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DEVELOPMENT OF A MODEL FOR ROBUST AND EXPLORATORY ANALYSIS OF THE RODENT BRIEF-ACCESS TASTE AVERSION DATA

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ABSTRACT

The rodent brief-access taste aversion (BATA) model is an efficient *in vivo* screening tool for taste assessment. A new E_{\max} (maximum effect attributable to the drug) model was developed and further investigated in comparison to three previously published models for analysing the rodent BATA data; the robustness of all the models was discussed.

The rodent BATA data were obtained from a series of experiments conducted with a bitter reference compound, quinine hydrochloride dihydrate (QHD). A new E_{\max} model that could be applied to both “lick numbers” and “lick ratios” was built and three published models that used lick ratios were employed for analysing the BATA data. IC_{50} , the concentration that inhibits 50% of the maximum lick numbers, quantified the oral aversiveness of QHD. One thousand bootstrap datasets were generated from the original data. All models were applied to estimate the confidence intervals of the IC_{50} s without symmetric assumption.

The IC_{50} value obtained from the new E_{\max} model was 0.0496 mM (95% CI 0.0297-0.0857) using the lick numbers for analysis, while an IC_{50} of 0.0502 mM (95% CI 0.0267-0.0859) was acquired with the lick ratios. Except from one published model, the IC_{50} values have a similar range for the 95% CI.

The new E_{\max} model enabled the analysis of both “lick numbers” and “lick ratios” whereas other models could only handle data presented as “lick ratios”. IC_{50s} obtained with these two types of datasets showed similarity among all models thereby justified the robustness of the new E_{\max} model.

KEY WORDS

Brief-access taste aversion, lickometer, bitterness, E_{\max} model, NONMEM

INTRODUCTION

Taste assessment has become an important element of pharmaceutical drug development after the release of the European Medicines Agency (EMA) “Reflection Paper on the Formulations of Choice for Children” (2006) (1). Pharmaceutical research laboratories and industry invest their funds and time for optimising the taste of drug formulations to increase patient acceptability hence compliance to the treatment, especially for patients with a chronic disease who need to take regular medication and for the paediatric population expressing more sensitive to unpleasant taste. Evaluating the taste of different derivatives of new chemical entities (NCE) such as their salts and formulations at early stage of the drug development process is therefore essential to achieve appropriate taste-masking which will potentially increase the acceptability for the patient.

Several *in vivo* and *in vitro* techniques such as human taste panels, e-tongue and animal preference tests are currently available to assess the taste of APIs (2). Among these approaches, the rodent BATA model has a great potential and has already shown very promising results comparable to human panel data (3,4). The BATA model has been widely used and documented in the literature for different purposes (3–9). In this animal model, rodents such as mice or rats, are mildly water-deprived and then put into a “lickometer” which records the number of “licks” that the rodents make to different concentrations of the

compound under test samples presented in several sipper tubes. Animals only have a very short period of time (between 5 and 10 sec) to lick each solution. Typically, a low number of licks compared to water will indicate an aversive taste. With this procedure, full aversion-concentration curves of lick rate can be obtained with very few animals. The comparison of the curves of several taste samples enables the determination of the compound with the least aversive taste.

In order to assess the taste of an active pharmaceutical ingredient (API) or to compare the taste of several compounds accurately, the determination of the concentration which suppresses 50% of the licks compared to water (IC_{50} value) is often taken as the key parameter for comparison. The data set chosen and the equation fitting the data are therefore very important for the determination of this parameter. Building a model which accurately matches the data points is a primordial step in data analysis as the acquisition of inadequate parameters could lead to different interpretations of the taste assessment results.

Though several models have been introduced for the analysis of BATA data, so far no standard model has been identified and the model chosen is based on practical considerations rather than any rationale mechanism. The lack of consensus in analysing the data can cause inconsistencies in explaining and comparing the results. As the data is interpreted in different ways among published studies, the comparison of IC_{50} data obtained from different models and/or different data treatments is difficult. Since these models can only handle data presented in the form of “lick ratios”, the information of the baseline could be lost; therefore the IC_{50} obtained could not be accurate. Thus, a robust model is required to analyse the results acquired from BATA experiments.

