Classical Questions in an Operant Learning Paradigm

Björn Brembs, Reinhard Wolf and Martin Heisenberg
Theodor-Boveri-Institut für Biowissenschaften
(Biozentrum) Lehrstuhl für Genetik
Am Hubland, 97074 Würzburg

I. Introduction

Sensory preconditioning, blocking, overshadowing, second-order reinforcement, these are all terms that were coined by learning psychology more than 20 years ago and are well established using classical conditioning setups. Classical conditioning is often described as the transfer of the response-eliciting property of a stimulus to a new stimulus without that property. This association between an unconditioned stimulus (US) and a conditioned stimulus (CS) can also be established when the animal is in control of the stimulus presentation (operant conditioning). We have not found a single report as to whether the above mentioned concepts also apply to operant conditioning. We used operant pattern and color learning in the *Drosophila* flight simulator (see central figure) to ask five basic questions:

(1) **US processing**: How do variations in US strength translate into associative strength?
(2) **CS Processing**: How do different CSs translate into associative strength?
(3) **Blocking**: Is it possible to systematically prevent a reinforced CS from being learned?
(4) **Second-order conditioning**: Can a well trained CS act as a US?
(5) **Sensory preconditioning**: Can a CS be learned without reinforcement?

II. Operant Conditioning at the *Drosophila* Flight Simulator

(1) We assayed **US Processing** by manipulating the intensity of the heat beam (US). The flies were trained to distinguish two orientations (upright and inverted) of four T shaped patterns (CS see central figure).

(2) For the **CS Processing** experiments, the coloration of the arena (CS2) was changed whenever the fly brought one of the two pattern orientations (CS1) into its frontal visual field (compound training, CS1+CS2+US). In the subsequent test phase, either the color filter was removed (patterns, CS1 alone), or the patterns were replaced by four identical vertical bars (colors, CS2 alone), or the pattern orientation associated with one color during training was reversed (nonsense CS). If similar positive scores in both CS alone tests and no learning in the nonsense CS test are obtained, it is concluded that no overshadowing occurred between the two CSs and both are learned equally well when presented in a compound.

Training	Test
CS1+CS2+US	CS1 alone
CS1+CS2+US	CS2 alone
CS1+CS2+US	nonsense CS

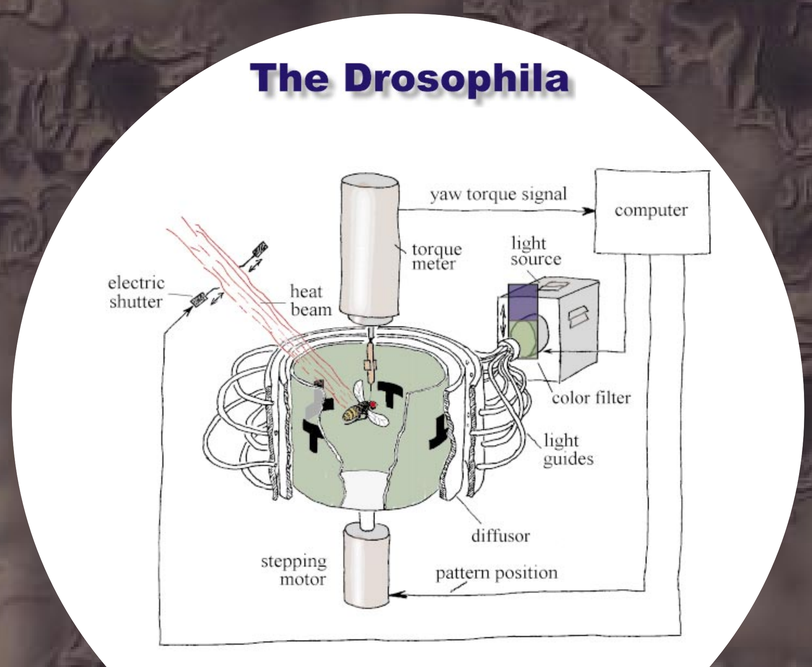
(3) We used a standard **blocking** scheme: The first CS was pre-trained to the maximum extent, then a compound training of equal duration followed. To assure the predictive value of the pre-trained CS, an intermitting test for the compound CS was introduced before the compound training. Additional controls were balanced for CS and US presentations.

