

Measuring Behavior 1998 Abstracts

A telemetric study in mice on protectors against doxorubicin-induced cardiotoxicity

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Doxorubicin is a very effective antitumour agent used in the treatment of various solid tumours. Apart from common side-effects in anticancer therapy, such as bone marrow suppression, nausea and vomiting, its clinical use is largely limited by the occurrence of a cumulative dose-related cardiotoxicity, which manifests itself as congestive heart failure. This observed cardiotoxicity is believed to be mainly caused by free (oxygen) radicals.

Flavonoids are a class of naturally occurring compounds, which are abundantly present in vegetables, fruits and beverages such as tea and red wine. Besides their relevance in plants, they are pharmacologically important for man, because of their high pharmacological potency. They are major ingredients of many traditional medicines and several health claims have been ascribed to flavonoids. In vivo experiments showed a dose dependent protection by the flavonoid monoHER. Using telemetry, changes in the ECG were recorded to monitor the cardiotoxicity. Transmitters were implanted into the peritoneal cavity of male Balb/c mice (20-25 g). The leads were placed subcutaneously. A possible increase in ST-interval in the ECG could be detected at an early stage as the animals were monitored during treatment.

Flavonoids have been shown to participate in several different mechanisms such as binding to enzymes and cell membranes and electron transfer in enzyme systems. The properties however, which are probably most promising for the use of flavonoids as modulators of doxorubin-induced toxicity are their radical scavenging and iron chelating properties. A drawback of monoHER therapy is the relatively high dose of 500 mg/kg needed to obtain complete protection. Therefore, current research is emphasizing on the development of new compounds with improved antioxidant properties and increased selectivity. The goal is to obtain more potent compounds than monoHER, that can be administered in lower doses.

Influence of the mineralocorticoid and glucocorticoid receptor on heart rate and blood pressure of the rat

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In the past it has been shown, by using tail cuff blood pressure measurements, that intracerebroventricular (icv) injection of the mineralocorticoid antagonist RU28318 decreases blood pressure (BP) and heart rate (HR) after 7 h. Direct BP measurements demonstrated that it was not a decrease in HR and BP but rather the blocking of an increase. After icv injection, the glucocorticoid antagonist RU38486 had the opposite effect on tail cuff BP, after 24 h BP was higher than in control animals. Also, RU28318 has been shown to have anxiolytic-like effects in an elevated plus-maze paradigm.

The aim of this experiment was to gain more insight, with the use of telemetry, into the effects of RU28318 and RU38486, especially to what extent they are induced by the experimental procedure. Wistar rats were equipped with an icv cannula and a TL11M2-C50 PXT ECG/BP transmitter or a TA11ETA-F40-L20 ECG transmitter (Data Sciences International). After a recovery period of at least 10 days the rats were icv injected with RU28318, RU38486 or vehicle. After 1.5, 6.5 and for RU38486 also after 23.5 h the rats were subjected to 32 °C for 30 min., after which they were restrained during 1.5 min and the pressure of the tail cuff was mimicked by slightly pinching the base of the tail. 7 h after icv injection of 100 ng RU28318, the stress-induced increase in HR was reduced by about 50 % from 128 bpm (vehicle) to 67 bpm (RU28318) ($p=0.0133$). 100 ng RU38486 icv increased the stress-induced increase in HR after 24 h from 80 bpm in vehicle treated rats to 123 bpm in RU38486 treated rats ($p=0.005$). The blood pressure during stress increased from 113 to 129 mmHg (vehicle vs. RU38486, $p=0.0187$). From the vehicle treated group it became apparent that rats show habituation when restrained 3 times within 24 h (peak HR 496, 420 and 406 bpm and peak BP 121, 112 and 113 mmHg after 2, 7 and 24 h, respectively) It can be concluded that central mineralocorticoid or glucocorticoid receptor blockade has a profound influence on both BP and HR changes during (restraint) stress.

Studying the Black Rat's (*Rattus rattus*) nesting behavior: methodological considerations

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Only few animals have a permanent home which they use throughout the year. The great majority only has one during the breeding season for protection of the young. In spite of this, given the opportunity, any rat, independent of sex and age, makes a multi-purpose nest and resorts to it for habitation, cover, sleeping, breeding and sometimes, as a feeding place. Maternal nest building seems to represent an autonomous process. The black rat (*Rattus rattus*) has a widespread distribution throughout the world. It is well-suited for arboreal life and is omnivorous with a preference for plant matter. Its behavioral flexibility is also displayed in its versatile nesting behavior. Given the rat's negative geotaxis drive and its notable climbing ability their nests are mostly "areal". In spite of this, it has been found that rats nest underground, on the ground and as a commensal dwelling in man-made buildings. More than that, sympatric rat populations which share a common niche with identical ecological conditions differ in their nest-building behavior. The nest-building flexibility is exhibited in the nest location, its shape, structure, size, building material, etc.

Previous extensive study in pine-tree habitat and experimentally manipulating rats' environment by means of man-made semi-artificial conditions, enable a comparison with several studies on rats' nesting behavior in diverse habitats throughout the world. This goal may be achieved by tracking with updated methodological and technical means, the nesting behavior of reciprocally interchanged rats which display a given specific building behavior habitat, to a habitat where rats nest in a different way. This work may provide an important contribution in clarifying the factors involved in different aspects of the versatility of the rat nest-building behavior.

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Comparison between maternal aggressive behavior and anxiety in female rats

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The present research aimed to evaluate the role of 5-HT_{1A} receptors in the modulation of maternal aggressive behavior and anxiety in female rats. Although, in a functional sense, parental aggression is a defensive protective behavior [1], the form and the topography of the behaviors displayed by a female rat with pups against a conspecific intruder share some similarities with the offensive behavior of males in colonies against subordinates and/or intruders. However, different from males, the aggressive behavior of lactating female rats comprises another strategy that is less contact-orientated which is characterized by the frontal attack (jump attack) directed to the head or snout of the intruder [2] and its form and topography have some similarities with the defensive explosive jump attack of a cornered rat by a predator.

8-OH-DPAT (0.2 µg/0.2 µl; 0.5 mg/0.2 ml and 2.0 µg/0.2 µl) was administered locally into the median raphe nucleus (MRN) and into the medial septal nucleus (MS). We performed two experiments. In the first experiment, female Wistar rats, 60-90 days of age, were divided in groups according to the brain area studied: NMR (N= 89); MS (N=88). The animals were maintained on a 12:12 light:dark cycle with the lights off at 16:30 hours and the experimental procedures were conducted during the dark phase, from 16:30 to 18:30 hours. The behaviors registered in the first experiment were: sniffing, locomotion, pup care, lateral threat, lateral attack, bite and frontal attack. The experiment was recorded on video tape. Afterwards, the tapes were coded by a trained observer, blind to the drug injected, using a special computer program. The mean frequency of the behaviors were compared among the groups by a factorial ANOVA and subsequently with Newman-Keuls Test ($p < 0.05$). The results showed that in the NMR the 8-OH-DPAT in the dosage of 0.5 mg and 2.0 mg/0.2 ml decreased frontal attack, lateral attack and biting the intruder, attack and bite as compared to saline injection. In the MS the 8-OH-DPAT in the dose of 0.5 mg/0.2 ml increased the lateral attack and bite the intruder. In conclusion, the 5-HT_{1A} agonist 8-OH-DPAT, when injected in the medial septum, has a excitatory effect on different components of maternal aggression. However, in median raphe nucleus this agonist has an inhibitory effect on maternal aggressive behavior of rats.

In the second experiment, virgin female rats (diestrus) were tested in the elevated plus maze. 8-OH-DPAT was administrated locally into the median raphe nucleus (N=83) and medial septal nucleus (N=91). The behaviors registered were frequency of scan and risk assessment. The percentage of time spent on open arm and closed arm were calculated. These behaviors were recorded. Afterwards, a trained observer, blind to the drug injected, recorded the behaviors using a special computer program. The mean frequency of the behaviors were compared among the groups by an ANOVA and subsequently by Newman-Keuls test ($p < 0.05$). The results showed that the 5-HT_{1A} receptor agonist 8-OH-DPAT in the median raphe nucleus (2.0 mg/0.2 ml) had an anxiolytic effect. However, in the medial septal nucleus (0.5 mg/0.2 ml) it increased anxiety.

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Measurement and analysis of head movement behavior during conversation

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To date, two different methodological approaches have been used to collect data on nonverbal behavior in conversation. On the one hand, interpretative methods involve raters having to identify a set of previously defined categories of behavior and to code the intensity of these behavioral manifestations using extensive coding manuals. On the other hand, transcriptions of behavior which contain detailed classifications of body movements use accurate procedures, e.g. picture-by-picture evaluations of videotapes, to deal with the complexity of behavioral manifestations.

In the present contribution, exact behavioral transcriptions of head movements are regarded as a prerequisite for investigating the function and meaning of nonverbal behavior. Additionally, a coordination of behavioral recording and analysis to verbal interaction and psychophysiological measurement is required which includes a time-related matching of all data channels in the range of milliseconds. In this respect, a method is developed which allows directly collecting data on head movements by using small ultrasonic transducers (buttons) which are attached to the head and the shoulders of an index person. The transducers are called up in the same order (in series), whereby positions are received every 40 ms. A microcomputer determines receiver-transducer distances and calculates three-dimensional positions by triangulation as a function of time, over a data-collection situation of variable length. Based on these data, evaluations are made which calculate angle-positions of the head (in rotation, lateral and sagittal dimensions), movement changes of the head (direction and size in rotational, lateral and sagittal dimensions), and intensities of head movements (cp. Figure 1). Further steps of the analysis present procedures to determine relevant changes in movements, to identify segments of movements, and to classify the quantifications of head movements according to the similarity of movement patterns.

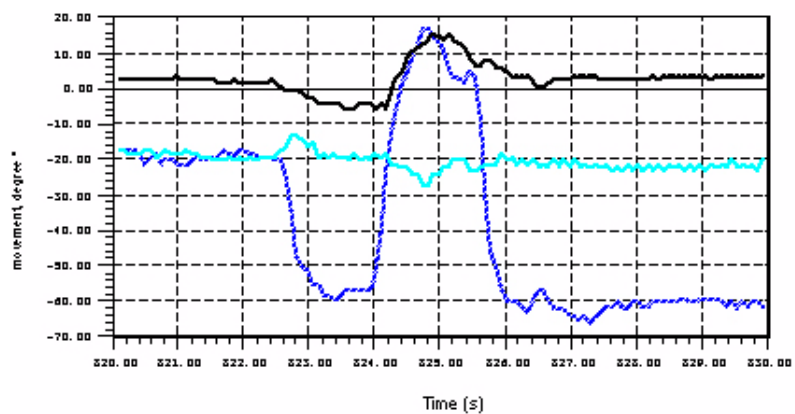


Figure 1. Head movements, angle positions in three dimensions lateral (—), sagittal (—) and rotational (—).

The measured patterns of nonverbal behavior can be accurately related to different features of the situational context (cp. verbal communication, vocal communication, cardiovascular activity [1]). The automated transcription and classification of movement patterns even allows the exploration of nonverbal behavior which still lacks descriptive identification. To estimate possible meanings of behavioral patterns, a heuristic is proposed which includes the situational context as the basis of interpretation (i.e. situations surrounding nonverbal behavior, e.g. verbal behavior in conversations).

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Assessing mechanical withdrawal thresholds of the rat paw with a new electronic algometer

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Von Frey filaments used for testing mechanical thresholds are mechanically unstable and their use is difficult to standardize. We have therefore constructed a hand-held electronic pressure algometer which corrects for these problems. The pressure algometer is connected to a computerized data collection system, allowing on-line display of the applied force as well as the application rate. Data stored on the computer can be replayed and further analyzed. Using this apparatus, we have measured the pressure induced withdrawal thresholds in rats with surgically induced neuropathy and compared the results with data obtained in the same animals by conventional use of Von Frey filaments. Five applications were made to each hind paw. The probe, with a circular tip of 1.0 mm diameter, was applied manually with a pressure increasing by approximately 0.05 N/second. Before surgery the withdrawal thresholds of the paws were normally distributed with a mean of 0.415 N, showing no significant difference between paws. During the two weeks of measurement after surgery, the mean withdrawal thresholds of the operated side were significantly reduced (range: 0.209 - 0.318 N), while the mean thresholds of the non-operated side remained at higher values (range: 0.432 - 0.491 N). Mean withdrawal thresholds of control rats without surgery were in the 0.380 - 0.520 N range, with no significant difference between paws or over time. The overall pattern of results were similar when Von Frey filaments were used but the variability was greater.

The visual feedback during application of the electronic algometer provides a means for standardized testing. Results obtained by two independent investigators aiming at the same application rate showed high inter-observer reliability, both between withdrawal threshold values obtained and between rates of application used. In conclusion, the electronic algometer allows detailed documentation of each experiment and provides an objective and accurate method for measuring the reactions of test animals to mechanical stimuli.

The Observer 3.0: the first study of the behavior of a couple mother-calf of *Tursiops truncatus*

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The development of the behavior and of the social interactions of a couple mother-calf of *Tursiops truncatus* (Cetacea, Odontoceta), belonging to a colony of 5 individuals housed in the Aquarium of Rimini, have been the object of a study carried out during the first year of life of the new borne (12 May 1995 - 12 May 1996). The method of sampling used has been "focal animal sampling" with 4 sessions at the day of video shot of 30 minutes for both the studied subjects. The sessions are carried out 2 times a week during the first 6 months of study and 1 time during the following 6 months. All through the 52 weeks of observation a total of 304 samples have been collected for both the items of the couple mother-calf, for the amount of 304h of observation.

Afterwards, the video data have been elaborated by means of The Observer 3.0 for Windows. The complexity of the nature and of the social relations of the analyzed subjects and the need to deepen the many aspects of the behavior of the couple mother-calf (evolution, allomaternal behavior, suckling, etc.), have made it necessary to adapt the reference ethogram to the characteristics of the computer program, in such a way as to optimize its use. Although our ethogram is divided in 5 behavioral classes, the configuration utilized with The Observer consists of a single behavioural class including a list of 84 behaviors; this is for the requirements of the study to have subsequent and not contemporaneous classes, so as not to alter the real duration of the individual behaviors. Moreover, the possibility of formulating only two modifiers (of which there are 71 divided in 7 classes) for behavior has reduced the amount of detectable information like, for example, in the case of the behaviors of contact between two individuals, for which the respective datum about the interested portions of the body has been partially lost. For each of the different examined aspects of our study it has reworked the standard configuration to obtain interfaces aimed at differentiated collection of data, afterwards the basic statistical analysis permitted by the software has been applied to these data, and the reports so obtained have been exported to other computer programs for a subsequent examination and statistical analysis (analysis of the variance, T-test, etc.).

In conclusion, the use The Observer 3.0 has provided an important contribution for the collection and for the analyze of data, even if many general problems connected with the behavioral complexity of the different species remain.

Analysis of sequential data: methods, techniques and software tools

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Whether beginning with live observation or videotaped recording, research based on systematic observation of behavior can be regarded as progressing through five phases. First, investigators form research questions and identify behaviors of interest. Second, they develop and pilot coding schemes that name and describe those behaviors. Third, guided by their coding schemes and whatever recording devices are available and appropriate, they record data. Fourth, investigators represent the recorded data in ways that facilitate analysis. And fifth, they analyze those data so as to address the questions that motivated the investigation initially. This talk focuses primarily on the fourth and fifth of these steps.

Computerized procedures and tools for data collection seem relatively well developed (e.g., Noldus' Observer). In contrast, general as opposed to specific computer programs or packages for reduction and analysis of observational data are rare. Bakeman and Quera [1, 2], reasoning that lack of a common format for sequential data has impeded the development of general purpose analytic programs, have defined a sequential data interchange standard (SDIS). For ease of use and to reflect procedures that have been used and found useful historically, five data forms are defined by SDIS. The five are event, state, timed event, interval, and multi-event sequences. Almost always behavioral sequences recorded by investigators can be expressed in one or another of these five forms.

Any standardization of sequential data that became widely used would prove advantageous. We think SDIS is a simple, useful, and flexible candidate. Observers can easily record data in this format directly or, when investigators possess recording equipment that uses a different format or wish to analyze data from an existing archive, it is a simple matter to write programs (e.g., in Basic or Pascal) that reformat existing data into the SDIS format.

Once sequential data are expressed using SDIS conventions, the considerable power of programs like GSEQ [2] can be brought to bear, including its ability to modify existing codes (e.g., recoding or lumping existing codes), create new ones (e.g., combining existing codes using standard and, or, and not logical operations; or defining new codes keyed to onsets and offsets of existing ones, such as the five seconds after the onset of crying, or the two intervals after the onset of smiling), and produce any number of cross-classified counts (e.g., the number of seconds within five seconds of the onset of infant crying that contain an onset of maternal

comforting). The remainder of this talk considers capabilities and limitations of GSEQ and the feasibility, once data are expressed in SDIS format, of writing additional, specialized programs to analyze SDIS data.

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“You wanna put a camera in our bedroom?” - Video observations of parent-infant co-sleeping in the home environment

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Research in US and UK sleep laboratories has demonstrated that a close behavioral and physiological relationship exists between mother-infant co-sleeping pairs which may protect infants against SIDS. However, in interview studies we have found that co-sleeping infants in the North Tees area of England are usually brought into bed with both parents, not just the mother. In this paper we present the initial results of an in-home observation study contrasting what happens to infants sleeping with both parents compared to sleeping with the mother only.

Participants were recruited through midwives, health visitors, baby clinics, and local media reports. Using low-light video cameras and infra-red lights we obtained all-night videos of dyadic (mother-infant) and triadic (mother-father-infant) co-sleeping parents and babies. Videos were coded using a behavioral taxonomy developed during our pilot study which allows us to quantify data on parent-infant sleeping positions, proximity, orientation, sleep state, and frequency and duration of interactions and interventions. Results presented are based on 128 hours of video-taped observations comparing 8 infants sleeping with their mothers only, and with both parents.

On both triadic and dyadic sleeping nights infants spent the majority of the night oriented towards the mother, regardless of the proximity or position of the father. Infants were also commonly observed in closer proximity to the mother than the father, and on triadic nights fathers were found to orient themselves away from the mother-infant pair. Mothers responded both more rapidly, and more frequently, to infants than did fathers. Differences observed between mother-infant interactions on the triadic and dyadic co-sleeping nights were minimal. From these preliminary data we conclude that the presence of the father in the bed has a negligible influence on the night-time relationship which exists between co-sleeping mothers and infants.

We also examined the video tapes for indications that sleeping with both parents may carry more risks for the infant than sleeping with the mother alone. All infants slept between both parents on triadic sleeping nights, and all were placed in a supine position. Using interval sampling we coded the position of bedding, pillows and parents relative to the infant's body, head and face in both co-sleeping situations. Quantification of the frequency of potential infant-risk events (smothering, overheating, rebreathing CO₂) indicates that infants were not at

increased risk when sleeping between both parents versus sleeping with the mother only. Further research will involve the integration of physiological variables.

Funded by Foundation for the Study of Infant Deaths, Nuffield Foundation & University of Durham.

UltraVox: an easy-to-use tool for automatic monitoring of ultrasonic vocalizations

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There is an increasing amount of research on ultrasonic vocalizations in bats, nocturnal primates and small rodents. Especially the vocalizations of the latter group play an important role in (etho)pharmacological research. In pharmacological screens, the ultrasonic vocalizations of rats and mice are used as therapeutic models for finding new anxiolytic drugs. For instance, it is assumed that rat pup vocalizations model separation anxiety which often proceeds panic disorder.

In principle, vocalizations can be analyzed in three different domains: time, frequency and amplitude. In pharmacological research the time domain is found to be most important. Neuroscientists wish to determine parameters like total time spent vocalizing and total number of calls, but also the interval between successive bouts and the interval between the calls of a single bout is of interest. However, analyzing ultrasonic vocalizations can be troublesome. To humans, these vocalizations are inaudible and a professional system with ultrasonic microphone, amplifier and recorder is too expensive for most research laboratories.

An alternative solution is UltraVox (figure 1). The UltraVox system is an easy-to-use tool for the automatic monitoring of the temporal structure of ultrasonic vocalizations. The system combines a bat detector, audio filter and data acquisition software. First, the bat detector shifts the high-frequency sound to the audible range. This sound is then fed into the audio filter. If the amplitude of the sound exceeds a threshold value, a signal is forwarded from the audio filter box to the PC. The UltraVox software converts the signal to onset and offset times of the vocalizations. The software is currently available under DOS. A Windows version is under development, a prototype of which will be demonstrated during the presentation.

