Modeling in-vitro functional assay of allosteric modulators

Biometrics and Reporting

T. Jacobs, H. Lavreysen, I. Biesmans

janssen
PHARMACEUTICAL COMPANIES
OF Johnson&Johnson

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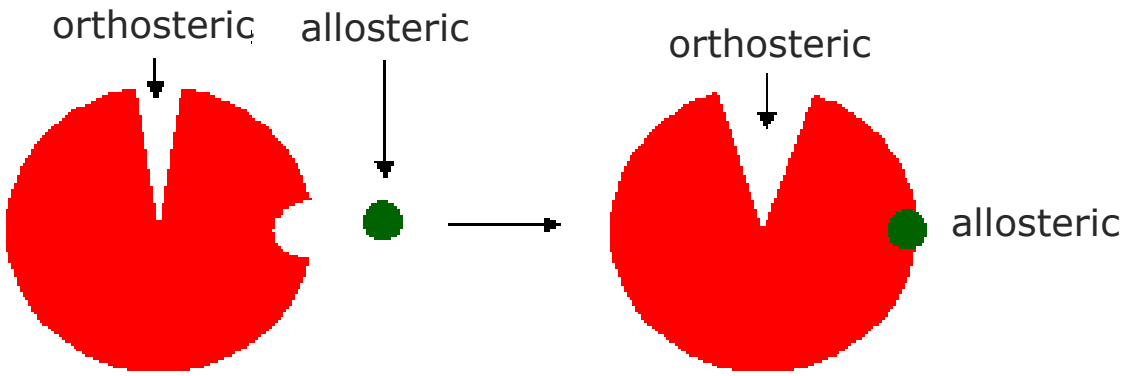
What's an allosteric modulator?

- does not bind to orthosteric site as full or partial agonists do
- has no or minimal agonist activity of their own
- increases the effects of natural neurotransmitters
- preserves the temporal and spatial integrity of the neurotransmission
- will only work in the presence of the (natural) agonist
- potentiates effect of a specific neurotransmitter (EC_{50} ↓, E_{max} ↑)

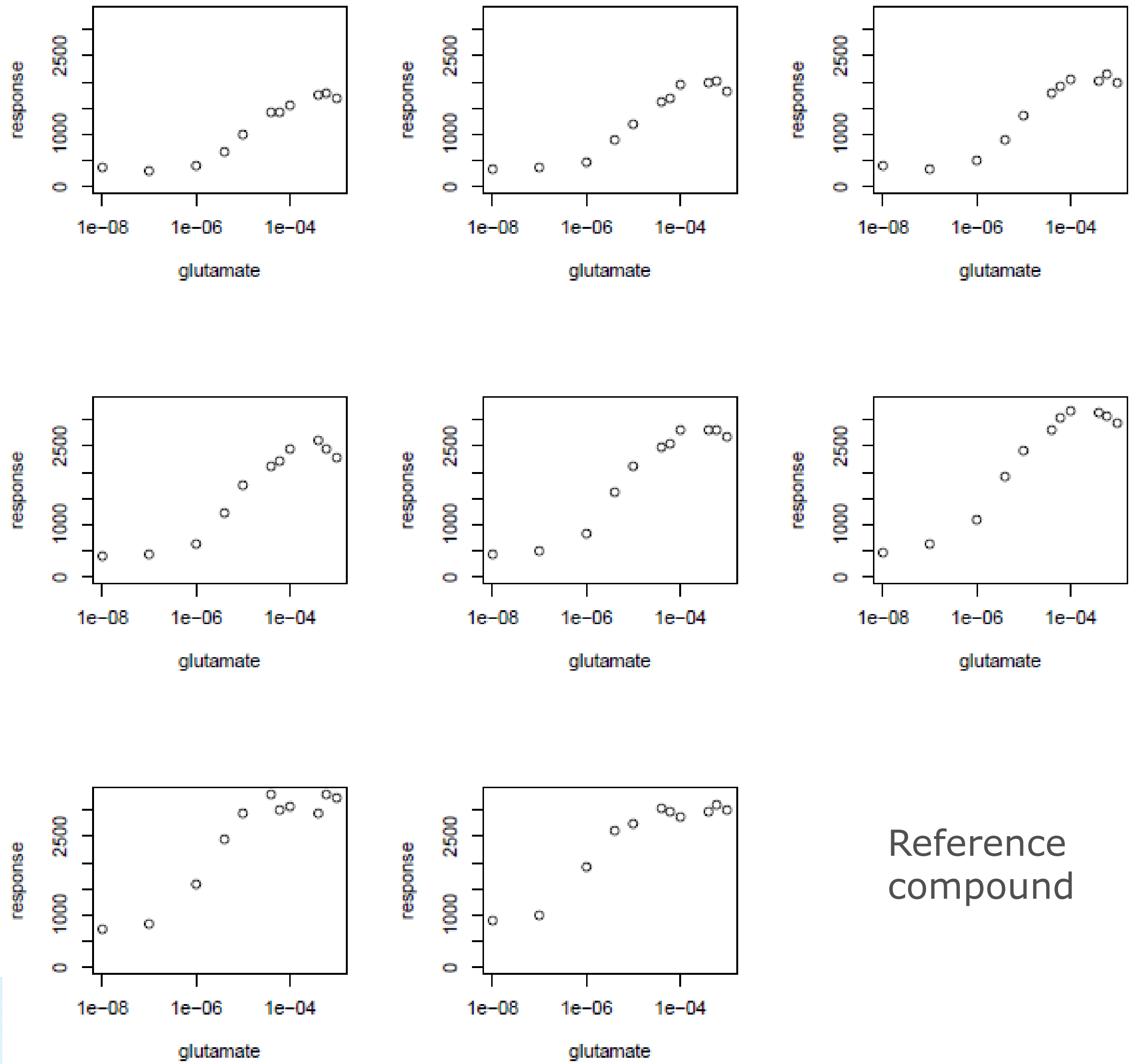
- Naively speaking: modifies the effects of a specific neurotransmitter by binding to a site in proximity of the neurotransmitter

