Supplemental Material

Data S1.

Statistical methods for repeated measurements

We model the outcomes at the $k = 1, \ldots, 7$ times during stimulation for subjects $i = 1, \ldots, 25$ in periods $j = 1, 2$ according to a linear mixed model described by the following formula:

$$Y_{ijk} = \alpha_k + \beta_k \cdot X_{ij} + \gamma_k \cdot Z_j + \delta_k \cdot X_{ij} \cdot Z_j + \varepsilon_{ijk}$$

The covariates are defined as:

$$X_{ij} = \begin{cases} 0 & \text{if subject } i \text{ received placebo in period } j \\ 1 & \text{if subject } i \text{ received treatment in period } j \end{cases}$$

and

$$Z_j = \begin{cases} 0 & \text{if } j = 1 \\ 1 & \text{if } j = 2 \end{cases}$$

so that at the kth time point during stimulation, β_k describes the treatment effect, γ_k the period effect, and δ_k a potential carryover effect (increased or diminished effect of treatment in the second period). The intercept α_k is the expected outcome for an average person with placebo treatment in the first period. As the outcome a priori varies with the stimulation all of the model parameters are potentially time dependent.

Model assumptions

The error terms $\varepsilon_{i11}, \ldots, \varepsilon_{i17}, \varepsilon_{i21}, \ldots, \varepsilon_{i27}$ are assumed to be *multivariate normally distributed*. The multiple error terms belonging to the same subject are a priori correlated and further correlation and error variance may depend on the specific times of observation. For this reason we model the error varaiance by an *unstructured covariance pattern*, that is we model the correlation and variance in the data *as is* with no further restrictions.

Test of carryover effect

For unambiguous reporting we would like to assume that it is the treatment effect is the same in both periods, that is that no carryover effects are present. Ideally this should be ruled out by ensuring an adequately long washout between the treatment periods. However, carryover effects may still occur due to e.g. differential compliance between the treatment periods.

To test for carryover effect, we test the hypothesis

$$H_0 : \ \delta_1 = \ldots = \delta_7 = 0 \tag{1}$$

In the linear mixed model, this can be tested by evaluating the second order interaction between time, treatment and period . However, in practice numerical problems may occur if the dimension of the unstructured covariance matrix is large relative to the number of subjects.

Test of treatment effect

Assuming that no carryover effects are present, the (global) treatment effect can be evaluated. We test the hypothesis

$$H_0: \ \beta_1 = \ldots = \beta_7 = 0 \tag{2}$$

In the linear mixed model, this can be tested by evaluating the first order interaction between time and treatment, but again convergence issues may occur.

Reduction of dimensionality

To reduce dimensionality we apply a computational trick.

First we add the corresponding outcomes from the two periods for each subject, that is, we consider the sums

$$S_{ik} = Y_{i1k} + Y_{i2k} = \begin{cases} 2\alpha_k + \beta_k + \gamma_k + \eta_{ik} & \text{if subject } i \text{ received treatment in period 1} \\ 2\alpha_k + \beta_k + \gamma_k + \delta_k + \eta_{ik} & \text{if subject } i \text{ received treatment in period 2} \end{cases}$$

where $\eta_{ik} = \varepsilon_{i1k} + \varepsilon_{i2k}$. Hence the hypothesis (1) can be tested by testing the interaction between allocation group (treatment first vs placebo first) and time in a twoway ANOVA-like linear mixed model with no main effect of allocation group.

Next we subtract the corresponding outcomes from the two periods for each subject. Assuming no carryover effects are present, we consider the differences:

$$D_{ik} = Y_{i2k} - Y_{i1k} = \begin{cases} \gamma_k - \beta_k + \zeta_{ik} & \text{if subject } i \text{ received treatment in period 1} \\ \gamma_k + \beta_k + \zeta_{ik} & \text{if subject } i \text{ received treatment in period 2} \end{cases}$$

where $\zeta_{ik} = \varepsilon_{i2k} - \varepsilon_{i1k}$. Hence we can test the treatment effect (2) by testing the interaction between allocation group and time in yet another twoway ANOVA-like linear mixed model with no main effect of allocation group.