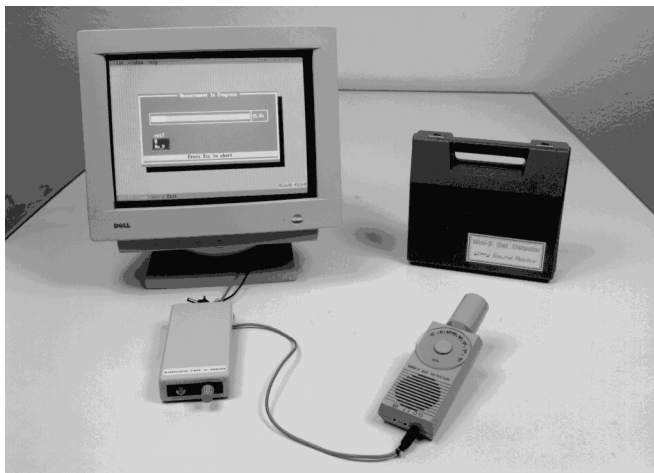


Figure 1. The UltraVox system: Mini-3 bat detector and carrying case (right), audio filter (front left) and computer monitor (back left) displaying the UltraVox software.

Although UltraVox has primarily been designed to monitor vocalizations in the ultrasonic range, calls of any nature can be automatically detected. For instance, the monitoring of audible sounds merely requires the use of a standard microphone instead of a bat detector.

Tuftsins analogue TP-7 and behavior of rats in the open field

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The influence of the tuftsins analogue TP-7 (heptapeptide, including Prp-Gly-Pro in its structure) on the behavior of white male rats (Wistar strain) in "unescapable" stress (an open field test consisting of 25 squares of 0.2x0.2 m each) underwent examination. The observation was held for three minutes for three days: 24 hours before, 30 minutes after and 24 hours after the tuftsins analogue (3 mg/kg in 0.2 ml distilled water) intraperitoneal administration. There was measured the motor activity: vertical and horizontal (the number of squares passed in the central area and peripheral zone, the number of passes across central area); time of freezing, grooming and orientation - searching activity (scenting the air in the corners of the open field). In comparison to control groups (1. without any treatment and 2. which received intraperitoneally 0.2 ml distilled water), tuftsins did not show a significant influence on behavior of rats, except prolongation of the time of orientation - searching activity 24 hours after its treatment ($p < 0.001$). Tuftsins had a tendency to shorten the freezing reaction and to decrease the number of passed squares both in the general and in central area. The administration of a heavy dose of TP-7 did not change Wistar rats' behavior on the administration day in a significant way. However, it had a sedative influence at 24 hours after its administration.

Stages of learning disturbances after haemorrhagic shock

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It is known that animal learning ability depends on the level of anxiety [1]. There is special interest whether such dependence influences on learning disturbances in postresuscitation period after haemorrhagic shock (HS) and what manner those disorders develop taking into consideration the starting functional CNS state. This research led to the finding that there are several stages in development of postshock learning disturbances.

Previously rats were placed in an elevated plus maze to determine their anxiety level. In 2 days anaesthetized rats were subjected to HS. Animals were rapidly bled to a mean arterial pressure of 40 mm Hg. Blood reletting or reinfusion was instituted to maintain this pressure for one hour followed by retransfusion with all shed blood. Rat learning was studied in a food-rewarded T maze (4 days of training sessions of 5 trials each) in a week and in a month after HS. Intact and operated rats were control. The program Statistica was used for data calculation. In the intact group, the correlation coefficients between starting anxiety level and learning parameters as latency and time of correct trials in the first day of training were 0.45 and 0.44, respectively. Probably the low correlation coefficients can be explained by the long time between starting test in elevated plus maze and learning in T maze, preliminary adaptation and handling. Such correlation was not in the other days. In the operated group there is no sufficient correlation between starting anxiety level and learning ability. At the same time in HS group sufficient correlation was found between starting anxiety level in one hand and latency, time of correct trials and number of correct trials in the other hand (Spearman coefficients: 0.52; 0.47; 0.49, respectively, $P < 0.05$) in 1st, 2nd and 3rd day sessions in T maze in a week after HS. Thus, learning disturbances in this period depends on starting CNS functional state. As long as behavior of intact animals depends on their starting functional state we divide animals to 2 groups concerning starting anxiety. All data in the other groups were standardized relatively these intact groups to determine learning disorders dependence on starting functional CNS state. Latency and time of correct trials increased in HS group by comparison with control groups on 3rd and 4th learning days in T maze in a week. However the number of correct trials in this group did not change relative to control. So the learning process slowed down. This is very likely to be caused by a change of perception. It is important only animals with low starting anxiety level had such disorders. In a month after haemorrhagic shock there were not correlations between starting anxiety level and learning disturbances. Besides learning curve of

rats subjected to HS in T maze during 4 days of training had strong divergence from that of intact and operated animals. In intact and operated groups latency and time of correct trials decreased and number of correct trials raised from 1st to 4th day of training while in HS group such process was not observed.

Thus, pathological processes developing in postshock period lead to the consolidation of memory track becomes weak. In conclusion measuring correlation coefficients between starting anxiety level and learning parameters in T maze showed existence of several stages in learning disturbances after HS.

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Observing and measuring fish behaviour in the laboratory and field

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At Dunstaffnage Marine Laboratory we are using a variety of video techniques to study the Predator-prey Interactions in Shallow Coastal Environments (PISCES). Field experiments use underwater video with infra-red illumination and time-lapse recording to reveal the tidal and diurnal migrations of fish on sandy beaches and rocky reefs. Laboratory experiments have combined high speed video (400 fps) recording with monitoring of physiological signals and sound to investigate escape and schooling behaviour. An infra-red dark-field video technique has also been developed allowing observation of fish larval behaviour *in situ* within culture tanks; variations in swimming behaviour have been correlated with growth rates of individuals. In other experiments a video "Spatial Actograph" utilises a computer and frame store card to log activity by time and location within an experimental arena to study habitat selection and responses to environmental gradients. This technique is particularly useful in situations with poor contrast - benthic fish that are camouflaged against a sandy substratum.

Intraperitoneal transmitter implantation in mice: effects on behavioural parameters and body weight

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Using biotelemetry several physiological parameters, such as heart rate, body temperature and ECG can be measured simultaneously in non-restrained, freely moving animals. The transmitter is placed in the peritoneal cavity through a ventral laparotomy. The weight of the transmitter without leads (about 3 gram) is quite high for a mouse weighing 25 gram. Also the volume of the implant (1,6 ml) has to be taken into account. Rough observation of transmitter implanted mice during experiments does not reveal abnormal behaviour. The aim of this study was to assess in an objective way the behaviour of mice carrying an intraperitoneal transmitter. Using an automated Laboratory Animal Behaviour Observation Registration and Analysis System (LABORAS) several behavioural parameters were monitored, such as eating, drinking, climbing, locomotion, grooming and resting without disturbing the animal. In this study male BALB/c (n=18) and 129-SV (n=18) mice were used in three different treatment groups: control animals (n=6), sham operated animals (n=6) and transmitter implanted animals (n=6). After one week acclimatization to a shifted light/dark schedule with a 30 minute dimming period the animals were treated in cohorts according to a random block design. After operation the animals were placed back in their home cage, placed by half on a heating pad for 24 hours and received buprenorphin (Temgesic⁷) as an analgetic for two days twice a day. In addition to standard food pellets and water, the operated animals received Solid Drink⁷ for four days, moistened food pellets and water containing 10% glucose for seven days. Behaviour of each animal was measured on LABORAS twice a week for one hour after the start of the dark/dimming period in the first week and for four hours in the second week. Body weight was determined each day. At the end of the experiment animals were killed and post mortem macroscopic inspection was carried out. Results of this study will be discussed.

Does the social representation of a receiver influence the structure of expert-novice interactions

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Previous research has shown that when 10 year old children trained to become experts in solving a sequential problem are placed in a situation of transmission to an untrained receiver (novice) of the same age, they interact through verbal messages and gestures using tutoring procedures adequate for informing the novice and regulating his procedure when solving the problem. We have analyzed (coded) the communication on the functional level using a list of behavioral categories that are combined to form “communicative events”. The behavior records were then analyzed structurally with the Theme program. In this research, the cognitive capacities of the receiver were not controlled and the expert had to discover and adapt to these during the interaction. We have used the same method but before entering the dyadic situation the receivers (novices) were presented to the experts as efficient vs. inefficient thus creating in the expert social representations regarding the receiver. Each expert had to deal successively (in a counter-balanced order) with receivers that were characterized as “good communicators” or “not very good”. This indication corresponded or not (i.e., for half of the subjects) to the results of a pre-test carried out among the future receivers. In this way four expert-novice groups were defined. The functional and structural analyses carried out in an identical fashion show significant differences between the experimental groups. In particular, in the cases when the novices do not correspond to the level indicated, one sees, by comparing the beginning and the end of the interaction, that the level discovered during the interaction is taken into account. We will present in a contrasting fashion the behavioral patterns found in each of the groups at the two indicated moments. This illustrates the role of the Theme program as a “Structuroscope” allowing the detection of fine structural modifications in dyadic interactions.

A simple method for measuring heart-rate in Guinea pigs during a standard handling procedure

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Introduction

In the course of a longitudinal study on social stress in female Guinea pigs [1] we developed a simple and noninvasive method to estimate the heart rate during a standard laboratory handling procedure. The method had to meet the following requirements:

- many animals (>20) had to be measured daily within a short time,
- the measure had to be highly standardized which means without manual restraint,
- because it was performed during a lifetime study, the procedure must not involve additional manipulations of the animals, and
- less financial and time effort.

Method

The investigated animals were familiar with a daily standard procedure which included weighing in a plastic basin since their first day of life. In order to avoid disturbing the animals we used this procedure as base for our heart rate measurement: The head of an electronic stethoscope (Bosch, Germany) was built in the weighing basin in a way, that it was directly under the chest of the Guinea pig (figs. 1 and 2). By using an underneath accessible adjusting device the stethoscope's head could always be adjusted in an optimal position without touching the respective animal with the hands. The electronically amplified heart rate sounds and the animal's identification were recorded with a commercial two-speed dictation machine (Sanyo, Japan).

A measuring period of 30s proved to be sufficient for reliable results. The evaluation of the heart rate (beats per minute) was done with the help of the program The Observer (Noldus, The Netherlands). The heart beat sounds were played with half speed while a trained person had to press a key synchronously with the beats until the program ended the recording after 60s.

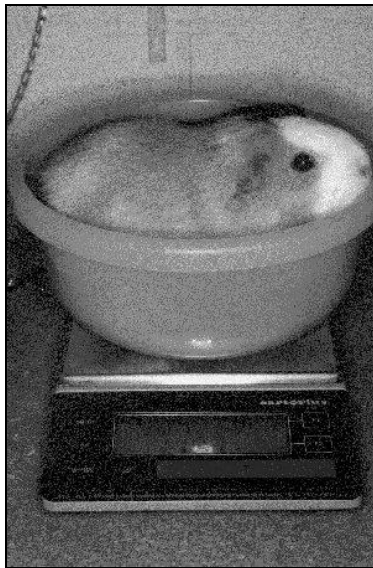


Figure 1. During the daily weighing procedure the Guinea pig remains calm in the basin.

Results

- The reliability of several measures performed by the same person was higher than 95%.
- The estimated heart rate of non-pregnant female controls is in good accordance with literature [2].
- The heart rate of female Guinea pigs rises during pregnancy.
- The heart rate in animals which were stressed by manipulation of their groups was significantly higher, than in controls.

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Figure 2. Placing the animal in the heart-rate measuring basin. The device is placed under the animal's chest.

Simultaneous measurement of blood-borne substances, heart rate, blood pressure, and behavior from freely moving socially housed nonhuman primates

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We devised a method for collecting blood samples by remote control from socially housed nonhuman primates without disturbing their routine and without letting them know when samples were being drawn. This allowed measurement, in a social setting, of plasma catecholamines and other substances that may fluctuate rapidly in response to psychosocial and psychological stimuli. Venipuncture, removal of animals from their group, and changes in the animals' routines alter plasma catecholamine levels. Blood can be unobtrusively drawn from tethered animals, but the vulnerability of an exteriorized catheter limits opportunities for social contact and exercise. We protected the catheter by containing it within a backpack worn by the subjects. Three blood samples per subject were collected each session, so baseline levels could be compared to levels at two points during events. Behavior was recorded on videotapes, which were coded concurrently for posture/locomotion and for other activities the baboons engaged in, such as grooming and aggression [1, 2]. Radiotelemetry was used to measure heart rate and blood pressure [3]. An integrated picture of physiological correlates of rank in captive male baboons emerged from application of this design to three stable groups. Each group contained one dominant and one subordinate male and 1-2 females. Cortisol levels from samples collected in the compound during quiet times were more than twice as high in dominant males than in subordinates.

Heart rate, blood pressure, and plasma catecholamines did not differ by rank during quiet times or when baboons watched food treats being prepared. When food treats were in the compound, heart rate, blood pressure, and plasma norepinephrine were significantly higher in the dominant males. The same trend was seen during non-contact exposure to an intruder. Heart rate and blood pressure increases were higher and more sustained in the dominant males, who also had higher plasma norepinephrine and epinephrine levels. Remote-controlled blood draws, radiotelemetry, and behavior coding made it possible to determine baseline levels of the physiological variables in a social setting, and to study the impact of events on behavior and physiology in dominant and sub-ordinate baboons.

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Computer-assisted method for the observation of the functional state of shiftworkers under prevalence of mental activity

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Shiftwork makes the necessity for the equal reliable and effective operator work at different times of day or night in contrast to the circadian variation of functions' activity that is imposed from outside and strengthened by experience. Operators in a balanced state are able to smooth away the incoming influences and maintain the performance at the due level. When the functional state deteriorates an operator can not resist the incoming circadian influences that results in pronounced fluctuations of mental performance. In this sense the significance of circadian variations in mental performance can serve as an integral criterion of the functional state of shiftworkers. The purpose of this study was to reveal and analyse the significant circadian fluctuations of the operator mental performance in shift rotation.

Eleven control room operators of a heat power plant (healthy men aged 28-53 with up to 19 years of work experience who worked in three 8-hours shifts) have been examined periodically over one year using a computer based system of performance testing [1]. At the end of the 1st hour of a work shift each operator was given 30 logic-combinatorial tasks on the PC screen (every next task being given after the previous task being solved). The task was to arrange 4 random numbers from 0 to 9 in order to create an increasing row, mentally rearranging the numbers for one position at a time. The response was the number of changes made; it was given by operator's pressing the corresponding PC key. The speed and quality of tasks solution were registered automatically. The test took about 3-5 minutes. Every test procedure was evaluated by calculation of 4 characteristics of Time of Task Solution (TTS) distribution - mean, deviation, asymmetry, excess - and a number of mistakes made (NM). The inter-shift differences in TTS and NM were analysed for a year as a whole and also 3 time points of a year: at the beginning (after vacation), in the middle and at the end (on the eve of vacation) of the individual work year according to the results of five tests on each shift (night, morning and evening) at each time point of a year. $N = 5 \text{ tests} * 3 \text{ shifts} * 3 \text{ year periods} * 11 \text{ subjects} = 495 \text{ subject-shift tests}$. Interrelationship of the alterations of characteristics of performance rate and quality during a year at the night, morning and evening shifts; variability of performance rate and quality (multi-day alterations and inter-shift amplitude of variations) were analyzed as well. The Student t-assessment of statistical reliability of differences and correlative analysis were used with a p-value of 0.05. The results obtained for a year as a whole and 3 time points of a year were compared.

Two operators showed inter-shift differences in mental performance for a whole year epoch of analysis ($df=28$, $p<.05$). Operator A (aged 31y with 1y experience) showed inversion of TTS inter-shift changes regarding the group tendency, the least TTS variability and the highest linear correlation of TTS and NM -- all over the group, that can be connected with body (over)tension. Operator B (aged 53y with 19y experience) showed TTS changes conformity to the group tendency, the most TTS variability all over the group and the absence of linear correlation of TTS and NM, that can be caused with decentralisation of regulatory interrelationship in the body, as a result of (chronic) fatigue. Two operators showed inter-shift differences ($df=8$, $p<.05$) in mental performance at the beginning of individual work year, four operators revealed that in the middle of a year, six operators -- at the end of a year, owing to development of unfavourable changes in the functional state. Operator A showed inter-shift changes in mental performance for a year analyse epoch also all 3 time points of a year. Operator B showed inter-shift changes for a year analysis epoch and did not show that for any 3 time points of a year. Six operators manifested inter-shift changes in mental performance at one or two time points during a year.

Computer-assisted analysis of inter-shift differences in mental performance can allow both to reveal operators who need prophylactic or rehabilitative measures and to ascertain the type of unfavourable changes in their body functional state for correction of these ones in due manner and time. This could help to maintain good health of shiftworkers and prolong their professional activity.

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Behavior and autonomic nervous system function as assessed via heart activity: the case of hyperarousal in boys with Fragile X Syndrome

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There are many scientific and clinical questions that require consideration of physiological responses concomitantly with behavioral responses. While in some cases, global assessments of physiological systems whose measurement provides a window into the system is sufficient, others require precise time-series analysis of moment by moment integration of behavior and physiology. The case of hyperarousal as a source of behavior problems in children with Fragile X Syndrome is one such example. Hyperarousal refers to the idea that these individuals have difficulty regulating arousal levels due to dysfunctional autonomic nervous systems (ANS). It has been suggested that the stereotypes and behavior problems often seen in these children may be behavioral attempts to modulate their physiological arousal. To test this hypothesis, it is necessary to record physiological markers of ANS arousal in real time, and in such a way as to permit integration with ongoing, real-time recorded behavior. In this presentation, we report the methods we have employed to test this hypothesis.

Heart activity, from which information can be derived about ANS function, is monitored with the Minilogger 2000 system (Mini-Mitter Co.), which employs Polar belts to transmit R-wave information to their logger. The Polar system is proprietary software that has very high accuracy in detection of R-wave peaks in a heart signal. The logger computes inter-beat intervals or summary heart rates, along with other information, and stores the data for download to a computer at a later time. Software permits extraction of ASCII files that can be imported into other software systems. Behavior is video-recorded for later coding, and an event-code marker is inserted into the logger file on tape to synchronize behavior and heart activity data. Behavior is coded with The Observer (Noldus Information Technology), which then integrates the heart activity data with the ODF file to provide information about mean heart period during each behavior scored as well as during each experimental phase. Because The Observer can integrate any time-series physiological data with behavior in ODF files, it is possible to use multiple heart activity measures to examine the sympathetic and parasympathetic nervous system responses to experimental manipulations and behaviors.

Applying these techniques to a sample of boys with Fragile X Syndrome (FraX) and a group of typically developing boys, we examined both raw heart activity data and spectral analysis measures of parasympathetic and sympathetic nervous

systems' contributions to the heart activity in relation to experimental challenges. We found that boys with FraX had lower parasympathetic activity, rather than higher sympathetic system activity. We also found that they were differentially responsive to experimental challenge. These results have important implications both for our understanding of the basic nervous system dysfunction in FraX and for the strategies likely to be effective in terms of pharmacological intervention with these children. These methods can be used in a variety of contexts to examine any physiological measures that can be collected as a time series.

Lab and field approaches to the study of activity rhythms of fossorial insectivores: an interesting as well as difficult task

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Highly specialized fossorial mammals, such as moles, may be important to understand the role of light as a *zeitgeber* of rhythms, both for the limited exposure to light and the regression of some of the structures apt to perceive it. Moreover, entrainment of circadian rhythms in subterranean animals is a particularly interesting task, as other environmental cues in addition to light could play an important role in the synchronization of organisms with the external periodic cycles. The search for an endogenous circadian rhythm of rest and activity has to be run in controlled conditions. However, it relies on a good knowledge of activity patterns in natural conditions. Here we describe two different approaches and techniques for investigating mole activity rhythms in the lab and in the field.

During lab studies moles must be housed in separate cages. Each cage consists of a nest box, a food box and a vertical box with plexiglass walls and earth inside. Different parts of the cage are connected with net or plain plastic runs. A program under MS-DOS written in JPI Modula-2 has been developed to monitor mole activity. The program interfaces to a hardware system which uses infrared (IR) barriers placed in couples along the paths and contact microphones which detect sounds coming from the soil in the mole cage. Both IR barriers and microphones outputs come to a microcontroller operated interface where signals are digitally converted and sent to the personal computer in the form of serial codes. The program continuously checks data from the serial RS-232 port and generates four files (one for each cage monitored) with raw data of IR barriers and microphones activation along with exact times of events generated. Sequential activation of IR barriers allows us to define movement direction and animal location. Activity state is determined by information coming both from location data and microphones activation. If a LED malfunctions this is reported by the program. This simple structure allows recording of rest/activity patterns of lab animals without the use of invasive techniques, can be run in dark conditions and with complicate settings.