A minimum number of licks was observed in the majority of rats tested, even when the drug concentration was extremely high. This indicated that there was a maximum effect of QHD in BATA experiments suggesting an E_{max} model is ideal to fit this kind of dose-

response relationship. The present study aims to introduce a new E_{\max} model to analyse both “lick numbers” and “lick ratios” obtained from eight BATA experiments using a compound with well-known unpleasant taste as a bitter reference, quinine hydrochloride dihydrate. Three published models were also applied to analyse the same data. A non-parametric bootstrap analysis was conducted to estimate the confidence intervals of all models' parameters without symmetric assumption. All models were then compared and discussed.

MATERIALS AND METHODS

Taste solutions

Quinine hydrochloride dihydrate was purchased from Sigma Aldrich (Sigma Aldrich, Dorset, UK). All the solutions were prepared identically prior the experiment in deionised water and used at room temperature (23°C).

Animals

Ten adult male Sprague-Dawley rats (Charles-River, Kent, UK) were used. Rats were housed in pairs in standard cages in a room that was maintained at $21 \pm 2^{\circ}\text{C}$ with $55 \pm 10\%$ humidity and with a 12:12h light/dark cycle. All training and testing occurred during the light phase of the cycle. Animals had free access to chow (Harlan, Oxon, UK) and tap water except for training and testing periods where a water-restriction schedule occurred (see BATA procedure). Throughout the experiment, daily food and water consumption were monitored. As a safety and welfare measure it was checked that their weight did not drop below 85% of their free-feeding weight. All the procedures were carried out in accordance with Animals (Scientific Procedures) Act 1986 (Project Licence PPL 70/7668).

BATA procedure

The commercially available lickometer “Davis MS-160” from DiLog Instruments (Tallahassee, Florida, USA) previously described elsewhere was used for this experiment (10). Each rat was water-deprived for 22 hours before each session (training and testing) and was then placed in the lickometer for a maximum session-length of 40 minutes. After each session, the rodents received tap water for rehydration. The first days of the protocol were dedicated to training: on the first training day the shutter was continually open, presenting a single tube containing deionised water; on the second training session the sixteen tubes contained deionised water. The training was followed by two or three testing days during which each rat was presented with different sipper tubes containing either deionised water or one of the six concentrations of QHD (0.01, 0.03, 0.1, 0.3, 1 and 3 mM). The trial began when the rat took its first lick from the sipper tube, and ended few seconds later when the shutter closed. A different sipper tube was positioned behind the shutter in preparation for the next trial during the inter-trial interval. Each trial was intercepted by a water rinse to minimise carry over effects from the previous solution tested. The order of presentation of the sipper tubes was randomised and each concentration was presented 4 times per session. The experiments were repeated on 8 different weeks intercepted by a one-week washout period.

Data treatment and model development

Figure 1 represents the work flow for data analysis.

