Division of Molecular Neuroscience
Institute of Anatomy and Cell Biology
Philipps University Marburg
Prof. Dr. Eberhard Weihe

*

Division of Neurochemistry
Department of Psychiatry
Leopold University Innsbruck
Univ. Prof. DI Alois Saria
Univ. Prof. Dr. Gerald Zernig

Validation of Runway

An operant model developed to detect reinforcing effects in rats

INAUGURAL-DISSERTATION

to become
doctor medicinae

at
Philipps- University Marburg

Written by
cand. med. Philipp Harbig
Marburg/ Innsbruck 2004

Gedruckt mit freundlicher Genehmigung der Medizinischen Fakultät der
Philipps Universität Marburg

Dekan: Univ.- Prof. Dr. Kohlmann

1. Berichterstatter:
2. Berichterstatter:

Tag der mündlichen Prüfung:

Acknowledgement:

- A. Prof. Gerald Zernig who introduced me into the scientist´s world and who helped me to understand the complex work in the lab; for his patience when he showed me how to use computer programme or do rat surgeries.
  Thanks, for inviting me to your wine- tastings and for being a friend.

- Prof. Alois Saria for afforing pleasure and a good working atmosphere in his neurochemistry laboratory;
  As well as for the „congress air " I smelled, because of his invitations.

- Prof. Eberhard Weihe for his friendly support in Marburg.

- Prof. Hartmann Hinterhuber who made my dissertation possible in the Department of Psychiatry.

- The whole staff of the neurochemistry lab:
  Iris, the good ghost of the lab; Margit for being the mother of the lab; Irene for her nice laugh; Bernd for the funny talks in the breaks; Kevan, Astrid, Mariana, Gudrun;
  Henna for being a good soul and friend; Ilka for being a companion; and all the others I have forgotten to mention.

- My parents for their tolerance, for making the year in Austria possible for me and for proof- reading.

- And finally my wife Maja who has stood by me in good times and bad. She has given me the power to cope with all the stress.

Contents

Page:

INTRODUCTION

Every culture has its own drugs of abuse which have gradually become part of society and were seen as a usual integrant. Down the ages drugs have appeared and disappeared.

At the present time we live in a fast moving, extremely individual, digital society, where interpersonal relations and feelings loose weight in society´s life. Drugs of abuse become more and more presentable in today's affluent society. Deficits in closeness are compensated in a new form of drugs, replacing missed feelings and interpersonal contacts for a short temporal moment.

" New , psychoactive drugs are custom-engineered for today's in people, when tired of the old-fashioned search for new ways of behaviour. Drugs become designed with desired effects especially suited for the new fashion." (*Saunders,* 1994)[1]."In a relativly close future cheap, from the human body easily digestible drugs will appear, so that humans can satisfy their zestfulness and dullness or control their productivity and relaxing phases whenever they want, without damaging themselves or society" (*Sahihi,* 1991)[2].

"Designer drugs are a milestone on the way to a fictive "intoxication- society", where the feeling, thinking and acting of the vigilantes is predominantly pharmacologically controlled" (*Cousto* 1997, *Linke* 1996, *Walder* and *Amendt* 1997, *Shugin* 1994)[3].

In the last few years laboratories all over the world have searched in the central nervous system for the chemical pathways which are activated or inhibited when drugs are abused. One of the most interesting findings was an increase of dopamine in the nucleus accumbens and area tegmentalis ventralis.

The neurobiological pathways which lead to pleasure, happiness and go along with the feeling of being reinforced, were seen in an activation of the mesocorticolimbic dopamine system. To this belong the limbic system, the tegmentum, the hippocampus, the frontal cortex, the corpora mammilaria and the amygdala. These structures work functionally close together with the hypothalamus, which regulates the hypophysis and controls vegetative functions of the body. Probably the nucleus accumbens is one of the central regulation centres in these complicated networks which precipitates the feeling of pleasure. From the nucleus accumbens dopaminergic dendrites lead caudal to the area tegmentalis ventralis and come in contact with dopaminergic nerves that go out from here. These dopaminergic pathways lead on the one hand to the nucleus accumbens and on the other to the frontal cortex, the tuberculum olfactorium, the corpus striatum and the amygdala. The nucleus accumbens and the tegmentum obtain also afferences from the limbic system (*Schmoldt,* 1998)[4].

The dopaminergic projections of the area tegmentalis ventralis in the nucleus accumbens and its direct or indirect activation of the area tegmentalis ventralis projections in the limbic system and frontal cortex lead in animals and humans to a better mood or the feeling of being positively reinforced.

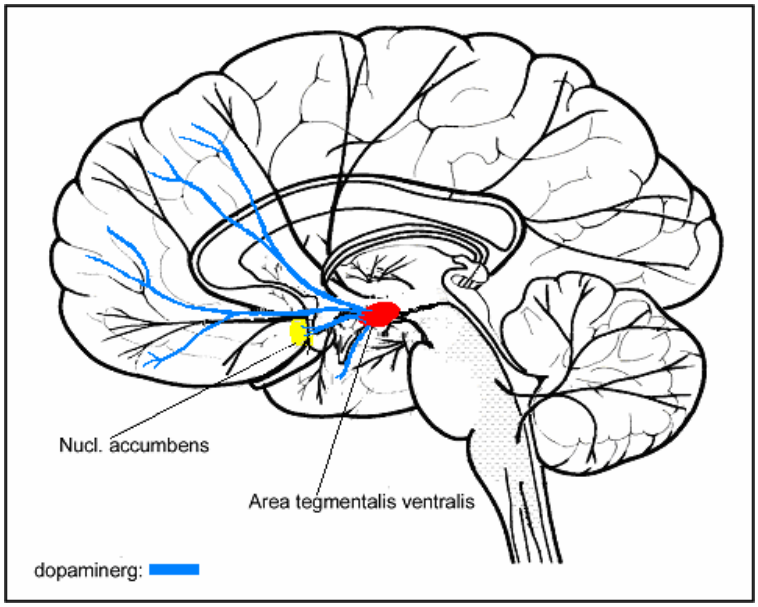

Fig.1: The dopaminergic reward system in the central nervous system (*Baumgarten* and *Grozdanovic* 1998)[5]

In vivo microdialysis with implanted quartz capillaries in animals (*Pfaus* et al 1990, Damsma et al 1992, *Pettit* and *Justice* 1989)[6], inhibiting trials with dopamine-receptorblockers or selective nerval poisons (*Robert* and *Koob* 1982)[7] underline the truth of this theory.

Noradrenergic, serotonergic and opioid releasing afferent nerves, which project to the nucleus accumbens can directly or indirectly stimulate the dopaminergic system via the modulation of activity (*Di Chiara* and *North* 1992)[8]. This can work via an inhibition of inhibitory nerves. Inhibitory influences of GABA nerves on dopaminergic nerve activation can be inhibited by opioids. This leads to a release of dopamine in dopaminergic nerves (*Di Chiara* and *North* 1992).

About “the therapeutic aspects unknowing predominates in many places and perplexity in consideration of new drugs, habits of consume and consumer population”(*R. Thomasius*, 1998)[9]. A difficult challenge for present scientists and doctors is to keep up with the rapid increase of drug abuse of today´s youth.

On the one hand prospective clinical research could improve the basic knowledge about dangers, intoxication levels and treatments. On the other hand animal research could unfold new attributes about drugs of abuse.

One basic approach in drug research is operant conditioning. We used this experimental approach in our laboratory to generate data with operant conditioning trials.