Field studies on activity patterns of fossorial insectivores are limited by the impossibility of direct observations, absence of a distinct neck for radio-collars, lack of adequate implantable micro-transmitters and of anaesthetic protocols. Moreover radio-tracking techniques often require exhausting schedules that cannot be protracted continuously for more than a few days, while a rigorous study on activity rhythms requires data logging over an extended period of time. We tested

different kinds of implantable micro-transmitters and we used automatic recording of field data by means of an event recorder connected to a receiver positioned over mole nests. Preliminary results are briefly discussed.

Workplace for Analysis of Task performance: towards a more realistic environment for experimental research on task performance

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In the current research field on task performance, a gap exists between laboratory approached research and task performance work conducted in a real life environment [1]. One of the factors for this, is that many of the real life tasks are conducted within a multiple-user environment.

The project “Workplace for Analysis of Task performance” (in short “Digital Workplace”) has been started as an approach to fill in this gap. The main goal is to develop a research laboratory in which multiple-user task performance research can be conducted without giving away the stability and reliability of laboratory task performance research. The laboratory is implemented on a PC platform. A prototype will be demonstrated during the conference.

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Customising “The Observer Video-Pro” to facilitate coding of behaviour by untrained observers (parents of children with disruptive behaviour)

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Observational and attending skills deficits have been identified in the parents of children with conduct problems who fail to improve after parental training. The established method of measuring these skills [3] involves parental coding of their child's behaviour from video tape. This is both costly and time-consuming and therefore not implemented clinically. The Bangor Project for Children with Disruptive Behaviours (School of Psychology, University of Wales, Bangor) wanted to evaluate Wahler's methodology against PCAMT (Parent-Child Autobiographical Memory Test), a brief assessment with standardised administration and scoring [2], to establish whether the latter is a comparable measure of parental observation and attending skills. If so, this could then be used in the clinical setting to predict those parents who would need extra training to enable their children's behaviour to improve.

The group at Bangor wanted an easy-to-use, mobile system for allowing both parents and professional observers to code behaviour during every 15-second interval. The parent would simply code child positive and aversive responses, and the trained observer would then subsequently score the same piece of video using a more complex coding system (a validated multicode observational system, the Standard Observation Codes (SOCIII, cf. [1]). Each pair of data sets would be compared to generate a positive and negative bias score for each parent (using t-tests of parental and professional ratings in 15-second intervals).

These problems were addressed by using The Observer Video-Pro, Noldus Information Technology's event recording and data analysis system, with a customised keypad (Tracksys Ltd). Thirty minutes of home-based family interactions were recorded using a VHS camcorder, which was then encoded via the Broadway card as an MPEG-1 (a high quality compressed digital video format) file and written to a CD-ROM. The researcher then returned to the family's home with only a notebook computer and the digital video was displayed using The Observer Video-Pro software. The event-recording screen was customised so that the video image alone was visible, avoiding extraneous information normally provided by the software influencing scoring behaviour. The parent used a customised keypad offering only 2 keys (to score aversive and positive child responses), linked to the PS/2 keyboard interface on the notebook. The keypad

was operated at the same time as the normal keyboard (allowing the researcher to control the start and end of observation sessions) and provided different audio feedback for positive and aversive behaviours. Preliminary results indicate that the system works well, and that parents find the test one which they can manage with relative ease. Data will be available by the end of 1998.

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LABORAS: automated behaviour classification of laboratory animals

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Introduction

At present, the analysis of laboratory animal behaviour is based mainly on video recording followed by visual observation or computerised screening of the recordings. Visual observation is time-consuming and scan sampling of a proportion of frames gives only a rough indication of the actual behaviour of the animal. Metris has developed a system named LABORAS (Laboratory Animal Behaviour Observation, Registration and Analysis System) for automated classification of behavioural patterns by analysis of sensor signals induced by the animal's movements. Each of the behavioural categories locomotion, sleeping, climbing, grooming, drinking and eating is characterised by a specific and unique movement pattern which is detected by the sensors and then processed by computer into the behavioural categories. Validation experiments have indicated that the technique is accurate and the results reproducible [1, 2, 3]. The system has several advantages, e.g. no visual observation needed, reduction of time needed for behavioural research and a flexible time window. The system is suitable for mice and rats.

System description

Basically, LABORAS consists of a sensing platform, a signal conditioning and data acquisition device and software for administration, control and behaviour classification.

Sensing platform

The sensing platform (Fig. 1) is constructed of low-mass material and positioned on two orthogonally-placed sensors and a third, fixed point. The cage with the laboratory animal is placed at a marked position. The platform is designed to accommodate Macrolon types II and III cages for tests with mice and rats, respectively. The sensors are placed so that all movements are optimally detected and the approximate location of the animal can also be determined. To reduce the inertia of the platform and to enable the detection of climbing behaviour, the wire top and food hopper is mechanically separated from the lower part of the cage.

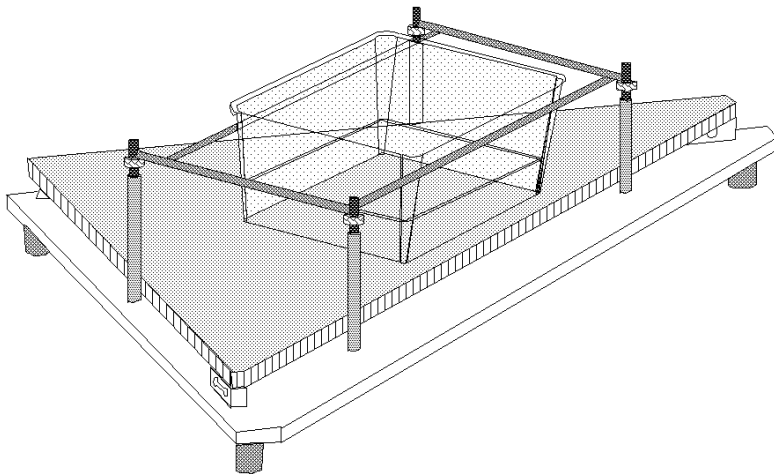


Figure 1. Diagram of the triangular sensing platform and a cage.

Signal conditioning and data acquisition device

Each sensor transforms the mechanical vibration into electrical signals which are filtered and amplified. Subsequently, the signals are sampled and stored on the computer's hard disk for later processing. Up to eight platforms can operate simultaneously.

Software for administration, control and classification

There are three software modules:

- Administration module: allows the user to manage experiment information (strain, treatment, housing, duration, climate, conditions, comments, etc.) before, during and after the test.
- Data acquisition and storage module: controls the hardware, data presentation during the test and storage of the signals sampled from the transducers. The output of this module is linked with the corresponding administration.
- Analysis and classification module: processes the data stored during the test. The analysis software searches for predetermined characteristic patterns in the stored data and translates the results in several user selectable formats for further statistical processing or presentation.

The analysis software has a modular design to enable addition of new specific behavioural categories or more detailed behavioural patterns.

Validation

Several LABORAS systems have accumulated an amount of data which is equivalent to more than 2500 h of experiment time. For validation purposes a

setup with 4 platforms was used, each housing one animal. Two tests were performed, one with mice and one with rats, each lasting 48 h. To evaluate the performance of LABORAS, different strains (C57BL and BALB/c mice; Wistar and Sprague Dawley rats) and sexes were used. Both tests were recorded using a video camera and a time-lapse video recorder for each platform. Each videotape was scored by three different observers independently. The scoring method applied was scan sampling with a sampling rate of once every 15 s.

Results and conclusions

The tests with mice show good results. For all four mice and four rats in the test LABORAS differentiated between climbing (mice only), sleeping (resting), locomotion, grooming, eating and drinking. On average the correlation between human observers and LABORAS appeared to be better than 85% [2, 3]. It can be concluded that LABORAS offers a new efficient and reliable method for automated behaviour classification and position tracking of small rodents.

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EthoVision Multi-Pro: improved animal identification during automatic multi-object tracking

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EthoVision Multi-Pro is a special version of the EthoVision tracking software that enables tracking of up to 16 unmarked animals in a single enclosure. During data acquisition the X,Y-coordinates of all objects that are visible in the experimental arena are measured and stored on disk. After acquisition, a reconstruction algorithm based on subsequent object positions recreates the movement paths of the individual animals. An initial prototype of this system was presented at *Measuring Behavior '96* [1]. Since then, intensive use of this system has resulted in a number of desired extensions to the software, designed to solve reported problems which are due to the nature of the tracking:

- Animals sitting close together are often detected as a single object.
- Often there are areas in the arena where the subjects can not be detected reliably. Examples of this are hides, insect traps or fodder areas. This results in the disappearance of the animal while it resides in such areas.
- Object size is usually small compared to the arena size: in low contrast areas of the screen this sometimes results in disappearance of the object for small periods of time and in detection of phantom objects.
- Confusion about the identity of objects at the moment that their paths intersect.

Some of these problems can be corrected automatically based on high level analysis of the animals' paths. Here the concept of *path segments* is introduced: a path segment is series of object positions that is continuous in time and during which no confusion with other animal occurs: it describes the movement of a single animal over a bout of time. A segment starts when a new object appears and ends when the object disappears. Segments are also separated at positions where confusion between animals can occur.

After the system has automatically detected these segments, several means are available to automatically eliminate the problems mentioned above:

- By discarding segments that are shorter than a preset minimal length, phantom objects are removed.
- The paths of animals that are detected as a single object are restored by duplicating segments.

- By defining *hidden zones* (areas where the animal can reside without being detected during analysis) it becomes possible to detect (dis)appearance of animals in hides, on traps, etc.

When the system confuses object identities, correct automatic reconstruction is not always possible. This is the case when multiple animals are located in a hidden zone and one of the animals leaves the zone or when the paths of multiple animals intersect. In these cases, the use of segments allows easy manual editing of the paths. Enhancements to the existing software allow the user to change the identity of an object for all locations within a single segment, linking of separate segments to a continuous path, deleting complete segments and assignment of animals to hidden zones when they are no longer detected.

The presentation will discuss the nature of the problems during tracking and the implemented solutions to these problems. Also, application of these techniques for applications with marked animals will be discussed.

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Video playback as a tool for investigating behaviour in the field

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Visual playback is a relatively new experimental approach that is growing in popularity due to recent technological advances in both video and computer animation techniques. Due to the type of equipment needed, video playbacks are usually conducted under laboratory conditions. The benefits to such a setup are obvious: environmental, technical and experimental conditions can all be controlled. However, carrying out playbacks in the field can have advantages - the subjects are more likely to give typical responses if they are tested under natural conditions, which will aid the interpretation of results. Despite this advantage, video playbacks are rarely attempted in the field due to the practical difficulties, such as lighting conditions, intrusion of apparatus, weather and performance of portable equipment.

We conducted a field study in Algarve, southern Portugal, using visual playbacks, to investigate the waving display of the European fiddler crab (*Uca tangeri*). Life-size playback stimuli (waving male, wandering female, male threatening and an empty mudflat) were presented to visually isolated male subjects. This poster discusses which of the many potential problems inherent in fieldwork need most careful consideration, how these critical problems were identified and several ways in which they could be overcome. We found that the problems of using video playbacks in the field are not insurmountable.

Use of The Observer for behavioral research at Zoo Atlanta's TECHlab

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TECHlab, Georgia Tech's laboratory for animal behavior, is a recently developed collaborative effort between the Georgia Institute of Technology and Zoo Atlanta. It functions as a think tank where graduate students in both psychology and architecture along with research affiliates from other institutions engage in research using advanced technologies in the areas of behavior, conservation, and education. Researchers at TECHlab currently use The Observer software on PCs, laptops, and handheld computers for the collection and preliminary analyses of behavioral data.

Topics

- Solitary behavior
- Social interaction
- Activity budgets
- Reproduction
- Infant development
- Behavioral management
- Enrichment
- Training
- Spatial distribution
- Conservation
- Visitor behavior
- Education

Species

- Gorilla
- Orangutan
- Drill, black & white ruffed lemur
- Black lemur
- Red panda
- Giant panda
- Lion
- Black rhino

- Sumatran tiger
- Flamingo
- Human

Projects_

- Longitudinal study of social behavior of drills
- Behavioral management of drills in captivity
- Reproduction in the black and white ruffed lemur
- Regurgitation and reingestion in the lowland gorilla
- Integration of an all-male juvenile gorilla group
- Space use in red pandas
- Comparative ethology of black rhinos in captivity
- Reproductive parameters of the giant panda in China
- Visitor behavior at the zoo
- Others

Relevant Magnetic Resonance Imaging in memory studies on healthy subjects

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At present studies investigate the relationship between memory and hippocampus with functional neuroimaging [2]. The morphometric magnetic resonance imaging approach was often performed to understand the pathology and the decrease of the performances. In our study we showed the evidence that this latter technique should give us interesting informations about memory processing on healthy subjects. The purpose of this research was to study the relationship between an explicit (EM) and implicit (IM) memory test and the hippocampal volume.

Seventy healthy adults were administered an established test of explicit and implicit memory: the word stem completion. A magnetic resonance imaging was performed for each subject on a 1.5 T signa unit (General Electric). The volumetric acquisitions were obtained with a spoiled gradient recalled acquisition at the steady state sequence (GRASS). Parameters of the sequence were 23/5/35/1; field of view was 22 cm, and matrix size 256x192. One hundred twenty-four contiguous sections were obtained of the entire head. To obtain measurements of the volume of the hippocampal formations, an accurate 3-D processing technique was used to segment the hippocampus [1].

There was a significant negative correlation between the EM test and both hippocampal volumes. The IM test did not correlate with hippocampal volume and total brain volume. These data indicated that MRI morphometric techniques were relevant not only for studying pathology but also for neuropsychological studies on healthy subjects.

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Teaching the observation of human behavior: combining printed media with computer software and digital video

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Since more than a decade, the practical course 'Observing Human Behavior' has been part of the first year of the curriculum at the Department of Psychology at the University of Groningen, based on a textbook written by J.P. van de Sande [1]. Due to technical developments in the field of computer-aided observation and the outcome of an evaluation by students, it has been decided to develop a substantially improved version of the course and supporting educational materials.

In the new course 'Observing Human Behavior' we have chosen for a combination of a theory book, a student workbook and a CD-ROM. The theory book provides the theoretical background for the practical course and has to be studied before students can begin with the workbook. The student workbook includes six modules with exercises. In these exercises the student gets acquainted with the principles and techniques of observation, learns how to design scientific research projects, works with practical examples of methodological and statistical procedures, and, finally, practices with The Observer[®] software. The CD-ROM contains a student version of The Observer, observational projects for The Observer and digital video material.

The student workbook offers instructions and questions related to the exercises. The student looks at a digital video clip and is asked to answer some questions. Then he starts an observation either with The Observer or with paper and pencil. Each module explains a particular concept of observation. Module 1 is an introduction to observational techniques and mainly focuses on how to best formulate a research question. In module 2 behavior is the main issue. Module 3 deals with methods of data collection and explains how to define the research design. Module 4 discusses reliability of scores, whereas module 5 deals with how to assess validity of scores. Module 6 considers some techniques of analyzing scores.

We have developed the course with the following design objectives in mind:

- **Quality of exercises.** The exercises in the course have been tested, reviewed and improved during 20 years of teaching, resulting in a series of interesting samples in which the relevant theoretical concepts easily get across. The computer and video technology has been tested thoroughly during the past

year. The digital video format allows highly interactive operation. Experience has shown that students really enjoy working with the material.

- **Flexibility.** The student is allowed to follow the course individually or while working together with several other students. If a student wishes to do the course on his own, he can find additional observational material in the student workbook to compare his findings with. Furthermore, teachers or students can also use their own examples. For this purpose, video material can be readily digitized and used with the course.
- **Variety.** The examples cover a wide range of topics within the field of psychology, including development, social interaction and language acquisition. The variety of applications to which the student is exposed enhances his knowledge about observational techniques.
- **Practical approach.** Statistical procedures are dealt with from a practical point of view. In each exercise students produce their own data. Being familiar with these data, they can more easily interpret the statistical measures pertaining to these data.
- **Sober user interface.** The course has a sober and scientific look-and-feel. There is no interactive multimedia shell around The Observer. While using The Observer, the student will find instructions on how to use the program in the student workbook. When not using The Observer, the student can view the digital video clips in a normal Windows environment. One of our aims has been to keep the package inexpensive, allowing individual purchase by students.

Some exercises will be demonstrated during the presentation. We hope you will take a look at the exercises and that you want to share your comments with us.

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A new computer program for the measurement of reaction times to simple visual and auditory stimuli

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We are presenting new software for the measurement of reaction times (RTs) to simple visual and auditory stimuli. An earlier program had been developed to measure stress conditions in basketball players in order to avoid spontaneous traumas following overtraining. Its supposed theoretical background is that stress modifies RTs, but in a different way when acoustic or visual stimuli are used to elicit them. RTs are supposed to differ also when we can elicit them from either brain hemisphere.

The computer program ST-CON (STress-CONtrol), by R. Cocchi and C. Carli (1993), was prepared to check up RTs. These can be elicited by hand answers to simple visual stimuli, as a variation of a 200*150mm rectangle from yellow to red. The same can be elicited to simple auditory stimuli as a beep lasting 55 msec. Every stimulus appears at random from between 1-15 seconds (start) from the beginning of the warning time. Stop is obtained by hitting the space bar. With some subjects (e.g. schizophrenics [1, 2]) the tester starts the warning time, otherwise the tested person can start it by himself. With this program we can measure RTs in msec, timing by functions that allow sequences of start-stop.

Four stimuli groups make up the test, carried out in a single session. This is the usual order: Visual stimuli - dominant hand; Visual stimuli - non-dominant hand; Auditory stimuli - dominant hand; Auditory stimuli - non-dominant hand. Each tested person has to do 15*4 or 30*4 valid trials, excluding Anticipations (< 80 msec for visual stimuli or < 70 msec for auditory stimuli) or Timeouts (> 279 msec). For each of the four groups the program gives the average RTs, the maximum, the minimum, the min-max differential, anticipations and timeouts figures. The program counts average, maximum, minimum and differential time only for the 15-30 trials considered as valid. In this way we can measure true RTs not biased by anticipations and timeouts. Immediate comparison is available with RTs range figures from a control group of normal people. Anticipations and timeout were considered a measure of impulsiveness or distraction, respectively. The program also allows the comparison between visual and auditory RTs and between left and right brain hemisphere RTs. Results on stress induced by alcohol challenge in disulfiram treated detoxified alcoholics [3] are presented.

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Image-analyzing systems for the study of spatial and postural transitions in rats

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In studies on rodent behavior, the available image-analyzing systems usually reduce the subject (rodent) to a single spot and provide its x and y coordinates: then, the distance, duration and velocity of the moving spot in space is calculated. In 1996 we have presented two new systems, including computer programs that provide quick, objective and new measures of the animal's progression in space [1]. The first, more classic system allowed a computerized and automated analysis of behavior on the open field, elevated plus maze and Morris water maze. Furthermore, the newly developed computer program allowed a quantitative objective and detailed analysis of exploratory behavior in a novel environment according to the rules of "home base behavior" as described by Eilam [2]. The second, more sophisticated system (CCD camera; video processor: Fast FPS60; computer: Pentium, Windows 95) allowed the computerized and automated measurement of lateral movements of the head, head-torso and head-torso-pelvis of freely moving rats [1].

We will now present an extension of the latter system. It allows a computerized and automated analysis of lateral, horizontal and vertical movements of the freely moving rat in space. The extended system has at least two broad applications. First, it allows a quantitative, automated analysis of distinct behavioral items. At the moment, we are able to distinguish items such as "head grooming", "torso grooming" and "genital grooming"; in addition, we are able to distinguish distinct postures such as "sitting", hunch back", "curved", "attend" and "stretched attention". When combined with the program that allows analysis of the animal's progression in space, the system allows a quantitative and objective analysis of nearly the entire behavioral repertoire. Second, the system allows a quantitative and objective analysis of the "Mobility Gradient" as described by Golani *et al.* [3]. This Mobility Gradient is an extremely useful tool in studies on ontogeny, ageing, drug-induced changes and recovery from brain damage.