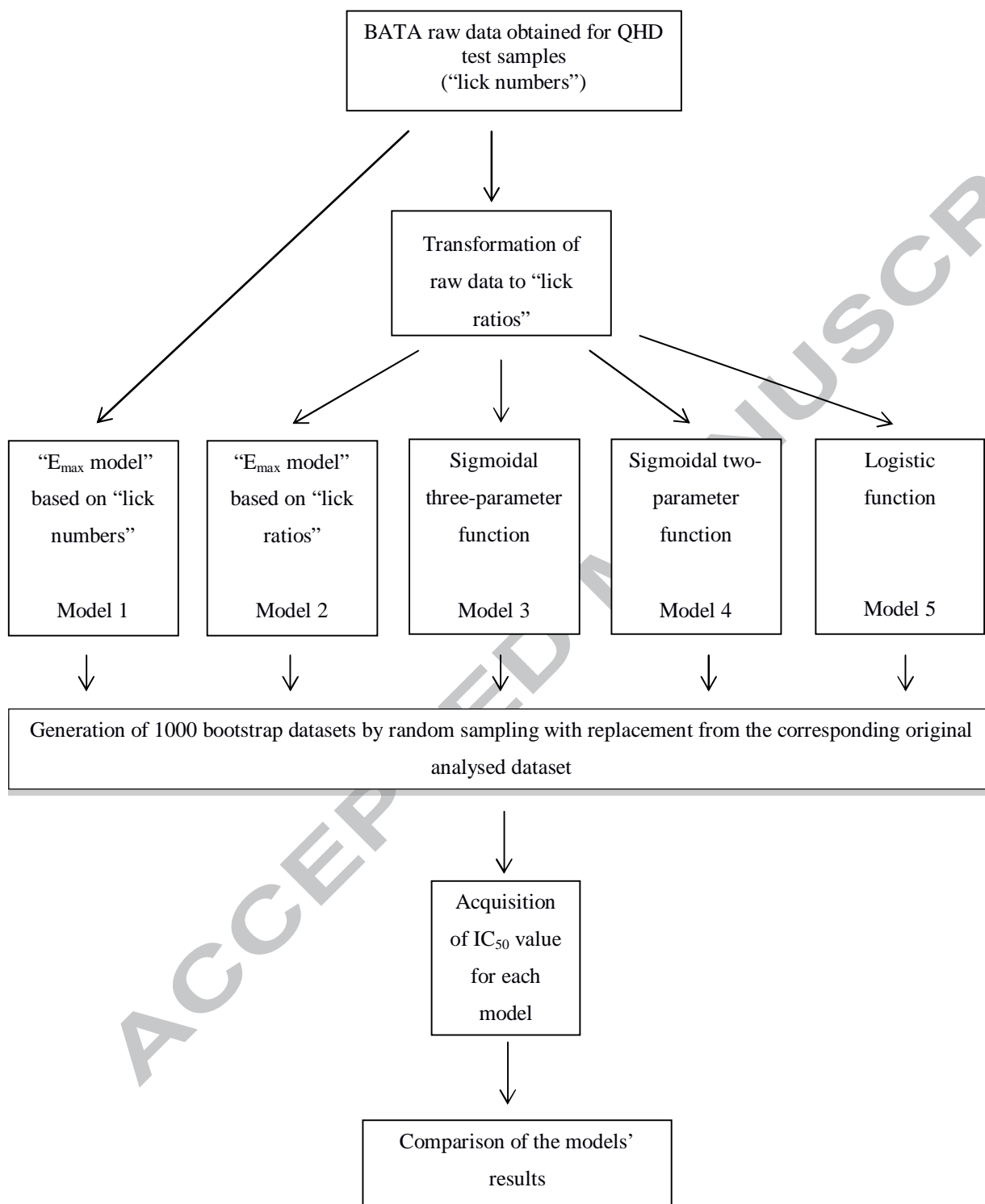


Figure 1: Workflow for data analysis

Data treatment

The “lick numbers” obtained for deionised water and six concentrations of QHD solution were recorded. “Lick ratios” (% inhibition of licking compared to deionised water) were calculated by dividing the number of licks of the test solution (QHD solution) by the average number of licks for deionised water. No data recorded were discarded.

$$\text{lick ratio} = \frac{\text{number of licks to each test solution}}{\text{mean number of licks to water}} * 100\%$$

E_{max} model

Based on the boxplots (Fig. 2) representing “lick numbers” (Model 1) and “lick ratios” (Model 2) versus QHD concentrations, the new E_{max} model was applied to describe both types of data change with concentration:

$$y = BL * \left(1 - E_{max} * \frac{X}{IC_{50} + X}\right)$$

where y represents either “lick numbers” or “lick ratios”, X is the QHD concentration, BL is the number of licks from deionised water, IC_{50} represents the concentration of QHD that produced the half-maximum response (i.e., a 0.5 “lick ratio” value or 50% suppression of “lick numbers”), and E_{max} is the maximum fraction of BL when the concentration is extremely high. For “lick ratios”, the BL was set to 1.

Other models published in existing literature

“Lick ratios” were fitted with three published models (11–13):

Model 3: Sigmoidal three-parameter function:

$$y = \frac{1 - d}{1 + \left(\frac{x}{IC_{50}}\right)^b} + d$$

Model 4: Sigmoidal two-parameter logistic function:

$$y = \frac{1}{1 + \left(\frac{x}{IC_{50}}\right)^b}$$

where x is the QHD concentration, b is the slope, IC_{50} represents the QHD concentration that evoked the half-maximal response, and d is the asymptotic minimum.

Model 5: Logistic function:

$$y = \frac{a}{1 + 10^{(x-10^{IC_{50}})b}}$$

where x represents \log_{10} of QHD concentration, a is the asymptotic lick response adjusted for water, b is the slope and IC_{50} is the QHD concentration that evoked the half-maximal response.