What's an allosteric modulator?



Case Study: functional assay

Study the in-vitro co-administration of an orthosteric neuro-transmitter and an allosteric modulator.



Reference compound

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Methodology

Based on receptor theory, one can prove that the data ought to follow following equation:

$$y = E_0 + \frac{E_m \left(\tau_A [A] \left(K_B + \alpha\beta [B]\right) + \tau_B [B] K_A\right)^\eta}{\left([A] K_B + K_A K_B + [B] K_A + \alpha [A] [B]\right)^\eta + \left(\tau_A [A] \left(K_B + \alpha\beta [B]\right) + \tau_B [B] K_A\right)^\eta}$$

Where:

- E_0: background noise in the experiment
- [A], [B]: orthosteric and allosteric compound concentrations, resp
- K_A, K_B: equilibrium dissociation constants
- α: cooperatively factor describing the allosteric effect on the other's binding affinity
- β: positive scaling factor
- τ_A, τ_B: ability to promote direct receptor activation (direct agonism)

Leach et al (2007), Melancon et al (2013), Gregory et al (2013)

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Note the similarities with the traditional 4PL model:

$$y = E_0 + \frac{E_m C^\eta}{EC_{50}^\eta + C^\eta}$$

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Benefit: if estimable, then directly interpretable parameters

Practical problems:

- more parameters to estimate
- starting values?
- is the data rich enough to estimate this?

Likelihood approach

How to obtain reasonable starting values?

- try and error: doomed to fail
- based on physiological knowledge if available
- likelihood profiling

Likelihood profiling:

```
proc nlmixed data=allosteric  maxiter=0;
parms E0=... emax=... logtauortho =... to ... by ... logKortho=... to ... by
  ... logKallos=... to ... by ... logtauAllos=... to ... by ... logalpha=...
  to ... by ... logbeta=... to ... by ... sig=... to ... by ... ;
  ...
ods output parameters=parm; run;
```

Explore where the optimum is located.

Time consuming!

Likelihood approach

Challenges:

- set hill function to 1
- too little information for emax, so fixed (instable due to outliers?)
- despite likelihood profiling no convergence could be attained

"The operational model can be fitted to experimentally derived data to provide estimates of some, or all, of its parameters"

Melancon et al, 2013

Conclusion: often too little information is available from the in-vitro experiment for the different parameters and the frequentist approach fails.

Abandon the idea and throw away the data?

