Occasion setting in *Drosophila* at the flight simulator

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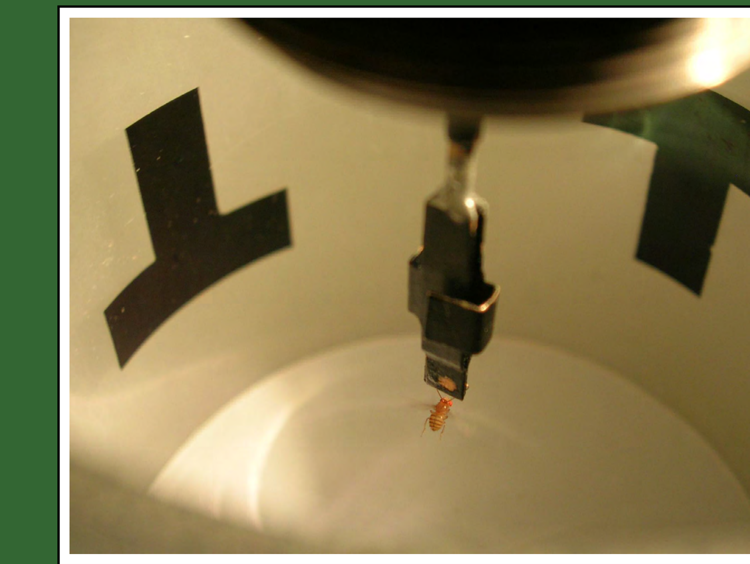


