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Random changepoint segmented regression with smooth transition

Julio M Singer[1], Francisco MM Rocha[2], Antonio Carlos Pedroso-de-Lima[1], Giovani L Silva[3], Giuliana C Coatti[4] and Mayana Zatz[4]

Abstract

We consider random changepoint segmented regression models to analyse data from a study conducted to verify whether treatment with stem cells may delay the onset of a symptom of amyotrophic lateral sclerosis in genetically modified mice. The proposed models capture the biological aspects of the data, accommodating a smooth transition between the periods with and without symptoms. An additional changepoint is considered to avoid negative predicted responses. Given the nonlinear nature of the model, we propose an algorithm to estimate the fixed parameters and to predict the random effects by fitting linear mixed models iteratively via standard software. We compare the variances obtained in the final step with bootstrapped and robust ones.

Keywords

Amyotrophic lateral sclerosis, fitting algorithm, mixed models, random effects

1 Introduction

Amyotrophic Lateral Sclerosis (ALS) is one of the most common adult-onset motor neuron diseases causing a progressive, rapid and irreversible degeneration of motor neurons in the cortex, brain stem and spinal cord. In the majority of cases, ALS occurs sporadically; in about 10% of the cases, it is caused by familial reasons. No effective treatment is available and cell therapy clinical trials are currently being conducted with ALS-affected patients. The SOD1 gene encodes an important antioxidant human enzyme and mutations in this gene represent one of the most frequent causes of ALS.

Among the different animal models for ALS, SOD1 mice are the most used in preclinical studies.[1] These mice overexpress the SOD1 gene bearing the G93A mutation, a point mutation also found in familial ALS. They remain symptomless for a certain period after which they start to develop muscle weakness, that increases until they become fully paralysed and die. Interestingly, in this animal model the disease progression exhibits a sex effect comparable to that observed in ALS patients. Males have a shorter lifespan and a clinical condition apparently more severe than females, and differences in electrophysiological parameters have also been reported.[2]

Treatment of ALS with stem cells is a current research topic. Mesenchymal stromal cells (MSC), especially those derived from adipose tissues, and pericytes have been used in studies of neurodegenerative diseases that focus on the reduction of the acceleration with which symptoms progress. In this context, we consider a study conducted in the Human Genome and Stem Cell Research Center, at the Biosciences Institute, University of

[1]Departamento de Estatística, Universidade de São Paulo, São Paulo, Brazil
[2]Departamento Multidisciplinar, Escola Paulista de Política Economia e Negócios, Universidade Federal de São Paulo, São Paulo, Brazil
[3]Departamento de Matemática – IST and CEAUL, Universidade de Lisboa, Lisboa, Portugal
[4]Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil

Corresponding author:
Julio M Singer, Departamento de Estatística, Universidade de São Paulo, Rua do Matão, 1010 São Paulo 05508-090, SP, Brazil.
Email: jmsinger@ime.usp.br

São Paulo, Brazil with the objective of comparing MSC cells and pericytes injected in SOD1-G93A mice with respect to their effects on the evolution of some symptoms of ALS. Details may be obtained in Coatti et al.[3]

Our objective here is to propose models to verify whether the treatment with stem cells delays the symptom onset and reduces the acceleration with which they develop.

2 The experimental setup

A set of sixty-six 8-week old SOD1-G93A mice was randomly divided into three groups with approximate balance between males and females. Given that 11 animals did not follow the study protocol (dying for not resisting the trauma caused by the treatment), the experiment was conducted with 34 female and 21 male animals. Animals in the first group (12 females and 7 males) were submitted to weekly injections of MSC cells, those in the second group (11 females and 8 males) to injection with pericytes while animals in the third group (11 females and 6 males) were submitted to the control treatment (*Hank's balanced salt solution* – HBSS). Progression of the disease was evaluated weekly up to each animal's death through clinical analysis by means of four variables, of which one of them (*rotarod*) is used to evaluate motor coordination and fatigue resistance. For that purpose, the length of time each animal could remain on a rotating cylinder of a *rotarod* apparatus (IITC Life Science model 755) was recorded. The initial speed of 1 r/min was increased constantly until a final speed of 30 r/min, after 180 s. Each animal was given three tries and the longest duration to fall was recorded. We consider this variable to illustrate the proposed statistical analysis with the following objectives:

(i) Identification of the moment when animals become symptomatic (symptom onset) for the six groups defined by the combinations of treatment (HBSS, MSC, pericytes) and sex (male, female).
(ii) Estimation of the acceleration with which the response deteriorates after symptom onset for each group.
(iii) Evaluation of the effect of treatment, sex and their interaction on the instant of symptom onset and post-onset acceleration with which the response deteriorates.

3 Statistical model and inference

Profile plots for the response along with LOESS curves are displayed in Figure 1.

A descriptive analysis of the behaviour of the response variable corroborates its expected stable level before the onset of the symptom (a decrease in the length of time during which the animal holds on to the rotating cylinder). Furthermore, individual differences in the moment where this occurs as well as differences among the acceleration with which the intensity of the symptom progresses are also visible.