Pre-Training	Test1	Test2	Training	Test
CS2+US	CS2=100%	CS1+CS2	CS1+CS2+US	CS1 alone
CS1+US	CS1=100%	CS1+CS2	CS1+CS2+US	CS2 alone

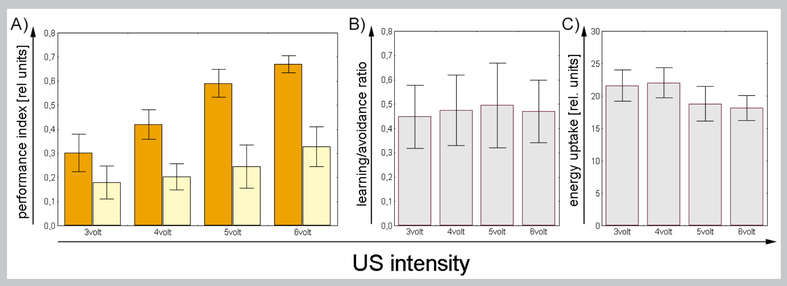
(4) In order to control for **second-order conditioning** occuring in our blocking experiment, we used several different procedures to find a second-order effect.

(5) For **sensory preconditioning**, the flies were allowed to fly in closed loop for 16 minutes (pre-conditioning). The coloration of the arena was changed whenever the fly brought one of the two pattern orientations into its frontal visual field (CS1+CS2). In the subsequent training phase, either the color filter was removed (patterns, CS1+US), or the patterns were replaced by four identical vertical bars (colors, CS2+US). In the final phase, the flies were tested for the CS

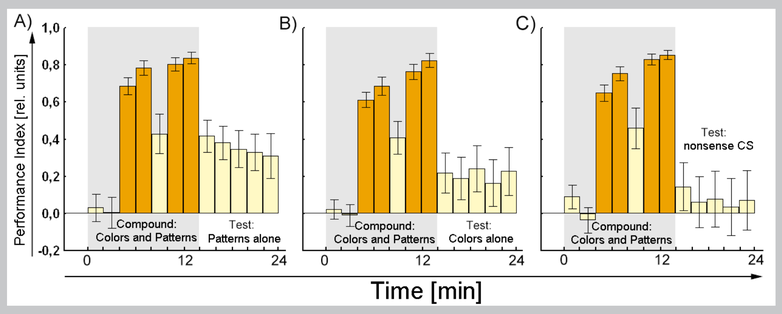
Pre-Conditioning	Training	Test
CS1+CS2	CS1+US	CS2
CS1+CS2	CS2+US	CS1



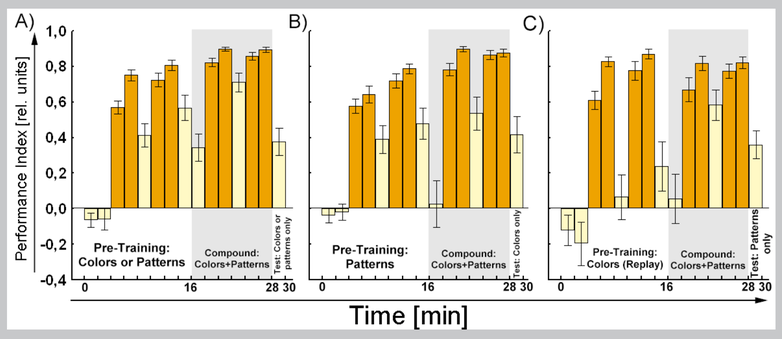
III. Operant Answers to Classical Questions



