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The Use of the First Order System Transfer Function in the Analysis of Proboscis Extension Learning of Honey Bees, *Apis mellifera* L., Exposed to Pesticides

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Abstract No attempts have been made to apply a mathematical model to the learning curve in honey bees exposed to pesticides. We applied a standard transfer function in the form Y = B3*exp(− B2 * (X − 1)) + B4 * (1 − exp(− B2 * (X − 1))), where X is the trial number; Y is proportion of correct responses, B2 is the learning rate, B3 is readiness to learn and B4 is ability to learn. Reanalyzing previously published data on the effect of insect growth regulators tebufenozide and diflubenzuron on the classical conditioning of proboscis extension, the model revealed additional effects not detected with standard statistical tests of significance.

Keywords Mathematical model · Pesticides · Honey bees · Learning curve

This paper describes a mathematical model of the learning process suitable for studies of proboscis conditioning (PER) in honey bees when bees are exposed to agro-chemicals such as pesticides. Although procedural variations exist in the way laboratories use the PER paradigm (Abramson et al. 2011), proboscis conditioning is widely used to investigate the influence of pesticides and repellents on honey bee learning (Decourtye and Pham-Delegue 2002). In the PER paradigm, harnessed bees receive odor (the conditioned stimulus) and sucrose (the unconditioned stimulus) pairings. After several pairings, the proboscis extends to the conditioned stimulus (CS) prior to the presentation of the unconditioned stimulus (US). Honey bees have been exposed to pesticides (or repellents) by imbedding them in the conditioned stimulus (Abramson et al. 2006), serving as either the conditioned stimulus or unconditioned stimulus (Abramson et al. 2010), and pre-exposing the honey bee with a pesticide prior to conditioning (Abramson et al. 2004). Surprisingly, no attempts to mathematically characterize the learning process following exposure to agrochemicals have been previously reported.

Mathematical models have much to recommend for the evaluation of pesticides and repellents. Mathematical models of the learning process conveniently summarize large amounts of data, guide, research and theory, and can characterize the results of various agro-chemical treatments across laboratories, species, and conditions. Mathematical models provide important information above and beyond that provided by standard significance testing.

Materials and Methods

Over the past several years, we have been developing a model of the learning process based on the first order system transfer function in the form $Y = B3\ e^{-B2\ (X-1)} + B4\ (1 - e^{-B2\ (X-1)})$ where X is the trial number and Y is the proportion of correct responses. The model contains three parameters: B2—the learning rate; B4—the asymptotic value of correct responses at X = Infinity and

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B3—the value of correct responses at the beginning of training (i.e. B3 = Y at X = 1). Since B2 = $1/\tau$, it means that 1/B2 is the number of trials required for achievement of 63% from the difference between B3 and B4.

We treat B3 as an estimator of the functional state of an animal at the beginning of training that includes, in particular, the value of prior learning based on previous experience and readiness to learn. Since the independent variable is the value of correct responses (i.e. a proboscis extension to the CS) the greater the value of B3, the higher is the readiness to learn. Thus, B3 is the 'readiness to learn'.

The learning mechanisms are reflected in the learning rate (B2) and asymptotic level (B4). In the case of PER conditioning, B4 is the maximal possible number of correct responses after a very large number of trials. The greater the value of B4, the higher is the ability to learn. Our rationale for using coefficient B4 is that the number of correct responses does not always increase to 1 (or 100%). That is why, in our view, it is impossible to pre-fix B4 to 1.0. Below we will designate B4 as **'**the ability to learn**'**.

Additionally, we restricted the value of coefficient B2. Coefficient B2 must exceed zero by definition of the mathematical model. The upper limit we established for B2 is equal to 5.0. It is known that the first order system reaches 99.3% of its asymptotic value during five time constants (Grodins 1963; Milsum 1966). Since B2 = $1/\tau$, it means that if B2 = 5, then an animal achieves its asymptotic maximum of conditioned responses in one session. In other words, we suppose that the exponential learning curve exists, if $0.001 < \text{B2} < 5.0$.

To illustrate the type of information obtained from our model we re-analyzed some of our previously published data on the effect of insect growth regulators Confirm®2F (Tebufenozide) and Dimilin® (Diflubenzuron) on the acquisition of honey bee PER learning (Abramson et al. 2004). In that study we concluded that exposure to Confirm®2F or Dimilin® affected the acquisition of learning. Honey bees were fed using a microsyringe 10 µl of Confirm®2F or Dimilin® 10 min prior to conditioning. Those in the Confirm®2F group received either 0, 16 µg/bee, 24 µg/bee, 32 µg/bee, 69.4 µg/bee, or 131 µg/bee of Tebufenozide. Honey bees in the Dimilin® group received either 0 µg/bee, 3.4 µg/bee, 8.5 µg/bee, 16 µg/bee, 32 µg/bee, or 69.4 µg/bee of Diflubenzuron. Each group contained 26 bees.