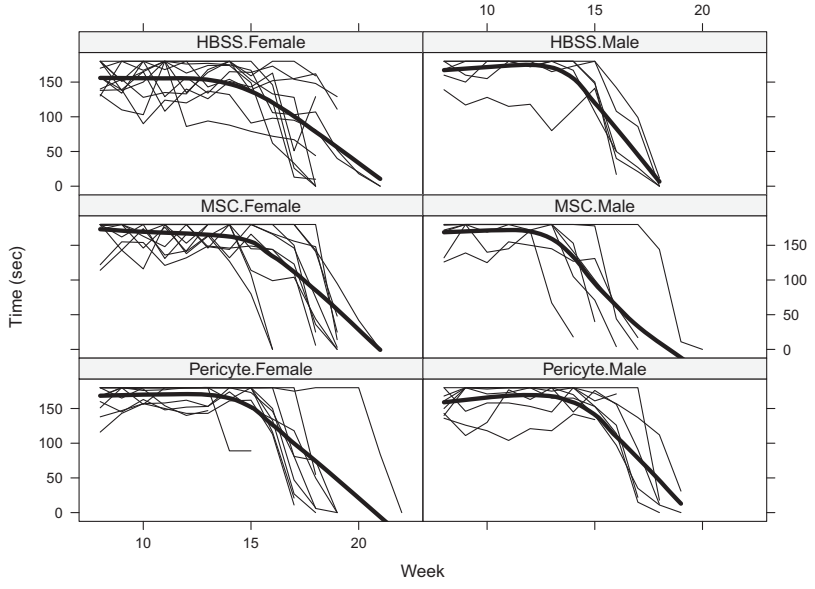

Figure 1. Profile plots for the response along with LOESS curves.

Given that such conclusions are in line with the expected biological behaviour, with a period where the response is stable, a random changepoint polynomial segmented regression model may be considered for the analysis. The changepoint corresponds to the point where the initial stable behaviour starts to change.

Such models have an attractive practical appeal in many fields and have been the object of statistical research for a long time as detailed in Muggeo et al.[4] These authors consider a frequentist approach as opposed to the commonly Bayesian perspective usually employed in the statistical literature.

We adopt a similar approach and consider an analysis of the ALS data based on the model

$$y_{ijk} = \alpha_{ij} I[t_k < \psi_{2ij}(\lambda_{ij})] + \gamma_{ij}[t_k - \psi_{1ij}(\lambda_{ij})]^2 I[\psi_{1ij}(\lambda_{ij}) \leq t_k < \psi_{2ij}(\lambda_{ij})] + e_{ijk} \quad (1)$$

($i = 1, \ldots, 6$, $j = 1, \ldots, n_i$ and $k = 1, \ldots, n_{ij}$) where y_{ijk} denotes the response for the j-th animal observed in the i-th group (defined by the combination of the levels of treatment and sex) at the k-th evaluation instant, α_{ij} is the corresponding stable level of the symptom, γ_{ij} is the coefficient of the quadratic term for the curve that governs the response behaviour post-changepoint ψ_{1ij}, with

$$\psi_{1ij}(\lambda_{ij}) = [L_1 + L_2 \exp(\lambda_{ij})]/[1 + \exp(\lambda_{ij})]$$

to restrict the value of ψ_{1ij} to the interval (L_1, L_2) in which the observations are recorded and ψ_{2ij} denotes the instant where the response is null. We assume that $\alpha_{ij} = \alpha_i + a_{ij}$, $\gamma_{ij} = \gamma_i + c_{ij}$, $\lambda_{ij} = \lambda_i + \ell_{ij}$ with $\mathbf{b}_{ij} = (a_{ij}, c_{ij}, \ell_{ij})^\top \sim N(0, \mathbf{G}_i)$ and $e_{ijk} \sim N(0, \sigma_i^2)$ independent of $\mathbf{b}_{ij}$. This is an extension of the models proposed by Muggeo et al.[4] where a smooth transition and a second changepoint are incorporated. Because of its nonlinear nature, the model must be fitted via iterative procedures.

The model takes the non-negative nature of the response as well as the possible non-constant speed with which the symptom intensity progresses post changepoint into account and therefore, it is more compatible with the biologic aspects of the problem than a simple linear–linear alternative. It is also more appropriate than some other non-linear function for which the stable level is approximated by an asymptote. Plots of observed profiles of two mice for which a linear–linear response curve does not seem reasonable are displayed in the left panel of Figure 2. A graphical representation of the components of the proposed model is depicted in the right panel.

For the sake of notational simplicity and without loss of generality, we drop the subscript i to specify the fitting algorithm.

Given that ψ_{2j} corresponds to the instant t_k where $\mathrm{E}(y_{jk}) = 0$, we have $I(t_k < \psi_{2j}) = 1$ and $I(\psi_{1j} \leq t_k < \psi_{2j}) = 1$ so that from equation (1), we obtain $\psi_{2j} = \mathrm{E}(y_{jk}) = \alpha_j + \gamma_j[\psi_{2j} - \psi_{1j}(\lambda_{ij})]^2 = 0$ and solving for ψ_{2j}, we get

$$\psi_{2j} = \psi_{2j}(\alpha_j, \gamma_j, \lambda_{1j}) = \sqrt{-\alpha_j/\gamma_j} + \psi_{1j}(\lambda_j)$$

implying that we actually need only three random effects in the model.

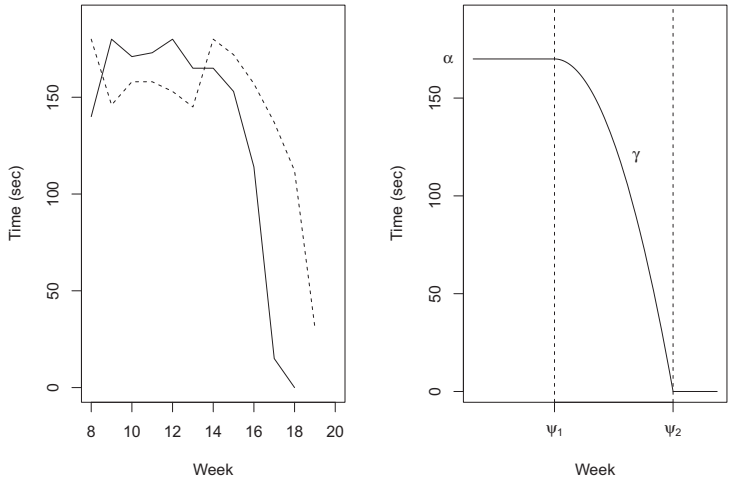

Figure 2. Profile plots for two selected animals and graphic representation of model (1). α: expected pre-changepoint response; ψ_1: instant of symptom onset; ψ_2: instant where animal is paralysed; γ: "acceleration" with which symptom progresses.