Statistical Model

Between subject variability (BSV) associated with all parameters for all models was tested using an exponential function as follow:

$$\theta_i = \theta_t e^{\eta_i}$$

where θ_i is the parameter for the i_{th} subject, θ_t is the typical value of the parameter in all rats, and η_i is a BSV with a mean of 0 and a variance of ω^2 .

An additive residual error structure was employed for all models as follow:

$$y_{ij} = \hat{y}_{ij} + \varepsilon_{ij}$$

where y and \hat{y}_{ij} represent the j_{th} observed and predicted response (“lick numbers” or “lick ratios”) respectively for the i_{th} subject, and ε_{ij} is the residual random effect assumed to be normally distributed with a mean of 0 and a variance of σ^2 .

Bootstrap evaluation

All models were evaluated using a non-parametric bootstrap method. One thousand replicate bootstrap datasets were generated by random sampling with replacement from the corresponding original analysed dataset. IC_{50s} for each dataset were obtained from each model. The robustness of the model was expressed as 95% CI of the estimate, by observing the 0.025th and 0.975th quantiles of the resulting bootstrap parameters' distributions from runs with successful convergence (14).

Software

The non-linear mixed effects modelling was performed using the software NONMEM® (ICON, Ellicott City, Maryland, version 7.3) in conjunction with a gfortran (64-bit) compiler using Perl-Speaks NONMEM® (PSN, version 4.2.0) as an interface to run NONMEM®. The first order conditional estimation with interaction (FOCEI) method was used throughout the model analyses. R (version 3.0.2) was used for plots.

Results

The water-deprivation length of 22 hours chosen for the experiments was adequate to motivate adult rats to drink from the different sipper tubes in the lickometer without any major sign of stress and dehydration. The rats did not show a decrease in the motivation to drink from the tubes during the 40-minute session over days or over weeks. Moreover, they did not lose more than 5.7 % of their initial weight after having being placed on the water-restriction schedule and always recovered their weight after the rehydration period and continue to grow normally. The training sessions were all successful and suggested a good confidence from the rats to drink in the lickometer and ensured they remembered the procedure over weeks.

Quinine is well known to be orally aversive to rats and humans and is generally used in taste studies as a bitter reference compound. Therefore, this compound was our model drug to describe the trend generally obtained in BATA experiments with drugs and formulations eliciting a bitter taste: a decrease of the number of licks when the concentration increases.

A total number of 5400 licks recorded for deionised water and 6 QHD concentrations were obtained from 10 rats. After averaging the values from deionised water, 4320 “lick ratios” for QHD solutions were calculated. As expected, the licking response to QHD produced monotonic decreases in rats' licking with increasing concentration of the bitter compound during each session. The concentrations chosen for QHD produced the full concentration-response curve (Fig 2). However, a large variability was also shown in both “lick numbers” and “lick ratios” plots since all the records were included.

The data were successfully fitted to all models and all resulting IC_{50} values (Table 1) were within the range of 0.03-0.1mM, which was in accordance with the boxplots shown in Fig.2. The IC_{50} values from the two E_{max} models were 0.0496 and 0.0502 mM for Model 1 and Model 2, respectively. Except Model 5, all the IC_{50} values were in the range [0.0488, 0.0539] mM. Among all IC_{50} pairwise comparisons, the values from the two E_{max} models were the closest pairs. Moreover, these two models had the same E_{max} value of 0.957, which means that 95.7% of the lick activities would be inhibited when the QHD concentration is extremely high.

All bootstrap datasets for the five models successfully converged. Table 1 included the results of the bootstrap analysis for the IC_{50s} of each model. The means of IC_{50} values from the bootstrap re-samples were similar to the estimations from the original dataset in all the models. Except model 5, the 95% bootstrapped confidence intervals of the IC_{50s} from the

other models were relatively small and similar. These narrow intervals indicated that these models have a good precision and robustness.