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Measuring the behavioral structure of rabbits in pharmacological experiments

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For examination of the influence of many pharmacological substances with psychotropic activity on the behavioral structure of rabbits we used two experimental models. In the first we investigated the spontaneous behavior, by event-recorded method in 2 hours, and in the second we studied behavior under stress conditions (between stimulation of the ventromedial hypothalamus nucleus, evoked every 10 min). The behavioral structure was estimated in the following phases (each of them changing aperiodically):

- tension (assuming the immobile standing position with increased muscle tension and quickened breathing),
- orientation-searching (sniffing at the experimental cage, watching the surroundings with either moving about or not, sometimes trying to leave the cage),
- comfort (assuming a resting position, mostly in a horizontal position, napping or falling asleep now and again),
- grooming (body cleaning with the tongue and paws, rubbing against the cage walls)
- food uptake (eating and coprophagy), and
- water uptake.

The spectrum of behavioral activity of the rabbits in either of the models - in comparison - showed a significant prolongation of the tension phase at the expense of all the others in the stress conditions ($p < 0,01$). The control experiments, however, have not shown any influence of a solvent (distilled water, starch solution) on the behavior of the rabbits. By administration of various pharmacological substances - potential anxiolytics (in different doses and in different ways: intravenously, intraperitoneally, intranasally, into the lateral ventriculus of the brain; and in different time before the experiment, dependent on the way of treatment), we have, first of all, investigated their influence on the behavior of the rabbits in the stress situation - "good" drugs shortened the tension phase and prolonged the other phases of the animals' behavior. The investigation under spontaneous conditions has provided information about possible negative side effects of some substances, for instance, sedation which was visible in prolongation of the comfort phase, weakened attention manifested in the

shortened of orientation-searching), or in the change of food tastes (shortened or prolonged water and food uptake). All substances taken by us into the experiment (fragment of the ACTH 4-10, cyclic analogue of ACTH 5-14, tuftsin analogue TP-7 including tripeptide Pro-Gly-Pro in its structure, derivative of benzodiazepine BD-158, new antioxidant CM-46 from the group of mercaptobenzimidazol) showed a significant anxiolytic effect in the stress situation ($p < 0,05$), but only TP-7 has not changed the normal behavioral structure of the rabbits in spontaneous conditions.

Quantification of search patterns in the Morris water maze

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One of the most frequently used spatial learning task in rodents is the Morris water maze. Image analysis systems have made it more convenient to quantify the performance of animals. However, to define the search/swim patterns of free swim trials in this task as persistent or random, a large set of parameters has to be chosen: like total distance swum, distance and time in quadrants, crossings of and latencies to former and “possible” platform locations in other quadrants, subdivisions of the maze into inner circle and rim along the walls, velocity, etc. These data sets have to be compared to finally describe a swim pattern as persistent, i.e. focused to the former platform location, or random.

During the past years we have used the water maze mainly to characterize the role of corticosteroid hormones in learning and memory. Specifically free swim trials proved to be an excellent tool to differentiate the involvement of hippocampal mineralocorticoid and glucocorticoid receptors, MR and GR, respectively [1]. A balanced activation of both receptor types is reflected by short latencies to platform (memory) and a persistent search pattern (strategy). In contrast, animals with suppressed MR functions showed a distorted and random search pattern, but still short latencies. This dissociation between memory and strategy and the required extended description of free swim trials listed above prompted us to search for more economic ways. Furthermore, we were interested if behavior during free swim trials before spatial training might be of predictive value for memory and strategy after spatial training.

To answer these questions, we used swim patterns of mice of several studies. Estimating the distance between the former platform location and each sample of the animal's position (EthoVision 1.70, Noldus Information Technology), *distance to zone* platform and possible platform positions proved to be *the* parameter to differentiate persistent from random search patterns. To allow comparison between experiments with e.g., different sampling or maze sizes, we established and validated that a percentage of distance to platform < 16 reflects persistent search. Random search could be further subdivided into a circling pattern close to and at a certain distance from the walls. Thus, the two parameters, *latency to zone* and *distance to zone* are sufficient to describe memory and strategy used in free swim trials. Furthermore, we did not find any relationship between behavior in free swim trials before and after spatial training. Our next goal is to quantify the “small loop” swim pattern often seen in mice. In terms of latency and distance measures,

this 'looping' can be as efficient as the "spatial" solution during training trials. Taken together, behavioral image analysis systems do not only increase the amount of data output, but their flexibility also allows to extract the essentials in an efficient way.

Supported by ISAO 679756-0270 and EC BiotecPL960179.

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Measuring human behavior in large groups: a review of methods and techniques

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The Experimental Centre of Eurocontrol (the European Organization for the Safety of Air Navigation) was built around what was then the first digital Air Traffic Control (ATC) Simulator in the world, and is certainly the most flexible. It is capable of simulating up to 40 working ATC positions simultaneously. A real-time simulation takes three to six weeks, and may involve 20 to 60 exercises, each about 90 minutes long. About 50 megabytes of data are collected by the system software for each exercise, giving exhaustive detail of the activity of the simulated aircraft, and the orders given by the controllers.

Air traffic controllers usually work in pairs on each sector. Although they employ speech communications and gestures to communicate, most of their work is mental, rather than physical. The task is not, strictly speaking, paced, although some activities are triggered by communications from aircraft or adjacent sectors. In normal operations, the controllers organize their workload to anticipate problems and maintain a continuously safe state. The ability of the controllers to cope with the workload is often the decisive factor in fixing the capacity of the system. We are therefore greatly interested in measuring the effects of carrying out air traffic control on the controllers. The methods used must be simple, reliable and labor-economic (we have, at most, five technical assistants to measure up to 40 participants). They must not be intrusive, and they must have some face validity, since controllers' co-operation must be requested, and cannot be demanded. Fortunately, controllers are fully aware of the need to improve methods, and will put up with considerable inconvenience if they are convinced it will have useful results.

Since 1970, we have investigated the following types of measurement, some repeatedly:

- Activity Analysis
- Communication Channel Use
- Debriefing
- Questionnaires
- Postural Analysis
- Heart Rate
- Sinusarrhythmia
- Electroencephalography (P300)

- Electroencephalography (Lateralisation)
- Electroencephalography (Alpha)
- Electroencephalography (Theta)
- Electroencephalography (Delta)
- Peer Assessment
- Voice strain analysis
- Secretions (Catecholamine)
- Secretions (Cortizol)
- Structured Interviews
- On-line Instant Stress Analysis
- On-line Subjective Workload Analysis Technique
- Post-exercise NASA-TLX assessment
- Secondary Tasks
- Embedded Secondary Tasks
- Skin Resistance
- Eye Movement (Eye-Mark Recording)
- Eye Movement (Analysis of Video Record)
- Eye Movement (Direct Observation)
- Subjective Fatigue Assessment
- Konzentrations-Leistungs Test
- Grammatical Reasoning Test
- Sleep Logs
- Blink Rate

This paper will present a systematic evaluation of these techniques, classified as measures of workload (stress) or effect on the controller (strain), potential observer effects, the time -sensitivity, start-up and running costs, staff requirement, portability, risks of failure or bias, speed and cost of analysis, ethical constraints, potential for automatic data collection, and our decision on the method in our context. References to EEC reports, papers and Web sites are provided for each. This paper is a summary of a revision, currently in progress, of the existing EEC Report No.164.

Developmental analysis of temperament in vervet monkeys

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The goal of the present longitudinal study is to develop objective methods which allow a definition of behavioral temperament in the African green monkey or vervet (*Cercopithecus aethiops sabaens*), and to determine the stability of these vectors over time. In a large captive breeding colony, newborn infants (in their birth cages) and mothers were observed systematically during the first six months of life. Within the social group, this study calls upon the methodologies standard to primate social behavioral analysis (focal animal approach), but the data is then viewed from the clinical perspective. Measures drawn from the standard primate social behavioral repertoire allowed evaluation of the frequency and quality of interaction with the mother, with peers and with other adults and of rank (in multi-infant cages). Similar social measures, as well as rank, agonistic behavior and measures of fearfulness were collected later in peer groups of 8 similarly-aged juveniles. Individual measures encompassed motility, state of arousal, modal affect, playfulness, fearfulness, exploration, etc. At 6, 9, 12 and 24 months of age, all subjects were individually assessed in a novel environment, intended in part as a complex analog of the more familiar rodent open-field test of exploration. Enrichment of this novel environment included poles, ropes, tunnels, traps and rewards. Each animal was scored with respect to motor capability, affective and physical response to the novel setting, exploratory behavior, level of inquisitiveness and problem-solving ability. Each animal was tested twice, 10 days apart, at each age point, to obtain a measure of habituation and change in performance.

The present report describes the characteristics and stability of social and individual behaviors in 78 newborns which have been tested at all time points. Preliminary results indicate that infants who consistently showed above average motility in their birth cages maintained a higher level of motility in the subsequent experimental conditions. However, those highly motile infants who also showed early signs of fearfulness in their birth cages, frequently displayed frozen immobility under the experimental conditions and showed no signs of habituation or improved performance ten days later. In addition, infants who expressed high levels of fearfulness in their birth cages, were also rated as highly fearful under the experimental conditions and showed no signs of habituation or improved performance in the repeat trial. Only those infants who were both highly exploratory in their birth cages and also had high scores for motility and outgoing, proactive behavior (e.g., initiating play fights, hanging on peer's tails, approaching the observer) maintained a high level of exploration in the test cage. Measurement

of CSF amines and metabolites, as well as circulating stress hormones, will contribute to the interpretation of these data.

The ultimate goal of this project, which will encompass some 300 newborns evaluated at all four time points, is to identify stable temperamental vectors for genetic analysis. Even in this preliminary evaluation, motility is shown to be stable from birth to age 2, unless it is dominated by fearfulness. Fearfulness is itself a stable trait from birth to age 2, and in the novel test environment, supersedes not only motility, but also such species characteristics as exploratory behavior, apparent curiosity, etc.

Supported in part by the Réseau Santé Mentale du Québec (FRSQ), the Medical Research Council of Canada and Behavioral Sciences Foundation.

Resampling approach to statistical inference: bootstrapping from Event Related Potential data

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Functional inferences from experiments in cognitive neuroscience are often based on sets of methodological assumptions which cannot be always fulfilled, as in recordings of cognitive brain potentials. The effect of these violations is not always corrected through the traditional statistical analyses and may lead to inconsistent results across studies. An example comes from Event Related Potentials (ERPs) studies where electrical brain response to stimuli presented in different experimental conditions are usually estimated, separately for each subject and each condition, through an averaging procedure. Averaging assumes the brain electrical activity as composed of a constant brain response (ERP) to the same stimulus under the same experimental condition and a zero-mean random uncorrelated spontaneous EEG. Estimated ERP data are then analyzed across subjects through statistical procedures which, however, do not guarantee for the reliability of the single estimates. If the assumptions which underlay the averaging procedure are not fulfilled, as it is usually the case, the lack of reliability of the estimated ERPs may lead to statistical results which are not reliable as well. Within-subjects bootstrap analysis [1] can be used to evaluate the reliability of neurophysiological and behavioral experiments. The bootstrap technique is a general resampling procedure which estimate the probability distribution underlying the sample data. We propose to use this approach within each experimental subject to test the hypothesis regarding the reliability of the differences between ERPs to different conditions.

In this study we applied bootstrap analysis to data from an experiment aimed at investigating the relationship between ERPs and memory processes [2]. ERPs were recorded from two groups of subjects engaged in a recognition memory task. During the study phase, subjects in group A were required to make an orthographic judgment on 160 visually presented words, whereas subjects in group B were only required to pay attention to the words. During the test phase all subjects were presented with the 160 previously studied words along with 160 new words and were required to decide if the current word was "old" or "new". To assess the effect of word imagery value half of the words had a high imagery value and half a low imagery value. Anova performed on ERPs showed that an imagery-induced modulation of the old/new effect was evident only for subjects which were not engaged in the orthographic task during the study phase. This result supports the hypothesis that this modulation is due to some aspects of the

recognition memory process and not to the stimulus encoding operations which occur during the recognition memory task. However, bootstrap analysis on the same data showed that the old/new effect on ERPs was not reliable for all the subjects. This result suggests that only a cautious inference can be made from these data.

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Using “The Observer” to train (and observe) observers

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Building on our joint experience of making the “Video Course in Behavioural Observation” [2], we got together to make a multimedia course to teach methods and techniques in behavioral observation. Our purpose is to provide a complete course designed primarily for undergraduate students of psychology and behavioral biology to enable them to carry out their own observational studies. Some of the material will also be accessible to senior school students. There will also be more advanced material suitable for postgraduate training, derived from the “GSEQ” [1] and “Theme” [3] packages for advanced sequential and temporal analysis.

The course will deal with the purposes, background, methods and techniques of behavioral observation, and will give explicit exercises in categorizing and coding behavior using a variety of digital video clips of several species, and in ways of analyzing the data obtained. The orientation of the course is ethological in its inception but broadened to include other approaches. The multimedia format will offer numerous advantages beyond the Video Course, including the following planned features:

- Much more interaction between the user and the program.
- Incorporation of relevant parts of “The Observer Video-Pro” (Noldus Information Technology), enabling film clips to be observed and coded on the one computer screen.
- Giving feedback on the user’s performance on the coding exercises in terms of degree of agreement with a “master file”.
- Structuring the carrying out of a small project in the field and guiding the write-up of the report on this.
- Encouraging reflection by means of self-assessment tests and exercises at appropriate points in the program.
- Providing a glossary of technical terms and concepts.
- Providing a full bibliography.
- Evaluating knowledge gained by means of a multiple choice examination at the end.

In addition to the examination the effectiveness of the program will be measured in order to develop it further by the following incorporated elements:

- Obtaining feedback from users and tutors by means of a user's evaluation questionnaire.
- Monitoring in detail the actual use to which the program is put by means of explicit tracking of the sequence and duration of visits to the different sections and subsections made by each individual user.

Our progress on this project beyond the prototype produced last year will be presented in a demonstration, and we hope conference members will try out sections of the program for themselves and give us the benefit of their criticisms and comments to promote the evolution of this project.

This project receives partial funding from the European Commission (Socrates project no. 25230-CP-1-96-1-GB-ODL).

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Magnetic resonance imaging and cerebral volumetric evaluation: comparison of four post processing techniques

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Recent developments in three-dimensional (3D) Magnetic Resonance Imaging (MRI) open new research horizons. These non-invasive techniques afford the possibility of studying *in vivo* cerebral structures of both patients and healthy subjects. Furthermore, MRIs gives an operational definition of cerebral structures. A study of the relation between variation in one or several cerebral structures and variation in behavior could be considered in neuropsychology. For this purpose, biometric qualities, i.e. reliability and validity, are required for cerebral volumetric measurements. Using MRI, 124 contiguous slices of the brain can be obtained and the brain can then be reconstructed in 3D for surface area or volume evaluations. In this study, images of brains from 10 healthy females aged 20.15 years (± 1.14 years) were analyzed to perform cerebral volume evaluations. The results of four techniques were compared. One is based on manual contour of the brain, two are semi-automatic and one is automatic. The cerebral volumes obtained by the two semi-automatic techniques did not differ statistically ($1233 \pm 86.2 \text{ cm}^3$ and $1218 \pm 110.5 \text{ cm}^3$), they nor did differ when the manual and automatic techniques were compared ($1305 \pm 100.6 \text{ cm}^3$ and $1311 \pm 82.3 \text{ cm}^3$). On the other hand, the two latter techniques gave volumes statistically different from those given by the two other. The intra-class correlation coefficients (ICC) in assessing inter-rater reliability were over 0.97. For correlation between techniques, three ICC were below 0.66 and not statistically significant. Only the manual contour and automatic techniques showed a significant correlation, giving a proportion of common variance (92%) that was satisfactory but not biometrically perfect. Although reliable, these techniques are not interchangeable. Neuropsychologists are interested in the relationship between behavioral variation and cerebral structures. This relationship could be studied from raw values of cerebral structure or from adjusted measures on brain volume. In order to compare results of studies using adjusted measures, a single and relevant evaluation technique of brain volume which will be the same for all researches is necessary.

The segmentation of free animal movement into natural units

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An animal's flow of behavior often appears to be segmented into elementary units. Parsing the behavior by setting some kind of a cut-off point might yield units that agree with the observer's intuition, but do not guarantee a classification according to natural morphogenetic units. We look for a division that is dictated by the geometrical and statistical properties of the data, rather than by some *ad hoc* choice. One strategy that we use is to define a numerical measure of motion, in which we check for the existence of a natural threshold value T . By this we mean that the set of values bigger than T has a statistical distribution markedly different from that of the values smaller than T . This strategy will be illustrated by the way we parse a rat's path into "stops" and "progressions".

At the level of body segments, the problem is to define and measure "action patterns" in terms of the complex coordination between the movements of all the segments, as well as parse them in time. Our strategy is to treat the movement at every point in time as part of a local cyclic movement, and measure the phase, amplitude and frequency of this cycle [1]. The relative phase (i.e., the phase difference) between concurrent movements is a good candidate for defining and recognizing the action pattern, while discontinuities in the amplitude and frequency mark the boundary in time between specific movements. For this purpose we developed special algorithms, and they will be demonstrated on ferret free locomotion.

This research was supported by the Israel Science Foundation founded by the Israel Academy of Sciences and Humanities.

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A novel nine-choice serial reaction time paradigm in rats: effects of nicotine on titrated performance

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Serial reaction time tasks are valuable paradigms for studying attentional processes and deficits. In contrast to the five-choice serial reaction time paradigm, to our knowledge no study has been published wherein attentional performance of rats was challenged by presenting nine choices. Our study investigated in rats whether a nine choice paradigm can yield consistent performance and is sensitive to pharmacological manipulation by nicotine.

Male random bred rats (Charles River, UK) were maintained at 85% of their free-feeding weights (425-590g). Training and testing was done in chambers (25x25cm) each with one concave wall with 9 apertures, each 2.5cm square, 4cm deep and set 2cm above floor level. Holes were equipped with photocell beams monitoring nose entrance and could be occluded by a metal cap. Precision food pellets were delivered at the opposite side of the box into a photocell-monitored foodtray. The holes and the magazine could be illuminated (green multifocal LED, RS) and each box had a roof-mounted house-light. Boxes were separately housed in a dark, soundproof compartment, ventilated by low-noise fans. Procedure and data collection were controlled by in-house-developed software (Arachnid; Paul Fray).

The behavioral procedure consisted of five phases:

1. Magazine training: With occluded apertures, food pellets were placed in the illuminated magazine.
2. Nose-poke training: In each of five sessions of 20 trials just one hole (1, 3, 5, 7 or 9) was exposed and illuminated in a pseudo-random order. A nose-poke was rewarded with a pellet.
3. Nose-poke training with light-tracking: As before, but reinforcement was conditional upon the cue-light in the aperture. After collection of the pellet a 4-8s intertrial interval (ITI) started. Responses in the aperture during the ITI were recorded as anticipatory responses and resulted in 10s time-out.
4. Five-choice-serial-reaction-time-task: Five holes (1, 3, 5, 7, and 9) were exposed in each trial and after an ITI only one hole was illuminated pseudo-randomly to ensure 20 illuminations in each aperture over 100 trials. A response within five seconds in the (correct) illuminated aperture was rewarded; an incorrect or a preservative response resulted in a time-out period. A tray-visit initiated the next trial.

5. Nine-choice-serial-reaction-time-task with titration: Nine holes were exposed and stimulus duration was eight seconds. Once performance was stable (three sessions in a row <25% omissions; >80% accuracy; >50 trials) stimulus duration became four seconds and titration started. A titration session consisted of six 18 trials blocks, each with two pseudo-random illuminations in each hole. After each block, stimulus duration was multiplied by 0.9 if accuracy was over 80% and number of omissions under 25%, else stimulus duration was multiplied by 1.1.

Variables analyzed included accuracy, latency-to-response, latency-to-magazine-visit, anticipatory responses; preservative responses; errors-of-omission. Drug administration: Nicotine (0.0; 0.05; 0.2; 0.8 mg/kg; n=6) was administered intraperitoneally 30 minutes prior to behavioral testing.

The acquisition curve of this task is shown in fig. 1: after three weeks of training in phase 5, animals obtained a mean stimulus duration of 0.544s (sem 0.0478s; range 0.30s-0.75s). None of the doses of nicotine had effect on performance, which suggests that animal's performance was both stable and optimal.