Following Muggeo et al.[4] and Fasola et al.,[5] the nonlinear model may be approximated by a first-order Taylor expansion of

$$f[t_k, \gamma_j, \psi_{1j}(\lambda_j)] = \gamma_j[t_k - \psi_{1j}(\lambda_j)]^2 I[\psi_{1ij}(\lambda_j) \leq t_k < \psi_{2j}(\lambda_j)]$$

Explicitly

$$f[t_k, \gamma_j, \psi_{1j}(\lambda_j)] \approx f[t_k, \gamma_j, \psi_{1j}(\hat{\lambda}_j)] + (\lambda_j - \hat{\lambda}_j)\frac{\partial f[t_k, \gamma_j, \psi_{1j}]}{\partial \psi_{1j}}\frac{\partial \psi_{1j}(\lambda_j)}{\lambda_j}\Big|_{\lambda_j = \hat{\lambda}_j}$$

with

$$\frac{\partial f[t_k, \gamma_j, \psi_{1j}]}{\partial \psi_{1j}} = h_j(t_k, \lambda_j) = 2\gamma_j[t_k - \psi_{1j}(\lambda_j)]I[\psi_{1j}(\lambda_j) \leq t_k < \psi_{2j}(\lambda_j)]$$

and

$$\frac{\partial \psi_{1j}(\lambda_j)}{\partial \lambda_j} = g_j(\lambda_j) = \frac{(L_2 - L_1)\exp(\lambda_j)}{[1 + \exp(\lambda_j)]^2}$$

Consequently, we may approximate model (1) by

$$\begin{aligned} y_{jk} \approx & \alpha_j I[t_k < \psi_{2j}(\hat{\lambda}_j)] + f[t_k, \gamma_j, \psi_{1j}(\hat{\lambda}_j)] \\ & - \hat{\lambda}_j h_j(t_k, \hat{\lambda}_j) g_j(\hat{\lambda}_j) + \lambda_j h_j(t_k, \hat{\lambda}_j) g_j(\hat{\lambda}_j) + e_{jk} \end{aligned} \tag{2}$$

Considering the pseudo observations defined by $y^*_{jk} = y_{jk} + \hat{\lambda}_j h_j(t_k, \hat{\lambda}_j) g_j(\hat{\lambda}_j)$, the model

$$y^*_{jk} = \alpha_j I[t_k < \psi_{2j}(\hat{\lambda}_j)] + f[t_k, \gamma_j, \psi_{1j}(\hat{\lambda}_j)] + \lambda_j h_j(t_k, \hat{\lambda}_j) g_j(\hat{\lambda}_j) + e_{jk} \tag{3}$$

suggests the following algorithm to fit equation (1)

1. Let $\psi^{(0)}_{1j} = \psi^{(0)}_1$ and $\psi^{(0)}_{2j} = \psi^{(0)}_2$.
2. Fit model $y_{jk} = \alpha_j I(t_k < \psi^0_{2j}) + \gamma_j(t_k - \psi^{(0)}_{2j})^2 I(\psi^{(0)}_{1j} \leq t_k < \psi^{(0)}_{2j}) + e_{jk}$ to obtain $\alpha^{(0)}$, $a^{(0)}_j$, $\gamma^{(0)}$, $c^{(0)}_j$, $\lambda^{(0)}_j = \log[(\psi^{(0)}_{1j} - L_1)/(L_2 - \psi^{(0)}_{1j})]$ and $\psi^{(1)}_{2j} = \sqrt{-\alpha^{(0)}_j/\gamma^{(0)}_j} + \psi^{(0)}_{1j}$.
3. Let $r = 1$.
4. Compute $y^{(r)}_{jk} = y_{jk} + \lambda^{(r-1)}_j h_j(t_k, \lambda^{(r-1)}_j) g_j(\lambda^{(r-1)}_j)$.
5. Fit model

$$\begin{aligned} y^{(r)}_{jk} = & \alpha_j I(t_k < \psi^{(r)}_{2j}) + \gamma_j[t_k - \psi^{(r-1)}_{1j}]^2 I(\psi^{(r-1)}_{1j} \leq t_k < \psi^{(r)}_{2j}) \\ & + \lambda_j h_j(t_k, \lambda^{(r-1)}_j) g_j(\lambda^{(r-1)}_j) + e^{(r-1)}_{jk} \end{aligned}$$

to obtain $\alpha^{(r)}$, $a^{(r)}_j$, $\gamma^{(r)}$, $c^{(r)}_j$, $\lambda^{(r)}$, $\ell^{(r)}_j$, $\psi^{(r)}_{1j} = [L_1 + L_2\exp(\lambda^{(r)}_j)]/[1 + \exp(\lambda^{(r)}_j)]$ and $\psi^{(r+1)}_{2j} = \sqrt{-\alpha^{(r)}_j/\gamma^{(r)}_j} + \psi^{(r)}_{1j}$.

6. Stop if some convergence criterion is satisfied, otherwise, let $r = r + 1$ and repeat steps 4–6.

This algorithm is an extension of the one proposed by Muggeo et al.[4] and essentially considers iterative fitting of standard linear mixed models by (restricted) maximum likelihood. At convergence, we expect a negligible difference between the third and fourth terms in the right-hand side of equation (2) and as a consequence, that the pseudo observations should well approximate the original ones.

