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Associative learning in the green toad (*Bufo viridis*)

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Abstract. In this experiment we investigate toad associative learning capacity and the retrieval capacity after two periods: hibernation and active life. Toads are able to associate real or fake food items to different environmental features. First association was performed between *Tenebrio molitor* larvae and the white box where the animals were fed; second association was between the worm-like stimulus and the gravel ground of the box where they were maintained. Toads are not capable to discriminate between the real food and the fake one. After two months of hibernation, toads were capable to remember the second association, and also after two other months of active life.

Key words: *Bufo viridis*, associative learning, discrimination, memory retrieval capacity, hibernation

Introduction

There are so many differences across species, in terms of their memory, that seem to correlate with the ecological conditions they face (Goodenough et al. 2009). A lot of experiments show that in special tasks animals has the ability to remember information and use it appropriately in a flexible adaptive manner (Olton, 1979). Learning is a process through witch experience changes an individual's behavior (Goodenough et al. 2009).

Behavior system is usually thought to be organized in time. Habituation is everywhere, from unicellular protozoa to humans and it is considered to be the simplest form of learning. (Wyers et al. 1973). It is a very useful tool for the study of cognitive processes in animals.

Classical conditioning occurs when an initially neutral stimulus (CS) is paired in close proximity with a biological significant stimulus (US) that elicits an unlearned, reflexive behavior (UR). Through CS-US association formation, the animal acquires a behavior (CR). As shows the Rescola-Wagner model, learning depends on the surprisingness of the US, and the strength of the conditioned response is stronger if the US is more intense (Annau & Kamin 1961, Morris & Bouton 2006).

Stimuli enter from the environment to sensory memory, and if they are attended to, they enter in another hypothetical store known as short term memory - the place where thinking occurs, and it have a limited capacity; but learning involves storage of information in long term memory that have an enormous capacity (Gleitman 1971). A surprising event gets extensive processing in short term memory and that increases its chances of being stored in long term memory, so an event is surprising only if it is not already present in the short term memory. An event that is already present in short term memory before it happens is said to be primed. So priming reduces surprise (Wagner 1976, 1978).

Some forms of conditioning are forgotten more quickly than others (Hendersen 1978; Thomas 1979). But forgetting does not necessarily mean a permanent loss of information from the memory store. There are two major causes of forgetting: interference – when the target memory can not be retrieved because something learned at some point in time interferes with the target memory; retrieval failure in which the target memory in not accessed because the context has changed (Bouton 2007).

The aim of this study was to investigate the associative learning capacity of green toads, the discrimination capacity between a real prey and a fake one, and the retrieval capacity after two periods: hibernation and active life.

Materials and methods

Study subjects

<u>Group 1.</u> Used for testing of associative learning after hibernation and active life.12 *Bufo viridis* (7 males, 1 female and 4 juvenile) were used for this experiment. They were collected in the vicinity of Turda in March 7, 2010. The experiment lasted 7 months (March 8, 2010- September 30, 2010).

Group 2. Used for testing the discrimination abilities of toads between *Tenebrio molitor* larvae and worm-like stimulus. Consist of six adult and subadult toads (three males and three females) and eight juvenile collected in the same place as the first group. The experiment lasted between May 1 and July 27, 2010.

Prey species

Live larvae of *Tenebrio molitor* and crickets were used to feed toads during the experiment.

Experiment design

Group 1. Testing of associative learning after hibernation and active life

Habituation period and Associative learning (1) began on March 9 and ended on April 19. Toads were housed in separate boxes with gravel on the ground and water in small pots. Cleaning was performed every second day: gravel washing and water refreshing. In the same day, individuals were fed with *Tenebrio molitor* larvae in white plastic boxes. After this period the expected result was that toads will associate the *Tenebrio molitor* larvae with feeding boxes white colored (first association).

Associative learning (2) took place from April 23 until May 13. Each second day, every toad was tested being offered a moving worm-like stimulus imitating the *T. molitor* larvae in the housing box with gravel on the ground. Toads attacked many times the fake warm. All attacks were recorded with a Digital HD Camera Recorder Sony HDV 1080i. The experiment stopped when no attack was recorded in a 1 minute period. After each experiment, toads were fed with *T. molitor* larvae in the white plastic boxes, and then they were moved back in the gravel ones. Boxes cleaning was also performed every second day. The experiment stopped when none of the toads attacked the worm-like stimulus. The expected result consists of the association between the worm-like stimulus and the gravel boxes (second association).

Hibernation. Soon after toads performed the second associative learning, individuals were hibernated in boxes with moist dead leaves (two animals in each box), in a refrigerator with a controlled temperature of 5-6° C, from 17 May to 16 July. After this period they were placed back in the gravel boxes and in 18 and 19 July their memory was tested being offered the same worm-like stimulus, in order to verify if they remember the second. Then they were fed with *T. molitor* larvae in the white boxes. All attacks were recorded with the Digital HD Camera.