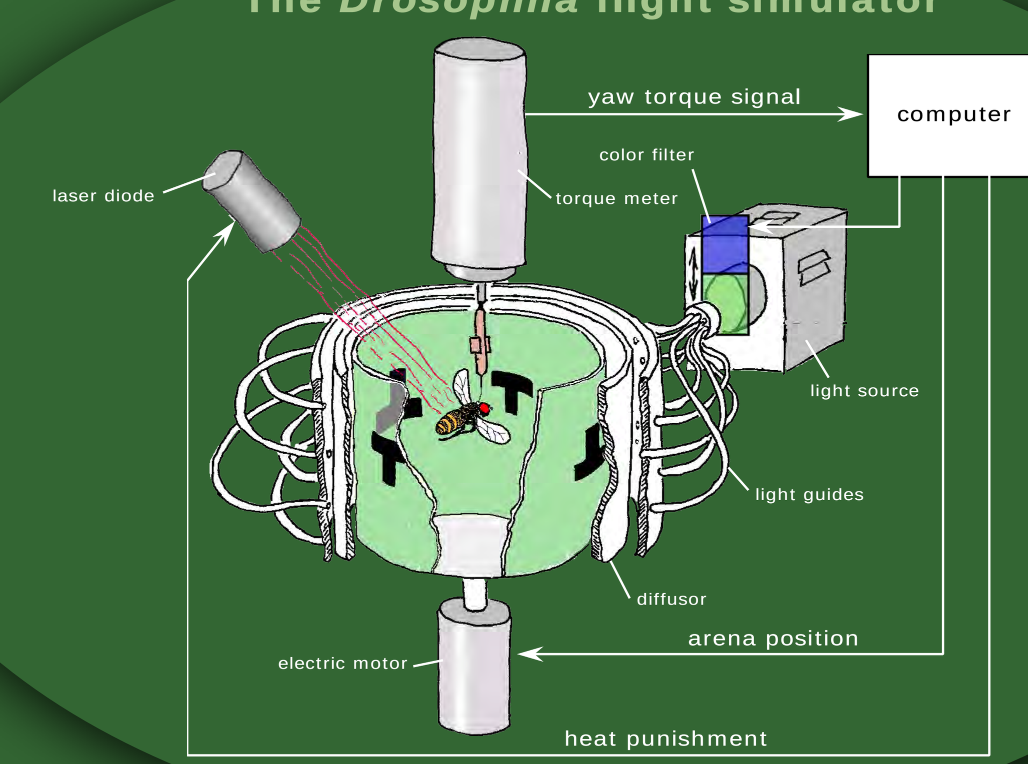
Introduction

Παντα ρει και ουδεν μενει - Everything flows, nothing stands still (Heraclitus). Rapid changes in environmental contingencies require flexible capacities through which organisms can come to expect biologically significant events (unconditioned stimuli, US) and modify the behavior in anticipation of those events if behavior is to remain adaptive. In a dynamic environment, some stimuli can predict the occurrence of single USs (conditioned stimuli, CS), others may indicate the nature of the CS-US contingency (occasion setters, OS) and again other may be present without having any relationship to the US whatsoever ('context'). Thus, in order to be able to form an accurate expectation of future USs, animals have to extract from the universe of sensory signals the actual predictors by separating them from non-predictive stimuli.
Tethered *Drosophila* can be trained to avoid heat punishment (US). We have used the unique environmental control the *Drosophila* flight simulator affords to highlight the role of the temporal relationship of initially neutral stimuli (context, CS and OS) and the US, and its consequences for the acquisition of predictive memory in wildtype and transgenic flies. In differential conditioning of visual patterns animals learn to avoid one visual pattern and to prefer another. Changing the background color between training and test periods does not disrupt performance Fig. 1a, 4c). The color remains constant during training and thus the patterns are the sole reliable predictors of reinforcement: the colors fulfill the definition of context. Wildtype flies can generalize the pattern memory across certain contexts, while flies with impeded mushroom body function fail in context generalization (Figs. 1a and 2a).

Questions:

1. Can enhancing the temporal relationship of the colors to the US change their status from context to occasion setters? (Fig. 1b-g)
2. Are the generalizing colors suitable as CS? (Fig. 1h, 5c)
3. How are the mushroom bodies involved in these tasks? (Fig. 2)
4. How does the choice of colors affect these experiments? (Figs. 3-6)
5. What do the answers to these questions teach us about the organization of learning experiments in general?