Although the algorithm may be applied to data with small sample sizes as in our example, in practice we noted that the quality of the fitted values depends on the initial values $\psi^{(0)}_1$ and $\psi^{(0)}_2$; in some cases, it does not converge

and in other cases, it may lead to local maxima. To bypass this problem, we applied the algorithm by choosing a set of 25 different initial values for $\psi_1^{(0)}$, ranging from the minimum to the maximum observed time points with $\psi_2^{(0)}$ set at the maximum observed time point.

In each case, we computed the mean squared difference between the individual observed and predicted values, and considered the values of $\psi_1^{(0)}$ and $\psi_2^{(0)}$ associated to the smallest mean squared difference as the initial value for the final run. Convergence was based on the difference between log-likelihoods; the convergence criterion was $\varepsilon < 10^{=5}$ for all groups, except for the HBSS group, for which $\varepsilon < 10^{=3}$. Given the unexpected behaviour of the response for some animals subjected to this treatment, the algorithm did not converge with stricter criteria. The results, however, are consistent with the observed data. It is worthwhile to mention that similar convergence problems were reported by Muggeo et al.[4] and Jacqmin-Gadda et al.[6] even though their examples involve much larger datasets.

The algorithm was implemented in R and the code as well as the data is available in the Supplementary Material.

In the last step, the algorithm produces approximate covariance matrices, $\mathrm{V}(\hat{\boldsymbol{\theta}})$, of the fixed parameter estimators $\hat{\boldsymbol{\theta}} = (\hat{\alpha}, \hat{\gamma}, \hat{\lambda})^\top$ which may be employed for inferential purposes. Since the interest lies in the changepoint parameters ψ_1 and ψ_2 instead of in the auxiliary parameters λ, we should consider inferences on

$$\mathbf{h}(\hat{\boldsymbol{\theta}}) = [\hat{\alpha}, \hat{\gamma}, \psi_1(\hat{\lambda}_i), \psi_2(\hat{\alpha}, \hat{\gamma}, \hat{\lambda})]^\top$$

Approximate covariance matrices of the transformed estimators $\mathbf{h}(\hat{\boldsymbol{\theta}})$ may be obtained via the Delta method (see Sen et al.,[7] for example) as

$$\mathrm{V}[\mathbf{h}(\hat{\boldsymbol{\theta}})] = \mathbf{H}(\hat{\boldsymbol{\theta}})\mathrm{V}(\hat{\boldsymbol{\theta}})\mathbf{H}(\hat{\boldsymbol{\theta}})^\top$$

with

$$\mathbf{H}(\hat{\boldsymbol{\theta}}) = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & (L_2 - L_1)\exp(\hat{\lambda})/[1+\exp(\hat{\lambda})]^2 \\ (2\hat{\alpha})^{-1}(-\hat{\alpha}/\hat{\gamma})^{1/2} & (2\hat{\gamma})^{-1}(-\hat{\alpha}/\hat{\gamma})^{1/2} & (L_2 - L_1)\exp(\hat{\lambda})/[1+\exp(\hat{\lambda})]^2 \end{bmatrix}$$

In particular, comparison among the fixed parameters to identify possible effects of treatment, sex and their interactions may be carried out via Wald tests.

Given the linear mixed model nature of the proposed fitting algorithm, an additional feature is that we may employ the diagnostic procedures outlined in Singer et al.[8] to check whether the adopted assumptions for the covariance structure and for the distribution of the random effects or of the random errors are reasonable.

4 Results

Since there are no parameters common to all six groups, we fitted the (saturated) model (1) via the approximation (2) to each group separately. Other covariates may be included in model (1) along the lines outlined in Muggeo et al.,[4] but this requires larger sample sizes to guarantee convergence of the proposed algorithm. Estimates of the parameters of model along with the corresponding standard errors are summarised in Table 1. Standard errors for the changepoint parameter estimates ($\hat{\psi}_{ij}$) were obtained via the Delta method from the standard errors of the estimates of the auxiliary parameters $\hat{\lambda}_{ij}$. Diagnostic plots for the male group treated with MSC are displayed in Figure 3 and do not show evidences against the adopted assumptions. In particular, the modified Lesaffre-Verbeke index plot suggests that the adopted covariance structure is acceptable for the mice in this group, with, perhaps, the exception of the fourth animal; given the exploratory nature of such plots along with the small sample size, it does not seem reasonable to modify the adopted covariance structure. The Mahalanobis distance *versus* sampling unit plot, on the other hand, does not suggest the presence of outliers. Furthermore, the Mahalanobis distance QQ plot and standardised least confounded residual plot along with the corresponding histogram do not show evidence against the Gaussian assumption adopted for either the random effects or for the

Table 1. Estimates and standard errors for the parameters of model (1) obtained via fitting the approximation (2) along with bootstrap and robust counterparts of the standard errors.

				Std error		
Parameter	Sex	Treatment	Estimate	Model	Bootstrap	Robust
	M	HBSS	166.4	10.0	8.0	9.1
Stable level (α)	M	MSC	170.2	6.2	5.4	5.8
	M	Pericytes	164.5	6.5	5.1	6.1
	F	HBSS	154.6	7.5	8.0	7.1
Stable level (α)	F	MSC	167.4	3.8	4.2	3.6
	F	Pericytes	169.8	3.7	4.4	3.5
	M	HBSS	−18.3	5.0	4.8	4.5
Acceleration (γ)	M	MSC	−36.7	11.4	13.9	10.4
	M	Pericytes	−23.1	12.6	13.9	11.7
	F	HBSS	−2.2	1.0	0.8	0.9
Acceleration (γ)	F	MSC	−26.6	6.3	8.9	6.1
	F	Pericytes	−37.9	7.2	12.3	6.1
	M	HBSS	13.9	0.3	0.3	0.2
Changepoint 1 (ψ_1)	M	MSC	13.3	1.0	1.1	0.9
	M	Pericytes	14.9	0.4	0.6	0.4
	F	HBSS	11.7	0.3	1.1	0.3
Changepoint 1 (ψ_1)	F	MSC	15.5	0.5	0.8	0.5
	F	Pericytes	15.6	0.7	1.0	0.6
	M	HBSS	16.9	0.3	0.5	0.3
Changepoint 2 (ψ_2)	M	MSC	15.5	0.7	1.0	0.7
	M	Pericytes	17.6	0.5	0.9	0.4
	F	HBSS	20.1	1.6	0.8	1.5
Changepoint 2 (ψ_2)	F	MSC	18.0	0.4	1.2	0.4
	F	Pericytes	17.7	0.6	1.0	0.6