The experimental model of operant conditioning is based on *Burrhus Frederic Skinner*(1953)[10]. He created the basic conditions of operant conditioning in systemical laboratory work by taking up ideas of *John B. Watson*, *Ivan Petrovitch Pavlov* and *Edward L.Thorndike*(1927)[11]. His laws of operant conditioning determined the experimental psychology and psychotherapeutic approach over decades (*Gregory* and *Zangwill*, 1987)[12]. Although most psychologists and behaviourists (*Margraf*, 1996)[13] vehemently criticized Skinners approach and delimited themselves from operant conditioning used in research and psychotherapy, operant conditioning is still an important experimental approach to quantify reinforcing effects of substances of abuse and other stimuli (*Schuster*, 1986, *Stolerman*, 1992, *Young* and *Herling*, 1986, *Katz*, 1989, *Woolverton*, 1987)[14].

However, one main problem in drug abuse research is to find an operandum for analysing behaviour of animals which spawns reliable data.
Every being, either researcher or animal is an individual character and influences the results in operant studies. This leads to „personal mistakes“ and falsifies data. E.g. one person uses a soap which smells aversive for rats, the other has a deep, calm voice and influences like this. Noises from machines or other laboratory experiments do the same.

Therefore, in recent years different operant models were constructed to detect reinforcing effects in rats, for example Skinner boxes. The aim of this research approach is to find similarities in rats´ behaviour in order to draw conclusions to human behaviour.
We tried to develop an operandum to detect differences in operant responding behavioural experiments so that every person in our laboratory could use it and come to the same results.
However, I tried to construct a cheap and simple operandum to detect reinforcing behaviours in male rats, with minimized „personal mistake “ and a large spectrum of use.
A very simple method is an alley which a rat has to pass through in a recorded time to receive a drug.
Alley-running has been used as an operant behavior in studies of both food and drug reinforcers. In particular, Aaron Ettenberg and co-workers (e.g., *McFarland* and *Ettenberg* 1998; *Geist* and *Ettenberg* 1996) [15,16] developed a runway especially suited for the investigation of intravenously administered drugs of abuse.

The aim of the project was to establish an operandum like the Ettenberg runway in our laboratory; we modified his device and validated this experimental approach with respect to the following parameters:

1.1 Effectiveness of food reinforcers

Before we started with our main experiments, we tried to find out which food reinforcer male Sprague-Dawley rats prefer most. Eight naive rats were tested on their food preference in a defined time span.
Sated animals were presented three different kinds of food, i.e., ***sweetened condensed milk*** (Nestlé,Vienna, Austria), ***salted and roasted peanuts*** (Ültje Erdnüsse, Bielefeld, Germany), and ***Kinder® chocolate*** (Ferrero, Innsbruck, Austria).

1.2 Discriminatory control of two olfactory stimuli

We tried to find out whether ***olfactory stimuli would provoke locomotion in rats*** and, in case they did, if an almond odor would do so to a different extent than an orange odor. A swab containing three drops of an essence of either almond (Dr.Oetker, Vienna, Austria) or orange (Bergland-Pharma, Salzburg, Austria) was put for five minutes into the lower right corner of a cage.

1.3 Different apparatus construction of Runway

Three different sized runways were built. The first one had a real start and goal area. The second and the third runway were straight alleys. But they had different measurements.
A new strategy was developed by replacing the sliding door between the different areas by infrared beams.

1.4 Demonstration of reinforcing effect of food reinforcers in the modified Runway

Thirty-six rats were tested in different groups on following parameters: 1.) speed of learning. We compared accelerated training versus one reinforcing session per day.
And 2.) how much the scientist influences the results. Three different scientists handled their own groups.
One of the scientists handled two groups (n= 12; 1.group Phil, 2. Ilka, 3. Margit, 4. Margit).

1.5 Effects of subcutaneous morphine

Subcutaneous morphine and saline were given. Some animals received food training first, and others started immediately with morphine injections.

METHODS

1.1 Effectiveness of food reinforcers

Subjects

Eight male Sprague-Dawley rats were obtained from Zentrale Versuchstieranstalt, Himberg, Austria (weighing 250-300 g on receipt). The rats were individually housed in cages located within a temperature-controlled (22-24°C) vivarium maintained on a 12-hr light-dark cycle (lights on at 7 a.m.).

Procedure

Before we started with our main experiments, we tried to find out which food reinforcer male rats prefer most. On two consecutive days, sated animals were presented with three different kinds of food, i.e., sweetened condensed milk (Nestlé,Vienna, Austria), salted and roasted peanuts (Ültje Erdnüsse, Bielefeld, Germany), and Kinder® chocolate (Ferrero, Innsbruck, Austria), approximately 1.5 gram of each, for 15 min. It was noted if any food was approached and consumed within the first minute, and, consequently, which of the three presented types of food was approached and consumed first. The total amount of food consumed at the end of the session was determined as well by weighing each food before and after the experiment.

1.2 Discriminatory control of two olfactory stimuli

Procedure

Six experimentally naive male Sprague Dawley rats were put in a single animal cage for 15 minutes. At the beginning of minute five, a swab containing three drops of an essence of either almond (Dr.Oetker, Vienna, Austria) or orange (Bergland-Pharma, Salzburg, Austria) was put into the lower right corner of the cage and exchanged for the other essence-drenched swab at the beginning of minute ten. Locomotion (i.e., distance covered) was measured with a Videotrack System (Champagne-au-mont-d‘or, France; large movement threshold, 5.0).

1.3 Different apparatus construction of Runway

Runway Apparatus

The first runway was built with identical start and goal boxes. Both were sized 25 x 25 cm, built like a cube and bigger than the alley (155 x 17 x 50 cm) between (Fig.2).

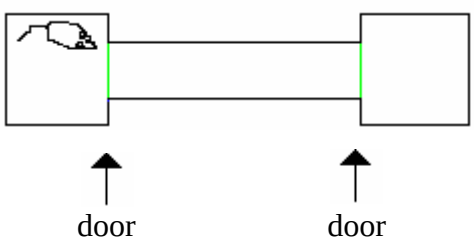

Fig.2: First runway seen from above

The second runway measured 250 cm x 25 cm and was 30 cm high, and compared to the first the runway it was a straight-arm alley without goal box and start box. These had been changed into a goal area and start area and were indistinguishable from the rest of the runway. The start area and the goal area each comprised 50 cm of the runway. The runway was built out of perspex which was covered with a black foil. Crossing of the finish line of the alley was detected by an infrared beam (Conrad electronics, www.conrad.de). The top was covered by a wooden board (Fig.3).

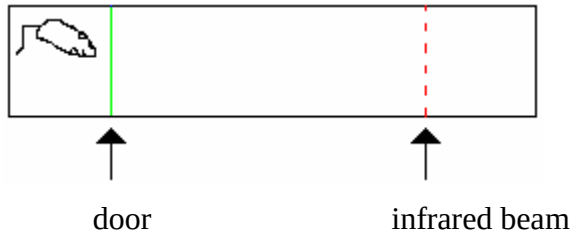

Fig.3: Second runway seen from above

The third runway (Fig.4) measured 200 cm x 10 cm x 10 cm; start area 30 cm and goal area 50 cm. It was built out of wood. The bottom consisted of metal mesh with square holes of 8 mm side length.
All runways had a sliding door at the start box. The first runway was built with a goal box door too, which was replaced in the second and third models by an infrared beam.

Fig.4: Third Runway, here shown with half opened top

1.4 Food reinforcment in the modified Runway

Subjects

The Subjects were 36 male Sprague-Dawley rats obtained from Zentrale Versuchstieranstalt, Himberg, Austria, weighing between 245 and 400g at the onset of the food deprivation. Twelve rats were individually housed. The

other 24 animals lived in four group cages (n = 6).They were all located within a temperature-controlled (22-24°C) vivarium, maintained on a 12-hr light-dark cycle (light on at 7 a.m.). They had unlimited access to water but were on a restricted food diet (5 gram pelleted chow/(rat*day)) intended to reduce and maintain its body weight at 85% of free-feeding values. Daily food rations were provided after the last operant session of the day.