Active life. After two trials, toads were placed in large terrariums (L=100 cm; l=50 cm; h=50 cm), each of them with 6 toads, for a two months period, from 19 July to 26 September. Terrariums were equipped with earth and live plants, and large water supply. Toads were fed every 5 days with crickets (*Althea domestica*) and *T. molitor* larvae. In September 26 toads were housed again in the gravel boxes and tested by offering the same worm-like stimulus imitating *T. molitor* larvae every second day, until September 30. They were fed with *T. molitor* larvae after each experiment in the white boxes. All attacks were recorded with the Digital HD Camera.

Group 2. Testing the discrimination abilities of toads between Tenebrio molitor larvae and worm-like stimulus

Habituation period and Associative learning (1) began on May 1 and ended on May 31. Toads were treated in the same manner as group 1.

Associative learning (2) took place from June 1 until June 21. Toads were treated and tested in the same manner as group 1.

Testing the discrimination abilities took place between 23 June and 27 July and consisted of testing toads in the feeding white boxes with the worm-like stimulus in order to verify if they are able to distinguish the real larvae from the fake one. After each second trial day, toads were fed in the same white boxes.

Results

Group 1

Habituation period *and* ***associative learning***

After being collected from field, toads need a period to familiarize with the laboratory conditions. They were maintained all time in the gravel boxes, thus determining their habituation to this type of ground. They learned to associate the white boxes to *Tenebrio molitor* larvae (first associative learning).

Considering that after 40 days they are familiarized with all laboratory conditions, toads were tested with a worm-like stimulus in the housing boxes (gravel boxes). At the beginning the attack rate was high (26.67), but it decreased dramatically reaching a very low value after four trials: (average of attacks in 29 April=1.08). This was the actual associative learning period: toads associated the warm-like stimulus with the gravel ground. The six following trials do not change significantly the average of attacks, but in this period toads reinforce their associative learning, so in the last trials none of them attacked the stimulus (Fig. 1).

Two hypotheses are to be considered here:

(1) Discrimination between the real larvae and the warm-like stimulus was realized;

(2) An association was realized between the warm-like stimulus and the gravel ground.

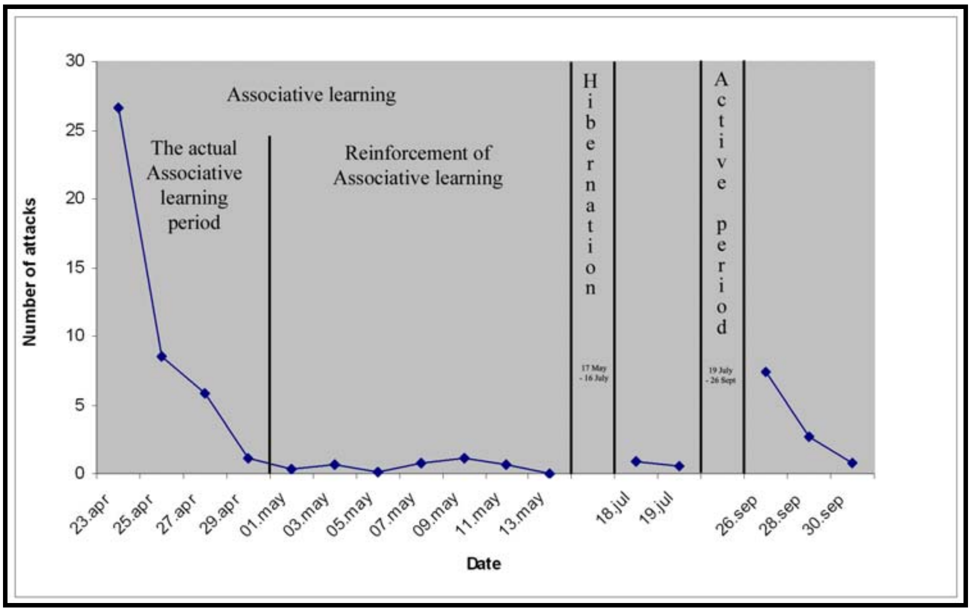

Figure 1. Average number of toad attacks on the worm-like stimulus in the gravel boxes, in different stages

We observed there are two types of behavior in these toads: individuals very reactive and individuals with low reactivity (Table 1). First ones had a high average of attacks for all 11 trials of the experiment (59.88 ± 19.90), and the second ones had a law average of attacks (17.75 ± 7.94). Both hypotheses are possible: (1) toads could learn after 17 attacks of a worm-like stimulus that it is a fake prey; (2) it is very probable they associate white colour of the feeding box with prey, and the gravel ground of the housing box with the fake warm.