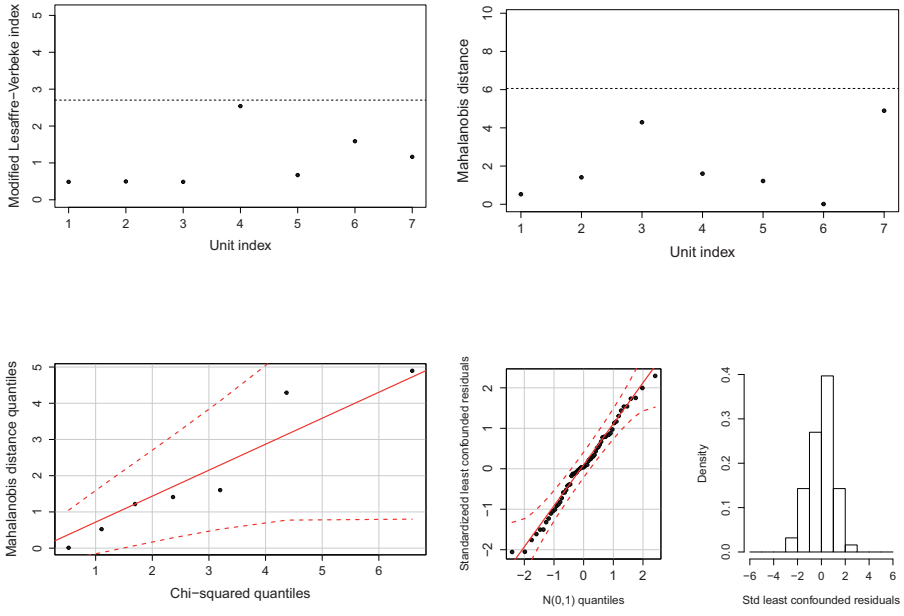

Figure 3. Diagnostic plots for males treated with MSC.

error component. Similar plots for the remaining groups show a similar picture. These plots may be obtained by applying the R functions provided by Singer et al.[8] as indicated in the Supplementary Material.

The estimated covariance matrices of the fixed parameter estimates available in the last step were employed for inferential purposes. This approach, however, is not exempt from controversy. In fact, Muggeo et al.[4] comment that the corresponding standard errors of the changepoint estimates underestimate the true standard errors and, based on a simulation study, suggest that bootstrap estimates should be employed instead. They consider a bootstrap procedure, but assume that the random effects are independent, which does not reflect the most common situation where the within subject covariance matrix is unstructured. It seems reasonable to expect a sharper progression of the symptom for animals with a delayed onset, suggesting that at least the random effects corresponding to the changepoint and acceleration parameters should be correlated. The estimated covariance components of the model, presented in Table 2, tend to confirm this hypothesis.

For each of the six groups, we generated 200 samples with the same number of response profiles, used model estimated fixed and dispersion parameters, namely, $\hat{\alpha}_i$, $\hat{\gamma}_i$, $\hat{\lambda}_i$, $\hat{\mathbf{G}}_i$ and $\hat{\sigma}_i^2$ and fitted model (1) via the proposed algorithm to obtain (parametric) bootstrap estimates of the standard errors of the associated fixed parameters. We also considered a robust version based on the suggestion of Liang and Zeger[9] for which the details are presented in Appendix 1. The standard errors of the fixed parameters obtained via the three approaches are displayed in Table 1. Both the estimates obtained via the proposed algorithm and via the robust version are quite similar with consistently smaller values for the latter. The bootstrapped version, however, does not suggest a consistent pattern. We conjecture that this is a consequence of assuming a trivariate normal distribution for the random effects a_j, c_j, ℓ_j. While this seems appropriate for the individual stable level parameters and changepoints, it may not be so for the individual acceleration component, $\gamma + c_j$ which according to the proposed model may be positive, a feature that is not biologically expected.

The results of a Wald test for the homogeneity of the six changepoints ψ_1 ($\chi^2 = 20.31, df = 5, p < 0.001$) suggest further analyses to identify the possible effects of treatment, sex and their interaction. A significant interaction between treatment and sex with respect to the ψ_1 changepoints ($\chi^2 = 20.31, df = 2, p < 0.001$) may be analysed via the multiple comparisons summarised in Table 3 and suggest that the onset of symptoms for the "typical" male in the control group (HBSS) is delayed by 2.2 [CI (95%) = 1.4, 3.0] weeks with respect to the "typical" female and that treatment with Pericytes (both sexes) or MSC (females) delays the onset of symptoms for the "typical" animal by 1.5 [CI (95%) = 0.7, 2.3] weeks with respect to the HBSS-treated "typical" male. The

Table 2. Estimates of the variance and correlation components ($\mathbf{G}$ and σ^2) obtained via model (1).