Procedure

For the first five days twelve rats (first group) did only one trial daily, in which the reinforcer food was presented. From day six on the rats ran two daily sessions, one in the presence and one in the absence of food. Between the sessions there was a break of two hours. It was randomised if the animals ran first for food or for no reinforcer.

A second group of 12 rats, a third group of 6 and a fourth group of 6 animals received accelerated training. For the first two days the rats were trained ten times in a row with milk and ten times with no reinforcer.
Group two started after two trials (food/ nothing) per day, like the first group.

The animal was placed behind a door in a start area. After 10 s delay, the door was removed and the animal had to traverse the alley to reach the goal area. To the animal, both the start and the goal area as well as the finish line were indistinguishable from the rest of the runway. ‚Runtime' was defined as the time between the removal of the start area door and the crossing of the finish line. In the second and third runway the crossing of the finish line

was detected by an infrared photobeam (Conrad electronics).

To avoid disturbing parameters, like voices, light and smells, the operandum was covered with a wooden board over the whole alley. Discriminative olfactory cues were counterbalanced and were hanging inside all three areas (start/ alley/ goal) of the runway.

According to Ettenberg and coworkers (*McFarland* and *Ettenberg* 1998; *Geist* and *Ettenberg* 1996), a decrease in runtime was taken as a measure of the reinforcing effect of the stimulus presented in the goal area. The food reinforcer was sweetened condensed milk (Nestle, Vienna, Austria) diluted 1:3 in tap water which the animals consumed out of a bottle-type dispenser located in the right corner of the goal area.

Experimental groups

GROUP	RATS	HOUSED	PRETREATMENT	1. TREATMENT MILK/ NO FOOD	2. TREATMENT MORPHINE/ SALINE
1 (PHIL)	**12**	**SINGLE CAGES**	5 DAYS (FIG. 4:-5;0) ONE SESSION/ DAY MILK	35 DAYS 2 SESSIONS/ DAY MILK/ NO FOOD	20 DAYS (FIG. 7) 6 RATS 2 SESSIONS MILK/ NO FOOD 6 RATS 2 SESSIONS MORPHINE/ SALINE
2 (ILKA)	**12**	**GROUP CAGES (n=6)**	2 DAYS ACCELERATED TRAINING 10 X 2 SESSIONS/ DAY MILK/ NO FOOD	30 DAYS 2 SESSIONS/ DAY MILK/ NO FOOD	10 DAYS 6 RATS 2 SESSIONS MILK/ NO FOOD 6 RATS 1 SESSION MORPHINE 10 DAYS 6 RATS 2 SESSIONS MILK/ NO FOOD 6 RATS 1 SESSION SALINE
3 (MARGIT; # 38-43)	**6**	**GROUP CAGES**	2 DAYS ACCELERATED TRAINING 10 X 2 SESSIONS/ DAY MILK/ NO FOOD		10 DAYS 3 RATS 1 SESSION MORPHINE 3 RATS 1 SESSION SALINE 2 DAYS ACCELERATED TRAINING 10 X 2 SESSIONS/ DAY MILK/ NO FOOD 10 DAYS 1 SESSION/ DAY MORPHINE 2 DAYS 1 SESSION/ DAY SALINE
4 (MARGIT; # 31-37)	**6**	**GROUP CAGES**	2 DAYS ACCELERATED TRAINING 10 X 2 SESSIONS/ DAY MILK/ NO FOOD 1 DAY 1 SESSION MILK		9 DAYS 1 SESSION/ DAY MORPHINE 10 DAYS 1 SESSION/ DAY SALINE 3 DAYS ACCELERATED TRAINING 10 X 2 SESSIONS/ DAY MILK/ NO FOOD 10 DAYS 1 SESSION/ DAY MORPHINE 10 DAYS 1 SESSION/ DAY SALINE
5	**24**	**SINGLE CAGES**	5 DAYS ACCELERATED TRAINING 10 X 2 SESSIONS/ DAY MILK/ NO FOOD	10 DAYS 1 SESSION/ DAY SUBSTANCE A/ B	10 DAYS 1 SESSION/ DAY SUBSTANCE B/ A

1.5 Subcutaneous morphine application

Sixty animals were divided into groups of n= 6 and treated with morphine. The groups were handled by four different scientists.
After establishing sweetened condensed milk as a reinforcer, animals were given the opportunity to traverse the alley for a subcutaneous injection of morphine (1 mg/ kg; first 10 sessions, 1 session/ day) and saline (following 10 sessions).
Twelve animals started immediately without accelerated food training (1. treatment) with subcutaneous morphine as the reinforcer (group three and four).

Group one received no accelerated training. From day 36 on the group was divided. Six animals did two sessions per day where morphine or saline was injected. The other six animals still received milk and no reinforcer.

Group two was also divided (n=6). Half of the group started on day 32 with one session/ day morphine treatment for ten days. The following ten days one session/ day saline.

The animals of group three were pretreated with 10 sessions/ day accelerated food training for two days. Then the group was divided (n= 3) and started immediately the second treatment (morphine/ saline) for ten days, followed by accelerated food training again for 2 days. For this the divided group was recomposed. For the next 10 days all animals did one session/ day where morphine was injected and one session/ day where saline on the last two days of their lives was injected (I want to mention that rats were

killed by guillotine at the end of all trials, because it's the most painless and fastest way).

In the same way like the other groups, group four was pretreated, but before morphine treatment started (nine days) the animals received milk inadvertently for one day longer. Instead of morphine the animals received one day longer milk. We decided on a second session with morphine on the same day. For the next ten days the rats received saline, followed by 3 days of accelerated food training. Then again 10 days morphine and 10 days saline.

Twenty-four animals (group 5) were tested in the newly-built runway (Fig.4.). They received all the same treatment. For the first 5 days accelerated food training and then 1 session/ day a double blinded substance A (10 days), followed by 1 session/ day substance B (morphine or saline).

The animals were gently taken out of the goal area and morphine(10mg/kgKG) or saline was given s.c.. After trial with no reinforcer, milk consume, saline or morphine treatment the rats were placed in their home cages. The 24 animals of the last trials stayed for two hours in single cages, when substances were given. Between the two sessions there was a break of at least three hours.

1.6 Statistical analysis

Some animals did not reach the goal area in one minute (cut off) or showed no locomotion at all. By statistical principle, the use of experimenter cut off introduces non-normality into the data set. Indeed, data analysis showed that runtimes value distribution was non- Gaussian. The value of numbers was too small. Accordingly, all statistical comparisons were based on non-parametric tests (Mann Whitney u- test).

RESULTS

1.1 Efficacy of food reinforcers

The analysis of the preferred food was the first step to validate the Runway.
Both in terms of the type of food consumed within the first minute and total amount of food consumed during the 15-minute session (Fig.5), sweetened condensed milk and roasted and salted peanuts were equally preferred to Kinder® chocolate.

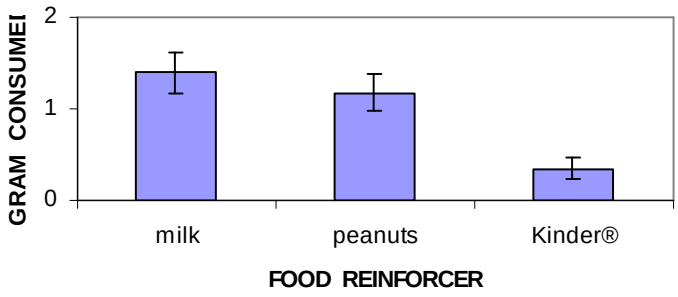

Fig.5: On the ordinate the total consume in grams and mean with standard deviation. On the abscissa the three compared food reinforcers, sweetened condensed milk, roasted peanuts and Kinder® chocolate are shown.

