Operant and classical conditioning of *Drosophila* at the flight simulator

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CONTENTS

*"Under carefully controlled experimental circumstances,
an animal will behave as it damned well pleases."*
Harvard Law of Animal Behavior

1 Introduction

The brain's primary objective is to carry out certain adaptive behaviors. It is fine-tuned by evolution to safely govern its carrier through life and to achieve successful reproduction. There are two alternatives to accomplish this task: by innate behavior programs (e.g. reflexes, stimulus-response chains etc.) adapted by evolution or acquired behavioral traits, adapted by experience.

This distinction is not new: in the 18th century, part of the empiricist philosophy of Locke (1689) was the assertion that individuals were born with a *tabula rasa* and only experience could establish mind, consciousness and the self. On the continent, Leibniz envisaged the self as a monad carrying some knowledge of a basic understanding of the world. The discussion as to whether nature or nurture are the driving force shaping cognitive abilities was for a long time considered to be interminable. Until the 1960s this dispute was still very vivid in the behavioral sciences: in the tradition of the English empiricists, Skinner's school of behaviorism postulated general rules for all types of learning, neglecting innate differences or predispositions. Lorenz was one of the protagonists of ethology in Europe, which focused on the inherited aspects of behavior. It was Lorenz who ended these antagonistic views of behavior in showing that there indeed are innate programs ("fixed action patterns") and predispositions in behavior where only little learning occurs. Today, it is largely agreed upon that nature and nurture are intimately cooperating to bring about adaptive behaviors. Probably only in very few cases ontogenetic programs are not at all subjected to behavioral plasticity. Conversely, the possibility of acquiring behavioral traits has to be genetically coded for.

1.1 Learning

Nevertheless, acquired behavioral traits can in principle be distinguished from inherited programs operationally: whereas many innate behaviors are displayed even in total deprivation of experience („Kaspar-Hauser" experiments), learned behavior is always absent under such circumstances. Consequently, learning can be defined as the process by which an organism benefits from experience so that its future behavior differs from that of a comparable organism lacking this experience.

Typically, studies of learning compare the behavior of two subjects at two times. At a time t_1 the individuals share the same experience and thus do not differ in performing the behavior in question. At a later time (t_2), the behavior of the same subjects is compared again: one of the subjects has in the meantime been exposed to the experience of interest (most commonly the presentation of one or several stimuli), whereas the other was spared this particular exposure and instead received a control treatment. Learning is assessed according to the difference in the behavior of the subjects in t_2.

By convention, learning is classified operationally into three types of stimulus presentation between t_1 and t_2:

1. Presentation of the stimulus alone. $\rightarrow$ habituation, sensitization (non-associative learning).
2. Presentation of the stimulus in relation to another stimulus. $\rightarrow$ classical conditioning (associative learning)
3. Presentation of the stimulus in relation to some of the organisms own behavior. $\rightarrow$ operant conditioning (associative learning)

Since the present work is concerned with a comparison of classical and operant conditioning, these are examined more closely.

1.2 Classical Conditioning

The term "classical conditioning" is used here to describe a type of associative learning in which there is no contingency between response and reinforcer. This situation resembles most closely the original experiment of Pavlov (1927), who trained dogs to associate a tone with a food-reward. In such experiments, the subject shows a weak or no response to a *conditioned stimulus* (CS, e.g. a tone), but a measurable *unconditioned response* (UR, e.g. saliva production) to an *unconditioned stimulus* (US, e.g. food) at t_1. In the course of the training, the CS is repeatedly presented together with the US; eventually the subject forms an association between the US and the CS. In a subsequent test-phase (t_2), the subject will show the *conditioned response* (CR, e.g. saliva production) to the CS alone, if such an association has been established and memorized. The subject is said to have learned about salient contingencies in the world. Control subjects usually receive unpaired CS and US presentations or CS and US alone. Such "Pavlovian" conditioning is opposed to instrumental or "operant conditioning", as described below (1.3), where producing a CR controls the US presentations.

Findings from Kandel and coworkers (Kandel et al. 1983; Hawkins et al. 1983; Carew et al. 1983; Ca-

rew and Sahley, 1986 and references therein) investigating the cellular and molecular processes underlying classical conditioning in *Aplysia* suggest that the US is 'replaced' by the CS during training: **simultaneous** stimulation of the sensory neuron receiving the CS (SN_1) from the sensory neuron receiving the US (SN_2), facilitates synaptic efficacy of the SN_1 presynaptically (Fig. 1). After a few conditioning trials, stimulation of the SN_1 **alone** elicits the reflexive behavior - the UR eliciting properties of the reinforcer have been transferred to SN_1.
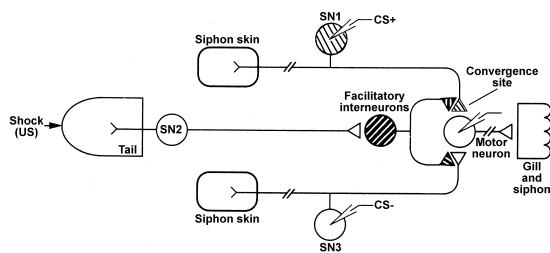

Fig. 1: Schematic diagram of the classical conditioning model inferred from evidence in *Aplysia*. According to the model, activity dependent presynaptic facilitation (ADPF) is induced by the conjoint firing of the sensory neurons of the CS+ pathway and the facilitatory interneurons mediating the US. After conditioning, firing of SN1 alone will activate the motor neuron. CS+ - conditioned stimulus contiguous with the US; CS- - conditioned stimulus unpaired with the US; SN1 - sensory neuron receiving the CS+, SN2 - sensory neuron receiving the US; SN3 - sensory neuron receiving the unpaired control stimulus CS-. (Redrawn from Glanzman ,1995)

1.3 Operant Conditioning

The term "operant conditioning" is used here to describe a type of associative learning in which there is a contingency between the presentation of response and reinforcer. This situation resembles most closely the classic experiments of Skinner (1938), where he trained rats and pigeons to press a lever in order to obtain a food reward ("Skinner-Box"). In such experiments, the subject is often able to generate a variety of motor-outputs. The experimenter chooses a suited output (the *response* R, e.g. pressing a lever) at t_1 to pair it with an *unconditioned stimulus* (US, e.g. a food reward). Often a *discriminative stimulus* (SD, e.g. a light) is present when the R-US contingency is true. After a training period (at t_2), the subject will show enhanced R - the *conditioned response* (CR, e.g. pressing a lever) - even in absence of the US if the R-US association has been memorized. The subject is said to have learned about salient contingencies between its own behavior and some part of the world. Such instrumental or operant conditioning is opposed to Pavlovian or "classical conditioning", as described above (1.2), where producing a response has no effect on US presentations. This fundamental difference has an important temporal consequence: whereas it is *per definitionem* of no value for the

subject to make any associations *during* classical conditioning - it will receive the US anyway - operant behavior provides the subject with a means to optimize its situation already during acquisition. In other words: classical conditioning can be perceived as passive learning from events in the past, while operant conditioning implies learning to behave in the present and the future.

This view is reflected in the work of Wolf and Heisenberg (1991), who have further analyzed the process of operant conditioning in *Drosophila* and propose a basic model of operant behavior (Fig. 2):

1. Operant behavior requires a goal (desired state).
2. In order to achieve the goal, a range of motor programs is activated (initiating activity).
3. Efference copies of the motor programs are compared to the sensory input referring to the deviation from the desired state.
4. In case of a significant coincidence, the respective motor program is used to modify the sensory input in the direction toward the goal.
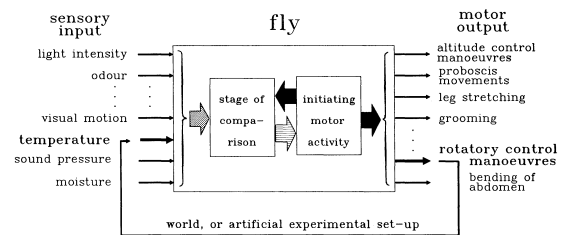

Fig. 2: General model of operant behavior. The brain generates a large variety of motor-outputs and cross-correlates them with one or several sensory inputs. If, for a certain combination, the correlation is sufficiently positive, the fly can manipulate this sensory input according to its needs. The long external arrow coupling 'rotatory control maneuvers' and 'temperature', depicts the situation of a fly in the *Drosophila* Flight Simulator. (From: Wolf and Heisenberg, 1991)

Consistent control of a sensory stimulus (i.e. the reinforcer) by a behavior may lead to a more permanent behavioral change (conditioning).

According to Wolf and Heisenberg (1991) *operant behavior* is the active choice of one out of several output channels in order to minimize the deviations of the current situation from a desired situation (1-4). *Operant conditioning* in these terms is expressed by persisting activation of this channel after the situation has changed (5). Mutant analyses in *Drosophila* have shown that these processes can also be genetically distinguished (Eyding, 1993; Weidtmann, 1993).

1.4 How Distinct are Classical and Operant Conditioning?

For a long time classical and operant conditioning were considered distinct categories of learning requiring distinct pathways in the brain. Some observations, however, seem to indicate that most

learning situations contain operant and classical components at various degrees.

Pavlov's hungry dog, for example, will show appetitive behavior towards the bell, if it has seen it while it was ringing during classical training even if it was immobilized at that time. More spectacularly, Chimpanzee males, trained operantly to insert coins into a food dispenser will trade the coins to equally trained females for sex, as they normally do with food.

A key to resolving the dilemma whether operant and classical conditioning can indeed be conceived as separate entities, might be to compare the different types of associations the subjects form between the various stimuli, the response and the reinforcer. According to Pavlov (1927), Kandel and his co-workers (Kandel et al. 1983; Hawkins et al. 1983; Carew et al. 1983; Carew and Sahley, 1986) and Hammer (1993), stimulus-substitution seems to account for the capacity of the CS to elicit the CR in classical conditioning. The association is assumed to be **stimulus-reinforcer** in nature. According to Skinner (1938), Mackintosh (1975) and Wolf and Heisenberg (1991), associations in operant conditioning are formed between the behavioral output of the organism and its stimulus situation. The association is assumed to be **response-reinforcer** in nature.

1.5 Drosophila in the Neurosciences

The key insight that all levels of functional organization from genes to behavior tightly interact, constitutes the basis for a very successful science: neurogenetics. Clearly emphasizing the inherited aspects of the phenomena studied in neuroscience, the primary model system for neurogeneticists is *Drosophila*. The rich repertoire of classical genetics together with very efficient molecular techniques, allows one not only to identify and clone new genes but also to assess their function at the molecular, cellular and systemic level. Exploiting these opportunities *Drosophila* offers has furthered to a great extent our understanding of the molecular mechanisms underlying such complex processes as classical olfactory conditioning in *Drosophila* (e.g. Davis and Dauwalder, 1991; Tully et al. 1994; Tully, 1991; Tully, et al. (1990).

Besides the genetic level, *Drosophila* provides several advantages for studying learning and memory behaviorally, compared to humans and other mammals: 1) a short lifespan in standardized vials reduces the inter-individual variance in experiential history to a minimum; 2) only minor ethical considerations have to be taken into account for experimental design; 3) relatively small experimental setups; 4) no social or linguistic complications; 5) the possibility to measure a large number of individuals.

The study presented here takes advantage of these features: *Drosophila* is used in an experimental situation that allows for minute control of the input the fly receives and the output it produces.

1.6 Drosophila in the Flight Simulator

In the flight simulator a single tethered *Drosophila* fruitfly flies stationarily in an artificial environment. Originally, open loop experiments, in which the fly's behavior has no effect upon its visual stimulus situation, were utilized for detailed examination of *Drosophila's* optomotor behavior (e.g. Heisenberg and Wolf, 1984; Heisenberg and Wolf, 1993). In this setup, however, the fly can be enabled to control some aspects of its visual input by coupling it to its motor-output (closed loop). Both the visual input and the motor-output are monitored on-line throughout the experiment.

Such a flight simulator setup is ideally suited for a detailed comparison of classical and operant conditioning, since various contingencies among behavioral output, visual input and the reinforcer - everything in exquisite control of the experimenter - can be established. In the present study, the environment consists of a cylindrical panorama arranged to center the fly within the cylinder. The motion of the environment is limited to the horizontal plane: only the rotational speed of the cylinder can be controlled by the fly's tendency to turn around its vertical body axis (yaw torque, see Fig. 3).

1.6.1 The Flight Trace

Upon observing a fly in the flight simulator, it is striking that the fly neither keeps the cylinder immobilized nor rotates it continuously: phases of fairly straight flight are interrupted by sudden turns at high angular velocity. Monitoring the fly's yaw
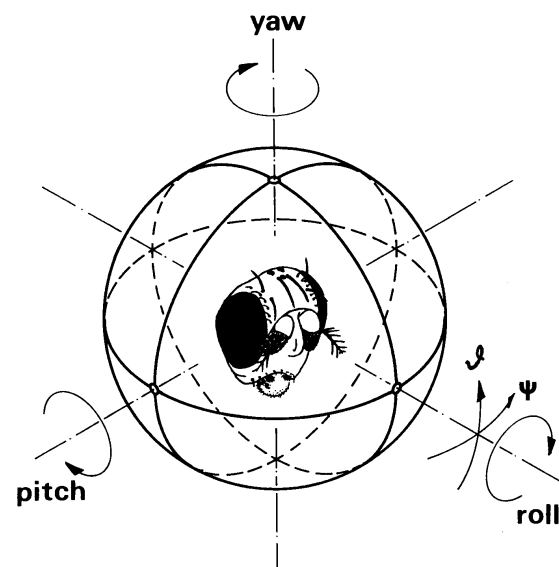

Fig. 3: The two spherical coordinates Ψ and ϑ of a fly's visual space and its three degrees of freedom for rotation (yaw, roll, pitch; redrawn from Heisenberg and Wolf, 1984).

torque, the turns are due to short pulses of torque (torque spikes, Fig. 4). The fly generates these torque spikes by reducing the wing beat amplitude by about 12° on the side to which it intends to turn (wing hitch; Götz et al. 1979; Götz, 1983). These sudden turns („body saccades") can be observed in free flying *Drosophila* as well. Based on previous work from Heisenberg and Wolf (1979), Heisenberg and Wolf (1984), Mayer et al. (1988) and Heisenberg and Wolf (1993), the present study assumes that the spikes are the primary behavior by which the fly adjusts its orientation in the panorama; further evidence in accordance with this assumption is discussed below (3.3).