		Covariance parameter						
Treatment	Sex	σ_α^2	σ_γ^2	σ_λ^2	$\rho_{\alpha\gamma}$	$\rho_{\alpha\lambda}$	$\rho_{\gamma\lambda}$	σ^2
HBSS	M	572.20	95.72	0.02	−0.34	−0.27	0.03	186.61
MSC	M	247.41	863.75	0.79	0.46	−0.11	−0.93	154.62
Pericytes	M	318.38	1196.81	0.18	−0.78	0.16	−0.75	174.04
HBSS	F	282.41	137.14	0.17	−0.16	0.79	−0.14	256.64
MSC	F	149.34	331.41	0.29	−0.31	0.25	−0.69	199.78
Pericytes	F	116.70	60.61	0.35	−0.85	0.15	−0.65	238.00

Table 3. Results of Wald tests for *ad hoc* comparisons of changepoints (ψ_1).

Comparison	χ^2	*df*	*p*-value
Sex within HBSS	28.08	1	<0.001
Sex within MSC	3.56	1	0.059
Sex within Pericytes	0.87	1	0.351
Pericytes = MSC(F)	1.23	2	0.539
Pericytes + MSC(F) = HBSS(M)	13.05	1	<0.001
MSC(M) = HBSS(M)	0.25	1	0.612
MSC(M) = HBSS(F)	2.41	1	0.121

changepoint for the MSC-treated "typical" male lies between those for HBSS-treated "typical" male and female but the small sample size does not lead to a significant difference in either case.

The results for a similar analysis of the acceleration with which the symptom progresses are displayed in Table 4 and suggest no difference between sexes as well as an increase in the acceleration of 20.9 [CI (95%) = 20.1, 21.6] sec/week^2 for the experimental treatments (MSC and Pericytes) relatively to that of the control treatment (HBSS).

Plots for the estimates of male and female "typical" animal response curves for the three treatments are displayed in Figure 4. The delay of symptom onset for males subject to HBSS with respect to the corresponding females as well as the extra delay of symptom onset for animals subject to Pericytes or female animals submitted to MSC is evident. The plots also suggest the similarity among the pattern of symptom progression for animals in all groups with the exception of females submitted to HBSS, which show a slower deterioration.

5 Discussion

We considered an extension of the algorithm proposed by Muggeo et al.[4] to fit a segmented regression model with smooth transition to data obtained from a study designed to evaluate the effect treatment with stem cells on the delay of the onset of an ALS symptom. The proposed model is appropriate for situations where the expected pre-changepoint response is constant. Furthermore, it allows for a varying speed of symptom progress by including a second-degree polynomial component post-changepoint, a feature suggested by those authors.

Table 4. Results of Wald tests for *ad hoc* comparisons of post-changepoint symptom acceleration parameter (γ).

Comparison	χ^2	*df*	*p*-value
Homogeneity	57.25	5	<0.001
Sex × Treatment	4.09	2	0.129
Sex	0.32	1	0.571
Treatment	14.64	2	<0.001
HBSS × MSC	9.40	1	0.002
MSC × Pericytes	0.01	1	0.908

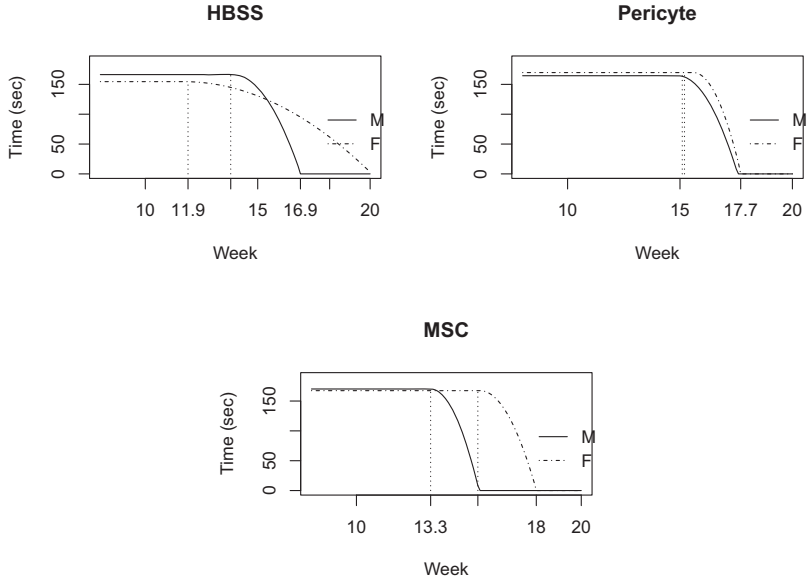

Figure 4. Estimated response curves for "typical subjects". Vertical dotted lines indicate instant of symptom onset (ψ_1).

Jacqmin-Gadda et al.[6] employed a similar model with the random changepoint governed by a log-normal distribution, independent of the distribution of the remaining random effects. They mention that consideration of dependency leads to unstable results due to numerical problems. This is also an issue raised by Segalas et al.[10] In our case, the relation between the random changepoint and the subsequent variation in the response seems reasonable since a delay in the symptom onset possibly accelerates its deterioration. We included such a dependency by considering an unstructured within-subject covariance matrix for all random terms and did not have problems in the estimation process, given the nature of the proposed algorithm that relies on iterative fitting of standard linear mixed models. This feature also allows the use of the algorithm with moderately sample sized data. Individual predicted responses are obtained with little additional effort.

Although the proposed model fits the data reasonably well, it may be further improved by considering the following modifications

(i) Either impose the restriction that $\gamma + c_j$ be negative in the fitting algorithm or adopt a different distribution allowing only negative values.
(ii) Consider a truncated (at 180) distribution for $\alpha + a_j$.
(iii) Consider a joint longitudinal and survival model to account for deaths occurring prior to animal paralysis.

Such features, however, preclude the use of standard linear mixed model methodology and require further research, possibly with the use of copulas to take the correlation between random components into account. At present we are trying to develop a Bayesian approach for such purposes.