1.2 Influence of olfactory stimuli on locomotion in rats

Any manipulation such as exposure to a novel environment, almond odor or orange odor produced the largest increase in gross locomotion within the first minute (Fig.6).

There was, however, no difference between the stimulation of locomotion and the two odors.
Animals locomote almost the same for almond odor as for orange odor.
All in all locomotion within the first minute is nearly two times higher when an odor is presented.
Further analysis showed that the order of presentation of the odor cue did not have an effect either.

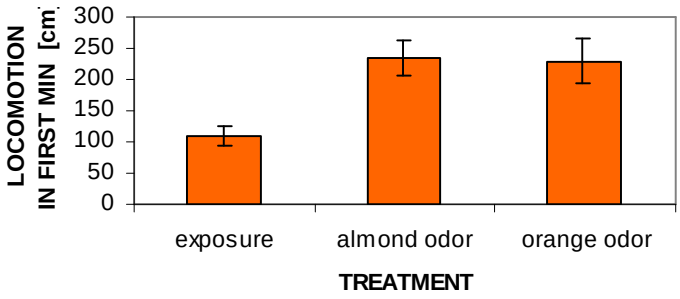

Fig.6: On the ordinate: Locomotion in the first minute (cm) in a common rat cage. On the abscissa: The treatment with different cue exposure compared to novel enviroment exposure and mean with standard deviation.

The locomotion within the fifth minute is shown in Fig. 7. The two different odors do not influence the rats like in the first minute:

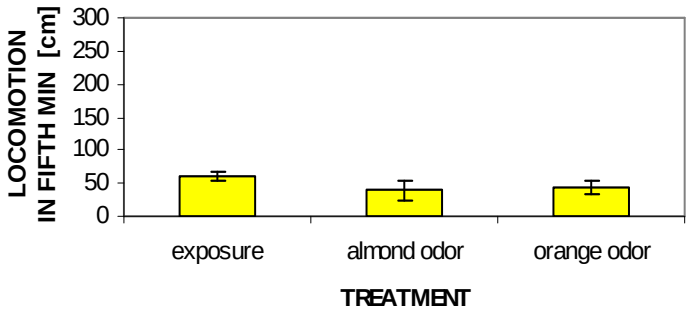

Fig.7: On the ordinate: Locomotion in the fifth minute (cm). On the abscissa: the different cue exposure and mean with standard deviation.

Our results suggest that the presentation of either an almond or an orange odor stimulates locomotion more than the exposure of the animal to a novel environment within the first minute. Furthermore, the lack of any locomotor stimulation during the fifth minute after the manipulation shows that (1.) the animals habituate to the new stimulus and (2.) that the chemicals producing the odor do not influence locomotion due to any direct pharmacological effect.

Based on these results we chose to stop the trials after one minute (experimenter cut off).

1.3 Effects of changing measure of Runway

Although the animals traversed the alley in all three runways, there was a big difference in results. The design of the runway had an influence.

The measurements of the runway were changed three times:

The first runway was built like a bone with a big start and goal box (Fig.2). We didn't mention the results of our first trials, because there was no significant evidence that any animal tested in this runway was reinforced by food or morphine. Most of the animals stayed in the start box.

The second runway which we used for our trials consisted of a straight alley (Fig 3).

The animals did not sit in a corner any more. Starting with the first day they explored the alley and it was possible to obtain significant results out of our trials (see 1.4).

Another important improvement was that we replaced the sliding door of the gaol box by an infrared beam (Fig.3).

The third runway was reduced from a wide alley into a narrow room, that rats felt more safe in a smaller cavity.

1.4 Demonstration of reinforcing effect of food reinforcers in the modified Runway

In accordance to the data published by Ettenberg and colleagues, when training was restricted for the first group (n =12) to two sessions per day 11 of the 12 tested animals traversed the alley significantly faster when food was presented within 15 days (Fig.8):.
We suggested that the olfactory cue acquired control over the animal's running speed.

Twelve rats received for the first five days only one session per day where the reinforcer milk was presented. From day six (0) on the animals ran two sessions per day:

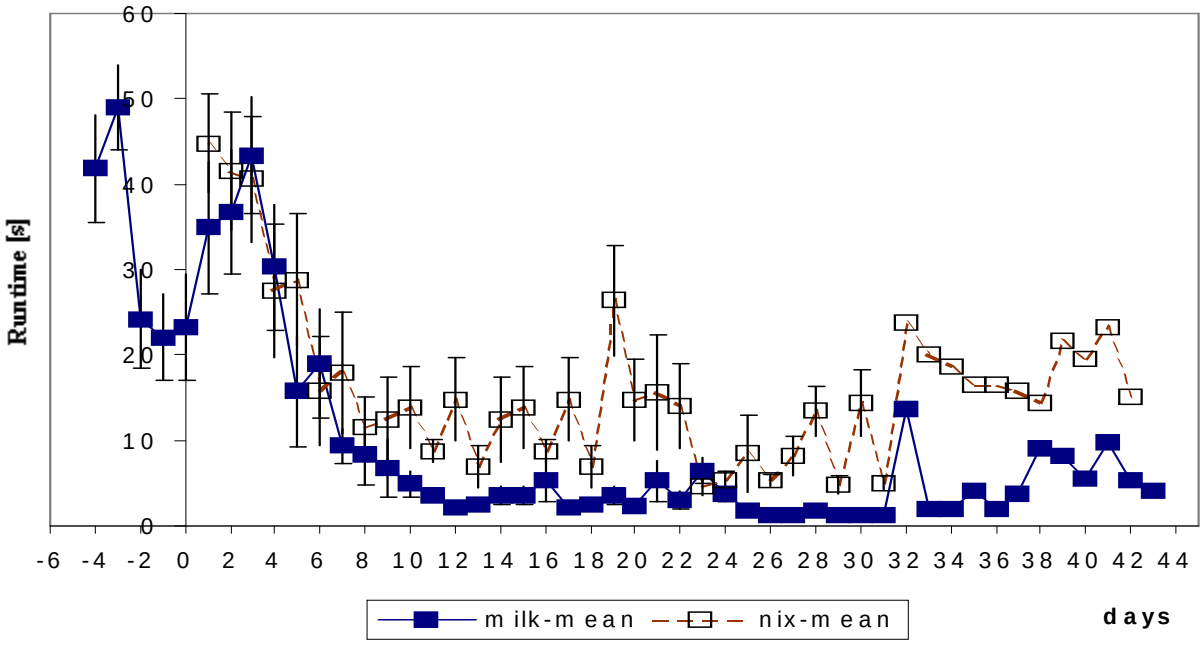

Fig.8: On the abscissa: Runtime/ sec.. On the ordinate: Days

Of the 24 animals subjected to accelerated training, seventeen rats ran significantly faster for food. During the first two days of accelerated training the animals seemed to learn to distinguish the odor cues. Runtime for milk decreased and runtime for no reinforcer increased (Fig.10).
However, the olfactory cues did not acquire discriminatory control in any of these 24 rats during the accelerated training (Fig.9).
When these animals were advanced to the 2-session-per-day training, they did not run faster in presence of the food-associated olfactory cue.

Twelve rats of group two received accelerated training for two days and started then with one reinforced and one baseline session.