The *Drosophila* flight simulator



Establishing the paradigm

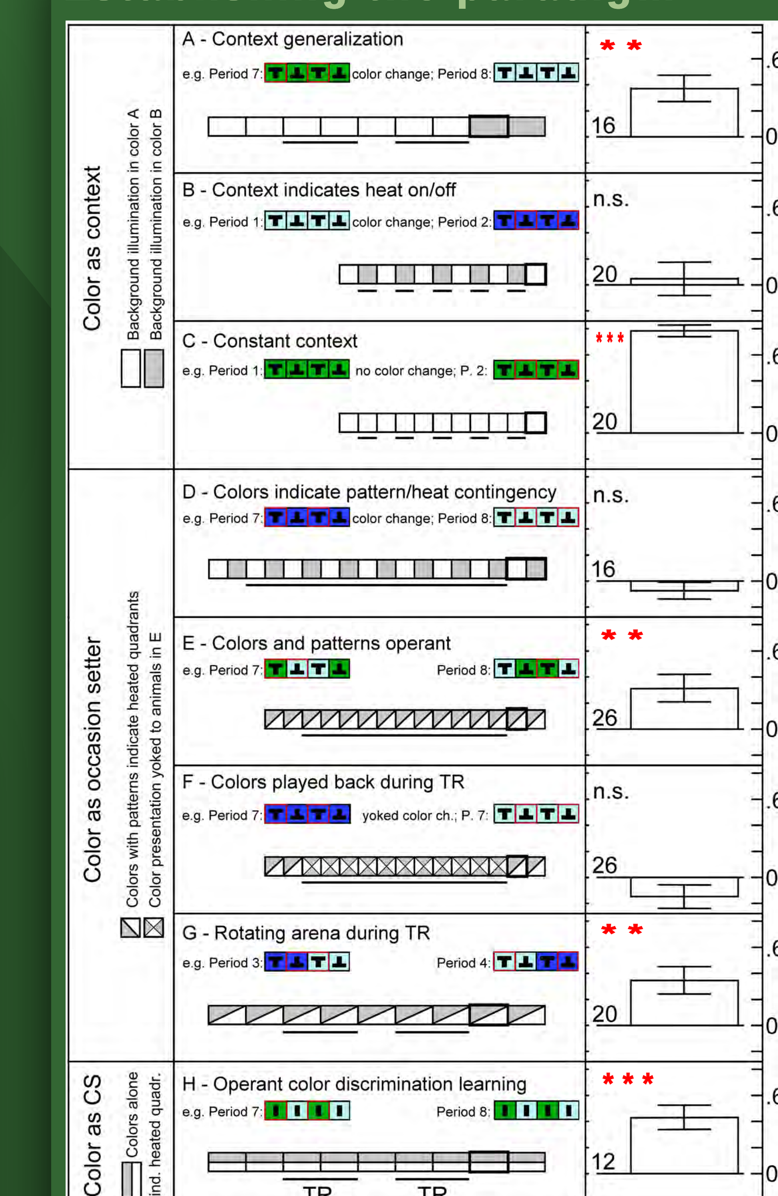
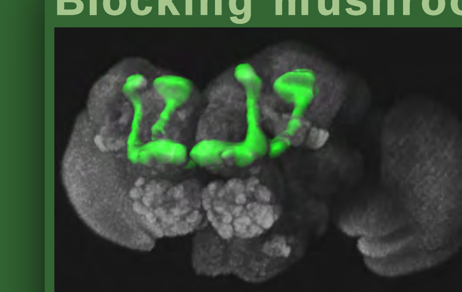
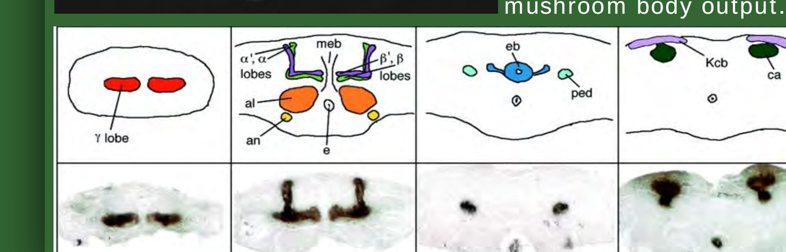


Fig. 1: Colors can be context, occasion setters and conditioned stimuli, depending on the temporal arrangement with the unconditioned stimulus.
A-C - Color as context. The color of background illumination during operant visual pattern discrimination learning changes according to the experimental schedule. A - Context generalization. A single change of background illumination after the final training period marks the beginning of a 2-min. test period for pattern memory in the new background color. The flies are able to show pattern memory in the new context. B - The color changes are concomitant with the change from training to test periods. Increasing the number of context changes with respect to A abolishes the generalization effect. C - Control group in which training and test periods alternate in constant background color. Alternating training and test periods as in B does not abolish the memory score.
D-G - Color as occasion setter. D - Color changes indicate the reversal of the pattern-heat contingency. Reversal learning cannot be facilitated by context changes. E-G: Colors change independently of the experimental schedule and indicate heated quadrants in conjunction with visual patterns. E -Flies can solve a fully operant occasion setting paradigm. F - Flies fail to solve an occasion setting paradigm, where the color presentations are yoked to the animals in E. G - Flies can solve occasion setting with classical training and operant test periods.
H - Color as CS. The colors used in this study can be discriminated by wildtype flies in an operant visual learning task, with the colors as conditioned stimuli.
Colored boxes with patterns illustrate the experimental design.
White/grey squares indicate 1-min. periods, rectangles 2-min. periods in the experimental time course. The performance indices of the highlighted test periods (bold) are displayed in the bar-graphs on the right. *** - p < 0.001; ** - p < 0.01; * - p < 0.05; n.s. - not significant. Numbers next to bar graphs indicate number of animals. Lines under experimental periods (indicated by 'TR' in H) denote training periods.
Colors: Blue or green and bluegreen.