Bayes approach: use of priors

"too little information for emax, so fixed"

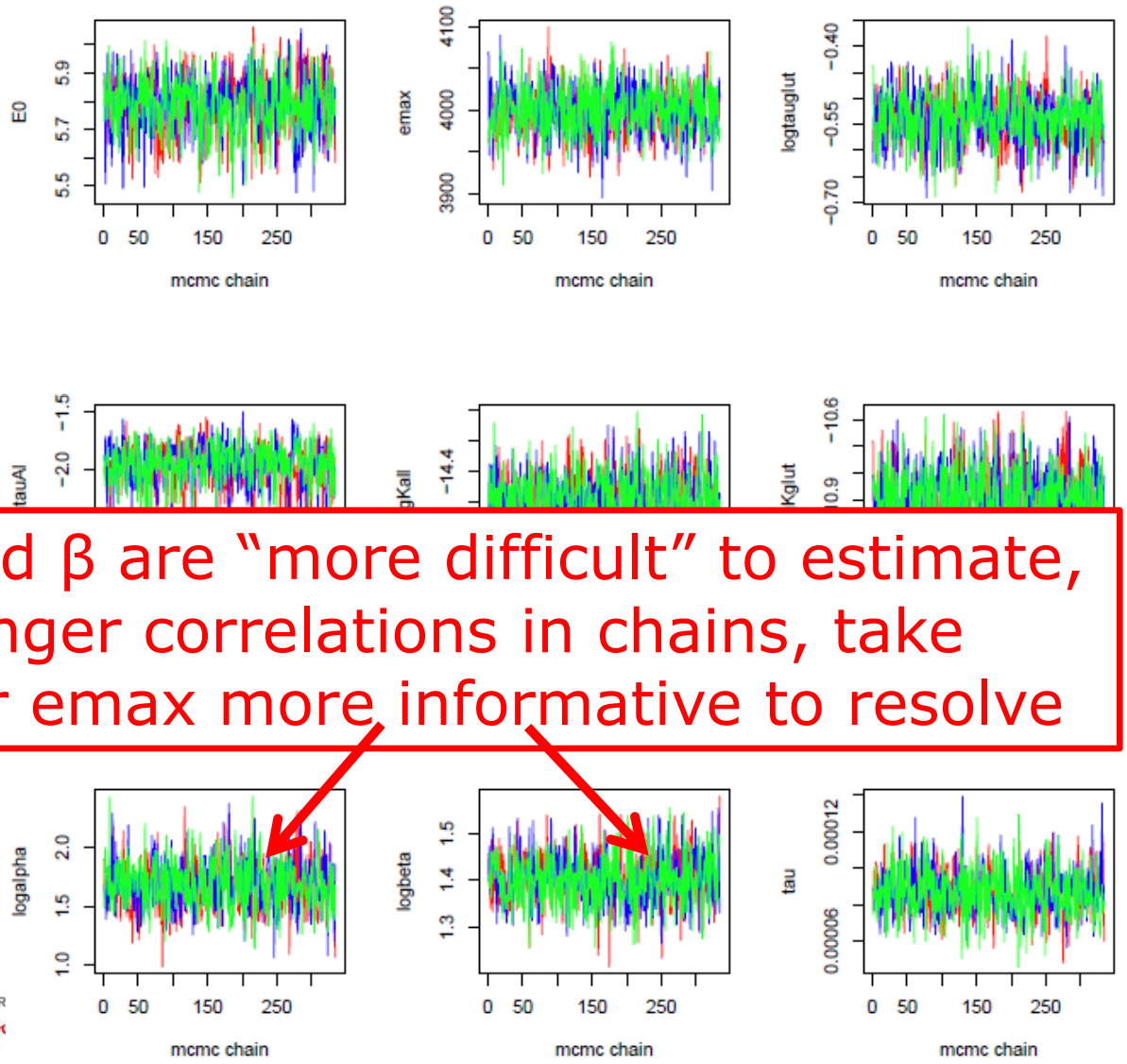
Alternative solution: give some reasonable feedback to the model, while allowing some flexibility with a weakly informative prior ("the emax is within a specific range").

Incorporate information from literature, likelihood profiling, etc regarding the orthosteric and allosteric compound (eg K_A) using (weakly) informative priors.