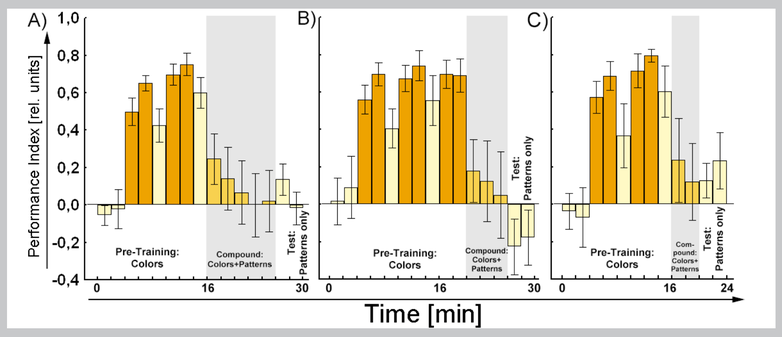
(1) **US Processing:** We were interested in the dependence of learning performance on the strength of reinforcement. To manipulate US intensity we increased the voltage running the microscope lamp that generates the heat beam in the flight simulator setup. A) Orange bars depict mean performance indices during training and yellow bars depict mean learning indices. B) The learning to avoidance ratio remains roughly constant over increasing US intensities. This might indicate a common dependence of avoidance and learning on reinforcement. C) We estimated the amount of energy uptake of the fly by multiplying the time the fly spent in the heat beam with the temperature measured at the focus of the beam after 10s of heating.



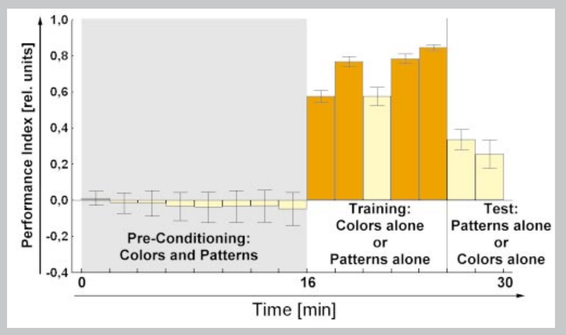
(2) **CS Processing:** After compound training of colors and patterns, either patterns alone (A) or colors alone (B) were tested. In both cases, significant learning scores were obtained, which could not be distinguished from each other statistically. To control for a direct overshadowing effect of one CS over the other, the relation between colors and patterns was reversed in the final test phase (C). No CS seemed to acquire a stronger associative strength when the other was present.
Orange bars - training; yellow bars - test.



(3) **Blocking:** In contrast to current learning theory, operant pre-training of one CS did not diminish the associative strength a second CS acquired when trained in compound with the first (A). The controls (B, C) are balanced for CS and US presentations and differ from the test group in the low predictive value the compound CS obtained (first test bar in the area shaded gray). No significant difference was found between test and control groups. Specifically, in no instance were the learning scores in the blocking groups lower than in either control groups or the respective CS Processing (2) groups.
Test groups were pooled, since no apparent difference in the performance indices was observed. Orange bars - training; yellow bars - test.



(4) **Second-order Conditioning:** One reason for the failed blocking experiment depicted in (3) might be the effect of second-order conditioning occuring in the compound training phase. This could not be corroborated since several different procedures did not yield a significant second-order effect.
Orange bars - training; yellow bars - test.



(5) **Sensory Preconditioning:** Unreinforced pre-exposure of the colors (CS2) and patterns (CS1) compound (area shaded gray) yielded significant learning scores in one CS (test patterns or colors alone) when the other CS had been paired with the reinforcer inbetween (training colors or patterns alone).
Data have been pooled since the performance indices in both experimental groups did not differ significantly.

IV. Summary

(1) Increasing the amount of reinforcement from a level already sufficient to induce a significant learning response (thus assessing **US processing**), resulted in an increase in learning performance. With increasing strength of the reinforcer, the flies kept the ratio between avoidance and subsequent learning score constant.
(2) Training the flies to a compound CS1CS2 (CS1: pattern, CS2: color. Both single CSs are learned equally well when trained without the other) results in similar learning scores for the single CSs in a subsequent test of patterns or colors alone (no overshadowing in compound **CS processing**).
(3) Most error-correcting learning rules predict that pre-training one of the CSs before a CS1CS2 compound training prevents subsequent learning of the second CS during compound training even though it receives the proper reinforcement. This **blocking** effect is known from most classical conditioning preparations. Our learning scores, however, were indistinguishable from the control experiments. This is at odds with all current learning theories.
(4) This result can not be attributed to **second-order conditioning** with the pre-trained CS as second-order reinforcer since this effect is negligible in our setup.
(5) Pre-exposure of a compound CS1CS2 without reinforcement before training one of the CSs leads to a significant learning score in a subsequent test of the respective other CS even though this CS has never been reinforced (**sensory preconditioning**).