The choice of initial values for the iterative procedures required to fit non-linear models is usually problematic. Models that include transition functions like those proposed by Bacon and Watts[11] and considered in Morrell et al.[12] are non-linear despite the linearity of each component and therefore require additional attention, given the associated numerical problems. Initialisation of the algorithm described in Section 3 is simple since it requires initial values only for the parameters ψ_1 and ψ_2. Although we chose these initial values by fitting the model to a

Table 5. Estimates for the parameters of model (1) obtained via fitting the approximation (2) along with corresponding estimates of averaged individually fitted and marginal parameters.

			Model		
Parameter	Sex	Treatment	Mixed (1)	Averaged	Marginal
	M	HBSS	166.4	166.5	165.0
Stable level (α)	M	MSC	170.2	169.8	173.2
	M	Pericytes	164.5	164.2	162.9
	F	HBSS	154.6	154.9	155.0
Stable level (α)	F	MSC	167.4	168.4	165.5
	F	Pericytes	169.8	171.5	170.3
	M	HBSS	−18.3	−33.0	−7.5
Acceleration (γ)	M	MSC	−36.7	−57.9	−2.2
	M	Pericytes	−23.1	−44.9	−9.9
	F	HBSS	−2.2	−14.7	−2.2
Acceleration (γ)	F	MSC	−26.6	−26.5	−6.1
	F	Pericytes	−37.9	−30.1	−2.7
	M	HBSS	13.9	14.1	12.9
Changepoint (ψ_1)	M	MSC	13.3	13.6	9.9
	M	Pericytes	14.9	14.2	14.2
	F	HBSS	11.7	13.4	12.3
Changepoint 1 (ψ_1)	F	MSC	15.5	14.8	14.3
	F	Pericytes	15.6	14.5	12.1
	M	HBSS	16.9	16.3	17.6
Changepoint 2 (ψ_2)	M	MSC	15.5	15.3	18.8
	M	Pericytes	17.6	16.1	18.3
	F	HBSS	20.1	16.6	20.6
Changepoint 2 (ψ_2)	F	MSC	18.0	17.4	19.5
	F	Pericytes	17.7	16.9	20.1

grid of values for ψ_1 and ψ_2 as described in Section 3, a non-parametric bootstrap procedure as the one proposed by Wood[13] and used in Muggeo et al.[4] could also be employed.

Finally, we mention that model (1) inherits the interpretational difficulty associated to nonlinear mixed models: the fixed parameters correspond to the response for a "typical" subject, i.e. one for which the random effects are null. Although the expected response may be obtained by integrating out the random effects in the likelihood, this does not lead to estimates of the population averaged parameters (the changepoint and the acceleration coefficient in our case). In an attempt to estimate such "population averaged" parameters, we considered two alternatives: (i) we fitted a standard mixed segmented regression model with an unknown changepoint to the data of each animal via the algorithm proposed by Muggeo[14] and averaged the corresponding parameter estimates; the results, presented in the penultimate column of Table 5, are similar to those obtained via the approximated version of

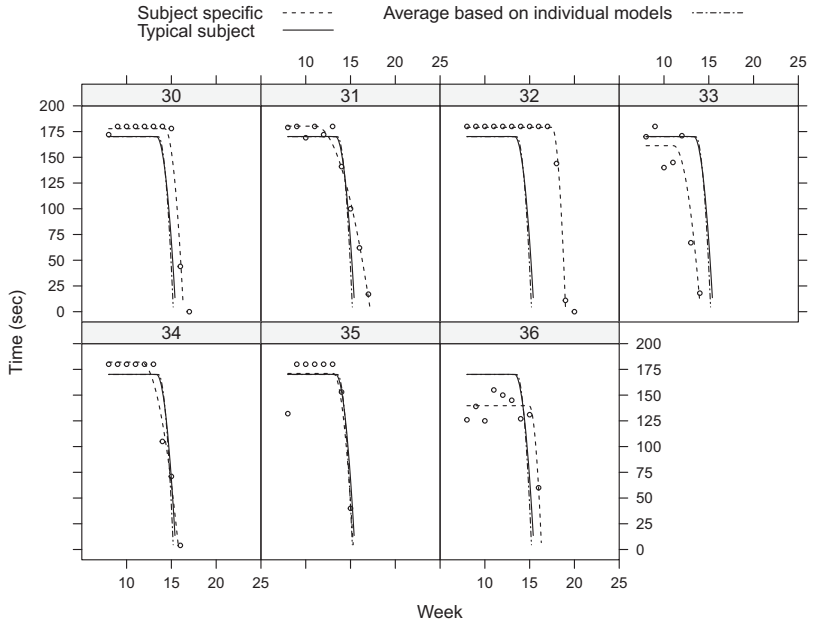

Figure 5. Predicted and estimated response curves (MSC males). Circles correspond to the actual observations.

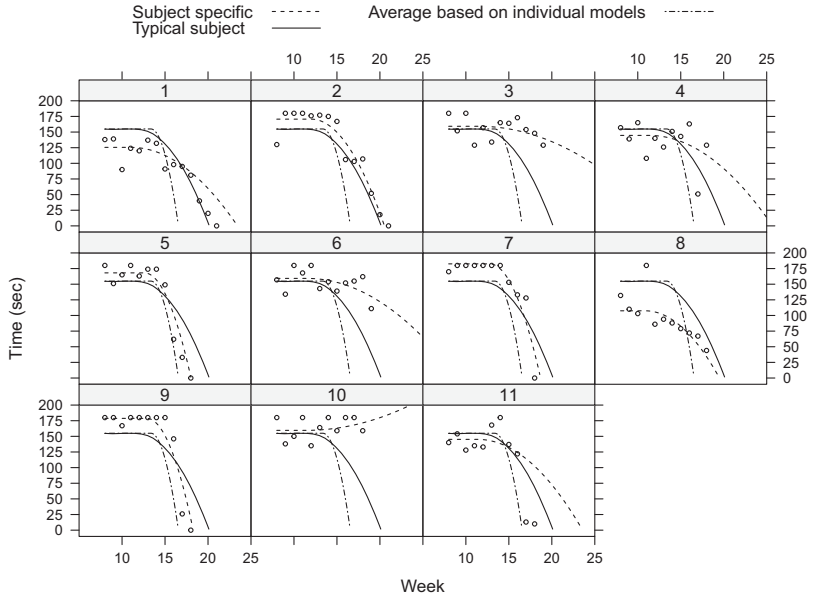

Figure 6. Predicted and estimated response curves (HBSS females). Circles correspond to the actual observations.