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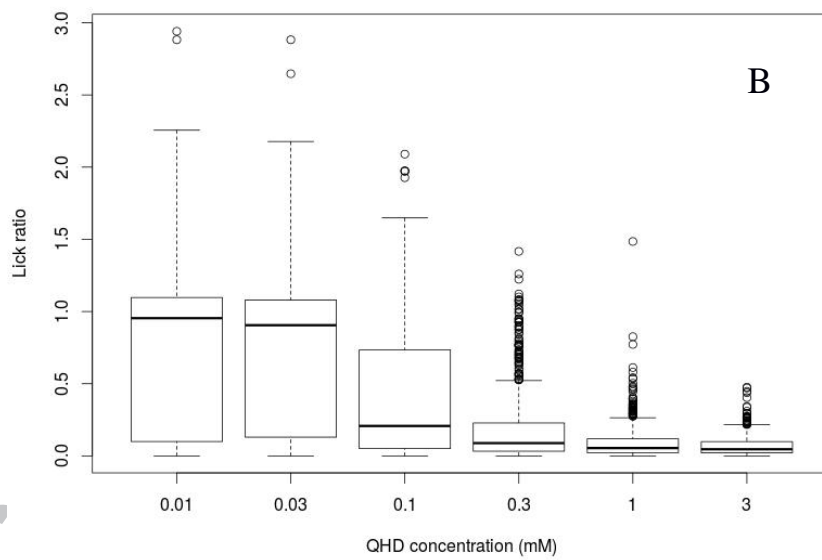
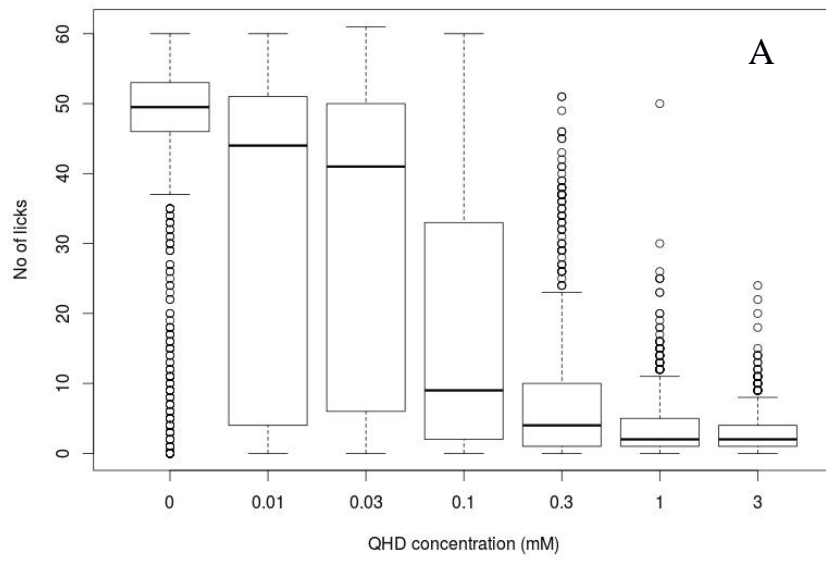


Figure 2: Boxplots of “lick numbers” (A) and “lick ratios” (B) from BATA experiments

Table 1: IC₅₀ values and 1000 bootstrap results from 5 models

	Model expression	IC ₅₀ (mM)	Bootstrap mean (mM) and 95% CI
Model 1	$y = BL * (1 - E_{max} * \frac{X}{IC_{50} + X})$	0.0496	0.0517 (0.0297, 0.0857)
Model 2	$y = 1 - E_{max} * \frac{X}{IC_{50} + X}$	0.0502	0.0510 (0.0267, 0.0859)
Model 3	$y = \frac{1 - d}{1 + (\frac{X}{IC_{50}})^b} + d$	0.0488	0.0517 (0.0288, 0.0848)
Model 4	$y = \frac{1}{1 + (\frac{X}{IC_{50}})^b}$	0.0539	0.0562 (0.0325, 0.0894)
Model 5	$y = \frac{a}{1 + 10^{(X-10^{IC_{50}})^b}}$	0.0864	0.0877 (0.0533, 0.120)

Discussion

Presently a new E_{max} model was introduced and was able to handle different types of data, namely “lick numbers” and “lick ratios”, in order to obtain reliable IC₅₀ values resulting from rat BATA experiments. A reference drug for bitterness, quinine hydrochloride dihydrate, was used. For comparison, the results from three other published models commonly used to process data from BATA experiments were also shown. The precision and robustness of all the models were evaluated and compared by one thousand bootstrap analyses. Our model appropriately fitted the concentration-response curves from both “lick numbers” and “lick ratios”.

