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

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Summary

Lick microstructure is a term used to describe the information that can be obtained from a detailed study of when individual licks occur, when a rodent is drinking. 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 insight into why an animal is drinking, such as if it is driven by taste or as a response to the consequences of consumption (e.g. feeling "full"). The simplcity of using LickCalc, requiring only a drag-and-drop of files, will make microstructural analysis accessible to any who wish to use it while providing sophisticated analyses with high scientific value.

Statement of need

`lickcalc` is a software suite that performs microstructural lick analysis on data files containing timestamps of lick onset and offset, in MedAssociates or csv/txt format. In addition to providing an overview of individual files (corresponding to a particular session for a single subject), results of the analysis can be added to a table that is exportable to Excel.

Microstructural analysis was first described in (Davis & Smith, 1992) and has since then been used 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 a great deal of interesting 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 in understanding how a manipulation 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 these data. Investing in lickometers can be costly, but there are an increasing number of alternatives to commercial products such as those produced by Med Associates. Several open-source lickometer designs are now available (e.g. (Frie & Khokhar, 2024; Monfared et al., 2024; Petersen et al., 2024; Raymond et al., 2018; Silva et al., 2024). 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 can now easily be solved by LickCalc. LickCalc does not require any special software or coding knowledge: all the user has to do is drag a file with timestamps of lick onset (and, ideally, offset) into the program and LickCalc will generate a detailed microstructural analysis, with a high degree of user control over key parameters.

microstructural analysis first described in (John D. Davis & Smith, 1992) used more recently to understand (Naneix et al., 2020)

Weibull analysis as described in (J. D. Davis, 1996)

Key features

The first chart shows 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 time 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 around 129, 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 will have smaller peaks at 258, 387, and so on), often beause of "missed licks" in which the mouse attempts to lick but its toungue 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). The second chart, of lick length, is only available when lick offset is 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. This may be because a "lick" is registered by something other than a tounge, such as if a rodent grabs the spout with its paws, or if a fluid droplet hangs between the spout and the cage and is thus able to complete the electical circuit. Concerns about data quality may be warrented with increasing number and duration of long licks. LickCalc displays both the number and maximum duration (s) of licks above the threshold that the user has set. There is also an option to remove these problematic licks from the dataset. The third chart shows a burst frequency histogram, 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 how many licks are in a 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 burst 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-tounge contact with the lickometer. Changing the minimum licks/burst parameter can perhaps filter out some of these suspect "licks." The fourth and final chart is a Weibull probability chart. The Weibull analysis, as described in (Davis, 1996), uses a mathematical equation to fit the 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 number of licks/burst parameter that is set by the user. The Weibull alpha means…. And the Weibull beta means…

Design and usage

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 ILI of 251-500 ms as separating "bursts" of licking, and pauses of more than 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 ILI of 1 s better reflects separation of lick bursts (Spector & St. John, 1998). In LickCalc, the user may set the ILI between 250 ms and 2 s. Another parameter that needs to be decided prior to the lick analysis is the minimum number of licks per burst. LickCalc allows between 1and 5 licks. The appropriate number of minimum licks/burst may vary depending on experimental set up, and the likelihood that a single lick respresents a lick rather than, for example, a tail 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. the mouse holding the spout with its paws) rather than a true lick. The user can decide whether to remove "long licks" or not. After loading a file and setting key parameters, LickCalc displays results, the detail of which are an additional advantage of using the program. Tables show 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, there are several charts 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.

Citations

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Figures

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