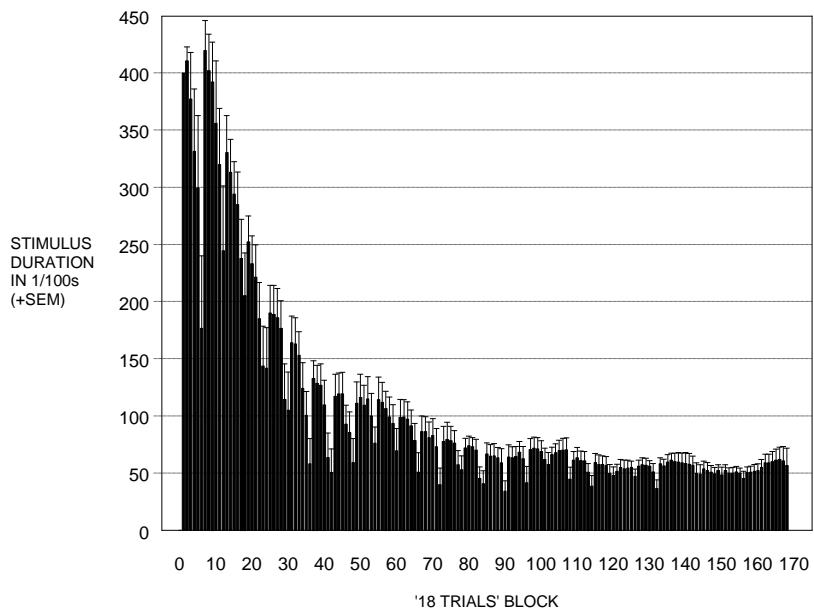


Figure 1. Acquisition curve of the titration phase for the nine-choice-serial-reaction-time paradigm. For each block of 18 trials the mean (+ SEM) stimulus duration is given in 1/100 s.

The nine-choice-serial-reaction-time task is a well-controlled paradigm that allows usual manipulations of attentional load (e.g., ITI variability; stimulus characteristics) as well as new protocols to quantify other aspects of attention (e.g., sustained) in rats.

The application of fluorescent powder to detect the use of space by the yellow necked mouse

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The research on the social system of an island population of the yellow necked mouse, *Apodemus flavicollis*, has been conducted since 1994 on the Crabapple Island (Mazuria). The data have been collected during trap sessions every six weeks from April to October. Each trap session lasted seven days (traps were checked at 07:00 a.m. and 07:00 p.m.). CMR (catch, mark, release) was used as the basic method to obtain information about sex, maturity and weight of individual animals. Yellow necked mice are shy and secretive animals and their above-ground activity is mainly nocturnal. Contrary to the bank vole (*Clethrionomys glareolus*) the second species on this island, they have low trapability and sometimes some of animals were absent from one or more sessions and then reappeared. It is known that the yellow necked mouse can climb trees. This suggests that it has a three-dimensional home range. To know more about this difficult species we started using ultraviolet reflective powder in 1997 to mark selected animals with different colors. This method - called 'powder tracking' - seems to be good for following movements of small mammals but it has not been used for this species under natural conditions. The dusted animals left trails visible in UV light at night. They were flagged and mapped during daytime. We have collected detailed information about spatial patterns of animal activity in different habitats without disruption caused by live trapping. Using of the same space by different animals in different seasons seems to be especially interesting.

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Analyzing individual differences in face-to-face interaction

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This presentation focuses on methodological problems in the analysis of individual differences in face-to-face interaction. Work in this area is complicated by the characteristics of interaction that render the standard psychological approaches to individual differences problematic.

Early work on individual differences in interaction applied the approach of psychological testing to participants' actions. The rate at which a certain action, such as gazing at the partner or smiling, was observed was taken as an indicator of some transient state or more enduring personal characteristic of the participant. These rates were calculated by counting the number or timing the duration of the action, and dividing by some broader number, such as the total time of the interaction or the total time speaking. It was argued [2] that these simple-rate variables could not be interpreted as representing exclusively the observed participant. Rather, because of the deep sequential interconnectedness of actions in interaction, the partner may be contributing substantially to a simple-rate variable intended to represent the participant.

An alternative approach to the analysis of individual differences is proposed based on the notion of interaction as a convention-based, or rule-governed, phenomenon (e.g. [1]). From this perspective it becomes possible to speak of grammars or structures for observed interactions. Sequential analyses can yield hypotheses concerning the actions, rules, and other elements of an observed interaction. These analyses can be significantly facilitated by Theme [3]. In many cases the rules for an interaction provide for various options, just as do the rules for chess or other game.

An hypothesis of an interaction structure can be represented as a flow chart. All possible paths through the flowchart can be identified. Each path represents one of the possible ways that participants can jointly construct an interaction within the constraints of the structure. Based on these paths or portions of them, it becomes possible to generate data from the observed interactions on a variety of phenomena, such as the number of times each path is taken, the number of times a particular option is exercised, and the number of times action sequences of particular interest occur.

It is proposed that data of the sort just described can be used for analyses of individual differences. That is, individual differences in interaction can be analyzed

in terms of the jointly constructed interaction, thus avoiding the problem of analyzing a participant's action apart from the sequential interaction context in which it occurs.

A variety of techniques involving computational modeling of flowchart phenomena will be illustrated, ranging from simple independent observations, through stationary Markov chains, to dynamic nonlinear models with feedback loops--models of the type whose output may display the type of sensitivity to initial conditions typical of mathematic chaos.

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Qualitative measurement and assessment of a hand posture in a virtual environment

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The hand is a communicable agent that operates under a wide range of scenarios in the physical world, but this study indicates a significant departure from conventional behavioral measurement. The hand, in this study is transposed, and directly operative in a 3-dimensional, virtual environment (VE), giving rise to an interdisciplinary study that encompassed cognition, motor behavior (motor learning and motor control) to ascertain grounds for problem-free hand postures, and hand performance in a VE. The VE offers a platform for quantitative measurement on the execution of a hand posture because it could map the activity in a regulated volume - Gesture Editor (Minimal Reality™) with a simple 3-dimensional 'virtual hand'. The manifestation of technical problems, that is system failure of the virtual world system, called for a contingency plan. The emphasis then became qualitative. A case study proceeded, adhering to ethical practice, in accordance with APA guidelines.

The case study employed an able-bodied, right-handed single male subject who was seated for Experiment I, and remained standing in Experiment II. The subject was tethered to and wore a Cyberglove™ - whole hand input (WHI) even though the system had expired. The WHI would in effect measure and record the displacement (abduction-adduction, flexion-extension) and rotation of one, or all extensors about the hand, and body of the hand (including the wrist) in accordance to which sensors mounted on the dorsal surface of the WHI were active. The investigator provided no other cue, other a visual stimulus to the subject in the form of an A4 sized photographic image of the required hand posture. The subject then aimed to reproduce the hand posture via their own hand-wrist-forearm performance [1] and maintain it for a period of not less than three seconds. A single video camcorder was used in two planes: sagittal and frontal in experiments I and II for analysis and review. On completion of the execution of a hand posture, the investigator posed pre-scrutinized questions to the subject on those preceding events. The qualitative investigation covered several issues with regard to a hand posture:

- Whole, i.e. complete hand patterns
- Discrete articulation, i.e. fine articulation of one, to at least three extensors
- Subjective appraisal on the ergonomics of an arbitrary hand posture-comfort/difficulty

- Memory, retention (forgetfulness) of a hand posture
- Questionnaire directed to the subject with regard to executing each hand posture

The qualitative nature of the investigation provided information on measuring, assessing and selecting a hand posture for the proposed implementation within a virtual environment.

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Effects of brain lesions on Morris water maze performance in rats: testing the reliability of an automatic video tracking system

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The entorhinal cortex (EC) has been regarded as a transition between the hippocampus and neocortex, providing the major source of afferent input to the hippocampus, a structure that is critically involved in spatial discrimination learning. We assessed the severity and duration of EC lesions, induced by different doses of the excitotoxin ibotenic acid (IBO), and the reliability of a video-tracking system, EthoVision, as a tool to measure Morris water escape behavior.

Male Møllegaard Wistar rats were randomly assigned to one of four experimental conditions (n=15 per group): bilateral sham or EC-lesion, induced by three stereotaxically guided injections of vehicle (0.9% NaCl), or of 1 mg/ml, 3 mg/ml, or 10 mg/ml IBO. The excitotoxin or vehicle was injected bilaterally in an application volume of 0.5 μ l per site. Behavioral testing took place in a Morris water maze with a diameter of approximately 150 cm. Testing started 10-14 days after surgery and was conducted in ten daily sessions with a 9 day resting period after the fifth day of acquisition. During each daily sessions the animals received four trials. After the fourth trial of the fifth and the tenth session, an additional trial was given as a probe trial in order to assess the spatial bias for the training quadrant. Performance on the Morris water maze task was registered automatically using the video tracking system EthoVision; in parallel, a manual scoring technique was applied.

Table 1. Product-moment correlation coefficients calculated per session across all rats ($n=60$) per session between distances traveled and the platform escape latencies, scored either automatically by EthoVision, or manually, by an experienced experimenter. The lowest and highest correlation coefficient is shown.

		Distance traveled		Escape latency	
		Manual scoring	EthoVision	Manual scoring	EthoVision
Distance traveled	Manual scoring	1.00 - 1.00			
	EthoVision	0.95 - 1.00	1.00 - 1.00		
Escape latency	Manual scoring	0.88 - 0.97	0.84 - 0.97	1.00 - 1.00	
	EthoVision	0.88 - 0.97	0.84 - 0.96	1.00 - 1.00	1.00 - 1.00

In order to validate the video tracking system EthoVision, product-moment correlation coefficients were calculated across the sixty animals per day between the two scoring methods for escape latencies and distances traveled to find the platform. The correlation analysis revealed that EthoVision produced highly reliable results (see Table 1).

The rats lesioned by 10 mg/ml IBO displayed retarded acquisition of the Morris water escape task during the first five daily sessions than the other three groups. However, this deficit was no longer apparent during the second series of five sessions, and all groups showed a strong bias for the training quadrant during the probe trials on the fifth and tenth day of training. This indicates that all groups had reached a similar performance level by the end of the fifth session. In the present study, entorhinal cortex lesions by IBO induced a dose-related, transient deficit in spatial orientation learning.

Effects of brain lesions on Morris water maze performance in rats: inter- and intra-experiment variability

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Post-mortem studies of the brains of Alzheimer's patients have shown that intraneuronal neurofibrillary changes and neurofibrillary tangles in the entorhinal cortex (EC) occur at a very early stage of the disease. These changes appear to correlate with the memory deficits present during the early progression of Alzheimer's disease, suggesting that these cognitive dysfunctions might be caused by disturbed hippocampal function due to degeneration of much of the architecture of the EC. Lesioning the EC in rats is considered as an animal model of this aspect of the early phase of Alzheimer's disease.

A series of nine experiments was performed in which the effects of putative cognition enhancers on the performance of rats with bilateral lesions of the EC were assessed in the Morris water escape task. Only the sham-lesioned and the ibotenic acid (IBO)-lesioned groups were used for the present inter-experiment comparison. All animals received bilateral stereotaxically guided operations of the EC. The sham-lesioned rats were injected with vehicle only, whereas the EC-lesioned rats were injected with IBO. The coordinates are depicted in Table 1.

Table 1. Stereotaxic coordinates used to induce entorhinal cortex lesions. The coordinates are with respect to the intra-aural line, the incisor bar was set at 3.3 mm above intra-aural line, and the syringe was lowered under an angle of 15° (mediolateral orientation). Per injection site, 0.5 µl vehicle or ibotenic acid (10 mg/ml) was applied through a Hamilton syringe.

Coordinates of lesion sites			
	First set	Second set	Third set
anterior-posterior	+ 1.0 mm	+ 1.5 mm	+ 1.2 mm
lateral	± 6.8 mm	± 6.6 mm	± 5.8 mm
dorsoventral	+ 3.11 mm	+ 2.74 mm	+ 2.37 mm

After a recovery period of about 1 week, the rats were tested in the Morris water escape task with four trials per session on five successive days. Observations were performed using the automatic video tracking system EthoVision. Lesioning of the EC induced learning deficits in all experiments. However, there were considerable differences between experiments with respect to the degree and time-course of the impairments. A certain degree of heterogeneity was also observed for the probe trial. All but one sham-lesioned group showed a strong bias for the previous training quadrant, whereas no such bias was apparent in the EC-lesioned rats.

Comparison of the Morris water escape performance shows that there exists a variability between shipments of rats, which cannot be neglected. All data were collected by the automatic video tracking system EthoVision. We have previously found in control experiments, where EthoVision and a manual scoring method were used in parallel, that the video tracking system yields highly reliable results.

Considering the inter-experiment variability, which we found in the present comparison study, it is of utmost importance to control and reduce intra-experiment variability. Reliable video-tracking systems such as EthoVision provide adequate tools to reach this goal. Although very reliable data can also be collected with simple manual scoring techniques, video tracking systems permit an in-depth analysis of rat's behavior in the water tank. Search strategies, for example, can only be identified if the X-Y-coordinates of the actual position of the animal in the pool for each sample are stored.

Remote video surveillance of wildlife behaviour

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Automatic video surveillance of wild animals in their natural surroundings allows a permanent record to be made of all activity within a pre-determined arena. The traditional method of direct observation, particularly when applied to group living animals, can lead to biases towards focal individuals. In addition, the proximity of a human observer may make the detailed study of extremely shy and/or nocturnal animals impossible.

We have developed a 12V DC battery powered, fully portable, weather-proof, monochrome, closed circuit television system. This is capable of filming automatically a selected arena of at least 10m by 10m for over 168 consecutive hours. Recording over such a long period is achieved using a time-lapse video recorder. Because of the continuous presence of the equipment at a site, habituation is more rapid than to a human observer. Without adjustment, the system can film in conditions of bright sunlight through to total darkness. Filming in the dark is achieved by exploiting the sensitivity of monochrome CCD cameras to infra-red (IR) light (and indeed one CCD chip has been designed especially for IR sensitivity). Arenas can easily be floodlit using infra-red light emitting diode (LED) arrays to provide sufficient illumination for good video filming, with the mammals being totally unaware of the IR light. The low power consumption of the LED arrays allows the system to operate without excessive battery requirements.

The system employs a combination of light switches, passive-infra-red (PIR) detectors (which trigger in the presence of animals), and timer devices to control the function and switching of lights and recording modes. A "vertical interval time code" (VITC) generator adds a computer readable time code to the tape as it is being recorded. This code is in real time - and therefore it is not affected by different time-lapse speeds of recording, and provides a resolution of up to 0.04 seconds. Therefore, given the correct computer software and hardware, analysis can be undertaken directly from the original tape. The system is based on "off the shelf" surveillance equipment and is designed to be compatible with all such technology, allowing for simple upgrading. Our experience is such that one person can set up and maintain at least six independent systems and so allow for simultaneous recording at multiple sites on a day-to-day basis. However, it is feasible to run the system from an alternative source, such as solar or wind

generated power, therefore allowing operation in remote situations, when daily attendance is not feasible.

We discuss the function and assembly of standard, low-cost equipment, which has enabled us to study the detailed behaviour of the European Badger *Meles meles* [1] and the Red Fox *Vulpes vulpes*. We also explain some of the more expensive technology, required for specialised applications.

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Performance and psychophysiological measures of fatigue in air traffic controllers

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Eight air traffic controllers carried out exercises using a TRACON II Air Traffic Control (ATC) Simulator. After a training and familiarization day, each controller carried out four simulation exercises, two low and two high traffic load. His performance during each exercise was recorded by the simulator, a self-assessment questionnaire for fatigue and a test of event-related potential (ERP) were applied and a sample of saliva was taken for cortisol analysis before and after each experimental session. The NASA-TLX was completed after each exercise and a test of alpha-rhythm attenuation was carried out at the start and end of each day. The NASA-TLX is the (US) National Aviation and Space Authority Task Load Index, a widely used self-assessment method, which combines six major aspects of workload to form a general index. It is widely used in Aerospace, Aviation Air Traffic Control and other studies of dynamic human operator activity.

Controllers rated the 'mental demand' scale of the NASA-TLX higher on the first, training, day. During measured exercises, their rating of their own performance was higher during the high traffic exercises, although their measured performance contradicted this assessment. The alpha-rhythm attenuation test showed no significant variation during the study, although subjective self-assessment of sleepiness and fatigue (different concepts) showed a clear circadian rhythm, in particular the 'post-lunch dip'. After the final session on day 2, however, sleepiness was reduced, while fatigue increased.

EEG spectral analysis of the delta, theta alpha and beta frequencies. During the learning day, an increase of theta was observed from low to high traffic, consistent with the suggestion that this is related to learning. (During the second, measured day, more high frequency EEG was observable in high load exercises.)

The Event-Related Potential, particularly P300, showed a significant post-exercise reduction after the high-traffic scenarios. Salivary cortisol showed a strong circadian component. In addition, although eight subjects are not really sufficient to establish a significant difference, controllers appear to form two groups, one showing high cortisol values, the other low values. The higher cortisol group performed better and rated their workload higher during the training day. During the measured exercises, however, the high cortisol group had better performance

in low workload, but showed a strong deterioration of performance in the high-load exercises.

The high cortisol group slept more, and felt more tired on waking. Apparently, they were more 'fatigueable'. Cortisol analysis, Event-Related Potentials and fatigue/sleep questionnaires are recommended for further use in Real Time (RT) simulations.

Because the TRACON/Pro simulator can be used with keyboard or mouse/pointer control, the controller need not speak during the exercise, which greatly facilitates EEG studies. Further studies are now being carried out using more EEG channels, to investigate inter-hemispheric differences as potential measures of the completion of training.

Methods for quantifying animal color variation

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While many theoretical questions in evolutionary biology depend on measuring animal color variation, few studies apply a formal definition of color and even fewer use a standardized means by which color can be assessed [1, 2, 3]. Previous studies have relied on either relative color assignments or standardized color chips for identifying an approximate measure of color. These methods lack an objective means by which scientists can define precise intra-individual and inter-individual color differences. Furthermore, these methods color may fail to detect subtle differences in animal color, which may be biologically meaningful to conspecifics and predators.

In this presentation, I will introduce the possible use of digital video cameras (DVC) for obtaining a sensitive and precise measure of color. DVCs capture high-resolution images which can be downloaded into basic software applications via a cross-platform interface (i.e. TWAIN). The software allows the user to highlight target regions and obtain a color measure based on the Pantone Matching System (PMS). The PMS provides a code for color samples according to three standard measures of color: the hue, value, and chroma. The "hue" refers to the colloquial color term, the "value" refers to the relative darkness of the sample and the "chroma" is the degree to which the sample is saturated with the hue [3]. Thus, DVCs can potentially provide an objective instrument which investigators can employ, to obtain precise, accurate and quantifiable measures of color.

I will discuss the advantages and limitations of the DVC system and compare these with more traditional tools for describing color. To conclude this presentation, I will offer practical uses and possible applications of DVC technology for investigating behavior in captive and field settings.

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The use of an infra-red sensor for automated oestrus detection in individually housed sows

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Oestrus detection of sows is an important activity within pig husbandry. It takes about 30% of the overall labor input, and financial losses related to non-productive days may vary considerably. Therefore, the principle of using an infra-red sensor has been evaluated to detect automatically oestrus of individually housed sows. The infra-red sensor used is a commercially available device, primarily intended for security alarms and which consequently had to be adapted for scientific use. The movements of the sow are converted to an analog signal by the infra-red sensor, and a mean value is calculated. The frequency range of the sensor is 0.01 Hz to 10 Hz and the maximal sensitivity is approximately 0.1 Hz. The bandwidth is 0.72-1.6 Hz and determines the range of detectable movements of 0.2 m/s to 0.5 m/s. An ultra-sound system is equally installed above the sow to get more information about the movements and an indication about the position of the sow (standing up or lying down). In the near future, the results of these measurements will be evaluated.

The infra-red sensor was mounted 50 cm above the front of the sow's body. Fifty-eight multiparous individually housed sows were monitored from the day after the piglets were weaned. Four parameters of body movement as quantified by the sensor's output voltage were investigated: mean daily activity, standard deviation of mean daily activity, minimal and peak value. The reference method was the standing behavior before the boar and the inseminator. 80% of the sows could be classified correctly when using mean daily activity as the selection parameter. Up to 86% were classified correctly when daily peak activity was also included. Positive and false positive sows could be distinguished at the 95% level by using a canonical discriminant analysis. Increase of mean daily activity, increase of standard deviation and increase of daily peak activity were statistically significant explanatory variables of a logit function predicting the onset of oestrus.

Reliability of observational data: obtaining different results with different estimation techniques

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Inter-rater reliability of observational data can be estimated by various methods, including: simple percentages of agreement; kappa and weighted kappa; Pearson correlations; comparisons of means (using t-tests or analyses of variance); and generalizability (G) theory techniques [1]. The "method of choice" in a given situation depends on a number of factors (e.g., type of measurement scale used); nevertheless, the researcher often faces two crucial dilemmas: 1) how to design an effective reliability study, and 2) how to analyze the data in order to maximize the information yield.