Blocking mushroom bodies



The mushroom bodies (left) are a prominent insect neuropil. We crossed the GAL4 driver line mb247 (below) to a line expressing the tetanus toxin light chain under the UAS promoter to obtain offspring with blocked mushroom body output.



Transgenic flies

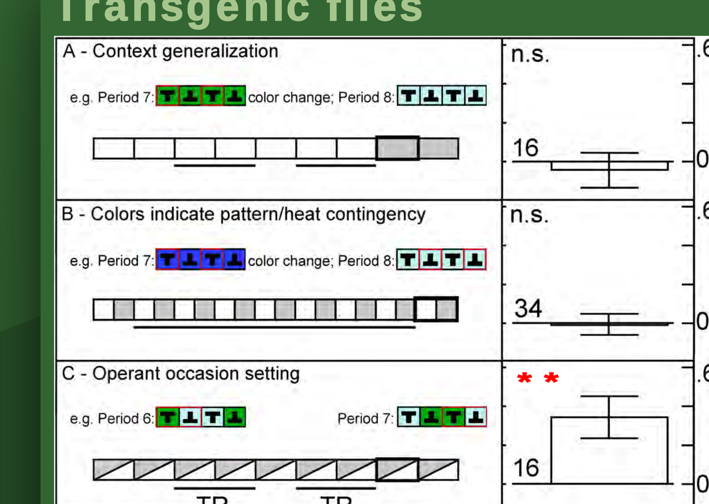
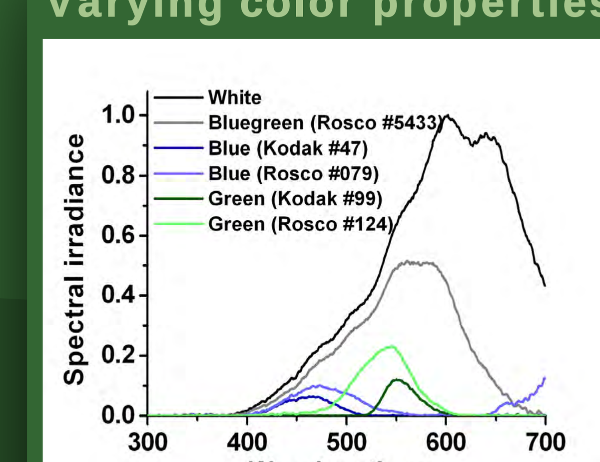


Fig. 2: Flies with blocked mushroom body output fail in context generalization and classical occasion setting but perform in operant occasion setting.
A - Context generalization as in Fig. 1a.
B - Colors indicate which T-Pattern is heated as in Fig. 1d.
C - Fully operant occasion setting as in Fig. 1e, but with altered period set-up and duration.
Colors: Blue or green and bluegreen.

Varying color properties



In order to investigate the physical boundary conditions for extracting predictive stimuli from the environment, we studied three pairs of colors in various learning tasks:
1) **Colors without overlap** in the spectral irradiance (Kodak blue and green).
2) **Colors with a partial overlap** (Rosco blue and green).
3) **Colors with full overlap** (Kodak blue or green with Rosco bluegreen).

After the experiments, brightness and chromaticity (see Table) were defined from receptor signals which were calculated by integrating spectral sensitivity of *Drosophila* rhodopsins and illumination functions. We assumed that the central photoreceptors mediate color perception and used a general model of color vision (Vorobyev & Osorio 1998 Proc.R.Soc.B).