A previous experiment (Brower et al. 1960) shows that *Bufo terrestris* is capable to associate a prey with a danger after only two trials.

Hibernation

Testing the associative memory after two months of hibernation was performed after moving each toad in a gravel box. After two days necessary for temperature accommodation, they were tested in the same conditions as before (Associative learning 2). Mann Whitney U test results between the averages of attacks in the reinforcement of associative learning period compared to post hibernation period: U=66.0; Z=-0.34; p=0.71 do not show significant differences. These results prove that two months of hibernation do not affect the associative learning.

Table 1. Sum of attacks of green toad (*Bufo viridis*) to worm-like stimulus in 11 trials of the experiment.

	Sum of attacks in the 11 experiments	
Nr. and sex of animal	Individuals with high reactivity	Individuals with low reactivity
1M		14
2M	38	
3M	44	
4M		28
5M	61	
6M		13
8J		16
9J	59	
10J	70	
11 M	97	
13F	71	
14J	39	
mean	**59.88**	**17.75**
stdev	19.90	7.94

Active life

After two months of active life toads were still capable to associate the worm-like stimulus to the gravel ground. In the first day of experiment, the average attack rate was 7.42 but in the next days the value decreased up to 0.75, proving that toads were capable to recall memory. If they had forgotten the association between the worm-like stimulus and the gravel ground, the rate of attacks would have to be

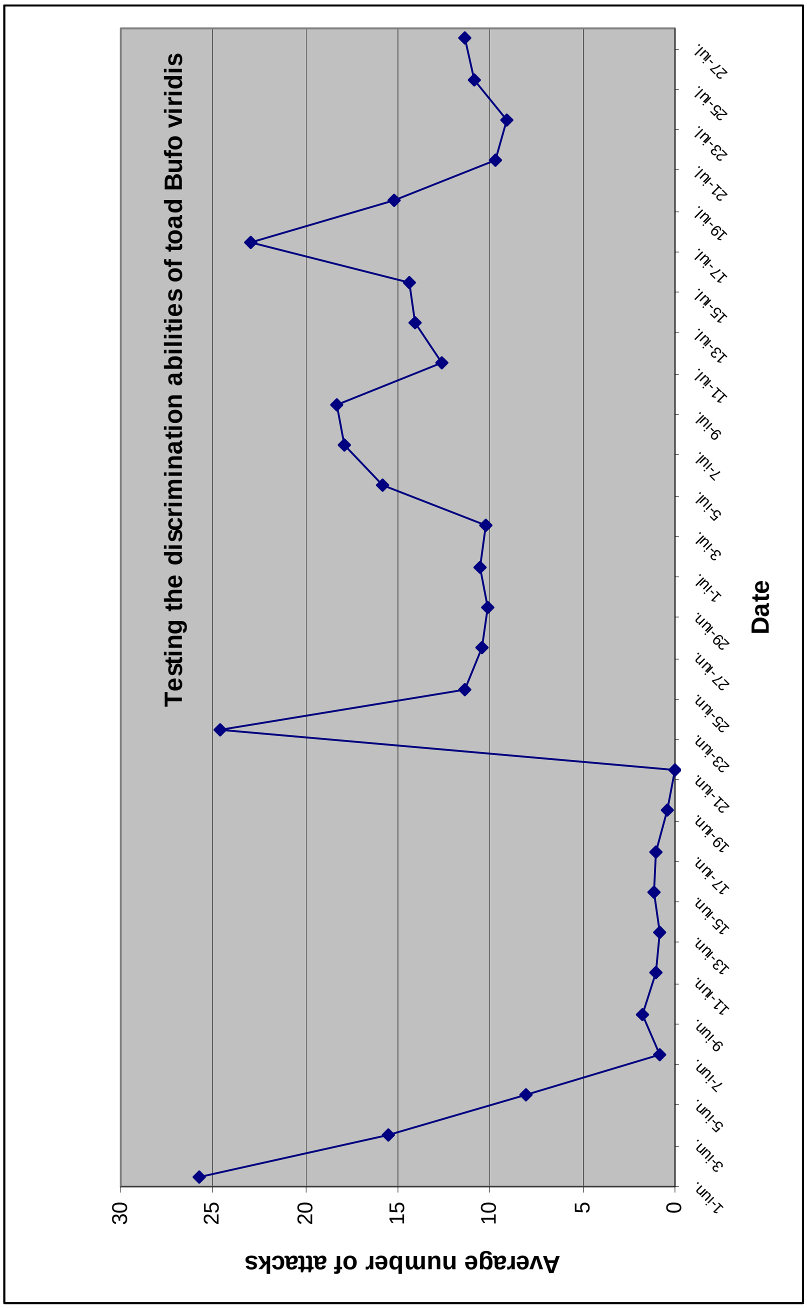

Figure 2. Average number of attacks on the worm-like stimulus in the gravel boxes (associative learning (2) and testing the discrimination abilities in toad *Bufo viridis*.

similar to that in the beginning of the experiment. Mann Whitney U test results show significant differences in the number of attacks between 23 April and 26 September (U= 11.5; Z=3.49; p=0.0004**).