In this presentation, two sets of data--one "hypothetical" and one "real"--will be used as the bases for illustrating the different results that can ensue from the use of various techniques available for the estimation of inter-rater reliability. Special attention will be given to the use of G-theory techniques, which represent a very comprehensive approach to reliability estimation. G-theory techniques allow the researcher to acknowledge and measure the multiple sources of error that exist simultaneously in any set of measurement data. The relative amount of variance attributable to a variety of factors--such as differences among raters, among items, across time or occasions--can be estimated separately. Further, variance due to the interactions among those factors also can be estimated.

The importance of making informed choices--choices based on full knowledge of what any specific estimation technique will yield in terms of information about the reliability of the measurement procedure--will be emphasized throughout the presentation. Discussion at the end will focus on the ways in which researchers can determine optimal types of reliability studies (and estimation approaches) for their particular types of data.

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Converging disparate data strings into a single behavior state indicator: conceptual and graphical methods for intra- and intersubject data

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Typically in observational research each behavior of a subject is assigned a code that represents theoretically relevant and contextually sensitive events. These discrete behavioral acts are then strung together to denote a process. With multiple interactants in the same field, the investigator has two choices: ignore subject interdependence and analyze as if the data strings are discrete; or treat the strings as interdependent and assign a unique code that represents the combined codes across subjects. Similarly, a single subject can have multiple simultaneous codes combined into a single code. In either situation, this combined code represents a state. This state code is a composite of information. If done well, a single string of these codes can adequately describe the process under scrutiny. Although numerous decisions must occur in constructing a state code, two are probably most important. First, can the base or underlying codes be quantified? Second, how does the state code construction affect subsequent data analysis and graphical presentation? Initially this paper will address these two primary decisions and provide some general guidelines about combinatorial processes. Several methods of combining codes will be shown using data drawn from couple interaction. Segments of video will be shown along with the resulting state codes. Next, the presenter will illustrate several data visualization techniques that help elucidate these state change processes over time. Although these decision rules and visualization techniques are illustrated with humans, data drawn from non-human interactions are equally applicable.

The behavior of the cockroach *Periplaneta americana* in "hot plate" and "open field" under the action of various analgetics

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The effect of opioid and non-opioid analgetics (promedol, metamizol, novocain) on American cockroach behavior was studied. The method of "hot plate" (45°C) combined with "open field" was adapted for the cockroach. The agents were injected into the insect's hemolymph. Estimation of behavioral effects (duration - 6 min) was based on the registration of horizontal locomotor activity (HA), latent period of first "rearing" (LP) and summary time that the insect spent on the vertical wall of the chamber (T). Promedol, in the dose of 0.12 mg/g, decreased HA with 31%, T with 20% and increased LP with 250%. It may be concluded that this opioid possesses high affinity corresponding to some type of receptors in the cockroach nervous system. Metamizol, in the dose of 2 mg/g, decreased HA with 66%, T with 62 % and increased LP with 380%. Novocain did not influence the insect's behavior in "hot plate", but disrupted the motor activity and movement of extremities in the "open field" (20°C). The developed method may be suggested as an alternative one (in the view of bioethic problems) for screening new biologically active agents.

The SYBAR motion analysis system: integrated recording and display of video, EMG and force-plate data

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Introduction

In rehabilitation medicine, the assessment of motor dysfunction is an important part of the diagnosis, for indication of therapy. Such assessment includes an analysis of motor disorders by careful observation of the patient who demonstrates his disability or a test of motor performance; e.g. walking. Muscle function can be measured by means of the EMG and total load of the lower leg is represented by the ground reaction force. However, the problem is how to represent these measures in a meaningful way to the physician who is familiar with observational analysis. To do so we developed SYBAR, which is a Dutch acronym for "system for movement analysis in a context of rehabilitation medicine" [1]. SYBAR is based on multimedia technology to integrate digitized video and digitized (physiological) analog signals.

Instrumentation

The EMG signal is recorded through a bi-polar leadoff, by two skin electrodes per muscle. This signal is rectified and low-pass filtered (smoothed) at 2.0 Hz. in hardware to obtain the Smoothed Rectified EMG (SR-EMG). The dynamics of SR-EMG approximates the dynamics of muscle force. The SR-EMG signals of several muscles together with six channels of the force-plate (that is immersed in the walkway) are digitized at 100 Hz. These values are stored together with the actual time-code, being the output of time-code generator that is triggered by the video signal. This time-code is also recorded as a part of the video signal, both as a Vertical Interval Time Code (VITC) and inserted as number into the video image. During off-line digitalization of the video, the images are synchronized with the digitized signals. When visualization of the force vector is needed, also a image of a three dimensional calibration objects is made, in order to relate world coordinates to image coordinates.

The display of the recordings is obtained by using SYBAR as a viewer (figure 1). User interaction is kept very simple. It employs regular playback and single frame functions. Random access by mouse clicking anywhere in the signal is possible. For a good interpretation of the ground reaction force, it is displayed onto the body (i.e. the video image). In this way, it is possible to estimate joint load for each position.

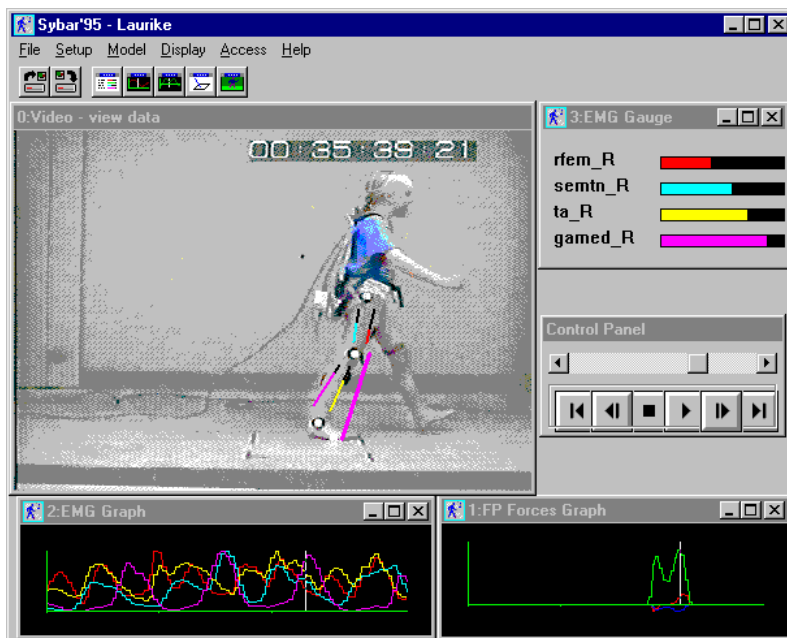


Figure 1. Typical screen of SYBAR used as a viewer.

Discussion

The SYBAR motion analysis system has been proven very attractive to physicians. The system does not replace the skills of the physician, but extends it by adding explicit information about muscle and joint function. Future developments comprise an integrated analysis of motion (based on feature extraction of the images) and an enhanced integrated online recording of signals and video.

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Affective/motivational aspects in adult/child assessment interactions

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In dynamic assessment Vygotsky's idea of the zone of proximal development is worked out by giving intellectual help in order to assess learning potential. This idea is now widely accepted in research into intellectual factors. We transposed this basic idea to non-intellectual factors. We have developed affective/motivational helping (or prompting) procedures that enable the diagnostician to give affective/motivational support to the child whenever the diagnostician observes that non-intellectual factors are affecting test performance negatively. The affective/motivational helping procedures consist of: (1) Introduction of tests, explaining increasing difficulty, explaining stopwatch, positive reaction on questions and actions of students; (2) Non task-specific instruction and questioning: Please work quietly!; Look well before you start!; Think first!; You have plenty of time!; Please start!; Did you think well?; Did you look well?; Are you sure?; etc.; (3) Praising and giving general feedback: Good!; You are doing well!; You did do a lot already!; etc.; (4) Mirroring emotions, verbal elucidation of disruptive behavior and emotions; attribution of success on competence and effort; attribution of failure on task difficulty and chance. This affective/motivational help was only given in the experimental condition when observation (circumscribed criteria) of the student showed: (I) Lack of concentration, (II) Impulsivity, (III) Passivity, and (IV) Fear of failure.

An experimental study on the effect of affective/motivational helping is presented. Participants in the study are 30 students (mean age 10 years) with complex learning problems of the Pedagogical Institute Leiden. A two group crossover design with random assignment was used. All 90 sessions were videotaped and computer-scored from video with The Observer (Noldus). The inter-observer agreement is sufficient.

Anova-analysis reveals a significant effect of affective/motivational helping on non-verbal analogical reasoning scores of 9 to 12 IQE points. Secondly a significant effect is found on the degree of systematic approach (systematic planning) with a block design task. In the third place 62% of students experienced that they worked more at ease with the diagnostician who did give affective/motivational help. In the fourth place we demonstrated significantly more verbal and nonverbal expression of emotions of the child in the assessment sessions where the affective/motivational help was given. These results are in line

with ideas of Vygotsky, Piaget, Wechsler and others, who are of the opinion that affective/motivational factors *go into* intellectual functioning. The fact that children express more of their emotions in the helping condition is in line with theories about expression of emotions as a way of stress-reduction [1]. Stress-reduction results in higher thinking achievements [2].

Future studies are planned in the Children's Learninglab about adult/child interactions during assessment and learning in which emotions are measured by behavior observation in combination with on-line registration of psychophysiological indicators of emotions.

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Analyzing system for the study of cognitive deficiencies in rodents

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The mechanic-opto-electronic system COGITAT (Version 5, Cognitron GmbH, Göttingen, Germany) records activities of rodents by a set of infrared beams which are situated at different levels in transparent tubes mounted within the holes of a board. The board with 25 deep holes containing the transparent tubes is framed by transparent walls and has a special entrance for the rats. Eight of the holes are baited with small hidden food pellets (40 mg) distributed in a distinct pattern not visible to the animals while walking. The animals learn during exploration in several daily sessions the pattern using their individual strategy. Spatial learning, working and reference memory were tested. Before entering the hole-board, the animal remains in its home cage for adaptation to the laboratory for one hour. A trial on the board is finished either when a fixed time is over or as soon as the animal has collected and eaten of the 8 pellets. Animals with a good knowledge of the pattern are able to collect all pellets within an average time of 20 s.

The software is running on Windows 95 or Windows NT. The operator uses a comfortable graphical interface to analyze and to display information about inspected holes on the surface of the plate, the number of visits deep in the holes, the number of collected and eaten pellets in a given time, inspections and/or visits of baited or unbaited holes (reference learning), reinspections and/or revisits of previously baited or unbaited holes (working memory errors). The data allow recognition of the searching strategies, the learning and memory capacities and the orientation capacities within the space using distal cues. The system is useful for investigations of rats, mice, and transgenic or knock-out mice. For demonstration here we used an animal model with rats that were affected by predamaged brains, with/without focal lesions in two different brain structures.

The model

Both oligemic episodes and increased iron concentrations in discrete brain regions have been reported to occur with age, and are thought to be associated with neurodegenerative diseases [1, 2]. Following bilateral clamping of carotid arteries (BCCA) in normotensive adult male rats for 60 min under pentobarbital anesthesia, animals subsequently received bilaterally 30ng FeCl₃ dissolved in 0.25µl buffer into the substantia nigra pars compacta [BCCA+FeCl₃SN], or 1.5µg FeCl₃ in 2µl unilaterally into the ventrolateral striatum [BCCA+FeCl₃Strvl]. BCCA does not result in delayed neuronal death [3]. Three months post surgery the animals

learnt to find all hidden pellets during 10 sessions (one per day) of 3 min, during 12 sessions of 1 min, and 12 sessions of 30 s.

Results

Using COGITAT, progressive changes in learning and memory were analyzed in time. BCCA rats found and ate fewer pellets than BCCA+FeCl₃SN rats during the learning period of 3min during 9 subsequent days. The opposite was found in BCCA+FeCl₃Strvl rats. The time to find all 8 pellets was shorter in the BCCA+FeCl₃SN rats than in the BCCA+FeCl₃Strvl rats. BCCA+FeCl₃SN rats showed most visits of unbaited holes on the 3rd day, but the rats learnt very fast to prevent the wrong searching strategy. BCCA+FeCl₃Strvl rats, however, showed an increasing number of reference memory errors during 8 days, and reached a plateau until day 10, whereas BCCA rats without additional iron in the striatum showed the same increased amount of reference memory errors as BCCA+FeCl₃SN rats, but learnt very fast to correct the unsuccessful searching strategy. BCCA+FeCl₃SN rats showed the most working memory errors.

Summary

The system COGITAT provides data from which specific deficiencies in different pathological situations can be recognized. The computerized and automatic analysis of the complete behavioral parameters with the statistic and graphic presentation offers an objective characterization of the system analyzed.

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Ethological analysis on sociometric matrices using MatMan for Windows

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Researchers interested in social structure, whether they study primate colonies, a flock of birds, groups of children, people in a meeting or any other group-living species, may wish to gain insight in the dominance hierarchy of the group studied. Behavioral interactions between the individuals can be represented in a sociometric matrix, in which each cell contains the frequency of interactions shown by one individual (the actor) towards another individual (the receiver). In order to facilitate the manipulation and analysis of matrices, MatMan has been developed [1]. MatMan allows the researcher to determine the dominance matrix representing the hierarchy within the group. Furthermore, an assessment of the strength, as well as the statistical significance, of the linearity in the observed dominance relationships can be obtained [3, 4]. The dominance matrix can additionally be reordered to reflect the social structure in the observed group of individuals [5].

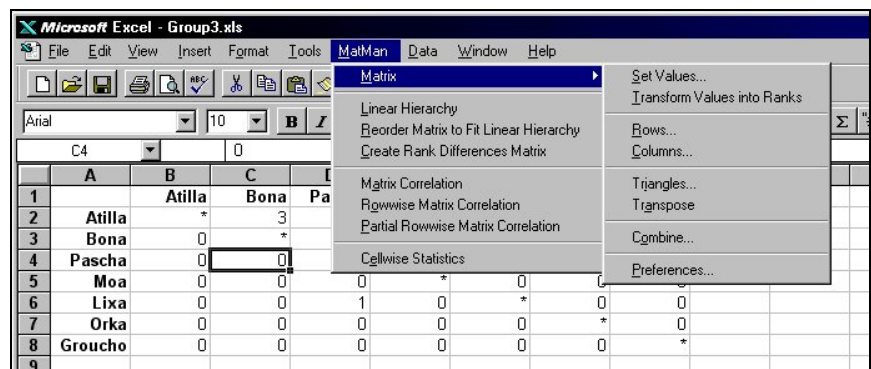


Figure 1. The application window of Microsoft Excel, showing the MatMan menu that provides matrix manipulation and analysis functions.

At the conference we will present the new Windows version of the program. MatMan for Windows has been designed as an add-in for Microsoft Excel. Matrices can be imported from other programs (e.g. The Observer) or typed in manually. Besides an elaborate set of options to manipulate the structure and content of a matrix, MatMan provides a set of matrix analysis functions. The functions are especially suitable for sociometric matrices, behavioral profile matrices and behavioral transition matrices allowing the user to investigate social dominance [4, 5], to apply matrix correlation methods [2] or to study the structure of behavior statistically.

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A simple method to measure the learned responses to plant chemicals by parasitoids

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We used a simple method to examine the most effective conditions to enhance learned responses in *Ascogaster reticulatus* Watanabe, an egg-larval parasitoid of the smaller tea tortrix, *Adoxophyes* sp. (Lepidoptera: Tortricidae). We also investigated the effect of both elapsed time and unrewarding experiences on the persistence of the learned response of these females. The strength of the response of a female was evaluated by the distance walked along the learned cues with her characteristic antennating response in Petri dish assays (Kainoh, 1997). We compared the learned response of females with zero to five oviposition experiences as well as females with various lengths of oviposition time. The learned responses of females tended to increase with the number of conditionings (1-3 times) and the oviposition time during conditioning (0-360 sec). However, responses were low after a single conditioning regardless of oviposition time. From these results, learned response through experience is essentially a gradual process in which the number of experiences and the oviposition time during conditioning are major elements.

The learned response of females gradually decreased as time elapsed after conditioning (rewarding experiences), while it quickly decreased after unrewarded experiences. Furthermore, we investigated the effect of additional rewarding experiences (re-condition) on the females that had ceased to respond to the learned cues after unrewarding experiences. Females receiving an additional rewarding experience recovered their response and were able to learn the previously-learned cues efficiently. This type of a flexible learning system would be useful for *A. reticulatus* females to help them utilize the most profitable cues that lead them to the next host.

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Testing individual cognitive performance of group-housed long-tailed macaques using touch-screen displays

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The performance of monkeys in learning tasks is generally measured in monkeys that are housed solitarily or in very small groups. Housed in this way, the animals can be handled easily and are considered to have a standardized environment. However, the generally poor housing conditions may affect normal development of cognitive abilities and lead to an increased responsiveness to mildly stressful events. By housing monkeys in larger social groups, with a social structure that resembles the natural situation, these problems can be overcome. A second important advantage of testing group-housed monkeys is that in this way the influence of consistent and long-lasting differences in individual levels of stress can be investigated. In stable groups of macaques the social position is the main determinant of stress in the animals, and the social position of females is generally stable for their entire lives. Knowledge of this position can be used as an additional variable in relation to the performance of the animal. As yet no researchers have been able to train a sufficient number of macaques living under such conditions to perform a learning task without invoking additional stress.

In this study we investigated the possibility to train female long-tailed macaques to voluntarily perform a computerized learning task. We developed a procedure in which twelve adult female long-tailed macaques could enter a small cage in which they could be isolated from their group mates for the duration of a test (10-20 min). As the animals were test-naïve, and not food-deprived, we investigated their ability and motivation to perform on two different learning tasks. All the animals learned to enter the test cage and to press on a touch-screen for an incentive. Initially, most of the animals had great difficulty in performing a delayed non-matching to sample test. Performance improved only slightly with adjustments to the discriminability of the pictures. On a subsequent discrimination reversal task, all animals succeeded in solving the task. The inability of the animals to solve the DNMS-task may be due to the difficulty of the task, the fact that the animals were inexperienced, or the rewarding scheme used. Probably, the animals first need extensive experience with testing equipment and procedures, before they can be given a difficult learning task. The conclusion of this study, i.e. that socially housed monkeys can be used for testing individual cognitive abilities, is relevant for researchers who are interested in the relation between social influences on cognitive performance, and may promote the welfare of the animals under study.

HEART: an objective and consistent way to determine the relationship between physiological and performance measures

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The National Aerospace Laboratory's HEART project aims at measuring workload quantitatively to uncover, identify and eliminate those instances in which the demands of required tasks would degrade human and hence system performance. Physiological measures are considered as appropriate for (in-flight) workload assessment applications because these allow an evaluation of dynamic workload variations over time.

Workload is strongly related to required and achieved performance, therefore during experiments many parameters need to be recorded. The possibility of inter-subject variability often requires that Human Factors (HF) experiments compensate for this fact by using a high number of experimental runs. Specific experimental designs are therefore used to be able to retrieve the required information like influence of certain procedures, or display features on the perceived workload levels/accessibility of information.

To facilitate the analysis process, the software program HEART (Human factors Evaluations, data Analysis and Reduction Techniques) has been developed. The program is especially aimed at bridging the analysis gap between actual data recordings and subsequent statistical analysis using commercial software packages like Statistica and SPSS. The HEART concept therefore supports many data input formats whilst standardizing both information coding and data output formats. In addition it allows easy inspection, correction (e.g. ECG artifact removal) and synchronization of data (e.g. physiological and performance data). The likelihood and impact of user selection and typing errors has been reduced through the structured way of working within HEART in combination with direct feedback of results.

Digital video: technical trends and opportunities for users of The Observer

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Modern observational research implies many questions about the right tool to use. One of these questions concerns the choice of the appropriate video medium. With the wealth of currently available options, manufacturers of video equipment do not make this choice easy. There are many different types of video media: analog video tape (VHS, S-VHS, Hi-8, etc.), digital video tape (DV, DVCPRO, DVCAM, etc.) and file formats for digital video on disk (MPEG-1, MPEG-2, AVI, QuickTime, etc). One, or perhaps a combination, of these video media could be the ultimate solution for one's observational studies. The cost involved with the use of digital video media has gone down drastically and appears to be leveling off. This means that digital video technology has come within reach of the behavioral researcher.