Occasion setting

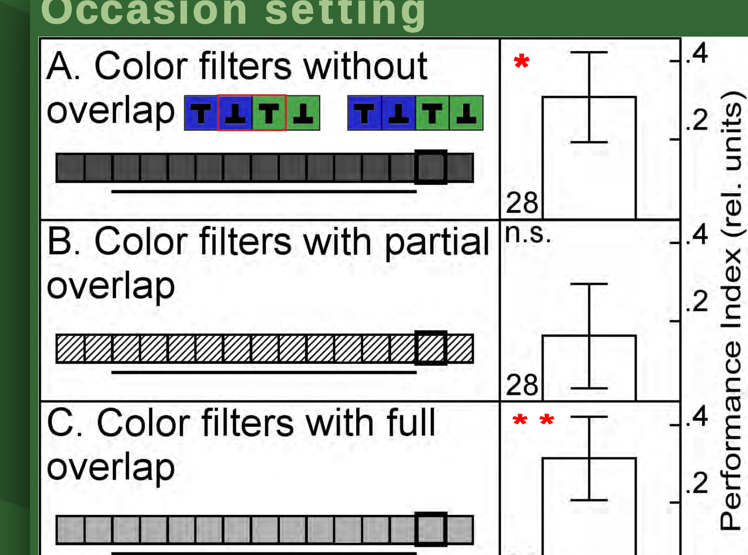


Fig. 3: Occasion setting with the three different color pairs. Only the color pair with partially overlapping spectra does not support occasion setting.

Context generalization

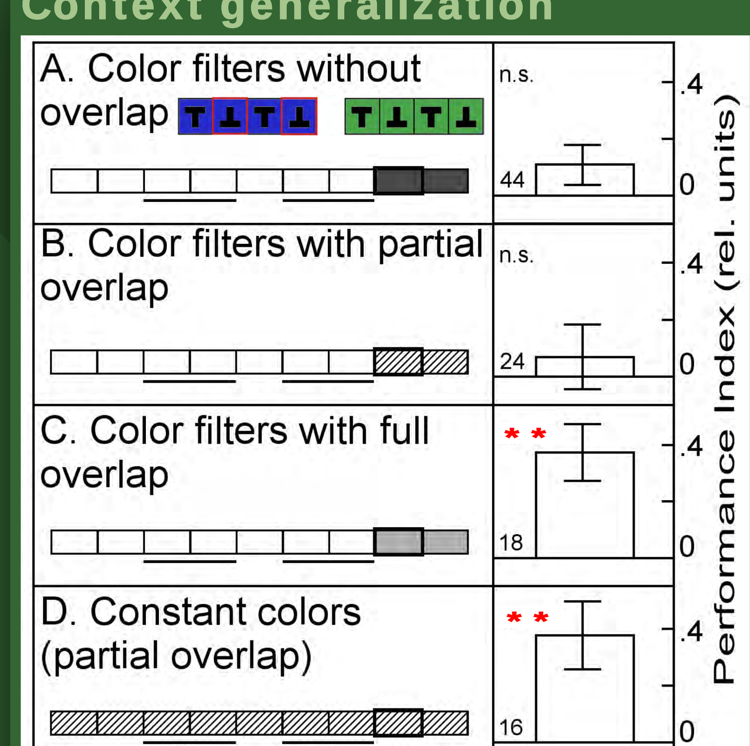


Fig. 4: *Context generalization.* A-C: Context generalization with the three color pairs (pattern training in one of the two colors and test in the other). Only the color pair with full spectral overlap supports context generalization. D - Pattern learning is unaffected if the background coloration is kept constant (color pair with partial overlap).