The data treatment prior to calculation of the important parameters resulting from BATA experiments e.g. IC₅₀ values might differ from one study to another. For example, in

some studies trials with two licks or less were disregarded (9) whereas in other works only licks equal to zero were excluded from the dataset (12); in other investigations all the data were taken into account (or not mentioned in the paper otherwise). This was done to avoid to falsely register licks or failure to taste the sample. However, a number of licks equal to 0 or 1 is not often due to the reasons cited above. A very aversive taste and/or odour of the compound being assessed could also lead to no licking. It is difficult to distinguish between the different reasons mentioned above. In our study, a compound perceived very aversive by the rats might not be licked at all from the sipper tube upon a second presentation during the same session. Consequently, the dataset chosen is of high importance, mainly for accurate comparisons across several studies as depending on the data selected, results will vary to a greater or lesser degree. In our analysis we took into consideration all the records obtained from the experiments.

Data from BATA experiments are generally displayed by concentration-response curves representing the means of the number of licks or “lick ratios” as a function of the concentrations of the compound tested and the standard error (SE) or standard deviation (SD) obtained with a number n of rats (3,5,7,8,11,12,15–18). This way of presenting the results can give a good overview of the results’ trend; however, it can result in some misleading in the interpretation, as it does not show the variability of the records. Moreover, the data recorded for each concentration group does not follow a normal distribution; therefore displaying the records’ means for each concentration group is not statistically appropriate. Boxplots (Fig. 2) are a more appropriate way of depicting the BATA concentration-responses, as more information is represented and available at one glance.

The existing literature often used “lick ratios” (calculated by dividing the lick numbers obtained from each concentration by the mean number of licks from water) into data analysis models. Since the “lick numbers” from water were not normally distributed (Fig.

1A), the average values did not represent their true central tendency. Moreover, “lick ratios” were based on the average values from water; thus, the variability from water was lost. In some articles it is even more inappropriate when the “lick ratios” were calculated by dividing the mean number of licks from each concentration by the mean number of licks from water (7,11,12,19,20).

The statistical tests that are usually undertaken to analyse BATA data e.g. Analysis of Variance (ANOVA) are used without always meeting all the assumptions required to run these tests such as a normal distribution of the data for each concentration group and the equality of group variances. Non-parametric tests can be more adequate to rigorously proceed to the analysis of the data. However, statistical tests can only be used to compare groups of the same experiment. Consequently, when the taste intensity of different compounds is compared, another method should be employed since statistical tests are not suitable to compare them from different experiments. For our research purposes, a parameter such as the IC_{50} enables to directly compare the bitterness intensity from several drugs. Furthermore, when the model was built, the results from other concentrations could be predicted.

Although some published models exist to analyse the BATA data, these models can only handle data presented in the form of “lick ratios” and most of their parameters are not interpretable. As shown in the results, the proposed E_{max} model can analyse both “lick numbers” and “lick ratios” and the model parameters from these two kinds of data were similar. Since “lick ratios” would lose information from water (the baseline) and then introduce some bias to the results, a model that can handle the original “lick numbers” is necessary. Nonetheless, if the results obtained with the “lick numbers” are close to the ones obtained with the “lick ratios”, it is more appropriate. Not only similar IC_{50s} were found from the E_{max} models when treating two kinds of data, but also IC_{50s} close to those from the other three published models were obtained. Moreover, every parameter from the new E_{max} model

had its own meaning and was easy to understand. The meaning of the parameter IC_{50} was the same as the one from other models. The baseline corresponds to the “lick numbers” only from water and the E_{max} refers to the maximum fraction of the baseline inhibited from the extremely high concentrations. Bootstrap analysis also demonstrated that the precision and the robustness of the new E_{max} model were also similar to, if not better than, those from other models.

Conclusion

In this study, the proposed E_{max} model successfully fitted two types of data, “lick numbers” and “lick ratios” obtained from rat BATA experiments. It was found that both types of data could be treated with E_{max} model to acquire the “bitterness equivalency” value, IC_{50} , thereby assess the taste and compare the taste intensity of different drugs. E_{max} model generated similar values to the ones reported in three other published models. Moreover, the new E_{max} model also showed good robustness from bootstrap analysis. In conclusion the new E_{max} model is proposed as a reference tool to bring a consistent approach for analysis of the data obtained from BATA studies.

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