Group 2

Habituation period and Associative learning (1) was identical to group 1.

Associative learning (2) was very similar to the same stage in the group 1 (Fig. 2). After eleven trials, none of adult or juvenile toads attacked the worm-like stimulus.

Testing the discrimination abilities. After first trial, the average attack number on the worm-like stimulus was very similar to the average in the first day of associative learning. This result proves that:

- toads are not capable to discriminate between real larvae and worm-like stimulus;
- toads associated food with the white box.

Beginning with the second day of experiments the average of attacks decreased to a value very variable from trial to trial, being confused because they received in the same white boxes real larvae and worm-like stimulus.

Toads use odor in orientation (Shakhparonov and Ogurtsov 2005) and their prey-catching behavior increases greatly in the presence of prey-odor (Dole et al. 1981, Ewert 1984, Ewert et al. 2001). Our experiments prove that if toads associated a food item to a certain feature of the environment, they do not discriminate visually or olfactory the real pray from the fake one.

References

Annau, Z., Kamin, L.J. (1961): The conditioned emotional response as a function of intensity of the US. Journal of Comparative and Physiological Psychology 54: 428-432

Bouton, M.E. (2007): Learning and Behavior. Sinnauer Associates Inc., Sunderland Massachusetts.

Dole, J.W., Rose, B.B., Tachiki, K.H. (1981): Western toads (*Bufo boreas*) learn odor of prey insects. Herpetologia 37: 63-68.

Ewert, J.P. (1984): Tectal mechanisms that underlie prey-catching and avoidance behaviors in toads. pp.247-416. In: Vanegas, H. (ed.), Comparative Neurology of the Optic Tectum. New York: Plenum.

Ewert, J.P., Buxbaum-Conradi, H., Dreisvogt, F., Glagow, M., Merkel-Harff, C., Rottgen, A., Schurg-Pfeiffer, E., Schwippert, W.W. (2001): Neural modulation of visuomotor functions underlying prey-catching behavior in anurans: Perception, attention, motor performance, learning. Comparative Biochemistry and Physiology, part A 128: 417-461.

Gleitman, H. (1971): Forgetting of long-term memories in animals. In: Honig, W.K., James, H. (eds), Animal Memory. New York, NY: Academic Press.

Goodenough, J., McGuire, B., Wallace, R.A., Jakob, E. (2009): Perspectives on Animal Behavior. John Wiley and Sons Inc.

Hendersen, R.W. (1978): Forgetting of conditioned fear inhibition. Learning and Motivation 9: 16-30.

Morris, R.W., Bouton, M.E. (2006): Effect of unconditioned stimulus magnitude on the emergence of conditioned responding. Journal of Experimental Psychology Animal Behavior Processes 32(4): 371–385.

Olton, D.S. (1979): Mazes, maps and memory. American Psychologist 34(7): 583-596.

Shakhparonov, V.V., Ogurtsov, S.V. (2005): The role of the native pond odor in orientation of the green toad (*Bufo viridis* LAUR.) youngs-of-the year. pp.209-212. In: Ananjeva, N., Tsinenko, O. (eds), Herpetologia Petropolitana, Proceedings of the 12th Ordinary General Meeting of the Societas Europaea Herpetologica: 12-16 August 2003, Saint-Petersburg, Russia.

Thomas, D.A. (1979): Retention of conditioned inhibition in a bar-press suppression paradigm. Learning and Motivation 10: 161-177.

Wagner, A.R. (1976). Priming in STM: An information-processing mechanism of self-generated or retrieval-generated depression in performance. pp. 95-128. In: Tighe, T.J., Leaton, R.N. (eds), Habituation: Perspectives from child development, animal behavior and neurophysiology, Hillsdale. NJ: Lawrence Erlbaum Associates, Inc.

Wagener, A.R. (1978): Expectancies and the priming of STM. pp.177-210. In: Hulse, S.H., Fowler, H. &, Hoing W.K. (eds), Cognitive aspects of animal behavior. Hillsdale, NJ: Erlbaum.

Wyers, E.J., Peeke, H.V.S, Herz, M.J. (1973): Behavioral habituation in invertebrates. pp.1-57. In: Peeke, H.V.S., Herz, M.J. (eds), Habituation Vol. 1, Behavioral Studies. Academic Press, New York.