1.6.2 Input/Output Analysis

Albeit it is hardly possible to account entirely for all stimuli any organism senses, it is probably safe to argue that all salient experience the fly receives in the flight simulator is controlled by the experimenter: 1) the fly is tethered and hence the visual field of the fly is either stationary or coupled to the recording device. 2) Most other stimuli (odorants, moisture, air-pressure, magnetic or electrostatic fields etc.) are to a large degree constant during the course of the experiment.

Consequently, most motor-output recorded is either initiated by the fly on its own (initiating activity [Heisenberg, 1983] or rhythmic activity) or induced by the experiment.

1.7 Classical and Operant Conditioning in Drosophila

The aim of this study is to compare the motor-output of two groups of *Drosophila* fruitflies in the flight simulator which both are trained to avoid a flight direction towards a given pattern in their environment. One group is trained operantly to perform the task, the other classically, according to the definitions in 1.2 and 1.3. In such an experiment, it is plausible that the conditioned responses might deviate as an effect of the different associations made during the different training procedures. More specifically, it can be expected that the operantly trained flies acquire 'new' behavioral strategies the classically trained flies lack or that the flies selectively activate and inactivate certain behaviors from a range of motor-programs while the classical group still uses the whole range. To investigate this, the assessment techniques measuring the performance of the behavior have to be identical in both the classical and the operant experiment. The experimental setup has to allow for exquisite control of stimulus presentation and response generation in order to 1) avoid complications with stimuli unintentionally connected with the experiment, 2) assure that the two experiments differ only in training and 3) detect differences in response generation with sufficiently high accuracy. The flight simulator
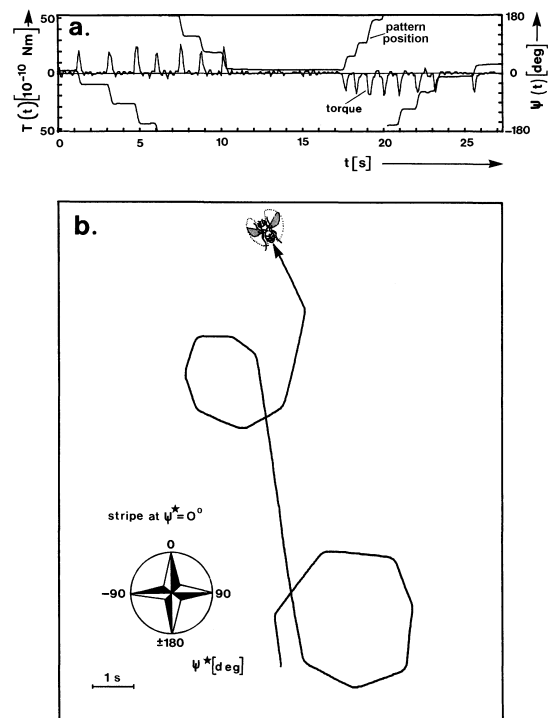

Fig. 4: Illustration of typical flight orientation behavior in the flight simulator. The pattern, in this case a single vertical black stripe, is assumed to be at infinite distance from the fly, i.e. the angular position of the stripe does not change during straight flight. a. Flight trace, consisting of torque and position trace, used to calculate the flight trajectory depicted in b. Forward flight velocity was assumed to be constant. It can be seen that the torque spikes in (a) correspond with the stepwise turning of the panorama. (Redrawn from Heisenberg and Wolf, 1984)

provides the means to achieve this goal. Moreover, the concept of studying 'microbehavior' (Heisenberg and Wolf, 1984) enables the student to pose his questions more specifically than in some setups often used by psychologists, where gross behavior is analyzed. In such preparations the expression of learning in gross behavior is most likely to be the product of a rather large amount of post-acquisition processing, complicating the interpretation of the data in terms of what has been learned.

2 Material and Methods

2.1 The Animals

Drosophila melanogaster flies of the wildtype strain "Berlin" were used throughout the experiments. The flies were treated according to a breeding regime developed by Reinhard Wolf (pers. comm.): in order to control larval density, flies oviposit over night on semolina pudding (Aurora Hartweizen-Grieß). Using a needle, larvae and eggs are collected the next day and transferred to a vial containing standard cornmeal-molasses medium, keeping the larval density at 6-9 larvae/ml medium. The vials are stored in an environmental room at 25°C and 60% humidity with an artificial 16hr light/8hr dark cycle. Newly eclosed flies are transferred to fresh vials on a daily basis and kept in the
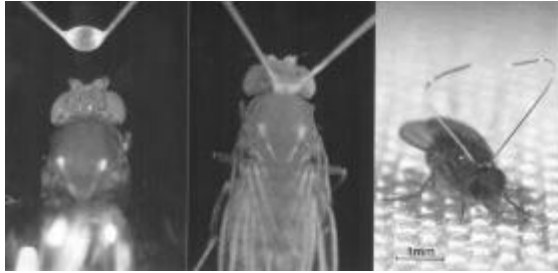
Fig. 5: The flies are attached to the hook of silver wire with a UV-sensitive light in a matter of seconds. The flies are little impeded by the wire; they can stand, walk, and fly with it. (From: Heisenberg and Wolf, 1984)

environmental room. Vials from which flies have eclosed for more than two days were discarded.

24-48h old female flies were immobilized by cold-anesthesia and glued with head and thorax to a triangle-shaped silver wire (diameter 0.05mm) the day before the experiment (Fig. 5). The animals were kept individually at 25°C and 60% humidity with a 16hr light/8hr dark regime in small vials and fed a saccharose-solution until the experiment.

2.2 The Experimental Setup

The core device of the setup is the yaw torque compensator (Fig. 6). Originally devised by Götz (1964) and repeatedly improved by Heisenberg and Wolf (1984), it measures a fly's angular momentum around its vertical body axis. The fly, glued to a small hook of silver wire as described above (2.1), is attached to the torque meter via a clamp to accomplish stationary flight in the center of a cylindrical panorama (arena, diameter 58mm), homogeneously illuminated from behind. Closing the feedback loop to make the rotational speed of the arena proportional to the fly's yaw torque (coupling factor $11°/s \cdot 10^{-10}$Nm) enables the fly to stabilize the rotational movements of the panorama (Flight Simulator mode). The position of an arbitrarily chosen point of reference on the arena azimuth delineates a flight direction of 0-360°. Arena position (i.e. flight direction) is recorded continuously via a circular potentiometer and stored in the computer memory
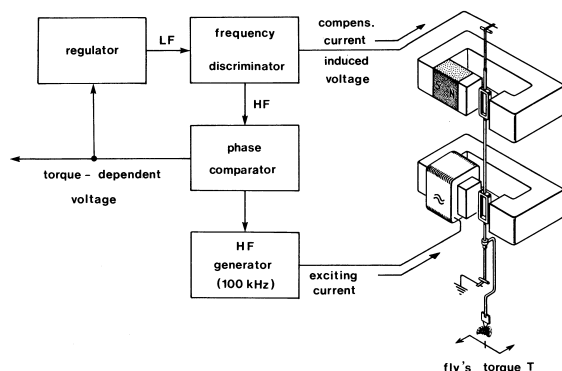

Fig. 6: Block diagram of mode of operation of the torque compensator for recording yaw torque of *Drosophila* in stationary flight. HF - high frequency; LF low frequency. (redrawn from Götz, 1964, where a detailed description of the mode of operation is given)

together with yaw torque (sampling frequency 20Hz).

Four black, T-shaped patterns of alternating orientation are evenly spaced on the arena wall (width $\Psi=40°$, height $\vartheta=40°$, barwidth=14°). Reinforcement, where applied, is made to be contiguous with the appearance of one of the two pattern orientations in the frontal quadrant of the fly's visual field.

The reinforcer is a light beam (diameter 4mm at the position of the fly), generated by a 6V, 15W Zeiss microscope lamp, filtered by an infrared filter (Schott RG1000, 3mm thick) and focused from above on the fly. The strength of the reinforcer was determined empirically by adjusting the voltage to attain maximum learning. In all experiments the heat was life threatening for the flies: more than 30s of continuous reinforcement were fatal for the animal.

The heat is applied by a computer-controlled shutter intercepting the beam (Fig. 7).

2.3 The Experiments

2.3.1 The Standard Experiment

In this paradigm the flight simulator establishes normal negative feedback between angular velocity of the arena and yaw torque (closed loop) throughout the experiment. This permits the animal to establish optomotor balance and to adjust certain flight directions with respect to the four T-shaped patterns on the walls of the cylindrical arena (Flight-Simulator mode). During training the fly is
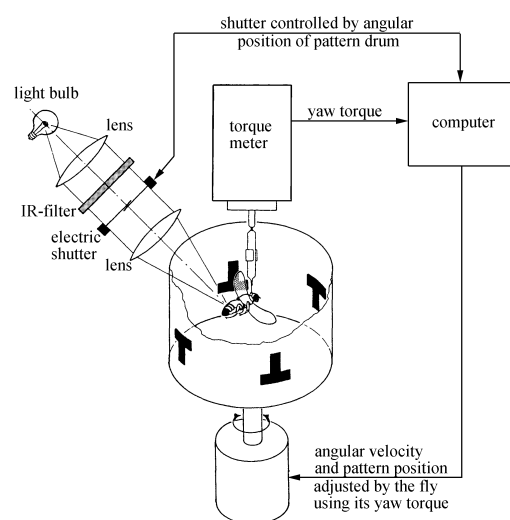
Fig. 7: Simplified diagram of the flight simulator setup. Yaw torque is continuously transduced into d.c. voltage by the torque meter. The computer couples this signal to the pattern drum by calculating the angular image deviations that the measured flight maneuvers would have caused on the fly's eyes in free flight. Thus the fly can control the angular rotation of the drum with its yaw torque: intended right turns of the fly rotate the arena counterclockwise, intended left turns rotate it clockwise - similar to a human pilot controlling the panorama in a flight simulator program with its joystick. The same computer controls the electric shutter intercepting the infrared light beam used as reinforcer.

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punished by an infrared light beam (input 6.9V), whenever one of the two pattern types is in the frontal quadrant of the visual field. The fly can control the appearance of the reinforcer by its choice of angular pattern position. During test, the heat source is switched off.

Each experiment lasts 9x2min (periods no. 1 through 9). After 2x2min. of unreinforced flight (pre-test, t_1), a training period of another 2x2min. is introduced; after the following 2min. test, the 2x2min. training is repeated. The experiment is concluded by a 2x2min. test-phase (t_2). At the beginning of each test-period the arena is rotated at high velocity to a random angular position.

A group of control-flies was subjected to the same experimental regime except for the reinforcement.

2.3.2 Classical Conditioning

In this paradigm, the flight simulator mode is interrupted (open loop) during training and the panorama is kept stationary with one pattern orientation in front of the fly: the fly's behavior cannot interfere with stimulus presentations. After 3s the panorama is rotated by 90° in 220ms, thus bringing the other pattern orientation into the frontal position. One of the two orientations is made contiguous with the reinforcer (input 5.3V). In the test periods the apparatus is switched to the flight simulator mode, and the animal's choice of angular pattern position is recorded without reinforcement.

The experimental regime is identical to the standard experiment and consists 9 periods of 2 minutes length (periods no. 1 through 9). After 2 periods of unreinforced closed-loop flight (pre-test, t_1), two training period of 2min. are introduced; after the following 2min. test, the 2x2min. training is repeated. The experiment is concluded by a 2x2min. test-phase (t_2). At the beginning of each test-period the arena is rotated at high velocity to a random angular position.

Again the control group received the same treatment, but was spared the reinforcement.

2.4 The Evaluation

Self-written programs computed the raw data from each 2min. period into several variables of individual means for each fly. The individual means were exported into ASCII-format for later analysis in a commercial statistics program.

2.4.1 The Flight Trace I: Arena Position

An analog to digital converter transforms the data from the circular potentiometer measuring the angular position of an arbitrary point of reference on the cylindrical panorama (arena position) into an sequential array of consecutive data points with possible values ranging from -2048 to 2047 (sampling frequency 20Hz). The zero value corresponds to an arena position at which all quadrant borders are at a 45° angle with respect to the fly's longitudinal axis. These raw data are stored in the computer memory (*position trace*).

2.4.1.1 Avoidance/Learning

Avoidance is assessed as the preference of a fly to keep one pattern orientation in the frontal position rather than the other. From the position trace the **preference index** is calculated as $(p_2-p_1)/(p_2+p_1)$, with p_2 being the number of data points corresponding to a position of the arena at which the pattern orientation not associated with heat was kept in the frontal quadrant of the visual field and p_1 denoting the remaining data points. A group of flies are said to have learned if their preference index at t_2 (the last two periods) differs significantly from that of the respective control group.