In this presentation, an outline will be given about analog and digital video technology (figures 1 and 2). In combination with The Observer, the trend towards digital video offers new opportunities in terms of video quality, presentation possibilities, speed of retrieval and ease of distribution among coworkers.

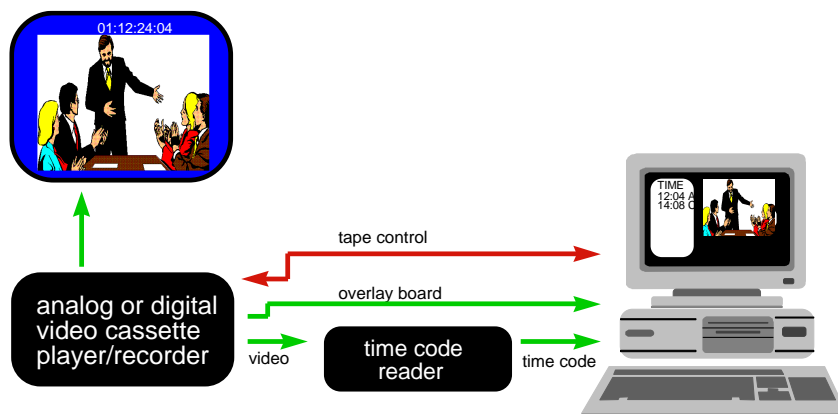


Figure 1. Diagram of a video analysis setup using analog or digital tape.

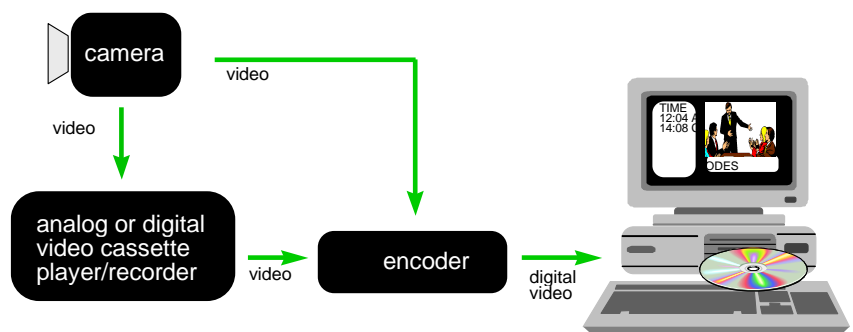


Figure 2. Diagram of an acquisition and analysis setup using digital video on disk.

Investigations of lying behavior of piglets in a preference farrowing pen with two nest areas (warm water bed vs. reference system) by The Observer / Video Tape Analysis System

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In the Institute of Animal Breeding and Genetics of Justus-Liebig-University Giessen a new heating and nesting system for piglets during suckling period was developed - the warm water bed. The advantage of this system could be that the piglets can lie on a warm and soft material according the species-specific demand.

Investigations were made in several rounds in an enlarged farrowing pen with two heating systems - the new warm water bed for piglets versus electrical infrared lamp with gum mattress or heated plastic plate. The piglets had the possibility to choose for lying between warm water bed or the other nest area. After every round the position of both heating systems was changed (warm water bed in the corner and other nesting area in the middle of the pen and vice versa). Once a week during suckling period (up to the 28th day of age) behavior of piglets in choice test was recorded by infrared camera and time-lapse VCR during 24 h. After recording, time codes were added the video tape by a time code generator box to use The Observer / Video Tape Analysis System for utilization. The number of piglets lying in one of the two nesting areas was counted and the time (begin, end) of every lying event over a 24 h period was registered.

Results

Ethological investigations showed that the warm water bed is preferred by piglets in a very high percentage. Between the 3rd and 5th day of age, the warm water bed is used by piglets for lying from 76 to 78 percent in 24 h (more than half of litter is lying on bed at same time). Only one percent in maximum (of 24 h period) the other nest area is selected by piglets for lying. The other time piglets have sucked or played in the pen. The position of warm water bed in the farrowing pen (corner or in the middle of the pen) had no influence on lying behavior of piglets. With increasing age of piglets, the duration of lying on warm water bed decreased with a wide variance between rounds. The percentage of locomotion and playing increases with increasing age. Between the 15th and 19th day of age piglets have spent 36 to 75 % of time (in 24 h) lying on the warm water bed. Piglets have used the other system for laying only up to 6 %.

Conclusion

The warm water bed is a new heating system and nest area for piglets which is in conformity with the species-specific demand for lying place (warm, soft). It is chosen by piglets for lying in a significant percentage compared to the traditional lying place.

Characteristics of cattle voice and their application to recognition of individuals

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The objectives of the present research are to analyze cattle voice in the time and frequency domains. Using the obtained results, it is tried to recognize the individual animal with the voice characteristics. The final goal of the research is the quality control of livestock during breeding by understanding the intelligent behavior of the animal. The voice generating process is described with the linear prediction filter and the parameters contained in this model are estimated with the maximum entropy method (MEM). Using these parameters, the spectral envelope can be computed and the formant frequencies are read from the resonance frequencies. The formant frequencies can also be computed through solving numerically the characteristic equation obtained from the all pole model of the linear prediction filter [1]. In this paper, we tried mainly to recognize the individual animal with the filter parameters.

The number of cattle whose voices were recorded was six, that is, three grown cows (78-133 months), one female calf (13 months) and two male calves (9 months). The variety of the cattle was Japanese Black. The voices were recorded with the precision microphone at 7 to 9 o'clock before feeding in the morning. The total power of each voice of the individual animal was computed as the variance. Based on the values of variances, the voice levels could be classified into three groups, that is, high, medium, and low levels. The voice groups of the high and medium levels were analyzed in this research. From the final prediction error analysis, the number of linear prediction parameters was set to 15. The formants were from 200 Hz to 1800 Hz and they were almost constant for one voice. The power spectra of one grown cow and male calf had the overtone structure in which the formants were three to six times of the fundamental frequency. The fundamental frequencies for the cow and calf were 500 and 200 Hz, respectively.

It was tried to recognize the individual animal on the feature plane on which the two dimensional feature vector was defined with two filter parameters selected from the 15 parameters of the filter. For the high level voices of two cow and one calf, it was possible to recognize with the simple hyper plane, that is the straight line. For the voices including the high and medium levels, it was difficult to discriminate these three cattle with the simple discriminant function as the straight line. In order to improve the discriminant efficiency, it may be necessary to increase the dimension of the feature space and to use the higher discriminant

technique. The formants estimated by solving numerically the characteristic polynomial of the all-pole model were significantly different from the resonance frequencies measured from the spectra calculated with the filter parameters. The cause of this discrepancy is unknown, but should be explored in the future, considering the number of the filter parameters and mathematical model of the vocal tract of the animal.

Reference

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Automated training of line drawing and object movement (in fingermaze and sorting tasks) on a touch-sensitive monitor in captive chimpanzees (*Pan troglodytes*)

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Line tracing

Two adult, captive, female chimpanzees (*Pan troglodytes*) were trained to trace lines presented on a touch monitor. Moving a finger over the monitor left a trace of electronic ink in the form of graphical symbols (filled circles). Tracing was analyzed on-line. Original training to draw in a controlled manner progressed from pressing individual dots on the monitor to moving the finger over the dots in a sweeping motion (Iversen & Matsuzawa, 1996). Training to trace multi-line stimuli progressed from tracing a single line to tracing two lines, then three lines, etc.

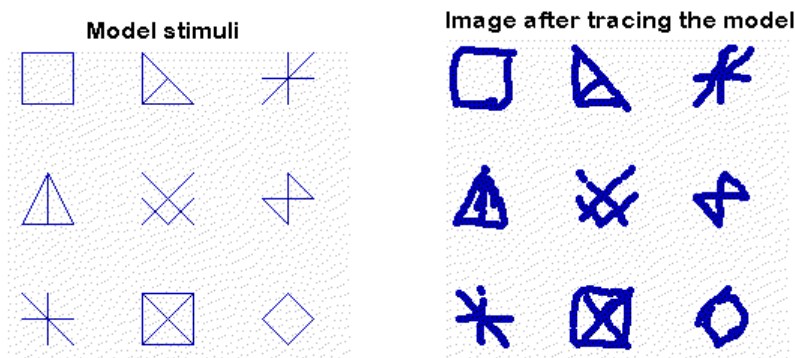


Figure 1. Examples of individual trials for one subject. Shown here are 9 stimuli presented one at a time in a single trial. The subject traces the stimulus and the electronic ink covers the lines. After tracing the subject presses a switch that enables the computer to analyze the drawing.

Figure 1 illustrates examples of the stimuli presented on the screen and what the screen looked like after tracing was completed. The stimuli were presented one at a time (each stimulus occupied a 15 by 15-cm area). When the subject pressed a switch next to the monitor the computer analyzed the drawing. Reinforcement was delivered if 95% of the stimulus was covered by ink and 95% of the ink covered the stimulus. Both subjects traced correctly on over 90% of all trials. Drawing became more efficient over time. Both subjects traced a variety of stimuli including all the letters of the alphabet. Both subjects also learned to trace lines of different

color on the same trial. Figure 2 illustrates one trial of color tracing. On a palette at the bottom of the screen, the subject chooses the color with which the finger draws. The experiments demonstrate that with gradually progressive automated training using a tracing technique, the chimpanzee can be induced to draw complex stimuli in a highly reliable manner.

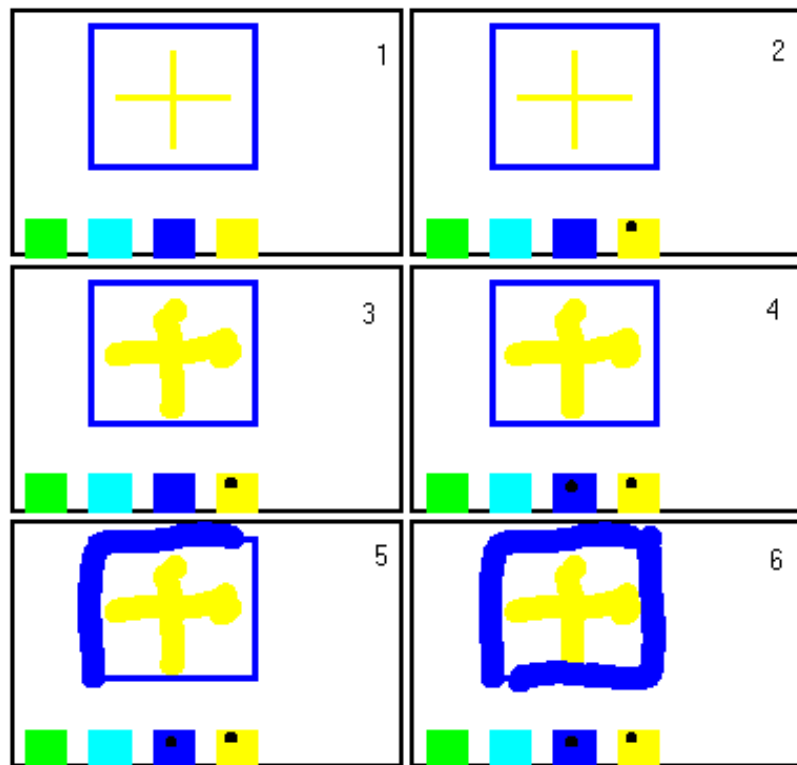


Figure 2. Color tracing, six steps of tracing for one trial. Frame 1 shows the model and the palette. Frame 6 shows the final product.

Object movement

The same two subjects were trained to move visual objects presented on the monitor with a finger. By placing the finger on an object, the subject could move the object on the monitor from one location to another by sliding the finger over the surface and the object followed the finger movement. With the *fingermaze task* the subject had to move a ball into a goal box. First, these were the only objects on the monitor. Next, obstacles were presented on the monitor that the ball could not go through. The subject had to learn to move the ball so as to avoid the obstacles.

Gradually the obstacles were made more complex and eventually formed a maze pattern. Both subjects learned to move the ball efficiently in the maze to minimize the number of times the ball hit an obstacle. Also, the subjects learned to avoid blind alleys. The analysis method allows for detailed assessment of movement patterns in the mazes.

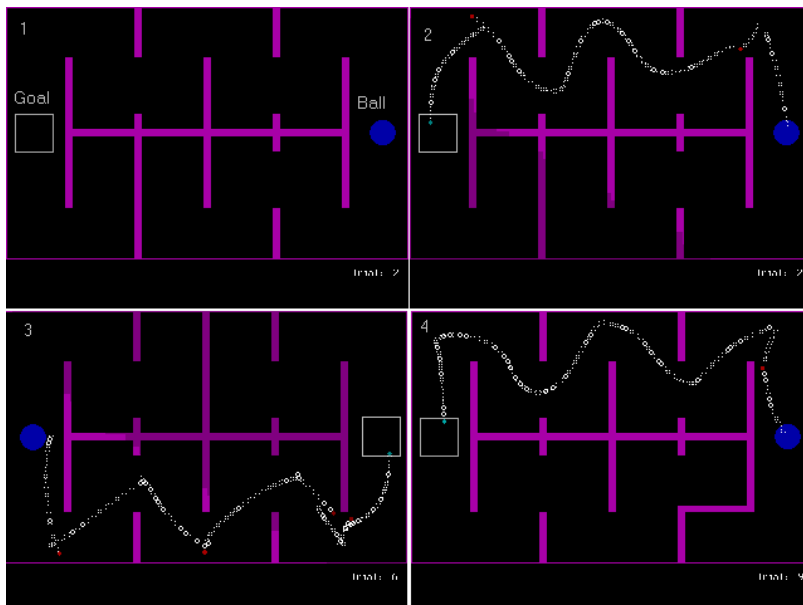


Figure 3. The fingermaze task.

Figure 3 shows some examples of maze patterns that the subjects solved. Frame 1 shows the maze and the ball and goal locations at trial start. Frame 2 shows the path the ball moved in the same maze. Frames 3 and 4 shows ball movement on other trials. The experiment shows how complex perceptual-motor skills can be examined in a completely automated fashion.

One chimpanzee was also trained on an *object-sorting task*. Figure 4 illustrates the task in a few trials. At trial start, the top of the screen shows some objects that may differ in color and shape. Frame 1 shows 4 objects in two categories. The bottom of the screen shows two horizontal lines, the trays. The objects have to be moved to the trays in any order but such that two categories are formed. Frame 2 shows the path of movement of each object. Although the objects are shown at the top in Frame 2, they were actually moved along the path and only appeared on the trays

by the end of the movement. When the objects are all down on the trays, a press on a switch outside the monitor will enable the computer to evaluate the sorting and present reinforcement if sorting is correct. Frames 3 and 4 show the object movement paths on two other trials. Experiments demonstrated that primate object sorting, which ordinarily is done with real objects and by manual training, could be fully automated.

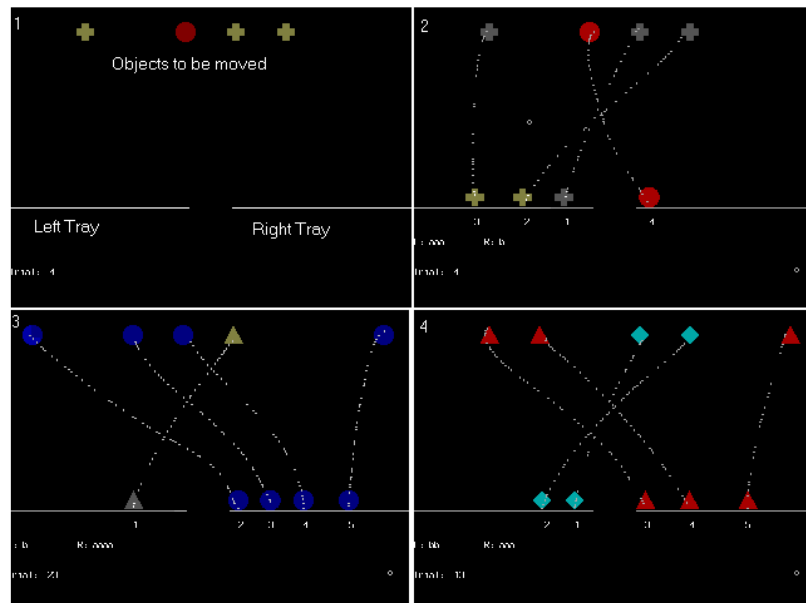


Figure 4. *The object-sorting task.*

The presentation will focus on the computerized methods of recording, analyzing, and training. Video examples of the methods will be presented.

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1. Iversen, I.H.; Matsuzawa, T. (1996). Visually guided drawing in the chimpanzee (*Pan troglodytes*). *Japanese Psychological Research*, **38**, 126-135. [In English]

On-line analysis of drawing and object moving on a touch-sensitive monitor: interaction between finger position and object location on the monitor

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The poster will describe the more technical aspects of the experiments described in the oral presentation at the meeting by Iversen and Matsuzawa [1]. The touch-sensitive monitor delivers an (x, y) pair at a given time interval and represents the location touched. Algorithms for how to present graphical symbols at the touched location for drawing will be presented along with demonstrations of detailed path analyses. For drawing and tracing, various algorithms have been implemented to determine on-line whether or not a drawing on a given trial should be reinforced. Examples will illustrate these automated contingencies of reinforcement. Methods for presenting the data will also be illustrated along with examples of direct control of a laser printer from the programming language. For experiments that involve object movement, algorithms will be presented for how one arranges for objects on the monitor to be moved in accordance with finger movement on the monitor. Programming of fingermaze training involved the implementation of algorithms that prevented the moved object from penetrating obstacles. Last, object sorting involved development of algorithms for automatic detection of object grouping. Examples will illustrate various aspects of the off-line data analysis of drawing and object movement paths. All programming for these experiments was accomplished with QuickBASIC 4.5.

Reference

1. Iversen, I.H.; Matsuzawa, T. (1998). Automated training of line drawing and object movement (in fingermaze and sorting tasks) on a touch-sensitive monitor in captive chimpanzees (*Pan troglodytes*). *This volume*.

An inexpensive method to automate the recording of direction and speed of individual bouts of wheel running in rats

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The speed and direction of wheel rotation can be monitored automatically by attaching a roller from a computer mouse directly to the axle of a running wheel. The modification of the computer mouse is very simple and does not require any modification of electronic components. Any programming language with mouse routines can be used to detect and analyze wheel rotation. Rotation of the wheel simply moves the cursor in the vertical or horizontal plane depending on which roller is attached to the wheel. Rotation was measured with QuickBasic 4.5 using the mouse routines from QuickPro (Crescent Software, Inc.) or Visual Basic 1.0 (DOS version), which incorporates mouse routines. Every 0.5 s the program assesses how far the wheel has turned within the past 0.5 s; the cursor position is reset after each mouse reading. For the mouse used, one full wheel revolution corresponds to a cursor displacement of 54 pixels. The measurement generates very simple data files with a string of numbers that represent speed and direction of running for each 0.5 s period. Positive values represent counter-clockwise (CCW) rotation and negative numbers clockwise (CW) rotation. Thus, 20 entries to a data file may read: 0, 0, 10, 15, 20, 18, 17, 9, -1, 0,0, -6, -9, -16, -32, -14, -4, 5, 0,0. The wheel is at rest, followed by positive acceleration (CCW) up to a peak speed of 20, followed by deceleration, followed by CW rotation with a peak speed of 32. Figure 1 illustrates the flow of wheel rotation for one Long Evans female rat during the first 15 min of a 1-hr session of free access to running. The letter A illustrates a bout of running lasting about 30 s with a peak speed near 1 meter/s. The rat runs in both directions and also “swings” back and forth in the wheel, as indicated at B. Different topographies of rotation such as a “bout” of running, a bout of swinging, a bout of “walking” can be defined precisely either online or offline. The methodology has been used successfully to monitor and analyze free running, running as an operant response, and running as a reinforcing response. The poster will show how to set up the equipment and some excerpts from the programs showing how to detect speed and direction. Data from actual experiments will illustrate the use of the method, which is tentatively called a Rotometer.

