lickcalc: Easy analysis of lick microstructure in experiments of rodent ingestive behaviour

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Summary

Lick microstructure is a term used in behavioural neuroscience to describe the information that can be obtained from a detailed examination of rodent drinking behaviour. Rather than simply recording total intake (volume consumed), lick microstructure examines how licks are grouped, and the spacing of these groups of licks. This type of analysis can provide important insights into why an animal is drinking, for example, whether it is influenced by taste or affected by consequences of consumption (e.g., feeling "full"). The simplicity of using `lickcalc`, a browser-based application with a simple interface, will make microstructural analysis accessible to any researchers who wish to employ it while providing sophisticated analyses with high scientific value.

Statement of need

`lickcalc` is a software suite that performs microstructural lick analysis on timestamps of lick onsets and/or offsets. Microstructural analysis was first described in Davis & Smith (1992) and has since then been used to understand diverse phenomena. In-depth reviews on many of these, and microstructural parameters used to study them, are available (Johnson, 2018; Naneix et al., 2020; Smith, 2001). Briefly, although much of the foundational work on drinking microstruture was on licking for nutritive solutions (e.g., sucrose solutions), microstructural analysis can also be used to study intake of water (McKay & Daniels, 2013; Santollo et al., 2021), ethanol (Patwell et al., 2021), and other tastants such as non-caloric artificial sweeteners, sodium and quinine (Lin et al., 2012; Spector & St. John, 1998; Verharen et al., 2019). Lick microstructure has been used to shed light on, for example, how licking is affected by neuropeptides (McKay & Daniels, 2013), enzymes in the mouth (Chometton et al., 2022), ovarian hormones (Santollo et al., 2021), nutrient restriction (Naneix et al., 2020), response to alcohol (Patwell et al., 2021), and diet (Johnson, 2012). The number of lick bouts over a session are thought to reflect postingestive feedback from the consumed fluid, wheras the number of licks in a bout are thought to reflect palatability of the solution.

Lick microstructure can provide nuanced information about why an animal is drinking. Often, changes in microstructure are accompanied by changes in total intake, but this is not always the case: sometimes, equal intake will be achieved by quite different licking patterns that indicate changes in orosensory and postingestive feedback (Johnson et al., 2010; Volcko et al., 2020). Analyzing lick microstructure is therefore highly valuable when trying to understand how a manipulation, X, affects appetite; if X causes an animal to feel more satiated after drinking, that may lead to a different interpretation than if X were to reduce the palatability of the solution. Because of the value of microstrucural data, many labs habitually record and analyze it. There are many others, however, that have not yet begun collecting and/or analysing these data. Investing in lickometers can be costly, but there are an increasing number of cost-effective alternatives to commercial products. As such, several open-source lickometer designs are now available (e.g. Frie & Khokhar (2024); Monfared et al. (2024); Petersen et al. (2024); Silva et al. (2024); Raymond et al. (2018)).

Recording individual licks with high temporal resolution is necessary for microstrucural analysis of drinking behavior, but another barrier to reporting microstructure is its analysis. This problem is now easily solved by `lickcalc`. `lickcalc` does not require any special software or coding knowledge: all the user has to do is load a file with timestamps of lick onsets (and, ideally, offsets) into the application and `lickcalc` will generate detailed microstructural analysis, with a high degree of user control over key parameters. Resulting data provide values for number of licks, number of bursts, and burst size (among others) – the values that are often reported and used to draw inferences about postingestive and orosensory feedback of the solution. But imporantly, several plots are also displayed that show information that helps with quality control of the data and challenges the user to think critically about which parameters they have chosen. In short, `lickcalc` makes microstructural analysis accessible to any with appropriate data, while providing detailed information needed for appropriate parameter selection and quality control.

Key features

`lickcalc` has several features that make it exceptionally user-friendly while providing sophisticated and detailed microstructural analysis. Some of these features include:

- **Ease of use:** Files of various formats can simply be dragged into the `lickcalc` software to trigger analyses. Parameters can be set manually using sliders, and results exported to Excel with the push of a button.
- **Flexibility:** The user sets key parameters appropriate for their experimental setup and data. Data can be analysed across the whole-session, within different epochs, or based on a trial structure.
- **Customization:** By using the configuration file, users can change default settings to match their preferences and avoid manually changing settings for each file loaded.
- **Results compilation:** Data from multiple sessions and/or individuals can be exported into a single Excel file, which streamlines analysis. A batch mode is also included allowing multiple files to be analysed simultaneously.
- **Detail of analysis:** One of the benefits of using `lickcalc` is the level of detail it provides. In addition to the properties often reported (e.g., burst number, burst size), `lickcalc` computes and displays attributes of the data that are important in establishing the quality of the data and determining appropriate parameters for its microstructural analysis. Four charts are:

    1) *intraburst lick frequency,* or how often certain interlick intervals within a burst of licking occur. While a rodent is licking, its tounge makes rythmic protrusions that are under the control of a central pattern generator (Travers et al., 1997). Rats typically lick 6-7 times per second (Davis & Smith, 1992), while mice lick at a slightly higher rate (Johnson et al., 2010). In addition to these species differences, there are also strain differences (Johnson et al., 2010; St. John et al., 2017). Because intraburst lick rate is under the control of the central pattern generator, it should remain relatively stable across mice and conditions (unless a manipulation is expected to cause changes in the central pattern generator). A typical chart for a mouse might show a sharp peak around an intra-burst ILI of ~129 ms, which corresponds to a lick rate of 7.75 Hz. Much smaller peaks are often present at the harmonics of the intra-burst ILI (e.g., a primary peak at 129 ms will have smaller peaks at 258 ms, 387 ms, and so on), often beause of "missed licks" in which the mouse attempts to lick but its tongue misses the spout. A large number of these, or other differences from the expected pattern of results, may indicate problems

with the experimental setup (e.g., if the animal fails to reach the spout frequently, then perhaps the spout is too far away).