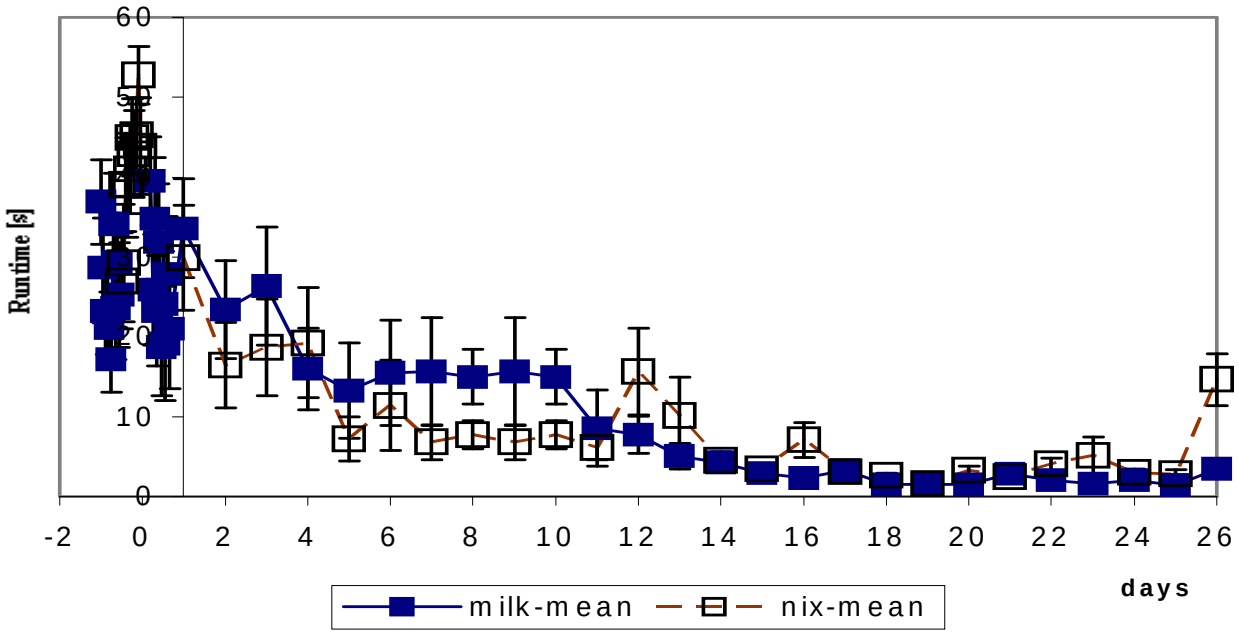

Fig.9: On the ordinate: runtime/ sec.. On the abscissa: Days

The days of accelerated food training out of Fig.9 (-2;1;0) are here shown more expanded in Fig 10. An increase of running speed for reinforcer and a decrease for no reinforcer:

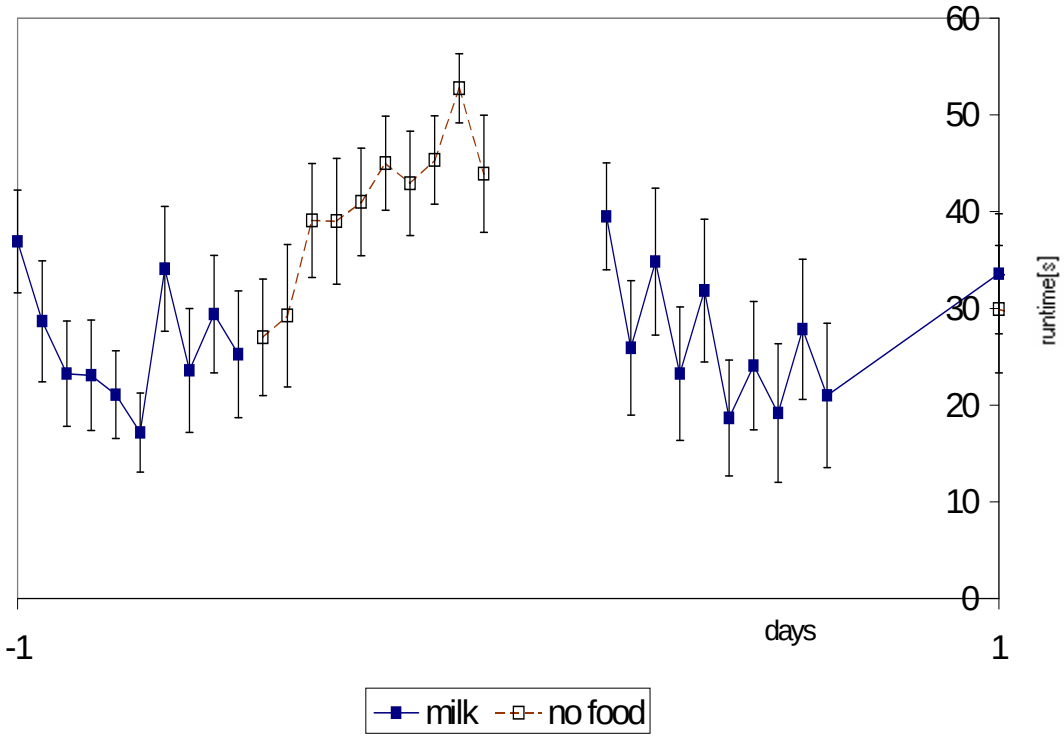

Fig.10: On the abscissa: Days.. On the ordinate: Runtime/ sec..

1.5 Effects of subcutaneous morphine on rats

At the beginning all morphine treated animals of group one ran faster for the substance than for saline, but still not as fast as the milk treated rats.
Group one did not receive accelerated training (Fig.11).

After 35 days of food reinforcment the twelve rats were split into two groups (n = 6). Six animals still received food or nothing, the other six were treated with morphine and saline:

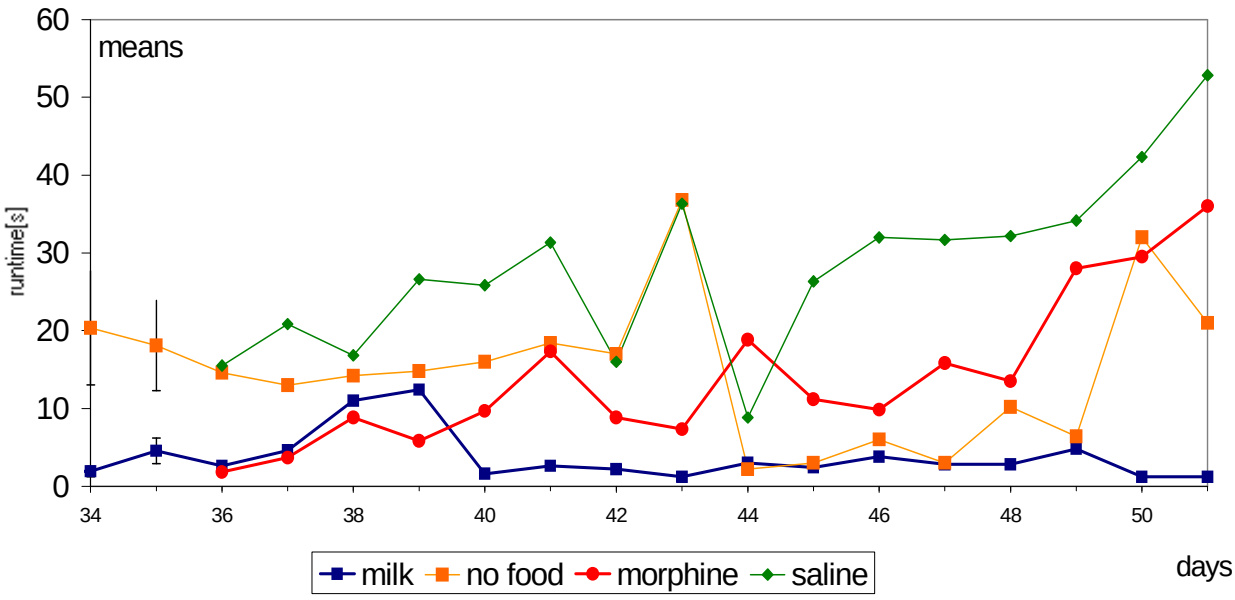

Fig.11: On the abscissa: Days. On the ordinate: Runtime/ sec..