The mean duration of periods of staying in one quadrant (dwelling times) can also be calculated from the position trace by dividing p_1 and p_2 by the number of stays in the respective sector.

2.4.1.2 Fixation

The ability to keep optomotor balance with one pattern directly in front of the fly (i.e. to fly straight towards the pattern) is assessed as the time the fly kept the patterns in the frontal octant of its visual field compared to the time the quadrant borders were in this position. In order to calculate a measure for fixation, the absolute values of the position trace data array are transformed with modulo 1024 to yield values ranging from 0 to 1024. From the resulting array (where now the two extreme values represent the centers of two adjacent patterns) the **fixation index** is calculated as $(f_1-f_2)/(f_1+f_2)$, with f_2 being the number of data points n fulfilling $256<n<768$ and f_1 being the remaining data points.

2.4.1.3 Quadrant Changes and Arena Rotation

As a measure for the activity of the fly during the experiment, the number of quadrant changes and the total amount of arena rotation are calculated from the position trace. Adding up the events where data points corresponding to one pattern orientation in the frontal position are followed by points corresponding to the adjacent pattern yields the number of **quadrant changes**. The amount of **arena rotation** is given by the sum of the distances between consecutive data points in degrees.

2.4.2 The Flight Trace II: Yaw Torque

An analog to digital converter transforms the data from the torque compensator measuring the fly's yaw torque into a sequential array of consecutive data points with possible values ranging from -2048 through 2047 (sampling frequency 20Hz). The zero value is adjusted individually for each fly by cali-
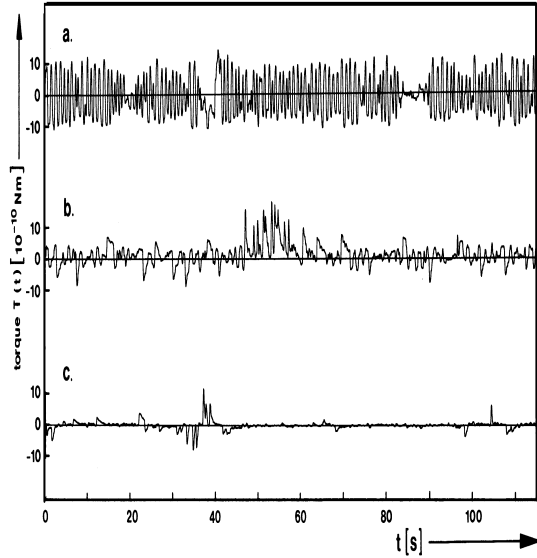
Fig. 8: Typical stretches of three common patterns of yaw torque flight trace. a. Oscillating mode. b. Noise mode. c. Quiet mode. (Redrawn from Heisenberg and Wolf, 1984)

brating the fly's maximum optomotor response to clockwise and counterclockwise turns of the arena to be zero-symmetrical; in the flight simulator this corresponds to flying straight ahead. These raw data are stored in the computer memory (*torque trace*).

2.4.2.1 Spike Detection

The spike detector used in this work takes advantage of the most prominent feature distinguishing spikes from the torque baseline (optomotor balance): their amplitude. Since the amplitude of the torque baseline is subjected to a considerable amount of inter- and intraindividual variation (Fig. 8), detection thresholds are computed from the torque trace every 600 data points (i.e. 30s of flight): the two peaks of a frequency distribution, gathered by arranging the data points corresponding to maxima and minima in the torque trace according to their frequency, delineate the interval inside which the torque baseline is assumed to lie (Fig. 9).
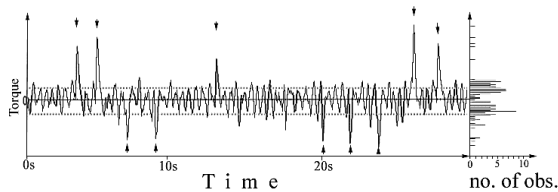

Fig. 9: Typical stretch of the yaw torque flight trace (left) with a distribution histogram of the torque maxima and minima (right). Detected spikes are indicated with arrowheads. Dotted lines denote the detection thresholds (see text).

A continuous array of $2<n<17$ data points of equal sign, the first of which exceeding the torque baseline is then considered a spike if it fulfills the following criteria:

1. $T_{max} > |1.4\tau_T + 5000/\tau_T|$, where T_{max} denotes the largest absolute value in the array and τ_T the ap-

pendant of the two thresholds delineating the torque baseline

2. $|T_L| < |0.2 T_{max}|$, or $\tau_1 < T_L < \tau_2$ where T_L denotes the last of the n data points and τ_1 and τ_2 denote the two thresholds with $\tau_1 < 0 < \tau_2$.

An array of data points containing two typical spikes is depicted in Fig. 10.
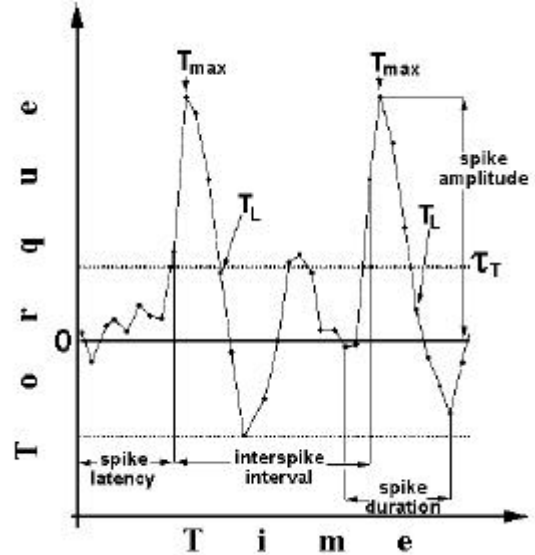

Fig. 10: Stretch of 33 yaw torque data points (i.e. 1.6s). Data points are connected by lines for better illustration. Dotted lines indicate detection thresholds. See text for details.

2.4.2.2 Spike Dynamics and -Timing

Once a spike is detected, its amplitude, duration and the time elapsed since the previous spike (or since the quadrant change, if one occurred between two spikes) is recorded (Fig. 10). From these data three indices are calculated: the **amplitude index** is calculated as $(a_1-a_2)/(a_1+a_2)$ where a_1 denotes the mean spike amplitude in the quadrants containing the pattern orientation associated with heat. The **latency index** is calculated as $(l_1-l_2)/(l_1+l_2)$ with l_2 being the mean time interval measured from a change into the 'hot' quadrant until the first spike in this quadrant. The **ISI index** is defined accordingly as the difference of the mean *inter*spike *in*tervals (ISI) in the 'hot' and the 'cold' sectors: $(d_1-d_2)/d_1+d_2)$; here d_2 denotes the mean distance between two spikes in the quadrant containing the pattern orientation combined with the reinforcer. Once the timing of the spikes is accounted for, one can calculate the number of spikes per time in each sector and define a **number index** as $(n_1-n_2)/(n_1+n_2)$ with n_1 denoting the spike frequency in the 'hot' sectors.

2.4.2.3 Spike Polarity

In addition to the force and the timing of the body saccades, the direction of the turns might be important for a fly when performing in the described learning paradigm. The polarity of a spike is defined as "towards pattern" if it leads to a rotation of

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the arena that brings the center of the nearest pattern closer to the very front, which is delineated by the longitudinal axis of the fly. Accordingly, the spike polarity "from pattern" brings the nearest quadrant border closer to the most frontal position.

After some preliminary calculations, the following variables have been taken into account: a **polarity index**, yields the fraction of spikes towards the pattern. It is defined as $(s_t-s_f)/(s_t+s_f)$ with s_t being the number of spikes towards the pattern and s_f the number of spikes away from the pattern. This index had to be calculated for 'hot' and 'cold' sectors separately.

The time elapsed from the entrance into a sector until the first spike away from the pattern is determined as well. Obviously, this is highly influenced by the timing of the spikes regardless of their polarity, so the difference is calculated between the latency index for all the first spikes towards the pattern and the latency index for all the first spikes away from the pattern. If this **polarity-latency index** is positive, the first spikes away from the pattern were generated earlier in quadrants where the pattern orientation is associated with the reinforcer than in the other sectors, spike *frequency* modulations included.

2.4.3 The Flight Trace III: Combined Evaluation

Since torque trace and position trace are measured simultaneously, the torque trace can be used to calculate the effects of torque on arena position.

Angular Displacement Size. As another measure of spike dynamics the angular displacement during each detected spike is calculated by the distance between two position-data points in degrees, the corresponding torque values of which denote the duration of the spike (Fig. 10).

Stepwise Arena Rotation. As a quantitative measure for the subjective impression of sudden arena rotations accounted for by spikes, the sum of angular displacements during spikes is compared to the sum of interspike displacement for each 2 min. period in a **rotation index**: $(r_s-r_i)/(r_s+r_i)$, where r_s denotes the sum of angular displacement during the spikes and r_i the sum of arena displacements between two spikes.

2.4.4 Spike Detection Efficiency

A subjective test for spike detection efficiency was performed with five members of the lab, only one of whom was familiar with *Drosophila's* body saccades: they were presented with the task to count the number of spikes in the torque traces of four deliberately chosen flies. The mean number of detected spikes in the first three periods was compared to the number the spike detector counted. Two of the flies were deemed to be 'regular' flies producing spikes that were very easy to distinguish from the

torque baseline (see appendix 1 and 2). The third fly was flying in the 'oscillating' mode and the fourth fly in the 'quiet' mode (see appendix 3 and 4).

2.5 Statistics

During classical training, the lack of contingency between behavioral output and sensory input leads to drifting of the torque baseline over most of the torque range of the fly. Therefore, no spike detection is possible in these phases. For this reason, all comparative studies were restricted to the five test-periods.

All between-group analyses were performed with repeated measures MANOVAs whenever more than one 2 min period were compared at a time. Wilcoxon Matched Pairs Tests were used to test single 2 min periods against zero. Correlational analyses were computed according to Spearman's Rank Order Correlation throughout.

3 Results and Discussion

It was mentioned in the introduction to this study that the different associations assumed to be made in the different training procedures - namely stimulus-reinforcer during classical conditioning and response-reinforcer during operant conditioning - might lead to different behavioral strategies to avoid the pattern orientation associated with heat. For instance, if the classically trained fly learned about the 'heatedness' of one of the pattern orientations, it might use the same behavioral repertoire to avoid this flight direction as is employed by the control group for spontaneous preference.

Conversely, operantly trained flies may have acquired a more effective (or at least different) way to avoid the heat during their training, selecting among many different behavioral strategies. In this case, the motor-output produced by those flies should be different from both the respective control-group and the classically conditioned group.

It is assumed - and data supporting this assumption is discussed below (3.3) - that torque spikes are the fly's primary behavior to adjust flight direction (Heisenberg and Wolf, 1979; Heisenberg and Wolf, 1984; Mayer et al. 1988; Heisenberg and Wolf, 1993). Therefore, all properties of the torque spikes are evaluated in this study: spike amplitude, spike duration, spike polarity, spike number, spike latency, interspike intervals etc.

3.1 Spike Detection

Fig. 8 gives a notion how highly variable the flight modes in different *Drosophila* individuals can be. As was shown by Heisenberg and Wolf (1984), size and shape of the torque spikes are under reafferent control. As will be shown below (3.5), size, timing and polarity of the spikes are subjected to conditioning dependent modulations. Because of this high

intra- and interindividual variability in general spike appearance, it is impossible to construct an error proof spike detector. Even the human eye is sometimes not capable of unambiguously discerning spikes from the torque baseline. In the end, only the fly 'knows' whether it has produced a spike or not. Fig. 11 shows this difficulty: the human subject is obviously following different rules to detect spikes according to the general appearance of the torque trace. The volunteers detected less spikes than the computerized spike detector if the task was judged to be 'easy' and spikes were clearly distinct from the baseline (Fig. 11, first and second fly). However, when the baseline was oscillating very much (see appendix 3) the number the spike detector produced was lower than the number of spikes the volunteers counted (Fig.11, third fly). The same was true if the baseline was very low and the spikes very small (see appendix 4).
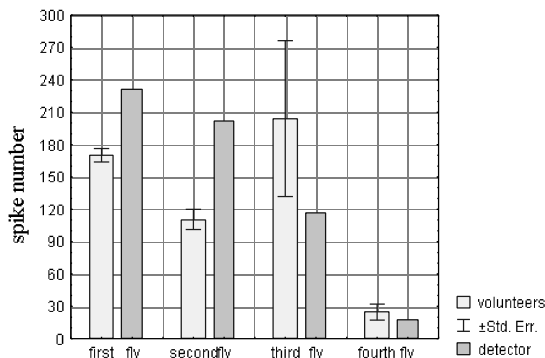

Fig. 11: Comparison of the spikenumber from four flies during the first three periods of flight between five volunteers and the spike detector used in this study. See text for details.