Color learning and generalization

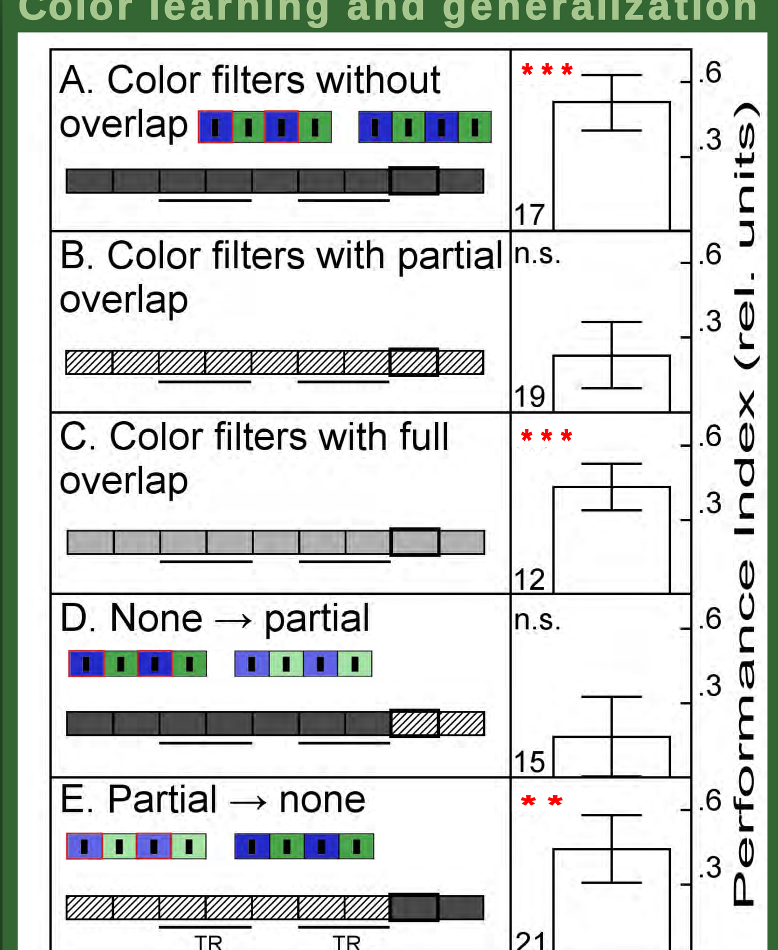


Fig. 5: *Color learning and generalization across different colors as CSs.* A-C: Color learning with the three color pairs. D-E: Reciprocal generalization between the color pair with partial and the one with no spectral overlap. Training one color pair and testing the other only yields a significant learning score only in one direction: If the colors with partial overlap are trained the animals can express the conditioned color preference only with the color-pair with no spectral overlap.