The conditioned stimulus was a 3 s presentation of cinnamon odor and the unconditioned stimulus a 2 s feeding of 1.8 m sucrose. Each animal received 12 acquisition trials. The time between trials was 10 min. The CS was presented first, and when the CS terminated the US was presented. Using ANOVA procedures we previously showed that both Confirm®2F and Dimilin® influenced the acquisition of honey bee learning. However, when we apply the model to this data set much more information can be extracted.

Results and Discussion

Effect of Confirm®2F on Acquisition of the PER

Exposure to Confirm®2F significantly decreased the learning rate (B2) in each concentration especially in bees given 32 µg/bee. Exposure to 69.4 µg/bee increased readiness to learn (B3) but this was not the case at any other concentration. Ability to learn (B4) was reduced in animals given concentrations of 24 and 131 µg/bee but not at other concentrations.

Effect of Dimilin® on Acquisition of the PER

With the exception of bees exposed to16 µg/bee, exposure to Dimilin® significantly decreased the learning rate (B2). The decrease was significantly pronounced in bees exposed to 32 µg/bee. Dimilin® also significantly decreased the ability to learn (B4) at each concentration, especially in bees receiving 24 µg/bee and 69.4 µg/bee Dimilin® did not influence on readiness to learn (B3) at any concentration.

Comparison of Effects of Both Pesticides

The main effect of Confirm®2F was to decrease the learning rate (B2). In contrast, the main effect of Dimilin® was to decrease the ability to learn (B4). These results suggest that Dimilin® is more dangerous to honey bees than Confirm®2F even though both insect growth regulators are considered "harmless" to honey bees. Readiness to learn (B3) was not affected in honey bees with the exception that bees given 69.4 µg/bee of Confirm®2F increased their readiness to learn.

It should be noted that fitting the learning curve with the first order system transfer function is not the only statistically sound approach to analyzing learning data. Any statistical technique that both adjusts for the inherent correlation structure and accounts for both the dichotomous data and the pattern of learning over time is appropriate. This includes "growth curve" analyses with asymptotic, increasing or decreasing functions.

Hartz et al. (2001) proposed a model based on logistic growth curve analysis for PER learning in honey bees. One rationale for the use of the logistic growth curve model is that it is available in many statistical packages and no sophisticated programming is necessary. A comparison of the logistic growth curve model and the first order transfer model advocated here revealed that our model provided better fits for their data and that our model can extract more information from the data in the form of our three coefficients. Our model also has the advantage that it has been successfully applied to learning in many animals including land snails (Balaban and Stepanov 1996), rats (Stepanov and Abramson 2008) and humans (Stepanov and Abramson 2005) including Luriya's test of learning Russian words (Abramson and Stepanov 2007).

Of particular interest is that we have used the model to characterize patients suffering from multiple sclerosis (Stegen et al. 2010), for patients with type 2 Diabetes Mellitus (Stepanov et al. 2010) and to characterize the learning of children given the California Verbal Learning Test—(Stepanov et al. 2011). We believe that this is an advantage of our model because it can be used to directly compare performance decrements produced by a variety of environmental factors and disease including environmental contaminates and to compare coefficients across species.

As with any model, extensive validation is needed. As discussed above our model has been validated with a number of organisms in a variety of situations. This is the first paper, however, that attempts to apply the model to pesticide data. It should be noted that to apply the model, a minimum of 7 conditioning trials must be used. Several studies in the pesticide literature use only 3 conditioning trials (El Hassani et al. 2005; Han et al. 2010) and so few trials do not supply the model with enough information for accurate modeling.

We believe that our model can be applied to many aspects of honey bee learning as it relates to pesticide exposure. Of particular interest is an examination of extinction (when the CS is no longer followed by the US) and discrimination learning (when two CSs are used with one of them paired with a US). An agro-chemical, for example, may not influence the acquisition of learning but its persistence when the US or reward is discontinued. Agro-chemicals may also affect the ability of honey bees to discriminate between stimuli.

In addition to classical conditioning, the model has been used to characterize the maze performance of rats exposed to various pharmacological agents (Stepanov and Abramson 2008) and we hope to expand the model to other areas of learning such as operant conditioning (Abramson 1994). We have not yet extended the first order system transfer function to include other dependent variables such as flight time.

Of particular interest is the application of the model to characterize so-called "green products" such as essential oil based pesticides which often make claims of being harmless to honey bees (Abramson et al. 2006). Our model opens the possibility of characterizing these, and other agro-chemicals, by their effect on learning rate (B2), readiness to learn (B3) and ability to learn (B4). These parameters provide additional information on the effect of an agro-chemical that standard significance tests cannot provide. For example, the application of the model to the insect growth regulators Dimilin® and Confirm®2F reveal that Dimilin® is more dangerous to honey bees because it affected the bees' ability to learn (parameter B4) although both growth regulators are considered "harmless."

Details on how to use the model including programming language for SPSS is available in Stepanov et al. (2010) and directly from the authors. We would be glad to assist any researcher who wants to apply the model to their data.

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