurements pre- and postadministration of MPH that laid the basis for a likelihood-based framework for formal inference. We computed model standard errors and confidence intervals using the inverse of the observed Fisher information matrix and a bootstrap procedure. The two types of standard errors and confidence intervals were in agreement. However, by using the bootstrap, we took account of the model lack-of-fit in the error assessment.

Our approach provides several advantages over current analyses. First, because the polynomial model captured well the time-varying nature of the mean respiratory rate present in nearly all of the animals both before and after injection of MPH, our analysis makes clear that the standard assumption of a constant mean respiratory rate in both conditions is not accurate. Second, the AR models rather than simple independent Gaussian error models gave the best descriptions of the serial dependence in the data across all of the animals in both conditions.

Third, by developing a detailed model for each study subject, we were able to make inferences about the effects of MPH on the respiratory rate of each animal using a z-test (11) that took account of both the time-varying nature of the means and the serial dependence in the recordings. We conclude that a positive effect of MPH on mean respiratory rate (see Tables I and II) is strongly present in each animal. Hence, it follows trivially that the positive effect is present for the group. The ranges of the 95% confidence intervals for the true mean differences across animals computed from the inverse of the observed Fisher information matrix and the bootstrap analysis agree and provide informative assessments of the magnitudes of the between animal differences in the effects of the drug. In this case, either of the two standard errors could be used to compute an appropriately weighted estimate of the mean difference across the group. These within and across animal assessments and inferences would not be possible from a group analysis based on a simple t-test or the sign test. Finally, the fits of the polynomial plus AR noise model (see Fig. 3) and the residual analysis (see Fig. 4) provide a quantitative assessment of a feature in the data (see Fig. 1) that has not been considered. The physiological effect of MPH is not only a time-varying increase in the respiratory rate but also, for most of the animals, an increase in the variability of those time-varying rates. Overall, our analyses offer additional details to the study of the effects of MPH on respiration [16].

Although scaling the time interval helped ensure the numerical stability of the polynomial fitting, use of spline models or orthogonal basis functions [26] offer another approach that may be more tractable across a wider set of experimental conditions and respiratory variables. The heteroscedastic nature of the dynamics in these respiratory rate recordings is not completely described in the current analysis and is the source of the lack-of-fit of the AR component. Using GARCH models [27], [28] to represent the correlated noise may offer one approach to improving this aspect of our analyses. Alternatively, for a more first principles approach, we can return to the plethysmography recordings and reanalyze these data using local regression methods or state-space modeling approaches to compute respiratory rates and make inferences using a single framework [26]. These methodological studies as well as the application of the current approach to other respiratory variables will be topics of future reports.

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Biographies

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Fig. 1.

Respiratory rate time series (solid curves) of the eight rats. Vertical dashed lines indicate the times of MPH administration. The pre- (post-) MPH injection periods range from 10 to 12 (30 to 35) min.

Fig. 2. AICc of 160 models for the eight rats for the before and after MPH injection conditions. Horizontal and vertical axes are, respectively, the AR order P and the polynomial order R . The dark blue cell in each panel is the model with the minimum AICc. All colors are displayed relative to the minimum AICc for the animal. AICc greater than the minimum AICc by 20 or more are red.

Fig. 3.

Respiratory rate time series (blue curve), estimated respiratory rates $\hat{y}_t$ (red), and the estimated time-varying mean rate $\hat{\mu}_t$ (green). Vertical dashed lines indicate the times of MPH administration.

Fig. 4.

Residuals (solid curves) from the model fits in Fig. 3 and the 95% confidence intervals for the residuals based on the empirical distribution of the residuals (dashed horizontal bands, 2.5th and 97.5th percentiles) and based on $\hat{\sigma}_\epsilon^2$ (shaded horizontal bands, $\pm 1.96 \hat{\sigma}_\epsilon$). Vertical dashed lines mark the times of MPH administration.

Fig. 5. Convergence trajectories of the mean estimates $\hat{\mu}(11)$ as a function of the cyclic descent algorithm iterations.

HYPOTHESIS TESTING OF DIFFERENCE IN MEANS USING OBSERVED FISHER INFORMATION MATRIX

TABLE I

Animal	1	2	3	4	5	6	7	8
(R,P) before (R,P) after	0.4 3.4	7.1 6.3	3.2 4.11	8.1 2.2	4.2 8.2	0.3 2.10	6.12 0.1	6.1 6.10
$\hat{\mu}_{\text{before}}$ (s.e.)	73.84 (0.66)	76.67 (0.91)	78.04 (0.38)	71.36 (0.60)	84.07 (0.93)	84.22 (2.99)	99.25 (0.79)	100.04 (1.97)
$\hat{\mu}_{\text{after}}$ (s.e.)	94.17 (0.60)	114.79 (1.19)	109.31 (1.00)	102.45 (0.94)	92.50 (0.77)	96.64 (0.79)	153.44 (3.11)	117.09 (0.93)
$\hat{\mu}_{\text{after}} - \hat{\mu}_{\text{before}}$ (s.e.)	20.34 (0.89)	38.12 (1.50)	31.26 (1.07)	31.09 (1.11)	8.43 (1.21)	12.42 (3.09)	54.20 (3.21)	17.05 (2.17)
test-statistic	22.80	25.44	29.18	28.05	6.95	4.01	16.89	7.84
p-value	$<10^{-16}$	$<10^{-16}$	$<10^{-16}$	$<10^{-16}$	$<10^{-12}$	$<10^{-5}$	$<10^{-16}$	$<10^{-15}$

TABLE II
HYPOTHESIS TESTING OF DIFFERENCE IN MEAN USING BOOTSTRAP METHOD

Animal	1	2	3	4	5	6	7	8
95% C.I. for $\hat{\mu}_{\text{before}}$	73.20 74.64	76.25 77.30	77.05 79.00	71.01 71.75	82.83 84.92	81.20 87.92	98.45 100.03	99.77 100.06
95% C.I. for $\hat{\mu}_{\text{after}}$	93.14 95.30	113.22 116.72	107.82 110.79	100.94 104.06	91.63 93.25	96.46 97.12	149.43 158.54	116.20 118.00
95% C.I. for $\hat{\mu}_{\text{after}} - \hat{\mu}_{\text{before}}$	19.07 21.50	36.54 40.08	29.34 33.11	29.57 32.79	7.12 9.79	8.69 15.71	50.37 59.74	16.18 18.12

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TABLE III
COEFFICIENT ESTIMATES AND STANDARD ERRORS FOR RAT 5 POST-MPH INJECTION

Coefficient	c_0	c_1	c_2	c_3	c_4	c_5	c_6	c_7	c_8	a_1	a_2
Estimates	96.09	-74.76	-109.05	136.57	564.10	-142.66	-909.62	46.39	457.25	0.1946	0.1483
(s.e.)	1.83	9.14	30.06	70.91	199.30	158.99	352.95	106.16	200.75	0.0645	0.0648