Abstract We propose an alternative statistical method, logistic growth curve analysis, for the analysis of associative learning data with two or more comparison groups. Logistic growth curve analysis is more sensitive and easier to interpret than previously published methods such as $\chi^2$ or ANOVA, which require the data to be collapsed into individual total scores or proportion of responses over time. Additionally, this type of analysis better fits the typical graphical representation of associative learning data. An analysis is presented where associative learning data from honeybees are analyzed using the three techniques, and the accessibility and power of the logistic growth curve analysis is highlighted.

Keywords Logistic growth curve analysis · Comparison of statistical methods · Associative learning · Honeybees

Introduction

The goal of many learning and memory experiments is to identify and characterize trends in cognitive skills for groups of individuals. One of the commonest paradigms used in such studies is classical conditioning (reviewed by Menzel 1999). Classical conditioning studies use a repeated exposure to a conditioned stimulus (CS) that originally had no obvious biological significance to the tested subjects, coupled with a biologically relevant unconditioned stimulus (US). Repeated exposures to the coupled CS-US result in increased performance on the tested learning criterion (usually an acquired behavioral response to the CS).

The data from these studies are a sequence of responses and non-responses for each animal. In previous reports, some authors scored the number of responses and did a t-test to evaluate the difference between groups; other authors calculated proportions of responses at each time point for each group and did a $\chi^2$-test on the difference between groups at each time point. Both methods address some aspects of the phenomenon, but ignore others. We advocate a third approach, logistic growth curve analysis, and argue its advantages. To illustrate the theory, we analyzed a single data set using the three different methods for comparative purposes.

Methods

Data set

Ben-Shahar et al. (in press) trained 56 honey bees to associate odor A (geraniol) with a sugar reward in a sequence of six odor exposures. If the bees learned to associate odor A with a reward, the proportion of bees responding would increase with time. The resulting data for each bee is a sequence of responses (R) and non-responses (N). For example, the response sequence of a bee to odor A of N-R-N-R-R-R would suggest that the bee had learned the association.

Bees were collected from two different behavioral groups, group X ($n=32$), and group Y ($n=24$), to determine whether they perform differently in this learning assay. The raw proportion of responses to odor A is plotted in Fig. 1. Visually, there appears to be a trend in the plots: bees from group X learn more quickly than bees from group Y. Initially, they respond more frequently, and reach the maximum level of learning by the third learning trial. The bees from group Y do not catch up with the bees from group X until the fifth learning trial.

The three analytic methods

Method 1: t-test on scored data

The data can be analyzed by collapsing the data to form either one observation per subject or one observation per time point. In order
to create one observation per subject, the data from each subject were converted to the total number of responses (e.g., Bitterman et al. 1983; Bhagavan et al. 1994; Pelz et al. 1996; Gerber et al. 1998).

Information is lost, however, when the data are collapsed in this way. For example, in an experiment with six trials, both the response sequences R-N-R-N-R-N and N-N-N-R-R-R give a score of three responses. The first case appears to be a random fluctuation, but the second case is suggestive of learning. In fact, in an experiment with six trials, there are 20 different ways that a subject can achieve a total of three responses.

A t-test of the difference in total number of responses per bee in groups X and Y was performed. Both X and Y groups had bees whose response totals ranged from zero to six.

Method 2: $\chi^2$-test on proportional data

These data can also be analyzed by converting the data to proportions of responses per group in each odor exposure (e.g., Ray and Ferneyhough 1997, 1999). Proportions were analyzed by using $\chi^2$-tests (with 1 df) for each odor exposure separately. A Fisher’s exact test was used when an entry in the table had $n \leq 5$.

Here, again, information is lost. Suppose there is an experiment with ten animals. Two out of ten animals respond initially, six different animals respond on the last trial, and there were random fluctuations in the middle. Overall, it appears the group of animals learned to respond because the proportion of responses increased. But there is no evidence of individual learning because different animals responded at different times. Additionally, there is difficulty in interpreting learning dynamics as a time-dependent process since each time point is analyzed as a discrete unit.

Method 3: logistic growth curve analysis

A common and intuitive way of presenting learning and memory data is to form “learning curves” by plotting the proportion of responses at each trial for a group of subjects as in Fig. 1 (e.g., Bitterman et al. 1983; Bhagavan et al. 1994; Gerber et al. 1996; Pelz et al. 1996). The learning curves from several experimental groups are frequently plotted on a single graph to allow visual comparison. Although the papers cited here presented learning curves, none of them used all the data from the curves to test the magnitude of the difference between the tested groups. Logistic growth curve analysis precisely tests the difference between several learning curves. It retains the structure of the responses, both on the individual and group level, and results in a statistical comparison of predicted curves resembling the empirical learning curves.

The data addressed in this paper (Fig. 1) consist of repeated measures of the same subject, where a measurement is a response or a non-response (coded 1 for response and 0 for non-response). In an attempt to estimate the curves seen in Fig. 1, the inclination may be to fit a line or curve to the data using linear regression. But a linear regression of these data violates several assumptions of the linear regression model. First, the proportions of responses to be modeled fall only between zero and one (equivalently, between 0 and 100%), so the assumption that a line or a polynomial can fit the data is incorrect because anything but a flat line will eventually cross either one or zero. Secondly, knowing the response of a subject at one trial gives a better idea of what its response will be at a later trial. That is, each consecutive response of a single subject is dependent on previous responses. For instance, if a subject were above average, we would expect it to stay above average. Statistically speaking, the responses of a single subject are correlated.

Logistic growth curve analysis, the combination of logistic regression and growth curve analysis, incorporates non-linearity and correlation into the analysis. Logistic regression models the probability of response over time, and its sigmoid curve lies between zero and one (Fig 2). Similarly to linear regression, it contains a slope parameter and an intercept parameter. The ratio of the intercept to the slope marks the point in time where half the subjects have responded. The slope parameter indicates the steepness of the curve. A slope between zero and one flattens the curve, and a slope of zero indicates no predicted change over time.

Growth curve analysis assigns a correlation structure to the data, allowing a positive correlation for observations from the same individual, while maintaining the observations from different individuals as independent. This correlation structure means that observations from a single subject are more related to each other than they are to observations from a different subject. The correlation estimate is then used to calculate the logistic regression parameters and their standard errors (Dunlop 1994; Littell et al. 1996).

We performed logistic growth curve analysis using PROC GENMOD in SAS, version 6.11 (Littell et al. 1996; see Appendix). A logistic curve fit to the data and then the correlation structure for each individual bee was estimated with generalized estimating equations, or GEE (for more information on correlation structure estimates see Littell et al. 1996, SAS 6.12 manual). Finally, the cor-