Apparently, there is quite an amount of subjectivity in the detection of spikes. This is of course also reflected in the programmed spike detector. In this case the number counted by the programmer was closer to the spike detector than the numbers of the five volunteers (not shown). It is important, that throughout the study *one* rule is followed in order to always compare the same turning maneuvers and that there are not too many turning maneuvers between the counted spikes which may corrupt the results (see below, 3.3).
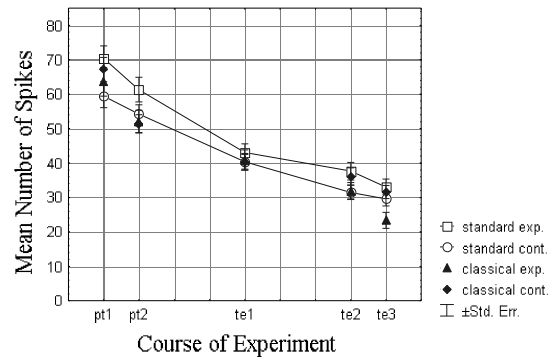
3.2 Spikes

Fig. 12: Mean spike numbers for all four groups (N=100 flies each) during unreinforced flight. Open symbols indicate standard groups, filled symbols indicate classical groups. *Lines are drawn for better illustration only.*

The mean spike number averaged over all 400 flies showed a significant ($p<0.001$) decrease in a linear fashion from 65 spikes in the first to 29 spikes in the last 2 min period (Fig. 12). Between-group variation was not significant. Heisenberg and Wolf (1979) have reported a spike frequency of 0.5-1 spike/s which is confirmed by the present study.

The average spike during unreinforced flight (periods 1, 2, 5, 8 and 9) had an amplitude of 1.19 (± 0.01 S.E.M) $\cdot 10^{-11}$ Nm, which seems rather well in accordance with the data in Heisenberg and Wolf (1979), Heisenberg and Wolf (1984) and Mayer et al. (1988). Fig. 13 shows only little variation in spike amplitude during the experiment albeit the pre-test values of the flies in the standard experiment are significantly higher than those of the control group ($p<0.001$ at t_1). However, the difference is only moderate compared to the absolute values (12%) and equalized during the course of the experiment.
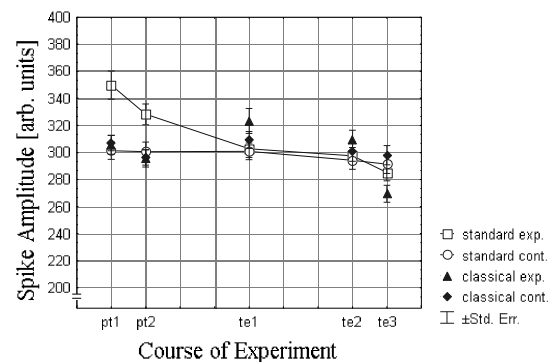

Fig. 13: Mean spike amplitudes in arbitrary units (1 unit = $3.9 \cdot 10^{-14}$ Nm) for all four groups (N=100 flies each) in the unreinforced periods. Note the high spike amplitude of the flies in the standard experiment at t_1 (periods 1 and 2). Open symbols indicate standard groups, filled symbols indicate classical groups. *Lines are drawn for better illustration only.*
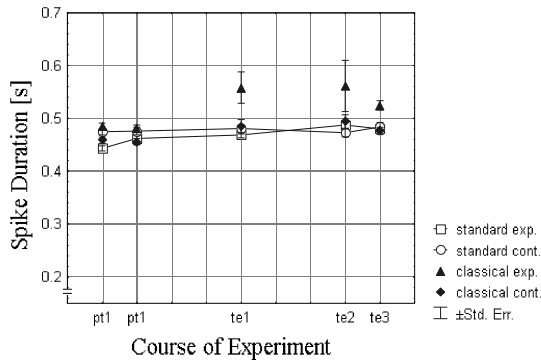
Fig. 14: Mean spike durations during periods 1, 2, 5, 8 and 9 for all four groups (N=100 flies each). The long duration in the classical conditioning group is obvious (see text). *Lines are drawn for better illustration only.*

The mean spike duration of all 400 flies was 0.48s (±0.004s S.E.M) and in three groups it did not show much variation during the course of the experiment (p=0.526, Fig. 14). Only the classically conditioned flies showed a prolonged spike duration after training (periods 5, 8 and 9), compared to the control group (p<0.02). This is considered to be attributable to the heat, as discussed below (3.6). The measured mean duration of a spike (0.48s) is well in accordance with data given in Heisenberg and Wolf (1979). Such rather long spike duration, however, is only achieved in stationary flight without proprioceptive feedback from angular acceleration or reafferent stimuli from air currents abruptly terminating the burst of torque (after 120-160ms) in free flight.

The mean angular displacement caused by detected spikes was 21.7° (±0.25° S.E.M) averaged over all 400 flies. This value was rather constant throughout the experiment and showed little between-group variation (Fig. 15). This is a little less than the „roughly 30°" reported in Heisenberg and Wolf (1979), however, it is not clear whether the number given in this reference was meant to describe wild-type strain *Berlin* or *Canton S* or *Drosophila* in general.

3.3 Stepwise Arena Rotation
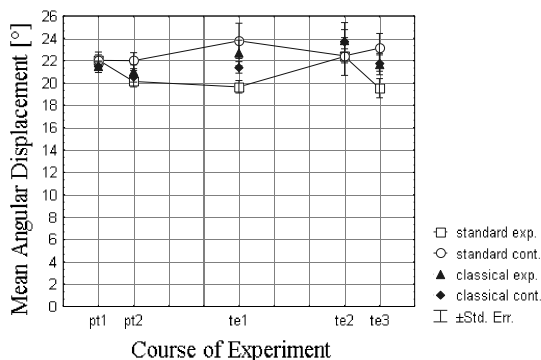
It was mentioned above (1.6.1) that the torque

Fig. 15: Mean angular displacement covered by the spikes of all four groups (N=100 each). *Lines are drawn for better illustration only.*

baseline is believed to correspond to optomotor response behavior (optomotor balance), whereas the body saccades (spikes) were mainly employed to adjust flight direction (Heisenberg and Wolf, 1979; Heisenberg and Wolf, 1984; Mayer et al. 1988; Heisenberg and Wolf, 1993). The rotation index was derived to quantify the amount of angular displacement accounted for by detected spikes in relation to the amount of displacement between the spikes (see above, 2.4.3). The rotation index did not reveal enough between group variation in the non-reinforced periods (periods 1, 2, 5, 8 and 9) to reject the null hypothesis that all four groups were samples from the same population. Therefore, the descriptive statistics of all four groups are given in Table 1.

	N	Mean	Confidence -95%	Confidence +95%	Median
RotInd1	400	0.35	0.33	0.38	0.39
RotInd2	399	0.32	0.29	0.34	0.36
RotInd8	396	0.25	0.22	0.28	0.30
RotInd9	386	0.22	0.19	0.25	0.25
	Min	Max	Lower 25%	Upper 25%	
RotInd1	-.67	0.82	0.23	0.53	
RotInd2	-.70	0.79	0.17	0.51	
RotInd8	-.85	0.86	0.07	0.44	
RotInd9	-.75	0.84	0.05	0.42	

Table 1: Descriptive statistics of the rotation indices (*RotInd*) in all flies at t_1 (periods 1 and 2) and at t_2 (periods 8 and 9).

Considering that only between 12% and 25% of the time of each 2min. period is consumed by spikes, the data presented in table 1 indicate that indeed most flies rotate the arena stepwise, i.e. the flight direction is fairly constant between the sudden turns caused by the spikes. For instance, in the first period more than twice as much arena rotation was caused by the spikes than by behavior in the interspike intervals (rotation index 0.35). The rotation index exhibits dependence only from the mean number of spikes per period (Spearman Rank Order Correlation 0.58, p<0.001 in the first and 0.50, p<0.001 in the last period). This dependence can be exemplified in two flight modes: in oscillating mode, the spikes (if there are any) are hard to detect and much orientation might be carried out by *omitting* optomotor waggles. In quiet mode, there are very few spikes and some orientation is accomplished by baseline drift (personal observation). Probably the minimal values in table 1 are examples of those flies. Of course, if a fly is producing many spikes, there is not much room for interspike navigation. As the overall spike number decreases during the course of the experiment (see above, 3.2), the decrease of the rotation index is not surprising. For the same reason, some flies are not accounted for later in the experiment: they ceased to produce spikes at all.

It seems that the rotation index is the lower estimate of the degree to which body saccades are used in

free flight for the adjustment of flight direction: 1) as was discussed in Heisenberg and Wolf (1979) and in Heisenberg and Wolf (1984) the three flight modes depicted in Fig. 8 may be instrumental artifacts disturbing spike evaluation. 2) Undetected spikes might contribute to 'interspike' orientation. 3) Spiking behavior correlates well with learning (preference indices) in the *Drosophila* flight simulator (see below, 3.6) and thus seems indeed to be responsible for the adjustment of flight direction.

However, some flies seem to use a behavior for choosing flight direction that is not covered by the spike detector: in about 25% of the flies at t_2 (table 1) the amount of summed interspike arena rotation reaches or exceeds the amount of summed arena rotation caused by spikes. Moreover, in studies of the *Drosophila* mutant strain ebo^{678} some flies showed orientation behavior without spikes, but by baseline drift (Ilius, 1992; Ilius et al. 1994) as could be observed during this study with wildtype flies in the quiet flight mode. Thus there are apparently two components of orientation behavior. In the majority of flies the dominant component is spiking behavior.

Therefore, the evidence from the rotation index is taken as confirmation of the findings from Heisenberg and Wolf (1979), Heisenberg and Wolf (1984), Mayer et al. (1988) and Heisenberg and Wolf (1993) where torque spikes have been shown to be endogenous motor patterns ('actions' and not responses to external stimuli) that are produced to adjust flight direction, whereas the baseline is assumed to contain the mechanism to establish and maintain optomotor balance in a responsive way and to be of minor importance for choosing flight direction.

3.4 Measurements at t_1

All the variables of all four groups were tested against the null hypothesis that they were drawn from the same population with a repeated measures MANOVA for the first two periods (pre-test, t_1). In those cases, where the null hypothesis could be rejected at $p < 0.05$ the differences were examined more closely. Four variables had to be taken into consideration (Table 2).

Due to time constraints, the non-reinforced control groups could not be measured simultaneously with the reinforced group (Table 3). Seasonal influences, for example, may have caused a drop in general activity in the non-reinforced groups. This drop is reflected in the differences in arena rotation and quadrant changes. The effect in spike amplitude is mainly due to the large spikes generated by the standard batch, many of which were conditioned before the other groups. Since spike duration is

	standard	*s-control*	*classic*	*c-control*
SpAmp***	339.0	301.0	300.7	302.1
SpDur**	0.45s	0.48s	0.48s	0.46s
ARot**	4742°	3646°	4206°	3691°
QuCh***	37.9	27.0	32.2	26.2

Table 2: Values for those variables, where the null hypothesis that the four groups were drawn from one population could be rejected, averaged over the first two periods. SpAmp - spike amplitude (in arbitrary units; 1 unit = $3.9 \cdot 10^{-14}$ Nm). SPDur - spike duration. ARot - total amount of arena rotation. QuCh - number of quadrant changes. * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$.

negatively correlated with spike amplitude (-0.17 at period 8 and -0.22 at period 9, $p < 0.001$ in both periods) the result in spike duration is not surprising. Taken together, the data indicate a decrease in overall strength and activity from the reinforced to the control groups. It has to be emphasized that none of the variables calculated with respect to the behavior in the differently treated (hot/cold) sectors showed any deviation.

	standard	*s-control*	*classic*	*c-control*
1st exp.	05-Oct-95	27-Feb-96	14-Jan-96	14-May-96
last exp.	26-Jun-96	12-Jul.-96	02-Jul.-96	12-Jul.-96

Table 3: Dates of first and last experiment in the respective groups.

3.5 Comparing the Standard Experiment and Classical Conditioning

In the standard paradigm, *Drosophila* avoids the heat during training very quickly and stabilizes the arena with the 'cold' pattern orientation in the frontal position, with short excursions into the heated sectors. There is a very prominent behavior to be observed during training: the entering of a reinforced quadrant during training is often followed by a volley of spikes, bringing the fly out of the heat (Fig. 16).

In the classical paradigm, *Drosophila* is confronted with the contiguity of one pattern orientation paired with heat during training and has no means preventing it from being heated. Observing flies when heated under open loop conditions reveals a behavior very similar to the volley of spikes depicted in Fig. 16: some flies produce spike volleys and a shift in the torque baseline during heating (Fig. 17).
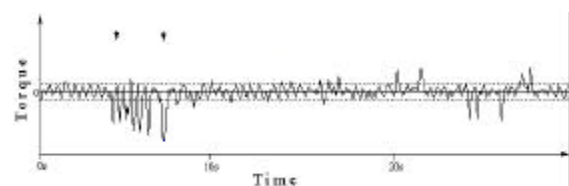

Fig. 16: Typical stretch of yaw torque flight trace during operant training with volley of spikes as the shutter opens (left arrowhead) until it closes (right arrowhead). In this trace another spike parameter modulation can be seen: the spike amplitude is larger in the spikes during the heat than after the closure of the shutter. Dotted lines denote spike detection thresholds.
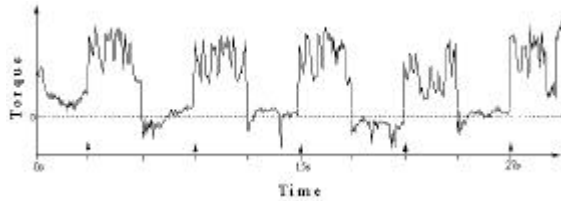
Fig. 17: Stretch of yaw torque flight trace of a single fly during classical conditioning. The pattern is switched every three seconds. The torque trace rises high above zero together with the generation of large, spike-like torque fluctuations whenever the heat is switched on (arrowheads). The dotted line indicates zero torque.