Group two started with accelerated food reinforcment for two days and then did two sessions per day (food/ no food) until day 30. With day 31 this group was treated ten days only with morphine and for the following ten days with saline (fig.12).

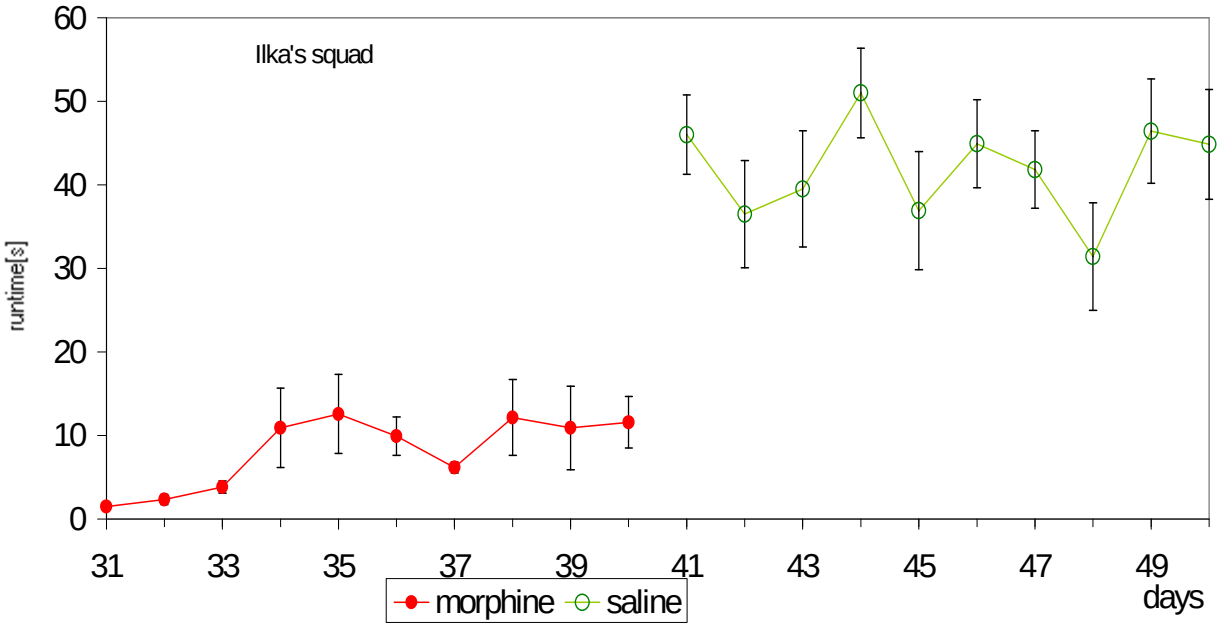

Fig.12: On the abscissa: Runtime/sec.. On the ordinate: Days

The results of the third and fourth group which were treated by the same person were not comparable with the first and second group.
(e.g. Fig.13).

For the first two days the animals received accelerated food/no food training (-1; 0) and then immediately started with morphine (10mg/ml)/ saline treatment:

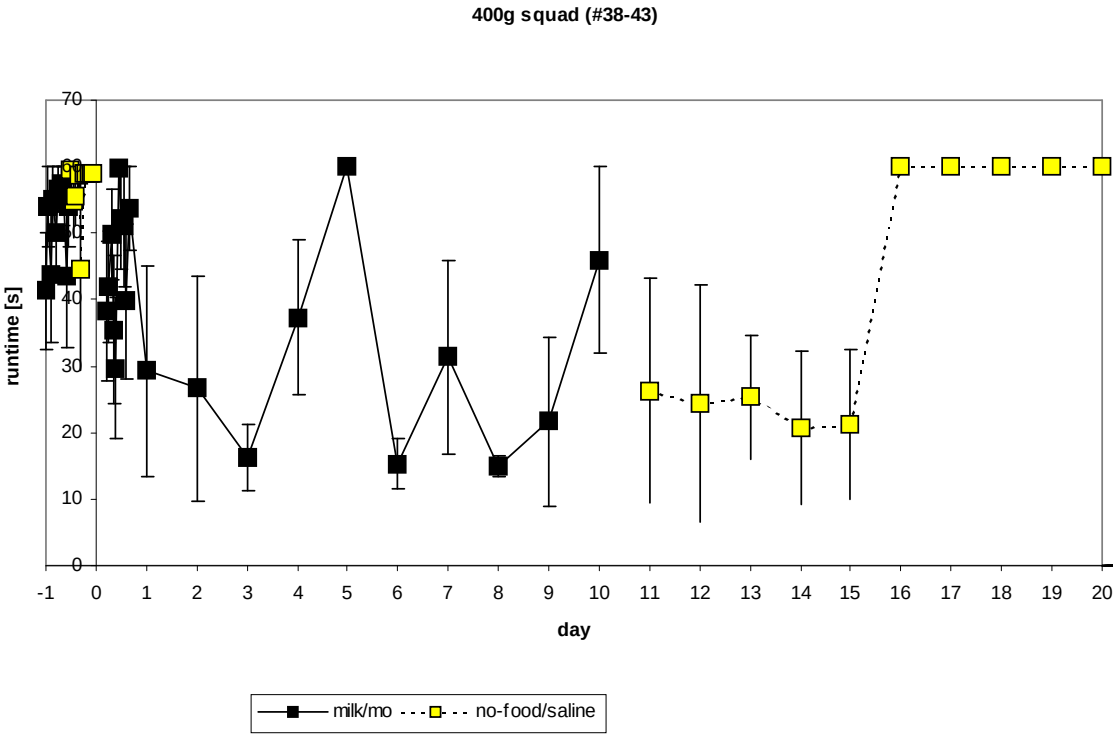

Fig.13: On the ordinate: Runtime/sec.. On the abscissa: Days

Compared to the first and second groups the animals showed contrary behaviour.
Runtime increased for morphine. To prove these results the animals received accelerated training again, followed by 10 sessions of morphine and 10 sessions of saline treatment (Fig.13), but the animals still did not distinguish the two different runs at all and seemed in the end to be too tired to traverse the alley.

The results of our present trials with 24 rats in the third-built runway underlined these suggestions:

Substances applicated under double blind conditions:

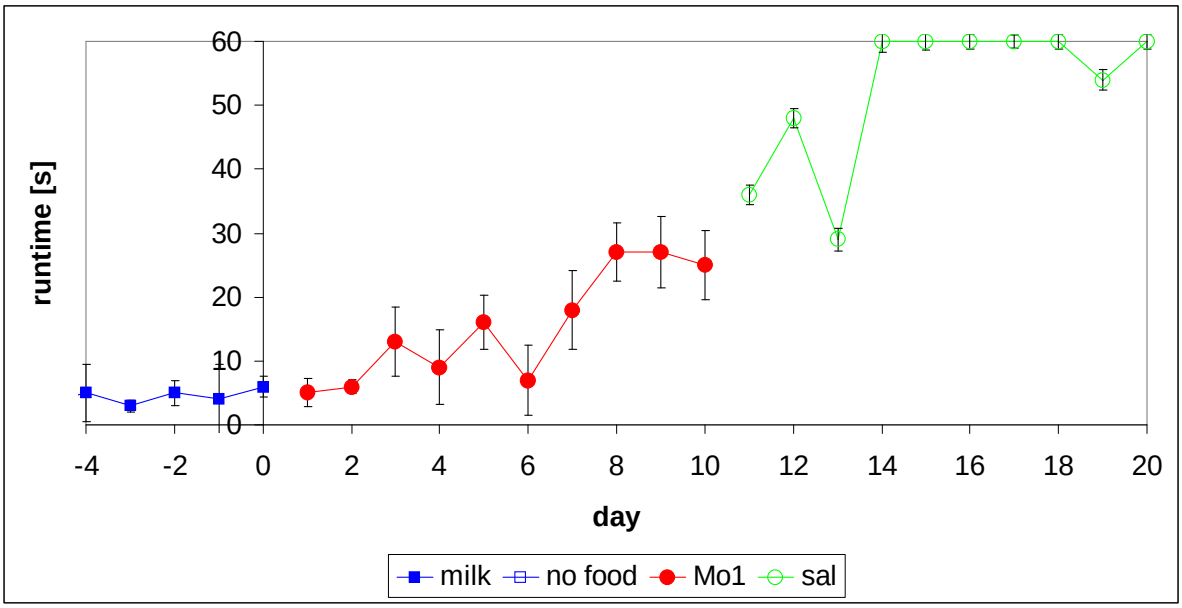

Fig.14: Morphine first injected

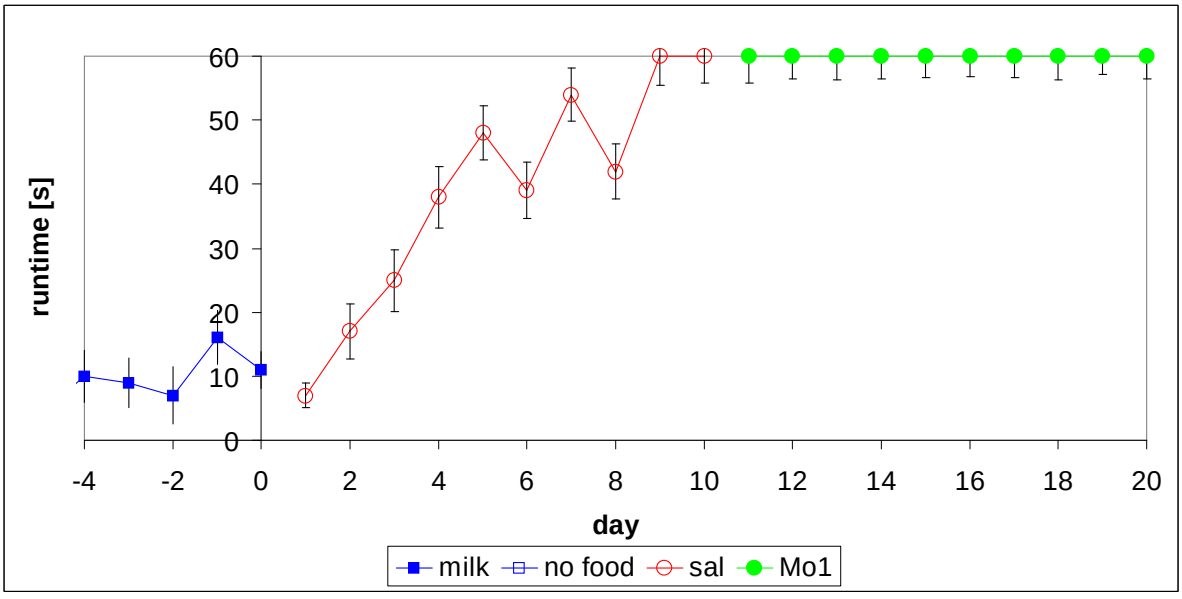

Fig.15: Saline first injected

In food reinforcement the results were like we expected, the runtime increased tremendously for sweetened condensed milk (1- 3 sec.). On the one hand morphine reinforcment did not show the expected results (see above).Maybe the subcutaneous injection was an irritating factor or withdrawal symptoms conduced to these unexpected results.

However, we suggest that there was a scientist effect in our data, because we did not blind the substances in our trials from the very beginning.

DISCUSSION

Following the analysis of the preferred food [1.1] the results prove that Kinder® chocolate which was established as a reinforcer in behavioural and neurochemical experiments (Bassareo and Chiara, 1999)[17] is not as strong as sweetened condensed milk or salted and roasted peanuts. Based on these new findings Kinder® chocolate as a reinforcer should be replaced by either sweetened condensed milk or salted and roasted peanuts.

Any manipulation such as exposure to a novel environment [1.2], almond odor or orange odor, produced the largest increase in gross locomotion within the first minute.

After five minutes animals get used to the new smell and locomotion decreases for both odors. This may lead to the suggestion that the animals have to get used to their environment quite fast.

These results do not conform with previous observations where “a highly significant main effect of stimulus condition confirming that subjects traversed the alley reliably faster when presented with the heroin- rather than the saline-predictive cue “ (Mc Farland and Ettenberg, 1998)[14]. It was also impossible to prove that “drug- paired stimuli come to activate the same neutral substrates activated by the drugs themselves “ (eg. Stewart, 1984)[18].

Concerning the dimensions of Runway [1.3] the results revealed that a straight alley, compared to a “straight-arm runway with identically sized start boxes and goal boxes “ (eg. Geist and Ettenberg 1997; Mc Farland and Ettenberg, 1998; N White, L Sklar and Z Amit, 1977) [19] changes the rats´

running behaviour in a positive way. We revealed that the corners of the start box helped the animals to orientate. It is apparent that a cubed start box give rats the feeling of being safer.

A second definite improvement in comparison with of Ettenberg´s runway was the replacement of the "vertically sliding door " by an infrared beam. With this change the irritating noise of the sliding door, that could influence rats´ running behaviour was avoided. Rats consequently did not demur any more to enter the goal area.

The present data are not in agreement with earlier work, that the application of "morphine had a positive reinforcing effect on the behaviour of naive rats " in a non- covered runway and that "the morphine animals were running faster than the saline animals by day 2 of the experiment, and the size of this difference increased over the next 3 days " (N White, L Sklar, Z Amit, 1977) [19].

In the beginning it seemed that the animals were reinforcerd by morphine and non- reinforced by saline. But on a second view it was clear that the scientist influence on the results was tremendous. For example in group 2: Most of the animals stopped traversing the alley on the first day (41) of saline application. Actually all rats excepted morphine treatment instead of saline.

In morphine reinforcment the results were not significant, but nearly every rat consumed sweetened condensed milk. It was equal if the breadboard construction was opened or closed or other factors made an unused atmosphere. The stronger and more common (e.g. food) the reinforcer was, the less important the influence from outside seemed to be. Uncommon reinforcers like morphine need to have a more sensitive breadboard construction, so that exterior influences

do not distract the animals, because outside influences result in higher deviances than before.

The bios of researcher influence and different behaviour of every individual involved in operant conditioning experiments is not to be denied. Every being differs in kind and habits.

A difficulty is that reactions on happenings result in different interpretations and behaviour. For example a scientist who is himself an individual character presages rats´ behaviour in conditioning trials and his more or less unconscious reaction influences the results.
Blinding substances is a good possibility to reduce those "personal mistakes ", but to cut off unconscious factors in conditioning trials, breadboard construction must be constructed automatically so that scientist influences are as small as possible. The animal should not have any contact (i.e. olfactoric, acoustic, visual, etc) with other beings and machines should standardize all trial- circles.
Humans should have a minimum of contact to the animals to get more exact results, animals should be kept in single cages and machines should overtake all jobs of the experimentalist.

PROSPECTS

In general, behavioural research and brain research are for sure an important basic approach; but main problems should be solved by preventive measures.

As mentioned in the introduction there is a lack of emotion and interpersonally relation lose weight in societies live.
"To a certain extent as our scientific understanding has increased, our world has become less human. Man feels isolated in his cosmos, because he has lost contact with Nature and his emotional identity " (*Jung*, 1964)[20].
What humans do and how they lately behave is a complicated reflection of living- circles. It is a network of family, friends, school, society, culture, etc.. These groups shape the character, but according to the possibilities everyone is looking for circles which fit his character. The choice of the circle depends also on how much unsuitable circles previously influenced a being.
If there is a lack of company humans turn introspective, anxiety increases and a dream world can evolve, in which it is possible to hide from outer problems. This can bring protection against stress factors, but can lead on the other hand to loneliness and isolation. In this case drugs of abuse are a risk and change the dream world into a prison.