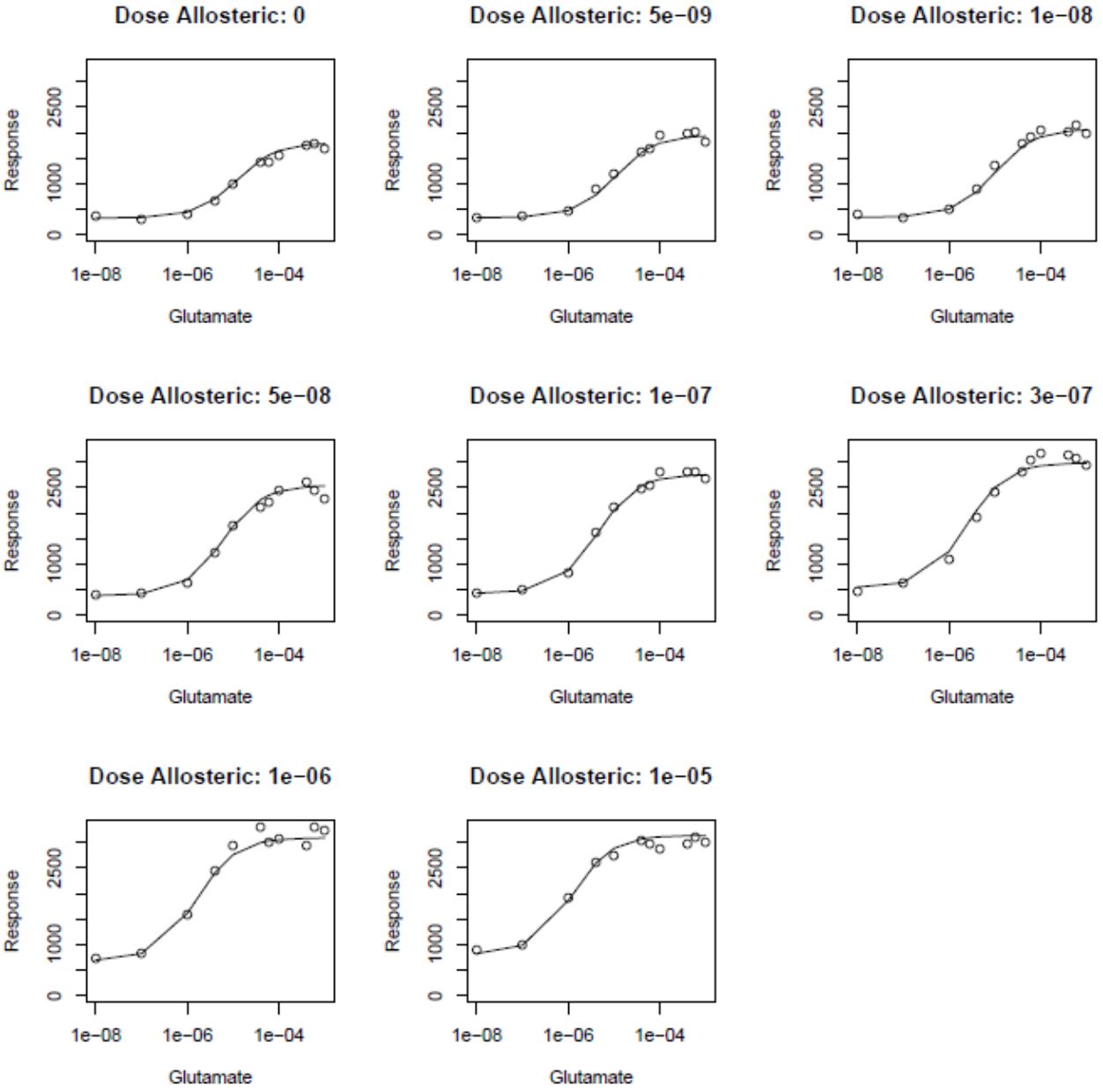
Next steps:

- check bayesian convergence, mcmc chains
- check model fit
- sensitivity check to the priors