3.5.1 Avoidance and Learning

If the heat is permanently switched off, flies of both test groups keep their orientation preference towards the previously 'cold' pattern orientation for at least several minutes (Fig. 18). In the operant groups there was no significant difference at t_1 (the first two periods, p=0.911), but the test group showed a significantly higher preference than the control group (p<0.001) at t_2 (the last two periods).

Even if a repeated measures MANOVA revealed a significant difference (p<0.03) in preference at t_1 (the first two periods) for the classical groups, the same analysis for all four groups (p=0.104) indicates that the sample flies were indeed drawn from the same population. Furthermore, the difference in
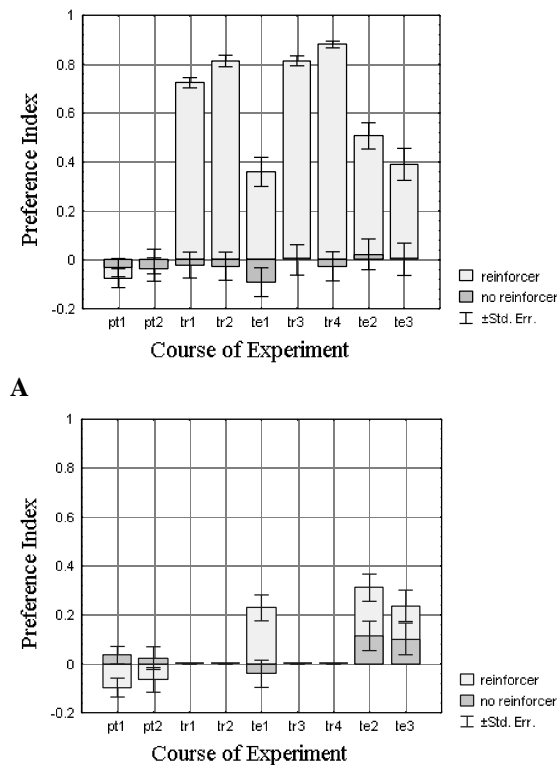
avoidance was of the opposite sign than that at t_2 (the last two periods, p<0.027).

Comparing the t_2 preference indices of the operant with the classical test group, the 'classical' index is significantly lower than the operant one (p<0.015; p>0.22 for the control groups). However, when the mean preference index at t_1 is subtracted from the indices at t_2 (to compensate for the initial individual pattern preference, see conclusion) the effect drops below significance (p=0.091).

In contrast to Dill et al. (1995), a comparison of the mean dwelling times (periods of staying in one quadrant) for the 'hot' and the 'cold' sectors with the respective spontaneous behavior reveals that the operantly trained flies modulate the average time they spend both in the 'hot' and in the 'cold' sectors, even during the last two periods (p<0.001 in both cases of the operant groups, Fig. 19A). This can also be seen in the mean spike amplitude in the different sectors and for spike timing, respectively (data not shown). Dill et al. (1995) had found that „the dwelling times in heat associated quadrants during test" were not significantly different from the control group by comparing averaged individual medians (not means as in this study) for each group. Since the frequency distributions of 'hot' and 'cold' dwelling times are very similar (Reinhard Wolf, pers. comm.), the contradiction is considered not to

A

B

Fig. 18: Mean preference indices for all four groups of flies (N=100 each). *Drosophila* learns to avoid one of the pattern orientations, if it was reinforced during the training periods (*dotted bars*). The control group, which did not receive any reinforcement only showed random avoidance (*hatched bars*). *A* - preference indices of the standard groups, *B* - preference indices of the classical groups.
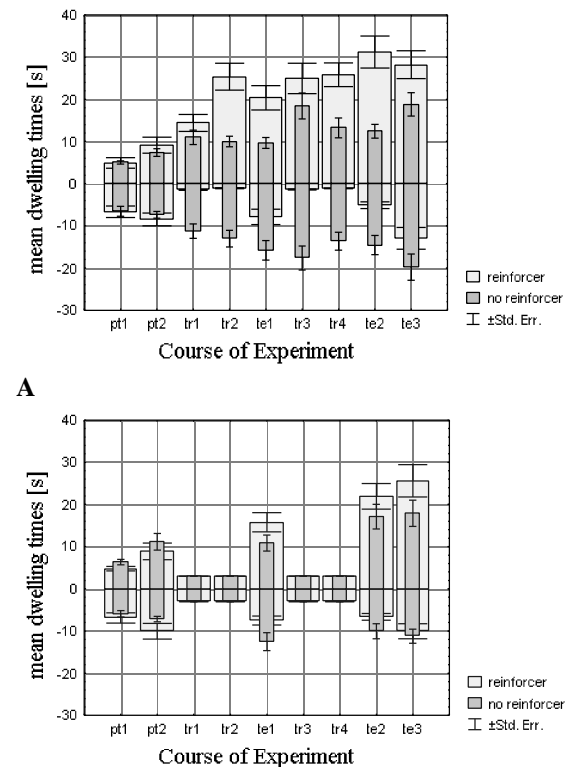

A

B

Fig. 19: Comparison of mean dwelling times for 'hot' and 'cold' sectors (N=100 flies in each group). Individual mean dwelling times were averaged for each group (*wide, dotted bars*: test group, *narrow, hatched bars*: control group). Negative sign indicates dwelling times in the reinforcer-associated quadrants. A - Standard groups, B - Classical groups.

be a statistical artifact. Rather the omission of dwelling times shorter than 1s in Dill et al. (1995) might have had an influence on the significance of the 'hot' effect: they might pull the mean 'hot' dwelling times significantly below control levels in this study. However, since comparing in a similar manner several of the variables discussed in detail below (3.5.2), produced lasting effects in the 'hot' sectors as well (data not shown), including the short dwelling times was probably the right choice.

Classically trained flies (Fig. 19B) also change the time they spent in one quadrant in response to the training, however to a lesser degree. Comparing the classical test and control group at t_2 (periods 8 and 9), the differences fail to rise above the significance level. Only comparing the 'hot' dwelling times at all three test periods yields a significant difference ($p<0.04$). Surprisingly, the 'cold' dwelling times, which subjectively seem to reveal a larger difference can still not be distinguished statistically using a repeated measures MANOVA over all three test periods ($p=0.10$).

3.5.2 Spike Dynamics and -Timing

While in neither of the groups spike duration was modulated in response to heat (data not shown), operantly trained *Drosophila* exhibited a small but highly significant modulation of spike amplitude, even when the heat was switched off during the last two periods ($p<0.001$, fig. 20A).

Although the classically trained flies do modulate their spike amplitude in response to the heat as does the operantly trained group (Fig. 20B), the difference fails to reach a level of confidence of $p<0.05$ at t_2. However, if one compares all three test-periods (nos. 5, 8 and 9) with the respective control values the modulation comes to lie at a reliable $p<0.03$.

The t_2 amplitude indices of the classical test group show no significant deviations from those of the operant test group ($p=0.35$). Furthermore, these amplitude indices are in both groups positively correlated with the respective preference indices at t_2 (Table 5) and before as well (data not shown). Most importantly, *Drosophila* seems also to modulate the spike amplitude spontaneously: even in the control groups is the amplitude index highly correlated with the respective preference index (Table 5).

In addition to spike amplitude, the flies use the timing of spikes to avoid the heat in the standard experiment: as noted above, often a volley of spikes is generated to get the fly out of the heat (Fig. 21). Comparing the spike latency in the heated sectors with that in the non-heated sectors, shows the reaction to the heat: the time until the first spike is significantly reduced in the 'hot' sectors (Fig. 21A).
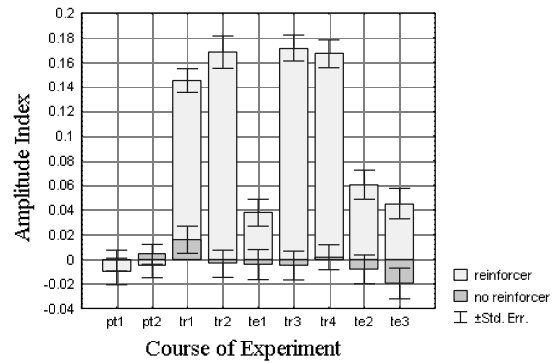

A
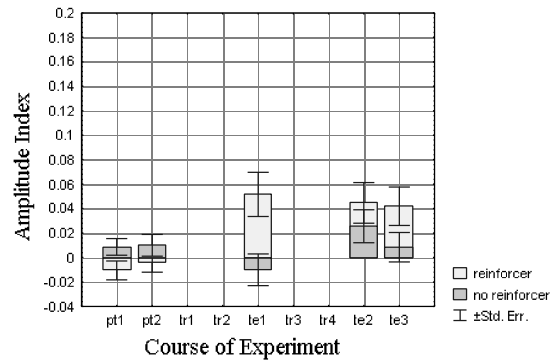
B

Fig. 20: Mean amplitude indices for all four experimental groups (N=100 flies each). The flies learn to generate larger torque spikes in quadrants with one of the pattern orientations, if they were reinforced in these quadrants during the training periods (*dotted bars*). This is the case for the operantly (*A*) and the classically (*B*) trained flies. The control groups, which did not receive any reinforcement modulated spike amplitude randomly (*hatched bars*).

This behavior is maintained even when the heat is permanently switched off. This is not significant for the last two periods alone, but for all three test periods a $p<0.041$ renders the effect reliable.

Since the latency indices of the operantly trained flies were already very low at t_2, it is not surprising that the latency indices of all three test periods from the classically trained batch rise only slightly above control-level (Fig. 21B). However, they could neither be distinguished from the operant batch with a significant reliability.

Interestingly, both groups show different correlations with the preference indices in the respective periods (Table 5). While there is some correlation in the operant groups - indicating that the latency until the first spike has a certain predictive value for avoidance and learning in these flies - there is no correlation between the latency indices and the preference of the classically trained flies at t_2. This seems to be an effect of the open loop pattern presentation, since this is also revealed in the classical control group (Table 5).

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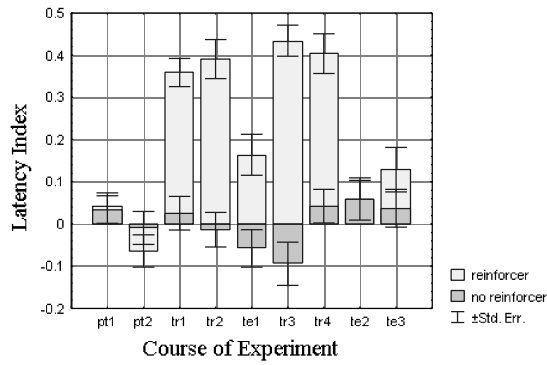
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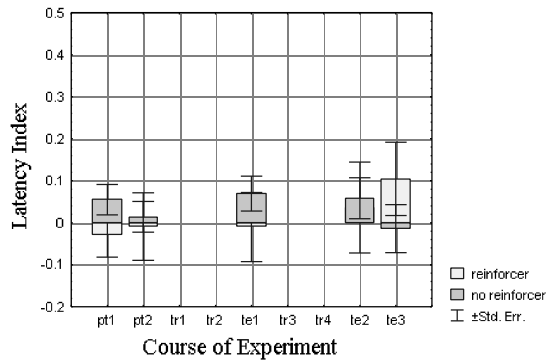
B

Fig. 21: The two test groups (*dotted bars*) show mean latency indices that suggest a modulation of the reaction time until the first spike in response to heat (see text). Most suggestive are the high training bars in the operant group (*A*). In the classical group (*B*), only in the last period, the first spikes are produced quick enough in quadrants with the heated pattern orientations to bring the index discernibly above the control group (*hatched bars*).

Even if the timing of the first spike were not modulated in response to the heat, the fly still has the possibility to make all subsequent spikes quicker to avoid the unpleasant flight direction. In the standard experiment, the interspike intervals seem indeed to be shorter in heated sectors than in 'cold' ones: flies that were able to control the appearance of the reinforcer produce shorter interspike intervals in 'hot' sectors compared to the control flies, even if the heat is switched off for the last two periods (t_2, $p<0.001$; $p=0.336$ at t_1; Fig. 22A).

Even if the plotted ISI indices of the classical test group (Fig. 22B) seem rather well in accordance with the findings in the operant test-group, they fail to rise above the required significance niveau. Nevertheless, both pre-test values being in the negative range and all test-values in the positive and above the control-values suggest a qualitatively similar although quantitatively less strong effect. Moreover, the corrected ISI indices at t_2 (mean ISI index of the pre-tests subtracted) cannot be distinguished statistically from the respective values in the operant group ($p>0.06$).

As the amplitude index, the distance and latency indices of the two test groups are highly correlated with the preference index at t_2 (Table 5). This is
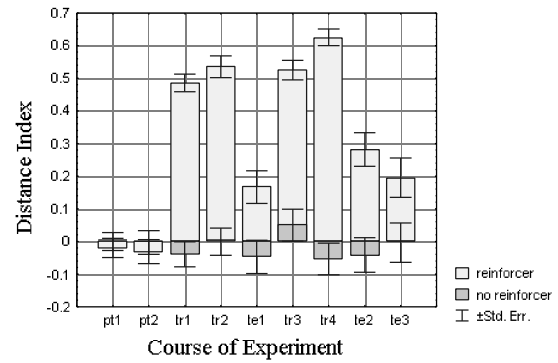
A
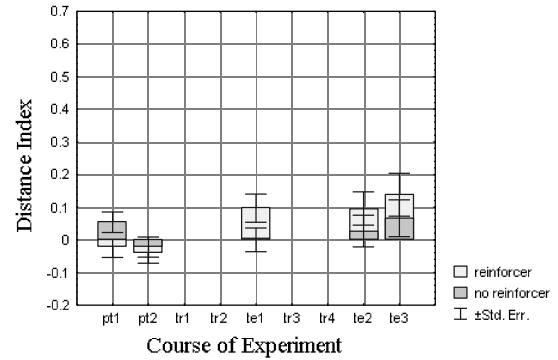
B

Fig. 22: Mean ISI indices for all four groups (N=100 flies each). *Drosophila* produces torque spikes more quickly when it is heated in closed loop (*A, dotted bars*). If the heat is switched off, the flies still generate spikes with shorter interspike intervals in quadrants with the 'hot' pattern orientation. The control group, which did not receive any reinforcement only showed random modulation of the interspike intervals (*hatched bars*).