Drugs of abuse are a problem of modern society. As more as humans lose contact to natural emotions and feelings, drugs of abuse will be an alternative for missed desires and feelings.
Kuntz (1997)[21] describes the "Raving Society " as an expression of an immoderate consume orientated "addicted society ".
Today´s In- people, who don not fit in this world of commercial illusion decide to take drugs, because they miss a natural emotional confrontation with themselves and the world around them. They are raised in a world where material

competition counts more for than interpersonal communication.

“drugs are taken because of their subjective positive effects in the beginning. Equivalent behaviour is shown in non-substance abuse, where cars, computers, video, sex or the job become a drug. “ (*P. Maertens*, 1999)[22]. Drugs are no longer used as expedient self-medication like seculars used them in former times.

It is necessary to intensify natural sensitivity and emotion in today´s society.

As more and more humans lose their natural emotions, drugs of abuse will be an alternative for missed desires and feelings.

SUMMARY

Drugs of abuse becomes increasingly present in today's affluent society. Immoderate drug abuse has risen to a deadly serious problem in today's youth.
Animal research with operant conditioning approach is one way of fathoming this problem and can contribute to gaining a clearer understanding of drug addiction.
In order to establish an operandum for detecting reinforcing effects in rats we constructed an alley based on the idea of A. Ettenbergs Runway.
The validation of the Runway was done step dy step. The first aim was to find the best food reinforcer [1.1] and to check if olfactory stimuli would provoke lokomotion in rats [1.2]. The construction of different-sized runways and modifications of Ettenbergs ideas[1.3] was the next step. Demonstration of reinforcing effects of food reinforcer[1.4] and morphine [1.5] was the last step.
The analysis of effectiveness of food reinforcers showed that rats traverse an alley significantly faster when they have received roasted and salted peanuts or sweetened condensed milk. Based on these new findings sweetened condensed milk should be used as a reinforcer in following food reinforcment training sessions.
Discriminatory influences of either an almond or an orange odor let rats locomote two times higher within the first minute, but not for a time of five minutes. There was no difference between the stimulation of locomotion and the two odors. The place of odor presentation had no influence on the stimulation of locomotion.

The different dimensions of the runway did influence the rats' running behaviour. The animals started to run through an alley earlier, when it was straight than when it had corners. A replacement of sliding doors by infrared beams was a definite improvement and reduced strongly disturbing influences.

Accelerated food training worked in a straight runway (Fig 10). The same results were recorded in two- sessions per day training, but here some rats did not distinguish between the two sessions and ran two times faster (Fig. 9).

The effects of subcutaneous morphine application was not as expected. The rats did not run significantly faster for morphine (Fig. 11, 12, 13, 14, 15). Milk as a reinforcer was always a stronger reinforcer than opioids.

References

1) Saunders N (1994) Ecstasy, pp.38ff. In: Walder P Ecstasy, Bilger, Zürich

2) *Sahihi A* (1991) Designer- Drogen. Die neue Gefahr, pp.9. Beltz, Wertheim Basel

3) *Cousto H* (1997) Ecstasy als Genußmittel. Plädoyer für einen gelassenen, aber hochinformierten Drogengebrauch. In: *Neumayer J, Schmit- Semisch H* Ecstasy- Design für die Seele? Lambertus, Freiburg

   Linke D (1996) Das Ich und sein Gehirn. Neurophilosophische Betrachtungen zur Gehirnforschung. Lettre Internationale 32: 26- 33

   Shulgin A (1994) Entaktogen- Forschung-- eine Gratwanderung. Weiten des Bewusstseins, Heft 3

   Walder P, Amendt G (1997) Ecstasy und Co. Alles über Partydrogen. Rowohlt, Reinbek

4) *A Schmoldt,* (1998) Ecstasy- Wirkungen, Risiken, Interventionen. Enke Verlag Stuttgart: pp 28- 29 Pharmakologische und toxikologische Aspekte; Rauschwirkung

5) *Baumgarten HG, Grozdanovic Z* (1997) Anatomy of central serotoninergic projection systems. In *HG Baumgarten, M Goethert:* serotoninergic neurons and 5- HT receptors in the CNS: 45. Springer Berlin, Heidelberg

6) *Damsma G, Wenstern D, Pfaus JG, Phillips A, Fibiger HC* (1992) Sexual behavior increase dopamine transmission in the nucleus accumbens and striatum of male rats: comparison with novelty and locomotion Behav Neurosci 106: 181- 191

   Pettit H, Justice J jr (1989) Dopamine in the nucleus accumbens during Kokaine selfadministration as studied by in vivo microdialysis. Pharmacol Biochem Behav 34: 899- 904

7) *Roberts DCS, Koob GF* (1982) Disruption of cocaine selfadministration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. Pharmacol Biochem Behav 17: 901- 904

8) *Di Chiara G, North A* (1992) Neurobiology of opiate abuse Trends Pharmacol Sci 13: 185- 193

9) *Thomasius R* (1998) Ecstasy- Wirkungen, Risiken, Interventionen. Enke Verlag Stuttgart: pp 6- 7

10) *Skinner BF* (1953) Science and hunam behavior. New York: Macmillan Publishing Co.

11) *Pavlov I P* (1927) Conditioned reflexes. In G. Anrep (Ed.), Conditioned reflexes, pp 34-35. Oxford, England: Oxford University Press

12) *Gregory RL, Zangwill OL* (1987) The Oxford companion to the mind. Oxford University Press, Oxford

13) *Margraf J* (1996) Lehrbuch der Verhaltenstherapie. Springer, Berlin

14) *Schuster CR* (1986) Implications of laboratory research for treatment of drug dependence. In: *Goldberg SR, Stolerman IP* (1992) Academic Press, Orlando, FL, pp 357-383

Young AM, Herling S (1986) Drugs as reinforcers: studies in laboratory animals. Academic Press, Orlando, FL, pp 9- 67

Katz JL (1989) Drugs as reinforcers: pharmacological and behavioural factors. Oxford University Press, Oxford, pp 164- 213

Woolverton WL (1987) Analysis of drug interactions in behavioral pharmacology. Lawrence Erlbaum Associates, Hillsdale, NJ

15) *McFarland K and Ettenberg A* (1998) Naloxone Blocks Reinforcment but Not Motivation in an Operant Runway Model of Heroin- Seeking Behavior. In: Experimental and Clinical Psychopharmacology, Vol. 6, No 4, pp 353- 359

16) *Geist TD and Ettenberg A* (1996) Concurrent positive and negative goalbox events produce runway behaviors comparable to those of cocaine- reinforced rats. Pharmacol Biochem Behav 57(1/2) pp145- 150, 1997

17) *Di Chiara G, Acquas E, Tanda G* and *Cadoni C* (1993) Drugs of abuse: Biochemical surrogates of specific aspects of natural reward? Biochemical Society Symposium: Vol.59, pp65- 81.

18) *Stewart J, de Wit H* and *Eikelboom R* (1984) Role of unconditioned and conditioned drug effects in self- administration of opiates and stimulants. Psychological review, 91, pp 251-268.

19) *N White, L Sklar* and *Z Amit* (1977) The Reinforcing Action of Morphine and Its Paradoxical Side Effect. Psychopharmacology 52, pp 63- 66.

20) *Jung CG* (1964) Man and his Symbols. Aldus Books Limited, London. pp 95

21) *Kuntz H* (1997) Mit Ecstasy auf der Suche nach dem verlorenen Glück- Du liebst alle und alle lieben Dich, Suchtreport 1:46- 51

22) *Märtens P* (1999) Pädagogische Ziele. In: Ecstasy- Wirkungen, Risiken, Interventionen, Enke Verlag Stuttgart, pp 164-165