2) *lick length* is only available when lick offsets are included in the data file. As with intraburst lick frequency, lick length should show little variability and the graph will have a sharp peak. Occasionally a lickometer will register longer licks than normal. A common cause of this is formation of a fluid bridge between the tongue and the spout during periods of high frequency licking. This can be prevented by moving the bottle further from the animal. In addition, other causes are if a rodent grabs the spout with its paws, or if a fluid droplet hangs between the spout and the cage and thus completes the electical circuit. Concerns about data quality may be warranted with increasing number and duration of long licks. `lickcalc` displays both the number and maximum duration of licks above the threshold that the user has set. There is also an option to remove these problematic licks from the dataset.

3) *burst frequency*, or how often certain burst sizes occurred. This is informative because burst size, by virtue of being a mean (mean licks per burst), does not take into account potentially relevant information about the distribution of licks in each burst. For example, a burst size of 80 could result from bursts all containing between 70 and 90 licks, or from many single licks and one or two bursts with a lot of licks. The latter case might raise some questions about how reliable the burst size value is. Although single licks occur, they can also be caused by non-tongue contact with the lickometer. Changing the minimum licks/burst parameter can filter out some of these suspect "licks."

4) *Weibull probability*. The Weibull analysis, as described in Davis (1996), uses a mathematical equation to fit data to a survival function. Although used by some (Aja et al., 2001; Moran et al., 1998; Spector & St. John, 1998), it is still relatively rare to find Weibull probabilities in microstructural analyses. The Weibull function can be used on several aspects of data, such as lick rate across a session, but in the `lickcalc` program the Weibull probability is calculated for burst size. It plots the probability that, given *n* licks, the mouse will continue to lick. This makes it sensitive to the licks per burst parameter that is set by the user. The Weibull α and β values reflect the slope and shape parameters, respectively. Slope (α) has been shown to vary with palatability.

Design and usage

`lickcalc` is hosted by UiT The Arctic University of Norway and can be accessed at lickcalc.uit.no. Alternatively, it can be installed locally following instructions in the repository. To use `lickcalc`, the user drags a file into the application, changes file format if necessary, and indicates which column contains the lick onsets (and, if applicable, the lick offsets). A plot is automatically generated that displays a histogram of licks across the session. Session length defaults to the time of the last lick but can be manually changed, or set in the optional config file. Session length can be set in seconds, minutes, or hours. The bin size (licks per unit of time) can be changed manually or in the config file. The user can toggle between the default histogram and a plot showing cumulative licks.

A microstructural analysis is, in essense, a division of individual licks into groups of licks. To perform this grouping, the user must set several parameters. One of these is the inter-lick interval (ILI), which is the minimum amount of time licks must be separated by in order to be considered separate groups. Early studies identified ILIs of 251-500 ms as separating "bursts" of licking, and pauses of >500 ms as separating "clusters" of licking (i.e., a cluster of licks is made up of several bursts of licking). Others have argued that an ILI threshold of 1 s better reflects separation of lick bursts (Spector & St. John, 1998). In `lickcalc`, the user may set the ILI to any value (values between 250 ms and 3 s provided by default but can be adjusted using the config file). Another parameter that needs to be decided prior to the lick analysis is the minimum number of licks per burst. `lickcalc` allows between 1 and 5 licks by default. The appropriate number of minimum licks per burst may vary depending on experimental set up, and the likelihood that a single lick respresents a lick rather than, for example, a paw touching the spout. Finally, in `lickcalc`, the user must set a "long-lick threshold" between 0.1 and 1 s. This parameter is only available when lick offset is included. Licks that are longer than the set threshold are counted as "long" and may indicate a problem (e.g., fluid bridges or a mouse holding the spout with its paws) rather than a true lick. The user can decide whether to remove "long licks" or not. All of these parameters can be set manually or through the config file. Four plots are generated (see *Key Features* section above), and tables are displayed showing values of several properties: total licks, intraburst frequency, number of long licks, maximum duration of long likcs, number of bursts, mean licks per burst, weibull alpha, weibull beta, and weibull r-squared.

To save these data, the user has two options. The first is to export an Excel file for the loaded data. The user sets the animal ID and chooses which data should be exported in addition to the summary of the microstructural analysis. These allow the user to recreate the plots displayed in `lickcalc` or perform further analyses. The second option for saving the data is to add the microstructural data to *Results Summary* table. The results in this table remain even as new data files are loaded, so the data from many sessions (and/or individual animals) can be exported into a single Excel file. In addition to the data from the whole session, the user can choose to divide the session into epochs, or to examine only the first n bursts, or perform a trial-based analysis (e.g., for Davis rig experiments). Each of these analysis epochs can be added to the table. The table contains the data and the analysis parameters (e.g., minimum burst size) used to generate them. Finally, a batch process feature is available allowing multiple files to be analysed using the same parameters.

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