In the classical groups (*B*), however, statistical analysis could not establish a significant difference between the test (*dotted bars*) and the control group (*hatched bars*). Nevertheless, the values are qualitatively in the proper range to assume that they perform the same behavior as the operant group, although to a lesser degree.

also the case for the spontaneous behavior measured in the control groups (Table 5).

Having demonstrated that at least the operantly trained flies indeed generate spikes more quickly in those quadrants associated with the heat, one can conclude that the spike frequency (number of spikes per time) is elevated as well, which can be seen in fig. 23.

At first sight (Fig. 23B) the response of the classically conditioned group seems to be the same as in the operant group. However, it fails to surpass control-levels to a sufficient degree ($p=0.17$ for all three test periods). However, omitting period 9 with the strikingly high control value, the modulation exceeds the control values of periods 5 and 8 ($p<0.02$).

Paralleling the conditions for the interspike intervals, the number indices of all four groups correlate with the respective preference indices (see Table 5) and the corrected number indices in the classical test group do not differ significantly from the values in the operant batch ($p>0.05$).
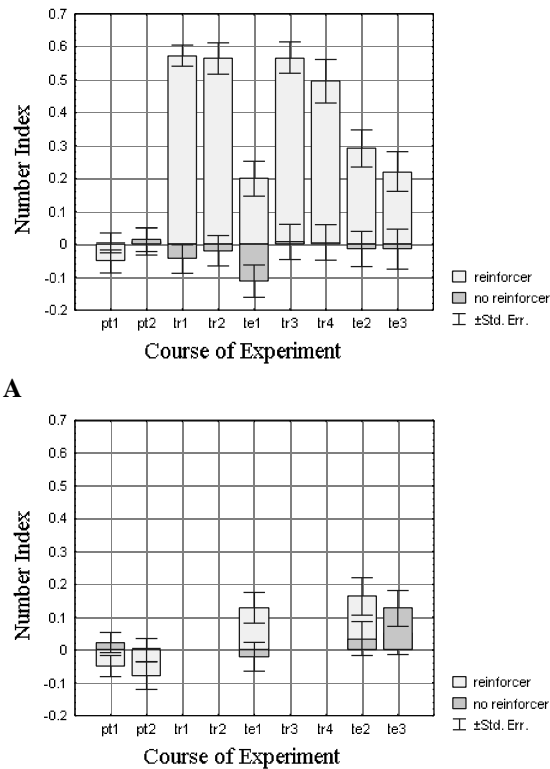
A

B

Fig. 23: The mean number indices in the operant groups (*A*) clearly reflect the behavioral strategy of making many spikes in previously punished flight directions: whereas the control group (*hatched bars*) fails to produce other than random spike frequency modulations, the test group (*dotted bars*) shows directed spike frequency modulation in the predicted way.
This is also visible in the classical groups (*B*), however to a lesser degree (see text).

Taking together all the data presented so far, it seems as if the major reason why the effects in the classical group appear to be less significant than in the operant group, is the unusually low preference in the two pre-test periods (t_1) of the classical group, together with the unusually high spontaneous preference in the periods 8 and 9 (t_2) of the corresponding control group. The finding, however, that the values of the classical group are not significantly different from the operant test group either is taken as evidence for the hypothesis that the effects are indeed of the same nature as in the operant group. Moreover, if one expects the control groups to exhibit symmetrical behavior, i.e. zero values in all indices, then one can test the index values of period 8 with a Wilcoxon Matched Pairs Test against zero. This test yields significant effects for the number index (p<0.01, period 8) and the amplitude index (p<0.03, period 8). The ISI index is very close to significance (p=0.067, period 8).

3.5.3 Spike Polarity

In this category, the crudest measure gave the clearest result: in the 'cold' sectors, more spikes are generated towards the pattern than away from it, whereas in the 'hot' quadrants the relation is zero or

reversed. This is still true if the heat is permanently switched off (Fig. 24).

A

B
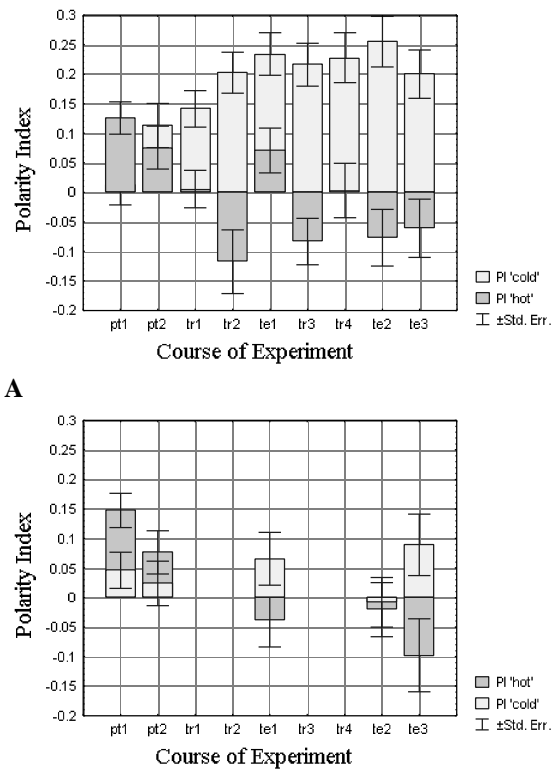
Fig. 24: Mean polarity indices (PI) for the operantly (*A*) and the classically (*B*) trained groups (N=100 flies each). In both groups one can notice a rise in PI in the 'cold' sectors (*dotted bars*) compared to a rather steep decline in the 'hot' sectors (*hatched bars*). One exception is period 8 in the classical test group.

In all the control groups, the indices for the respective sectors were indiscernible and came very close to 0.1 (Fig. 25). Comparing operant test and control groups at t_2, both 'cold' and 'hot' indices were significantly different: 'cold' polarity indices were higher in the test than in the control group (p<0.02) and 'hot' indices were lower (p<0.002). Both 'hot' and 'cold' indices show significant correlations with the respective preference indices (Table 5). As can be expected from the low absolute differences in the polarity indices (Fig. 24) these correlations are

Fig. 25: The mean polarity indices (PI) for both control groups. Open symbols indicate the operant group, filled symbols the classical group. *Lines were drawn for illustrational purposes only.*

A

B

Fig. 26: The mean polarity-latency indices for the operant test groups (*A, dotted bars*) indicate, that on average the first spikes away from the pattern are generated earlier in quad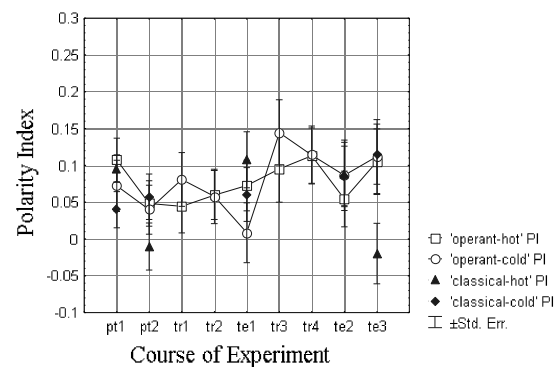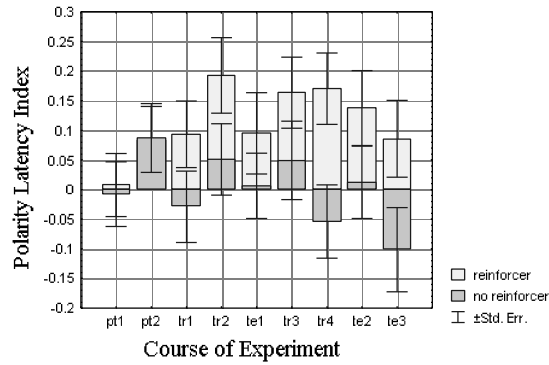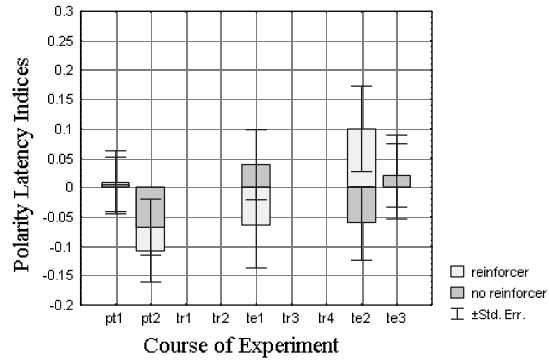rants where the pattern orientation is associated with the reinforcer than in the other sectors (*hatched bars* - control group). In the classical groups (*B*), there was no detectable pattern of modulation of spike polarity-latency neither in the test group (*dotted bars*), nor in the control group, which did not receive any reinforcement at all (*hatched bars*). Only in period 8 The flies from the classical test group generated the first spikes away from the pattern earlier in quadrants where the pattern orientation is associated with the reinforcer than in the other sectors.

lower than those for the polarity independent variables (Table 5). Furthermore, the unproportionally large training values commonly observed in the polarity independent variables are missing in the polarity indices, indicating that this parameter is not modulated in a responsive way.

As the spike polarity effects are already quite weak in the standard experiment, they diminish even more after classical conditioning. Evaluating the polarity indices for the 'hot' and the 'cold' sectors (Fig. 24B) still shows a steep decline in the polarity index for the 'hot' quadrants, the effect in the 'cold' quadrants is less impressive, however. Consequently, only the 'hot' effect is statistically safeguarded against the controls ($p < 0.02$, taking all test periods into account). Nevertheless, as in the standard experiment, the 'hot' and the 'cold' polarity indices are significantly correlated with the preference indices (Table 5).

Comparing the two test groups at t_2 yields the same results: the 'hot' effect is not different in both groups, the 'cold' effect, however, is significantly larger in the standard group ($p < 0.02$) even if the t_1 values are subtracted. This is also reflected in the

difference in fixation as will be discussed below (3.5.4).

In the operant group an even more subtle but nevertheless significant effect can be observed in the polarity-latency indices. (Fig. 26A). The difference between the test and control group is clearly to be seen throughout the experiment and at t_2, when the heat is switched off, it is still statistically reliable ($p < 0.04$). As with the polarity indices, unproportionally large training values are absent, indicating a gradual development of directing spike polarity.

In the classical group an effect of spike polarity latency can only be seen in period 8 (Fig. 26B). All other periods show the same random fluctuations as the control group.

Since the absolute values are so small and the errors are relatively large, it is not surprising that the two test groups do not differ significantly from each other at t_2 ($p = 0.305$).

In all four groups no correlation between the polarity latency indices and the preference indices can be detected (Table 5). This behavior seems thus to be acquired independently of the expression of learning.

3.5.4 Variables Measured Independently of Quadrant Treatment

In both operant groups fixation increased during the course of the experiment, whereas in the classical groups only the control group showed an increase in fixation (Fig. 27).
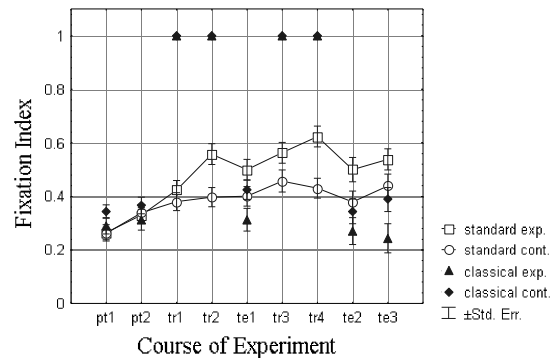
Fig. 27: Mean fixation indices of all four groups. Note that the training indices of the classical groups are always 1.0 due to the static presentation of the patterns.

Compared to the operant group, the flies that were presented with static patterns fixated the patterns significantly worse at t_2 (periods 8 and 9; Table 4), whereas there was no difference at t_1 (Fig. 27). Since the two respective control groups do not reveal any significant differences in fixation, this effect has to be attributed to the different reinforcement procedures. For the same reasons, the shift in the amount of total arena rotation at t_2 has to be due to the training procedures (Table 4). Classical and operant groups showed no difference in the number of quadrant changes (Fig. 28).

17
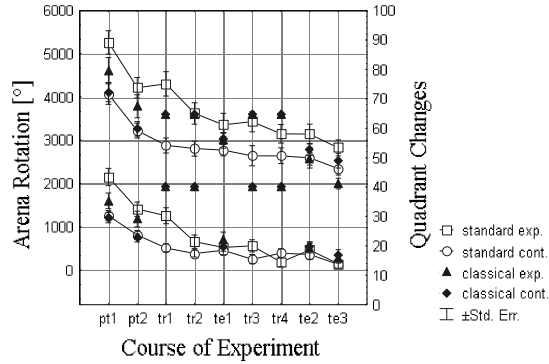
Fig. 28: Combined presentation of total amount of arena rotation (upper four lines, left abscissa) and number of quadrant changes per period (lower four lines, right abscissa). Note the fixed values for the classical training periods.