Compound conditioning

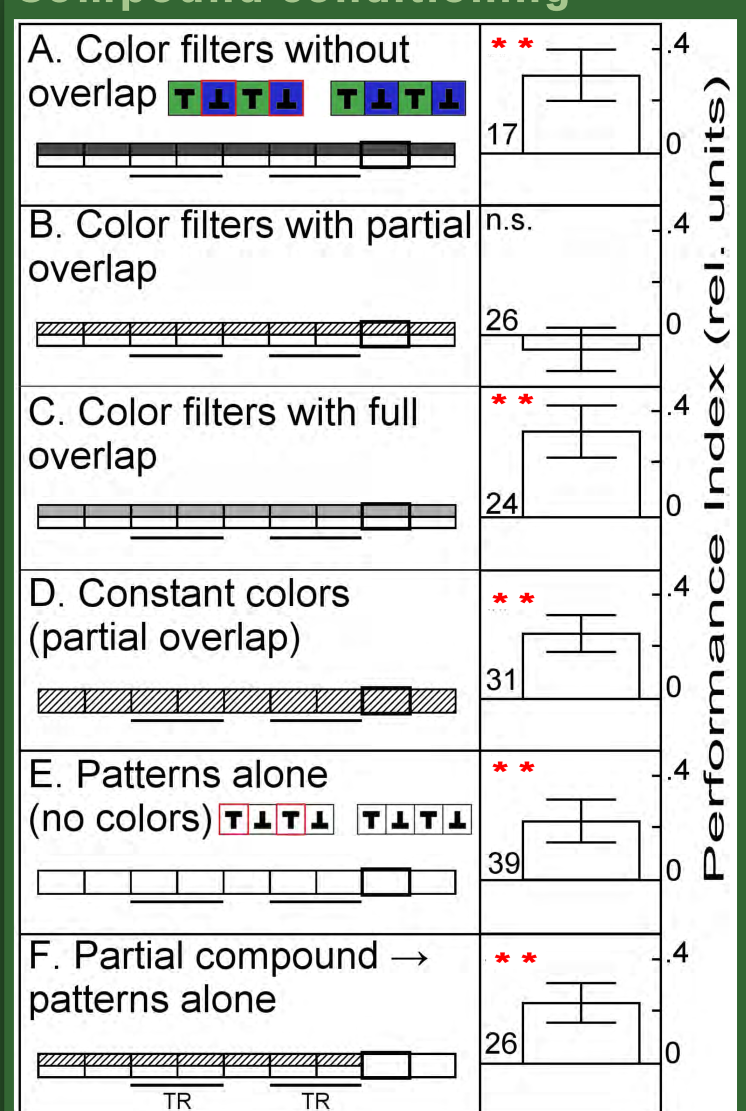


Fig. 6: *Color and pattern compound conditioning.* A-C: Pattern/color compound conditioning with the three color pairs. D - Pattern learning in constant colors (partial overlap). E - Pattern learning without colored background. F - Training in pattern/color compound (partial overlap as in C) and final test with the color filter removed (as in E).

Answers:

1. Can enhancing the temporal relationship of the colors to the US change their status from context to occasion setters? (Fig. 1b-g)
Answer: Yes, the classification of a stimulus depends mainly on its temporal relationship with the reinforcer (Fig. 1a, e, g). Even flies without mushroom bodies are able to learn the occasion setting task, albeit not better than wildtype flies (Fig. 2).
2. Are generalizing colors suitable as CSs? (Fig. 1h, 5c)
Answer: Yes, even colors across which pattern memory can be generalized are suitable as CSs and can be differentiated (Fig. 1h, 5c). On the other hand, not all non-generalizing colors are suitable as CSs (Fig. 5b).
3. How are the mushroom bodies involved in these tasks? (Fig. 2)
Answer: It appears, mushroom bodies specifically enhance the stability of memory traces despite changes in the stimulus situation and do not decrease the ability to detect such changes.
4. How does the choice of colors affect these experiments? (Figs. 3-6)
Answer: Performance in the various learning tasks was highly dependent on the spectral properties of the background colors used. However, spectral properties alone cannot explain all of the variability. The flies' subjective perception of the colors has to be computed for a full picture. Colors carry both color and brightness cues which result in a unique percept but can mediate different parts of the behavioral output.

Stimuli	Color difference	Log Brightness difference (Σ R7-8)	Log Brightness difference (Σ R1-6)	Overlap	Color	Brightn.
Kodak blue - Kodak green	6.9	0.78	1.52	none	large	large
Rosco blue - Rosco green	3.3	0.17	0.07	partial	small	small
Kodak blue - Rosco bluegreen	2.9	1.93	1.77	full	small	large
Kodak green - Rosco bluegreen	6.0	2.71	3.29	full	large	large
Rosco blue - Kodak blue	1.7	0.59	0.65	full	small	small
Rosco green - Kodak green	5.4	1.54	2.09	full	large	large

For generalization across two colors, their spectra must overlap sufficiently. With full overlap, generalization occurs despite even relatively large color or brightness differences. With only partial overlap, even small color and brightness differences prevent generalization (Figs. 4, 5e). Possibly, this overlap is required in order to stimulate a comparable set of receptors. For discrimination of two colors (Figs. 3, 5a-c, 6), there has to be a large brightness or color difference. Spectral overlap seems to have only minor effects on discriminability. Colors with partial spectral overlap and only small color and brightness differences can be distinguished (Fig. 4b, 5d), but can themselves not be learned and may even prevent the expression of pattern memory (Fig. 6b, f). This is reminiscent of similar results using different pairs of visual patterns.
5. What do the answers to these questions teach us about the organization of learning experiments in general?
Answer: The formation of memory templates after conditioning is more complex than initially anticipated. Great attention must be paid to the details of the physical properties of the stimuli present in a learning experiment and of their temporal relationship to the US.

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