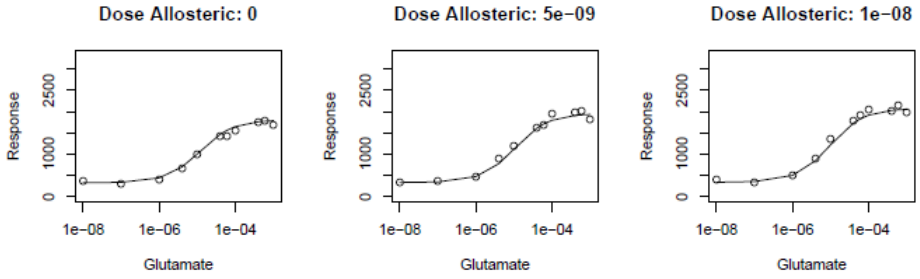
Bayes approach: mcmc chains



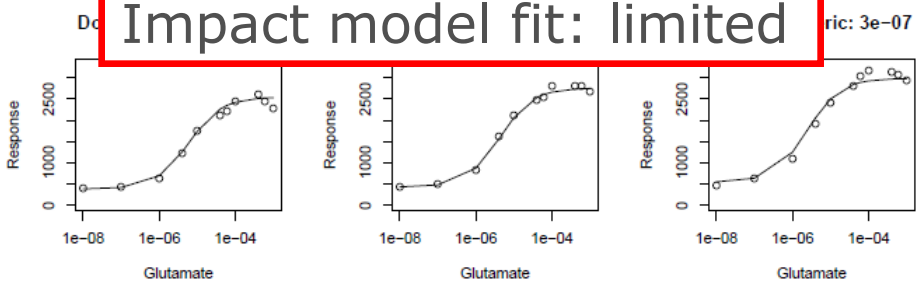
Bayes approach: model prediction

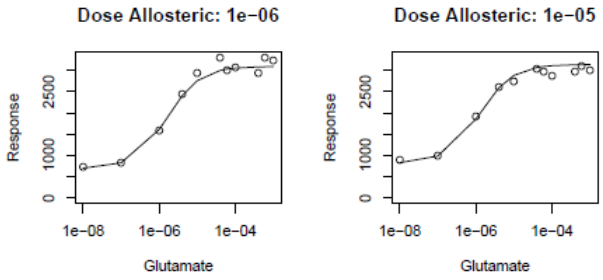


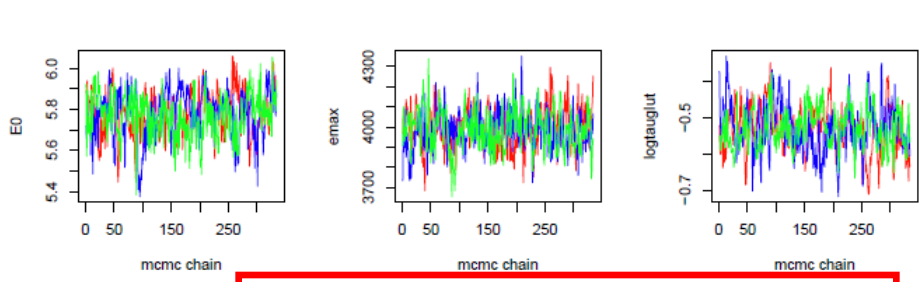
Bayes approach sensitivity analysis: priors and initial values



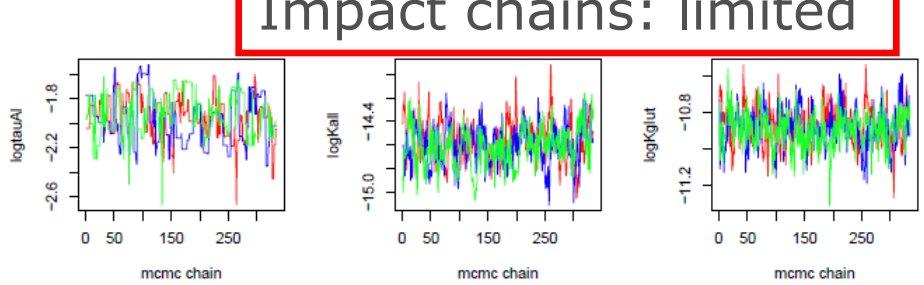
Impact model fit: limited

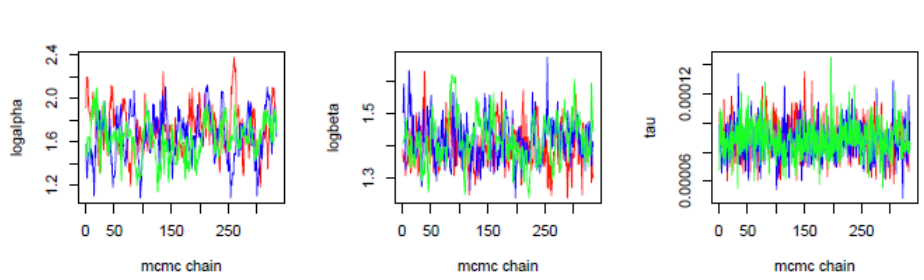






Impact chains: limited





Parameter estimates

	Est. (95%CI)
log(E0)	5.79 (5.57 ; 5.97)
Emax	3999 (3941 ; 4057)
Log(Kall)	-14.6 (-14.9 ; -14.2)
Log(Kglut)	-10.9 (-11.1 ; -10.7)
Log(alpha)	1.69 (1.28 ; 2.13)
Log(beta)	1.40 (1.30 ; 1.52)
Log(tauAl)	-1.97 (-2.40 ; -1.66)
Log(tauglut)	-0.539 (-0.642 ; -0.438)

Conclusion

Giving reasonable feedback to the model using (weakly) informative priors allows answering the scientific question. It is a perfect intermediate solution between allowing parameters to vary freely and fixing them to a specific value.

The mcmc chains suggested for which parameters (Emax, α, β) the data was less appropriate.

Although helping the model to converge, the predictions and parameter estimates are reasonable, but some caution remains.

Acknowledgements

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- An Vermeulen

References

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