Decreasing numbers of quadrant changes and amount of arena rotation (Fig. 28), together with decreasing spike numbers support the view that the flies become acquainted with their situation and calm down during the course of the experiment. The force of the body saccades, however, remains constant throughout the experiment (Fig. 13).

The finding that the operantly trained flies fixate best confirms earlier experimental data from Dill et al. (1995) where it was demonstrated that flies prefer flight directions in the middle of the 'cold' quadrants when they have been heated in the other sectors. It seems as if this increase in fixation is an acquired behavioral strategy and not the consequence of decreasing overall activity (see below, 3.6). While at t_1 the fixation index is weakly (about -0.2) but significantly correlated with spike number and total amount of arena rotation (i.e. activity) this correlation ceases at t_2. It is therefore not surprising that the flies that show the highest fixation index at t_2 generate more spikes and cause more arena rotation than the others (however, this group was already more active at t_1).

If fixation were an actively selected behavioral strategy, the poor fixation in the classically conditioned flies might be due to the avoidance of the 'hot' pattern orientation (i.e. anti-fixation) while fixation in the 'cold' sectors is within control limits. This view is discussed more thoroughly below (3.6).

	standard	s-control	classic	c-control
FixInd***	0.52	0.41	0.26	0.37
Spikes*	35.4	30.5	27.7	33.9
SpDur**	0.48s	0.48s	0.54s	0.49s
ARot*	2986°	2470°	2285°	2658°

Table 4: Values for those variables, where the null hypothesis that the four groups were drawn from one population could be rejected, averaged over the two last periods (t_2). FixInd - fixation index; Spikes - number of spikes per two minute period; SpDur mean spike duration, ARot - total amount of arena rotation. * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$.

It cannot be ruled out, however, that the poor fixation is due to fatigue caused by the high amount of heat each fly is receiving during classical training: the flies get weaker as they are dehydrated in the course of the training. Consequently, they generate less and smaller spikes (Figs. 12 and 13) as well as a reduced amount of optomotor balance (quiet mode, Fig. 8). Heisenberg and Wolf (1979) report that 'non-fixation' is particularly common in flies using the quiet mode of flight:

„[...] the stripe may suddenly start to be shifted to any position and may be kept there quietly for some time. We call this behavior 'non-fixation'."

Picturing this quiet mode might also explain the extremely long spike duration in the classical test group: it leads to a later reversion of the torque slope after each spike which is the confinement of spike duration as defined above (Fig. 10). Consequently, spike duration is negatively correlated with arena rotation, spike number and spike amplitude (not shown).

3.6 Measurements at t_2

As discussed above (3.5.2), the flies primarily modulate two polarity independent characteristics of turning maneuvers in order to avoid certain flight directions in the flight simulator: spike amplitude and spike timing. Two sources of evidence support this view: the comparison of the test values with the respective spontaneous values generated by the control flies (see above, 3.5.2) and a correlational analysis in all four groups with the preference values (Table 5).

	standard	s-control	classic	c-control
AmpInd8	0.60***	0.60***	0.40***	0.45***
AmpInd9	0.60***	0.68***	0.46***	0.46***
LatInd8	0.09	0.45***	0.15	0.14
LatInd9	0.40***	0.25*	-0.01	0.14
ISIInd8	0.79***	0.68***	0.72***	0.83***
ISIInd9	0.76***	0.90***	0.83***	0.79***
NumInd8	0.56***	0.70***	0.62***	0.76***
NumInd9	0.75***	0.75***	0.61***	0.84***

Table 5: Spearman Rank Order Correlations with the preference index at t_2. AmpInd - amplitude index, LatInd - latency index, ISIInd - ISI index, NumInd - number index. * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$.

The only obvious difference between the operant and the classical groups to be detected in Table 5 is in the latency indices. This is not surprising, since only one spike is counted for each stay in one quadrant while the fly is generating many more afterwards and therefore has many more options to choose flight direction. Small disturbances in flight behavior due to rather long phases of open loop might exert a large effect in such a variable. Furthermore, even in those cases where the correlation is significant, the values are lower than all the other correlations. From the data presented so far one can infer that flies that have high preference indices produce more and larger spikes in the 'hot' than in the 'cold' sectors, even if the heat is switched off, regardless of their training procedure. Performing a correlational analysis among the different parameters yields results that strengthen this view: those flies that generate more spikes in the 'hot' sectors do also tend make them larger (significant positive Spearman Rank Order Correlations at t_2 between the ISI index, the number index and the amplitude index in all four groups, data not shown). This is also the case for spontaneous behavior (see table 5): the flies use the same motor-output to express their endogenous preference for a certain flight direction. This in turn leads to an important corollary: both training procedures might modulate the endogenous preference of each fly.

Another piece of evidence pointing in this direction are some of the variables measured independently of quadrant quality (spike numbers, fixation index and quadrant changes): in the test groups they are significantly correlated with the respective preference indices, whereas in the control groups they are not (Table 6). Does this mean that flies that are calmer and fixate better are also better learners?

	standard	s-control	classic	c-control
Spikes8	-0.27**	-0.01	-0.27**	-0.10
Spikes9	-0.04	0.02	-0.14	-0.20
FixInd8	0.53***	-0.10	0.34***	0.17
FixInd9	0.36***	-0.15	0.34***	0.11
QuCh8	-0.48***	0.06	-0.47***	-0.19
QuCh9	-0.23*	0.00	-0.33***	-0.16

Table 6: Spearman Rank Order Correlations with the preference index at t_2. Spikes - number of spikes per two minute period; FixInd - fixation index; QuCh - number of quadrant changes per two minute period. * - p<0.05; ** - p<0.01; *** - p<0.001.

This inference is not necessarily true, of course, since performing the same analysis with the absolute preference indices yields identical results for all four groups (not shown). In other words: all the reinforcer does is switching the endogenous preference in all flies to the same direction. This is mimicked by using absolute preference indices. Then of course it becomes clear why reduced activity and better fixation leads to higher scores: the lower the activity is, the longer the dwelling times in each sector and the higher the preference indices - independently of whether the fly has learned anything.

This outcome would be expected if fixation were correlated with the expression rather than with the acquisition of memory.

Among all the variables measured independently of the differently treated (hot/cold) sectors, the null hypothesis that all groups were still from the same population had to be rejected in four variables: fixation index, spike number, spike duration and total amount of arena rotation (Table 4). Interestingly, the fixation indices indicate that the flies trained in closed loop fixate the pattern more closely to the very front (i.e. generate more spikes towards the pattern) than do the flies presented stationary patterns during training. As discussed above (3.5.4), this effect is largely due to strong deviations among the two test groups (p<0.001), whereas the control groups do not differ (p=0.449).

After evaluating a number of spike polarity dependent variables, only the number of spikes towards the pattern compared to the number of spikes away from the pattern yielded results that could be related to learning (Table 7).

	standard	s-control	classic	c-control
PolHot8	-0.49***	-0.30**	-0.34***	-0.27**
PolHot9	-0.41***	-0.50***	-0.48***	-0.43***
PolCold8	0.49***	0.42***	0.31**	0.49***
PolCold9	0.51***	0.40***	0.20	0.28**
PolLat8	0.02	0.17	0.11	0.11
PolLat9	0.05	0.19	0.02	0.14

Table 7: Spearman Rank Order Correlations with the preference index at t_2. PolHot - 'hot' polarity index; PolCold - 'cold' polarity index; PolLat - polarity latency index. * - p<0.05; ** - p<0.01; *** - p<0.001.

As fixation index and overall polarity index are of course highly correlated (mean Spearman Rank Order Correlation at t_2 0.46, p<0.001), the polarity indices in the differently treated sectors are especially telling: In the 'cold' sectors, more spikes towards the pattern were generated than away from the pattern and *vice versa* in the 'hot' quadrants. However, there was no 'training effect' i.e. unproportionally large values for the training periods, indicating that this behavior is largely independent from the reinforcer. This was the case for all the variables connected with spike polarity (not shown). In the light of spike polarity, the poor fixation of the classical test group compared to the operant test group might indeed reflect different behavioral strategies acquired by the different training procedures: during operant training the flies learn that the centers of the 'cold' quadrants are 'safe' (Dill et al. 1995). During classical training, the sector borders are not perceptible for the fly - the flies are punished with the pattern in the centralmost position. They might even learn to avoid this central position. This experience might be more salient to the fly than the unpunished position of the other pattern orientation. The data on spike polarity points in this direction: while in the 'cold' sectors the polarity index of the classical test group does not rise above
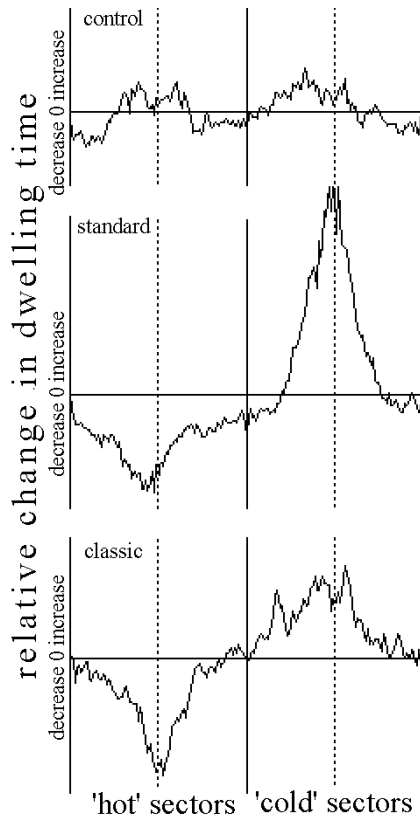
Fig. 29: Illustration of the changes in dwelling time distribution. From the position trace, a dwelling time/position histogram for 'hot' and 'cold' quadrants is calculated for both pre-test (t_1, periods 1 and 2) and test (t_2, periods 8 and 9). The difference between these histograms at t_1 and t_2 is depicted here. Dotted lines indicate the centers of the patterns, vertical lines quadrant borders. The horizontal lines depict zero change in dwelling time from t_1 to t_2.

the control level as does the operant group, the drop in the 'hot' sectors is significant and indistinguishable from the operant group. Fig. 29 illustrates this accurately: in the 'hot' quadrants both groups equally decrease fixation (i.e. decrease the number of spikes towards the pattern), while in the 'cold' sectors only the operant group decreases its stays near the borders and increases fixation. The flies in the classical group increased dwelling times in the entire 'cold' sector.

This difference is also reflected in the results of a correlational analysis among the measured behavioral parameters: while spike polarity at t_2 in the 'hot' quadrants was significantly correlated (negatively) with indices describing modulation of polarity independent spike parameters in all four groups, spike polarity in the 'cold' sectors was not correlated with the other indices in the classical group (Table 8).

	standard	s-control	classic	c-control
AmpInd^H	-0.18	-0.29**	-0.27**	-0.11
ISIInd^H	-0.43***	-0.45***	-0.36**	-0.22*
NumInd^H	-0.23*	-0.32**	-0.30**	-0.30**
AmpInd^C	0.23*	0.34***	0.14	0.10
ISIInd^C	0.44***	0.32**	0.21	0.43***
NumInd^C	0.33**	0.36***	0.14	0.31**

Table 8: Spearman Rank Order Correlations with spike polarity at period 8. Superscripts indicate with wich polarity index the variable was correlated: H - 'hot' polarity index. C - 'cold' polarity index. AmpInd - amplitude index, ISIInd - ISI index, NumInd - number index. * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$.

The modulation in the polarity of the first spike was negligible: a mean decrease of 0.06 in the probability that the first spike is towards the pattern in the 'hot' compared to the 'cold' sectors was the largest value obtained (standard experiment). The overall probability was over 0.8 that a first spike in any quadrant is „towards pattern".

Summarizing the data concerning the direction of turning maneuvers, it seems as if modulation of spike polarity is one but not a primary behavioral strategy for orientation in the *Drosophila* flight simulator. 'Simpler' strategies such as modifying the force or the frequency of the body-saccades seems to account for more of the avoidance behavior than directed orientation in space.

4 Conclusion

Suppose all the assumptions made in the introduction to this study are correct - namely that different associations are made in operant and classical conditioning and our paradigms represent operant and classical conditioning - then there is only one possibility why the behavioral strategies of the four groups are so strikingly similar: the range of behaviors a fly uses for flight orientation seems to be very limited and rather hard wired; the components of this behavior are tightly interconnected. Therefore, the CR in the classical conditioning paradigm is the same as the response conditioned in the operant paradigm and there is only very little room for acquiring 'new' strategies. An observation mentioned above (3.5) is in favor of this notion: very similarly to the volley of spikes depicted in Fig. 16, some flies produce spike volleys and a shift in the torque baseline when heated under open loop conditions (Fig. 17). In this view, the significant difference in fixation/spike polarity might be a cue as to how the expression of learning is accomplished in the *Drosophila* flight simulator: modulating spike dynamics and -timing irrespectively of the spikes' direction seems to be a set of very basic and interdependent behaviors that is activated whenever the fly is asked „stay or leave?" while the directional usage of spikes is a more sophisticated behavior that becomes important when the fly is asked „how can I stay?" or „how can I leave?". In the classical conditioning paradigm studied here, the latter ques-

tions are never asked, since the patterns are presented stationarily during training. In the standard paradigm, however, the question *how* to stay away from the heat is of great importance. Nevertheless, the 'knowledge' how to perform that task is only acquired slowly during the experiment - there are no training effects in spike polarity indicating a rather fixed, responsive behavior. Unpublished data from Reinhard Wolf (pers. comm.) lead him to the idea of a two-process theory, too.