model (1) with the exception of the acceleration parameter γ, probably because of the effect of outliers. (ii) We also fitted a marginal segmented regression model with an unknown changepoint using the estimated covariance matrices obtained from the proposed model via generalised least squares to the data; the results, displayed in the last column of Table 5, present the same attenuation characteristic described by other authors under different nonlinear setups (see Diggle et al.,[15] for example). This is particularly evident from the estimates of the acceleration parameter γ, the values of which are considerably smaller than those obtained via model (1), leading to a delay with which the response becomes null. This attenuation is also visible in the LOESS curves presented in Figure 1, where the last response of a single mouse may distort the general behaviour.

Plots of the predicted (via the segmented mixed model) and estimated profiles (via mixed and averaged segmented regression models) for animals in the MSC male and HBSS female groups, respectively, are displayed in Figures 5 and 6. Similar plots for the remaining groups are presented in the Supplementary Materials. The similarity between the curves for the "typical units" and for the approximate "population averaged" for male animals in the MSC group is probably due to the behaviour of the observed data which follows the pattern dictated by the model for all units. This is not so for the HBSS female group, where the observed data for units 3, 6, 8 and 10 do not follow the expected pattern, implying that, perhaps, the proposed segmented mixed model should be modified by requiring the acceleration parameter to be negative. Nevertheless, we refitted model (1) to the data for this group, obtaining the estimates (standard errors within parentheses) $\hat{\alpha} = 159.5\,(8.0)$, $\hat{\gamma} = -3.4\,(1.0)$, $\hat{\psi}_1 = 12.1\,(0.4)$, $\hat{\psi}_2 = 19.2\,(1.4)$ and $\hat{\sigma}_2 = 198.42$, which are compatible with those obtained with complete data displayed in Table 1.

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Orcid ID

Julio M. Singer https://orcid.org/0000-0001-6515-9643

Supplemental material

Supplementary material contains additional figures and is available in a zip file.

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Appendix 1

As before, for the sake of notation simplicity, we omit the index i associated to the n_i animals in group i and consider model (3) defined in terms of the pseudo observations y^*_{jk}. Defining $\hat{\boldsymbol{\beta}} = (\hat{\alpha}, \hat{\gamma}, \hat{\lambda})^\top$ and letting $\mathbf{y}^*_j = (y^*_{j1}, \ldots, y^*_{jm_j})^\top$ denote the vector of the m_j pseudo observations at the last iteration of the algorithm associated to the j-th animal, the robust variance of $\hat{\boldsymbol{\beta}}$ is

$$\mathrm{V}(\hat{\boldsymbol{\beta}}) = \hat{\mathbf{A}} \left[\sum_{j=1}^{n_i} \mathbf{X}_j^\top \hat{\mathbf{V}}_j^{-1} \mathrm{V}(\mathbf{y}^*_j) \hat{\mathbf{V}}_j^{-1} \mathbf{X}_j \right] \hat{\mathbf{A}}$$

where $\hat{\mathbf{V}}_j = \mathbf{Z}_j \hat{\mathbf{G}}_i \mathbf{Z}_j^\top + \mathbf{I}_{m_j} \hat{\sigma}_i$, $\hat{\mathbf{A}} = \left[\sum_{j=1}^{n_i} \mathbf{X}_j^\top \hat{\mathbf{V}}_j \mathbf{X}_j \right]^{-1}$, $\mathrm{V}(\mathbf{y}^*_j) = \sum_{j=1}^{n_i} (\mathbf{y}^*_j - \hat{\boldsymbol{\mu}}^*)(\mathbf{y}^*_j - \hat{\boldsymbol{\mu}}^*)^\top$, $\hat{\boldsymbol{\mu}}^* = \sum_{j=1}^{n_i} \mathbf{X}_j \left[\mathbf{X}_j^\top \hat{\mathbf{V}}_j^{-1} \mathbf{X}_j \right]^{-1} \mathbf{X}_j^\top \hat{\mathbf{V}}_j^{-1} \mathbf{y}^*_j$

$$\mathbf{X}_j = \mathbf{Z}_j = \begin{bmatrix} 1 & 0 & \hat{h}_j(t_1, \hat{\lambda}^*_j) \hat{g}(\hat{\lambda}^*_j) \\ 1 & 0 & \hat{h}_j(t_2, \hat{\lambda}^*_j) \hat{g}(\hat{\lambda}^*_j) \\ \vdots & \vdots & \vdots \\ 1 & t_r & \hat{h}_j(t_r, \hat{\lambda}^*_j) \hat{g}(\hat{\lambda}^*_j) \\ 1 & (t_{r+1} - \psi^*_1) & \hat{h}_j(t_{r+1}, \hat{\lambda}^*_j) \hat{g}(\hat{\lambda}^*_j) \\ \vdots & \vdots & \vdots \\ 1 & (t_{m_j} - \psi^*_1) & \hat{h}_j(t_{m_j}, \hat{\lambda}^*_j) \hat{g}(\hat{\lambda}^*_j) \end{bmatrix}$$

λ^*_j denotes the value of λ_j at the last iteration, $\psi^*_1 = \psi_1(\lambda^*_j)$ and r denotes the order of the observation time where $t_r = \psi^*_1$.