The important implication of this hypothesis is that for both groups, in essence, the same has been learned, namely that a certain orientation of the patterns is 'hot' and has to be avoided (i.e. a stimulus-reinforcer association has been formed). In surplus, the flies of the operant group have learned how to avoid the pattern more effectively. This is well in line with the expectations.

Concentrating on the first, more basic process of avoidance, it can be inferred from the behavior of the control groups that our type of conditioning merely switches or confirms the sign of the individual fly's spontaneous preference. In addition to the data on the flies' behavior presented so far, it can be observed that the preference at t_1 is carried as a 'socket' throughout the whole experiment (not shown). Then the procedure of subtracting the pretest values (t_1) from the test values (t_2) is admittable. After this subtraction, only *one* of the indices calculated in dependence of the differently treated (hot/cold) sectors showed significant differences between the two test groups: the 'cold' spike polarity indices (Figs. 24 and 29). All the other parameters showed the same modulation. If modulating the direction of spikes is considered a more **sophisticated** strategy, maybe acquired after learning the 'basic' avoidance task, then the flies apparently employed the *same* behavioral output irrespectively of their training to perform the **basic** task of avoiding the pattern orientation associated with the reinforcer: the flies from both groups generate many, large spikes when the previously heated pattern orientation is in the frontal sector of the visual field.

If the lack of significant differences in the basic responses generated by the flies were due to the same associations made during the different training procedures, several questions have to be asked: how classical is the standard paradigm? Is the distinction between operant and classical merely operational? What is learned during conditioning?

4.1 How 'Classical' is the Standard Experiment?

Using recorded movements of the arena (together with the heating schedule) from a previously trained fly (master) during open loop training periods in a second fly (replay-experiments, Wolf and Heisenberg, 1991) fails to elicit a pattern preference comparable to that observed in the paradigms used in this study. The recorded sequence of patterns associated with heat sufficed to produce a significant learning score in the master-fly, so the operant component clearly is required. *The classical component of pattern sequence associated with the reinforcer is not sufficient to explain learning in the standard paradigm.* However, it is not clear how the important operant component might exert its effect. Maybe the control of the reinforcer facilitates a stimulus-reinforcer association; it might be easier to perceive contingencies with one's own behavior than among some stimuli totally independent of oneself.

If there is a 'classical' (learning about contingencies in the world) association formed in the standard experiment, the assumptions made in the beginning have to be questioned. Either one can not consider an experiment 'purely operant' as soon as a single sensory stimulus is contingent with the reinforcer and the subject learns about this stimulus. Or operant conditioning is accomplished by the formation of two (or more) associations. Obviously, the view of singular associations that are formed in the investigated learning tasks was oversimplified.

4.2 Classical and Operant Conditioning: Merely an Operational Distinction?

If it was so, that whenever a single contingent sensory stimulus is present, the operant component were only facilitating the process of learning about the object that is transmitting the stimulus, then all learning in nature can only be categorized as classical. There is no natural situation one can think of, where only the reinforcer is present. If this inference is correct, why is 'pure' operant conditioning (i.e. where only the reinforcer is present; for experiments see e.g. Cook and Carew, 1986; Cook et al. 1991; Wolf and Heisenberg, 1991) feasible at all? So-called non-associative learning might provide some useful insights, how salient situations might be processed in general: when rats, habituated to a mild electric shock to their feet are transferred to a different experimental context (e.g. a larger or smaller cage) the habituation is undetectable. Apparently, the rats associated the shock with their environment, since transferring them back to their training cage restored habituation (unindexed poster on the 24th Göttingen Neurobiology Conference, 1996). This interpretation might lead to the idea of one common mechanism, underlying all types of learning.

4.3 What is Learned During Conditioning?

If there is a common mechanism underlying all types of learning, what associations are formed during conditioning?

In the context of operant conditioning, it has to be determined if the introduction of a discriminative

stimulus into a 'purely' operant paradigm significantly alters the mode of acquisition of memory. One crucial experiment to find out to which degree a classical component is integrated in an operant paradigm, seems to be to train an individual operantly to use one output channel to optimize its stimulus situation (reinforcer and stimulus) and then test it by coupling a different output channel to the same environment (stimulus without reinforcer). Such an experiment has not been performed, yet.

In the context of classical conditioning, the issue is which properties of the contiguous events become encoded and associated. One consequence of the notion that stimulus-reinforcer associations were formed during classical training has gathered much attention in the literature: if simple stimulus substitution were to account for learning, the CR should be identical to the UR. Several observations seem to indicate that this is not the case. Rabbits for instance, respond with swallowing and jaw movements during training in a salivary conditioning paradigm, but fail to show these behaviors during test (Sheffield, 1965; cited in Rescorla and Holland, 1982). Or the CR might include behaviors not present in the UR: Pavlov's abovementioned dog that showed appetitive behavior towards the bell is one example of motor activity towards CSs paired with food, although activity is not part of the response to food itself. Pigeons peck visual signals for USs that do not elicit pecking, such as water delivered directly into the mouth (Woodruff and Williams, 1976; cited in Rescorla and Holland, 1982) or heat (Wasserman, 1973; Wasserman et al. 1975; cited in Rescorla and Holland, 1982). Spear et al. (1990) cite Pinel et al. (1980) where conditioning is expressed by suppression if a tone predicted the US (shock) but by active hiding if the US was signaled by a prod.

As noted above (4), some flies observed during this study confirm the evidence in the literature: they produced spike volleys and a shift in the torque baseline when heated under open loop conditions (Fig. 17), clearly to be classified as an UR to the heat. This behavior disappears completely if the heat is switched off (Reinhard Wolf, pers. comm.); a trace of it, however, can be detected in closed loop: the spikes produced in quadrants with the previously heated pattern are larger and closer together than in the other sectors.

Moreover, it has been shown that conditioning still does occur if the stimulus-properties of the US are suppressed: stimulation of the VUMmx1-neuron in bees can serve as substitution for the sugar-reinforcer (Hammer, 1993). Suppression of the response-evoking properties of the US, for instance by applying response attenuating drugs such as curare does not prohibit learning either (Solomon and Turner, 1962; cited in Rescorla and Holland, 1982), ruling out direct stimulus-response associations in classical conditioning.

If the view of singular response-reinforcer or stimulus-reinforcer or stimulus-response associations is so oversimplified, what is happening in the brain of a conditioned organism?

Assume an animal struggling for survival: every sensation might provide a clue how to escape a predator, find a mate, explore new food patches, hiding places, etc. In every second it is confronted with potentially dangerous or advantageous situations. The possibility to predict such situations must convey an enormous selection pressure. A very effective way to accomplish this task would be an evaluation mechanism, judging *situations* according to their 'beneficence' for the individual. With such a mechanism *salient* internal and external stimulus-arrays extracted from the situation would receive situation-specific rankings on a value-scale in terms of 'good', 'bad' or 'neutral'. The probability of performing a given behavior in a certain stimulus situation is the manifestation of a more or less complex superposition of stimulus-rankings, motivation and initiating activity. In this picture learning corresponds to linking neutral or unknown stimuli to already ranked ones, whenever a sufficient cross-correlation between them is detected. In 'pure' operant conditioning, the internal representation of behaviors (efference copy, von Holst and Mittelstaedt, 1950) is linked to the ranking of the reinforcer if the correlation coefficient between them is sufficiently positive. If more stimuli are contingent with the reinforcer, they receive adequate rankings as well. The richer the environment, the more complex the net between the stimuli becomes. An *a priori* ranking of stimuli and behavioral representations constitutes the basis for URs, fixed action patterns, and the species-specific salience and associability of certain stimuli. As mentioned above, many of these rankings are assumed to be situation specific. Situation-specific in this case means the situation in which the ranking has been acquired. For instance, if the reinforcer was food, the contiguous behavior(s) or event(s) would receive a food-specific ranking rather than a mate- or danger-specific ranking. Prolonged training intensifies the links (associations) between the stimuli. This might lead to such prominent behavior as described above for the chimpanzees.

Such an informal model is in conformity with several attentional models for acquisition (e.g. Rescorla and Wagner, 1972; Mackintosh, 1975), describing overshadowing (Pavlov, 1927) or blocking (Kamin, 1969), since the distance of stimuli from the value 'neutral' might convey them with the appropriate attentional properties. It fully accounts for differences in UR and CR by not only transferring the US value to the stimulus, but also by linking the rankings of different US features to the appropriate behavioral rankings (situation-specificity). Sensory preconditioning (in rats: Rescorla and Cunningham, 1978; in bees: Müller et al. 1996) is predicted by

this model as the ranks of contiguous (or similar for that matter) stimuli would become linked.

Such a model would imply that as soon as one salient sensory stimulus is presented contiguous with the reinforcer in an operant conditioning paradigm, the subject will use all output-channels to respond appropriately during test.

5 Summary

The assumptions for this study were as follows: in operant conditioning a response-reinforcer association is formed, whereas in classical conditioning the association is stimulus-response in nature. Using the *Drosophila* flight simulator, the motor-output of two groups of *Drosophila* flies - one presented with stationary stimulus-reinforcer pairings (open loop training), one enabled to control the presentation of the pairings (closed loop training) - was compared. It was assumed that the open loop group was classically, the closed loop group operantly conditioned. If this were correct, there might be a difference in the behavioral strategies an operantly trained subject might acquire compared to a subject classically trained to perform the same task. It can be concluded from the gathered data that *one* of the observed behavioral strategies was significantly enhanced in the operantly but not in the classically trained flies: choosing a flight direction as far away from the reinforced stimulus as possible.

It appears, however, that an individual during conditioning is not confined to making singular associations but is rather evaluating complex stimulus situations. This leads to the transfer of reinforcer properties to the unconditioned stimulus even in the operant paradigm. An experiment in which the subject uses one behavioral repertoire (e.g. walking behavior) to control the reinforcer and a sensory stimulus during training and another (e.g. flight control behavior) to control the conditioned stimulus during test, deserves special interest in resolving this issue.

6 Acknowledgements

7 Zusammenfassung

Unter der Annahme, daß ein Subjekt bei operantem Konditionieren eine Kreuzkorrelation zwischen eigenem Verhalten und seiner Stimulus-Situation bildet und bei hinreichend positiver Übereinstimmung eine Antwort-Verstärker Assoziation knüpft, bei klassischem Konditionieren jedoch Eigenschaften des konditionierten Stimulus durch Eigenschaften des Verstärkers ersetzt werden (Stimulus Substitution) und somit eine Stimulus-Verstärker Assoziation vorliegt, wurde in dieser Studie das Verhalten von zwei Gruppen von *Drosophila* Fruchtfliegen am Flugsimulator verglichen. Den Fliegen der einen Gruppe wurden zwei stationäre Musterpaare zusammen mit einem Verstärker in „open loop" präsentiert, die Fliegen der anderen Gruppe konnten die Muster- zusammen mit der Verstärkerpräsentation selbst kontrollieren (closed loop). Es wurde davon ausgegangen, daß erstere Gruppe klassisch, letztere operant konditioniert worden sei.

Bei Richtigkeit aller Annahmen war eine Selektion von 'effektiven' Strategien im Vermeideverhalten der operant trainierten Gruppe zu erwarten. Aus den gewonnenen Daten kann geschlossen werden, daß *eine* der beobachteten Verhaltensstrategien nur von den operant trainierten Fliegen selektiv aktiviert wird und nicht von den klassisch trainierten, nämlich eine Flugrichtung mit *größtmöglicher* Entfernung vom bestraften Musterpaar einzuschlagen.

Die Annahme singulärer Assoziationsbildung erscheint jedoch übersimplifiziert. Offensichtlich werden alle mit dem Verstärker in Zusammenhang zu bringenden Sachverhalte ausgewertet. Dies führt auch im 'operanten' Paradigma zu einem Transfer von Verstärkereigenschaften auf den konditionierten Stimulus. Von besonderem Interesse zur Klärung dieses Sachverhaltes wäre ein Experiment, in dem im Training ein Verhaltensrepertoire (z.B. Laufverhalten) den Verstärker und einen sensorischen Stimulus kontrolliert und im Test ein anderes Repertoire (z.B. Flugverhalten) zur Kontrolle des konditionierten Stimulus eingesetzt werden muß.

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Appendix 1: Flight trace of fly no. 1 that was evaluated by five volunteers for spike numbers. Thick curve: position trace, thin curve: torque trace. Shaded areas: 'hot' sectors. Each block contains a two minute period.

Appendix 2: Flight trace of fly no. 2 that was evaluated by five volunteers for spike numbers. Thick curve: position trace, thin curve: torque trace. Shaded areas: 'hot' sectors. Each block contains a two minute period.

Appendix 3: Flight trace of fly no. 3 that was evaluated by five volunteers for spike numbers. Thick curve: position trace, thin curve: torque trace. Shaded areas: 'hot' sectors. Each block contains a two minute period.

Appendix 4: Flight trace of fly no. 4 that was evaluated by five volunteers for spike numbers. Thick curve: position trace, thin curve: torque trace. Shaded areas: 'hot' sectors. Each block contains a two minute period.

Ich erkläre hiermit, daß ich die vorliegende Arbeit selbständig verfaßt und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe.

Würzburg, den 30.08.1996