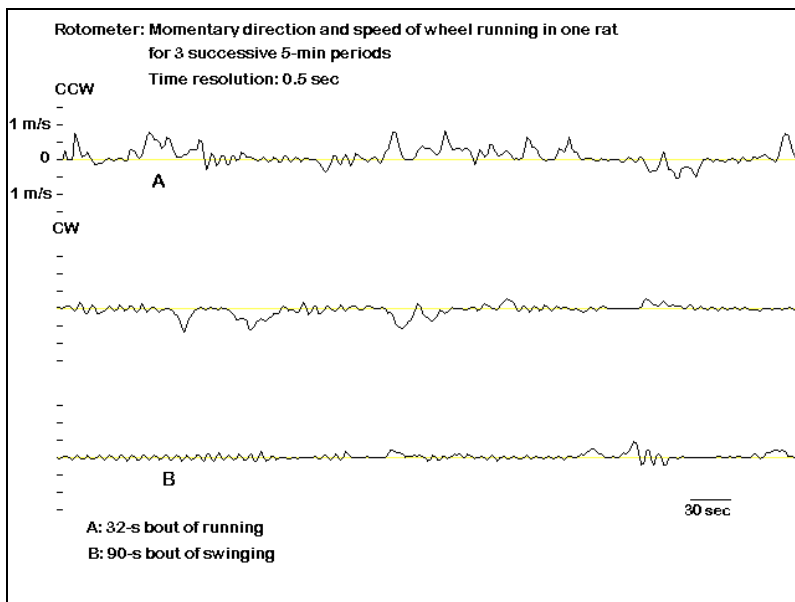


Figure 1. The flow of wheel rotation for one Long Evans female rat during the first 15 min of a 1-hr session of free access to running. The letter *A* illustrates a bout of running lasting about 30 s with a peak speed near 1 meter/s. The rat runs in both directions and also “swings” back and forth in the wheel, as indicated at *B*.

Biotelemetric measurement of the ECG in growing pigs: effects of social stress on heart rate, heart rate variability and occurrence of cardiac arrhythmias

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Social stress is a common phenomenon in pig husbandry. In this experiment, social stress was produced by a food competition test with a penmate and a resident-intruder test with an unacquainted pig. We studied the effects of social stress on heart rate, heart rate variability and occurrence of cardiac arrhythmias in growing pigs, and determined the relations between the outcome of the food competition test and the heart rate responses to the food competition test and resident-intruder test.

Twelve crossbred barrows (10 weeks of age) were used in the experiment. Heart rate was measured by biotelemetry. At 8 weeks of age barrows were implanted with a transmitter (type TA10CTA-D70; Data Sciences, St. Paul, MN, USA) allowing stress-free measurement of the ECG. The autonomic control of cardiac electrical activity was estimated by measuring the heart rate variability (e.g. [3]). Behaviour during the tests was recorded on video tape and analysed using the program "The Observer" (Noldus Information Technology, Wageningen, The Netherlands).

Agonistic behaviour was scored during interactions of the pigs. The outcome of the agonistic interactions during the tests was determined using the outcome of a procedure calculating the social rank index [2]. If the number of victories was larger than the number of defeats, the pig was considered to be the winner of the test. In case of the reversed situation, the pig was considered to be the loser. The outcome of the tests was always clear, i.e. the difference between the number of victories and the number of defeats always was large. Pigs that were defeated in the food competition test had a higher heart rate ($p < 0.10$), but no significant differences in heart rate variability parameters were observed between defeated pigs and winners. All pigs were defeated in the resident-intruder test with an unacquainted heavier pig, however, large individual differences in heart rate responses were observed. Pigs that were defeated in the food competition test were more sympathetically activated in the resident-intruder test than pigs that won the food competition test (i.e. the heart rate variability was significantly lower

for defeated pigs ($p < 0.05$), and had a higher heart rate in the resident-intruder test than winners ($p < 0.10$).

As it has been shown that the outcome of a food competition test is correlated with the social status of pigs [1], the social status may determine in the heart rate responses to the food competition test and resident-intruder test. Occurrence of cardiac arrhythmias did not increase during the tests as compared to baseline recordings. When heart rate is used as an assessment of stress, the results suggest that pigs that were defeated in the food competition test may experience more stress during the food competition and resident-intruder test.

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3. Sgoifo, A.; de Boer, S.F.; Westenbroek, C.; Maes, F.W.; Beldhuis, H.; Suzuki, T.; Koolhaas, J.M. (1997). Incidence of arrhythmias and heart rate variability in wild-type rats exposed to social stress. *Am. J. Physiol.*, **273**, H1754-H1760.

Detecting patterns in complex behavioral processes with The Observer and Theme

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Human interaction can be viewed as a co-ordinated and synchronized system of behavior. The present type of analysis corresponds to a view of behavior organization as the repetition of a particular type of intra- and inter-individual (hierarchical/syntactical) behavioral patterns. Each of these patterns (called T-patterns), is characterized by significantly similar time intervals between its components over pattern occurrences. These patterns have a hierarchical structure, as each pattern component can be a simpler pattern of the same kind. Therefore, the pattern definition and the corresponding pattern detection method focus on relationships between the occurring times series of various behavioral event types. Such series also constitute the data for the pattern detection software, called Theme, which has been developed especially for the detection of such patterns in behavior. Theme has mainly been used for the analysis of intra- and inter-individual real-time behavior records with varying number of actors. It is able to work scale-independently with time resolutions of seconds, days, month's etc. and with any kind of behavior. Theme can be used alone but using an especially developed interface it can also read and analyze the relevant types of observational data files (.ODF) created with The Observer.

To demonstrate this approach two studies are presented here, a soccer game and a boxing game analysis. The soccer game was coded with The Observer using categories such as player and ball positions and key game events such as goals, shots on goals, corner kicks, completed passes, intercepted passes, ball possession, etc. The boxing game was coded with Theme using categories such as position of boxer and relevant movements, such as left hook, right hook, knock down, etc. Results, from both studies, are demonstrated, both graphically and statistically, and examples of detected patterns are displayed and related to the results of the soccer game and the boxing game. The new Theme – The Observer interface will be demonstrated to Observer users. The results already suggest the possibility of discovering new kinds of profiles for both individuals and teams using the detected behavioral patterns in combination with elementary statistics. Moreover, some answers are already suggested to questions like: “Are there certain patterns that are related to doing well?”, “Are there certain patterns that could be labeled bad?” or “What responses seem to be evoked by certain actions or sequences of actions?”. Coaches could possibly use this kind of structural information when selecting players or when searching for the opponent's “weak spots”.

Computer-based analysis of physiological data in natural situations

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The measurement and analysis of physiological data is part of the project of the Swiss National Science Foundation "Emotional processes in natural situations: a computer-based algorithm supporting the analysis of their physiological and expressive components" by M.L. Käsermann, A. Altorfer and K. Foppa. One of the project's main tools is the program VASC Analyzer which can automatically analyze large quantities of data for individual single-case studies or interpersonal comparisons. About 30 minutes of a conversation between two human subjects are examined. During the session, the spoken contents are recorded and the peripheral blood flow (PBF) is measured with a photoelectric sensor (plethysmograph) attached to a finger. The PBF data correlates highly to the heart beat and the constriction and dilatation of the small blood vessels that result from changes of arousal of the subject.

The usual procedure to analyze peripheral blood flow or heart beat is to measure the Inter-Beat-Interval (IBI) which means that a person with e.g. 60 heart beats per minute delivers 60 data points per minute. If the resolution of the data has to be higher, then the values between the peaks are extrapolated as a rough estimate. However, there lies much more information between the peaks. The program VASC Analyzer can extract the periodic information between the peaks of any wave of data. First the PBF data is transformed into the Vascular-Volume-Data (the quantity of blood which is circulating through the small finger blood vessels over time). This is done with a "Sequel Analysis" which operates with a combination of highpass and lowpass filters (fig. 1). These Vascular-Volume-Data display a greater density of measured, not estimated information like IBI algorithms.

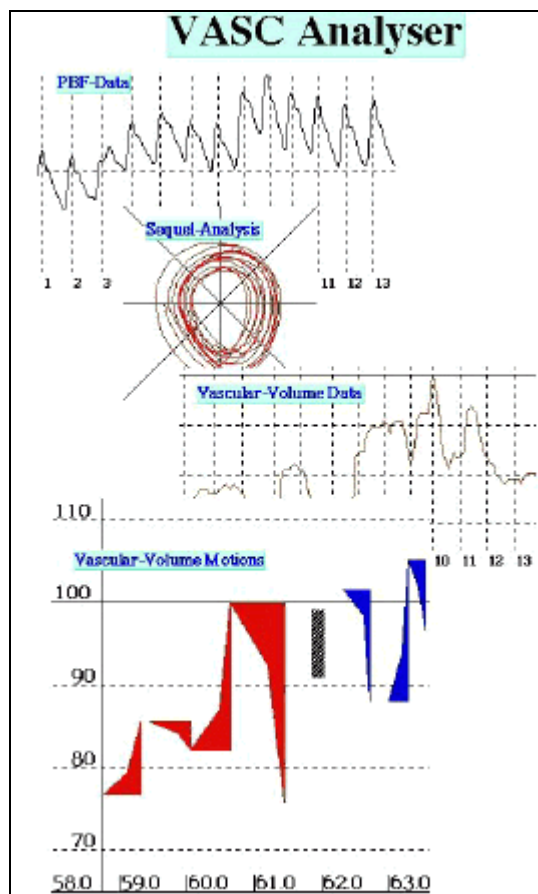


Figure 1. Procedure of analysis, peripheral blood flow (PBF), sequel analysis, vascular volume changes, and vascular volume motions.

An important principle of VASC Analyser is to continuously build higher levels of abstraction without losing the primary information. The lowest level is the PBF data and the highest level are classes of similar Vascular-Volume Motions which still show the time of occurrence, the initial values and the change of values of each member of the class. This is essential if these classes are to be linked to the original speech or video-tape. VASC Analyser starts at pointing to relevant Vascular-Volume-Data Events with a motion-detection algorithm. These events are significant changes of the Vascular-Volume-Data flow. It is also capable of locating biologically unlikely events like artefacts or missing values. Local processes

of arousal can consist of a group of several succeeding Vascular-Volume Events and are called Vascular-Volume Motions. The most important part is the classification of these Vascular-Volume Motions. Several algorithms of pattern and template matching are being used to build classes of similar Vascular-Volume Motions. Every motion within a class is kept together with its occurrence in time. In this way it is possible to focus on episodes in the recorded conversation with similar Vascular-Volume processes and episodes with extraordinary values. In this respect, communicative interaction and psychophysiological processes can be investigated by using methodologically linked systems of analysis which concern verbal utterances and emotional arousal [1].

Reference

1. Käsermann, M.L.; Altorfer, A.; Foppa, N.; Jossen, S.; Zimmermann, H. (1998). Measuring emotionalisation in everyday face-to-face communicative interaction. *This volume*.

Comparing frequency-based with sequential discourse analysis: convergence or divergence?

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Discourse analysis in psychology and education can be distinguished into two broad categories: qualitative and quantitative. In the case of the latter, discourse is firstly segmented and these segments are subsequently assigned to a number of codes. The frequencies of occurrence of these codes are used as scores for further statistical analyses. Exclusive consideration of frequencies is problematic on two counts. Firstly, an utterance always addresses and responds to previous utterances and, thus, is dialogically related to them. Secondly, the same utterance in two different contexts may have potentially different meanings, and, therefore, the local context has a bearing on the meaning of the utterance [4]. It is argued that sequential analysis of discourse provides a very promising alternative to analyzing classroom discourse because the dialogic relationship of each code with the others is maintained as well as the meaning of every code is preserved by considering specific codes preceding and following it. The importance of sequential analysis of discourse is illustrated by examining data from an empirical study. Twenty grade ten students were divided into two groups of ten and were tutored by their teacher in how to solve correlational problems. Students in one group used paper and pencil, while students in the other used a spreadsheet. All tutorials were videotaped, transcribed verbatim and subsequently coded by two trained judges using a coding scheme we developed for the purposes of the study. The main question we were trying to answer was whether the two groups differed as far as the teacher and student codes were concerned. The discourse data was subjected to a conventional frequency-based statistical analysis as well as to sequential analysis [1, 2, 3]. A number of statistically significant differences emerged when the groups were compared using the code frequencies as a criterion. In sharp contrast, a drastically different picture of the data surfaced with sequential analysis, some discourse patterns being either unique to each group or more likely in either of them. These divergent findings are discussed and some implications for discourse analysis in the educational and psychological scene are drawn.

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Measuring emotionalisation in everyday face-to-face communicative interaction

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Communicative interaction in face-to-face situations involves subtle processes of emotionalisation, that is, it is characterized by a continuous variation of positive and negative departures from a neutral mood. The aim of our research is to show, first, that these small-scale changes of emotionalisation do occur in normal as well as in pathological discourse, and, second, that they contribute to an individual's actual and global well-being. Specifically, with regard to communicative exchanges with psychotic index persons, we attempt to show that these mood variations and how the psychotic individual is able to cope with them is relevant for the course of a given mental illness.

In order to grasp variation of emotionalisation we concentrate on three types of variables:

- physiological measurement of vessel volume changes registered by photoplethysmographic equipment [2],
- behavioral measurement of changes of head movement behavior [1], and
- behavioral measurement of vocal changes during communicative interaction [3].

We conceive of these variables on the one hand as being expressive of a given emotional-activational state of the index person and on the other as being instrumental in guiding the partner's and the index person's own subsequent communicative behavior. Furthermore, we have evidence that in turn communicative reactions are fed back and may modulate (i.e. neutralize) a given state of emotionalisation.

Within this theoretical framework we are confronted with a number of methodological problems which, as a rule, are not encountered in experimental settings. However, problems concerning the control of artefacts, the coordination of data from multiple behavioral channels, the definition of relevant units and magnitude of variation are specifically discussed in the contributions of Jossen *et al.* (physiological measurement), Altorfer *et al.* (head movement measurement) and Zimmermann *et al.* (vocal measurement). Here, the discussion is restricted to a more fundamental problem pertaining invariably to each of these domains. It is the question of how, in the absence of an experimental manipulation which normally

delimits baseline from intervention-dependent data, meaningful segments entering a comparison across situations may be created.

Answering this question is indispensable for working with data from natural settings. To come to grasp with the definition of classes of equivalent events in natural communicative interaction, we adopt the following approach: The background of all of our analyses is formed by a minimal model of communicative exchange. Within this model three utterances of two participants A and B are assumed to be functionally related in the following way. A's first utterance A1 is an offer to be interpreted by B; B's utterance B1 contains an interpretation of A1, and A's second utterance A2 is based on an evaluation of the expected with the observed B1; in case of actual discrepancy, A2 (ideally) contains a correction. Within this framework, critical communicative events (deviations from or violations of communicative standards, e.g. B's interruption of A; B's silence after A's offer etc.) can be defined independently of any other variable (esp. independent of the physiological and expressive ones we assume to be affected by communicative violations).

The application of the minimal model of communicative exchange to the analysis of specific research questions has proved to be fruitful, as will be demonstrated by a review of some pertinent results.

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Alarming cascade in Giant honeybees (*Apis dorsata*)

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Asian Giant honeybees (*Apis dorsata*) nest in single combs which are covered by several layers of bees forming a curtain. When moderately threatened the bees flick their abdomens, synchronously performed by a great number of individuals, which is said to repel potential predators effectively. Such colony responses can be evoked by visual stimuli like approaching wasps or birds, or even by wasp-like dummies (as we used). In selected video documents (in total: 5 hours observation) movements of bees were visualized using the Optimas[®] image analysis tool. For that, movements were thresholded in 40 ms steps by using arithmetical, filtering and morphological operations combined with statistical processing.

Abdominal shaking is performed only by bees of the roofing layer of the curtain in all regions of the nest, it is initiated by a single wing stroke of 80-160 ms duration followed by an abdominal thrust of another 200-250 ms; still position is achieved again after further 200 ms. The abdominal shaking movements of thousands of bees are tightly coordinated, they form a cascadic colony response in temporal and spatial terms, emanating from one or more centers, radiating over a nest (e.g. with 80.000 individuals) within one second.

Such fast response patterns are initialized at particular sites of the nest, where groups of guard bees obviously respond more rapidly and powerfully than others. Visual and mechanoreceptive sensation is here the obvious basis to let this colony process shoot up within 300 ms (revealing a maximum waving area of 130 cm²; the horizontal span of the small experimental nest was 80 cm).

While the wave affects more and more parts of the nest in the declining phase during the subsequent 600 ms, the total of "shaking" areas and dispersal speed decrease. Shaking activity is distributed saltatorically, whereas sub-centers are likely to amplify and distribute the process. The dummy's direction is significantly ($P < 0.001$) correlated to that of the shaking wave, but only 20 cm around the primary trigger center. Shaking is disseminated differently in different nest areas; in the mouth area near the rim, coupling is stronger (0.6) than beneath the mouth (< 0.2). Shaking is spread fastest (< 1 ms/cm) horizontally and close to the primary trigger center, and slower (> 20 ms/cm) in the periphery. Such kind of directivity is probably caused by the posture of curtain bees which are hanging side to side with the abdomen down and the head up.

Visualization of multiple influences on flight behavior by the Viscovery® data mining tool

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Viscovery® SOMine (Self Organizing Maps) is a powerful tool for exploratory data analysis and data mining. Employing Kohonen neural networks, it puts complex data into order based on its similarity, and shows a map from which the features of the data can be identified and evaluated. This tool is very fast on large data sets, through the implementation of new techniques (such as SOM scaling) the speed in creating maps is notably increased compared to the original SOM algorithm. Designed also for scientific research, it supports analysis of non-linear dependencies, parameter-free clustering, association and prediction, non-linear regression pattern recognition and animated system states monitoring.

With this data mining tool we mapped non-linear relationships in multi-dimensional behavioral data assessed from honeybees under free-flight regime: *Apis mellifera* in Graz [3] and *Apis dorsata* in New Delhi [2]. In the experiments we trained fully sighted and ocelli-occluded bees to come to an artificial feeder. On the way from the nest to the feeder (feeding flight) and from the feeder to the nest (homing flight), respectively, the bees had to pass a flight chamber with a stationary side light (which was on or off). Hereby, we considered the following flight parameters: the direction of the bee's long axis (L), the flight course (C), the deviance of L and C from the target direction (Lt, Ct), the angular velocities of L and C (dL, dC), the deviance of L from C (Lc = yawing), ground velocity (Vg) and acceleration (Ag). The behavioral responses were mapped by Viscovery® SOMine (a) using the original data and (b) using contracted data processed from the original ones by conventional statistics (arithmetical means and standard deviations) and by case statistics (considering probabilities of positively or negatively oriented responses, respectively [1]).

For both honeybee species it is proved that occlusion of ocelli will not cut down orientational abilities, it (significantly) enhances or reduces reactivity under specific surrounding conditions (side light stimulation, spatial position in the flight chamber). However, the differences in behavior found between feeding and homing condition, between side light on or off, regarding the spatial influences of length or width position in the flight chamber are more dominant than those between trained fully sighted and ocelli-occluded honeybees.

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Behavior of rats in the complex maze: typology of cognition activity in normal and brain-damaged animals

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To model human limbic brain pathology (which is described as a defect of consciousness) in rats, we have used spatial learning (SL) in the multi-alternative maze, where we compared intact and operated groups (massive removal of brain amygdala). The behavior was registered by hand computer method (two observers) continuously during 18 sessions per day, each lasting 13 minutes. 15 “cognitive” and 24 “emotional” components were taken into consideration. SL included re-entering the maze; therefore, the number of cycles, associated with the efficacy (E) of food rewarding, was chosen as a main parameter of SL. To overcome redundancy of data, a selection of the original data was made. According to increase of E velocity, all individual variants were clustered in three different types of SL (exponential, logistic and no-developed ones). Comparison between normal types versus amygdalectomized, using own software, showed that there is a disturbance of the “emotional profile” for every type after amygdalectomy rather than the defect of SL itself. The specificity of the last was revealed only by applying information analysis, elaborated in psycholinguistics for text decoding, to our material. For us it includes transcription of all behavioral data for every session as a continuous “symbol text”, when a locomotor act in the space of the maze is symbolized by some letter to differentiate semantic information. For this approach a final realization of the SL is a sequence of 14 “letters”. Previous intermediate working stages may be represented as change of different letter combinations. Frequency analysis of these combinations is supposed to reflect cognitive processing. Applying information analysis allows one to reveal abnormality of cognition activity after amygdalectomy as well as discrepancy of cognition processing for above mentioned types in normal animals.

Design and analysis of experiments with single subjects

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Environmental enrichment is a very important issue for zoos and is becoming more important for farm animals. Evaluation of the effect of enrichment is mostly done by behavioral measurement, but it is often difficult to apply statistics, due to low numbers. However, statistical techniques are available, for instance single case analysis and meta-analysis, that - alone or in combination - could replace analysis of variance and can be applied to a single animal. Characteristic of the mentioned techniques is a very careful preplanning of the experiment, i.e., the number and the order of treatments given to the subject(s). Our research in farm and zoo animals is concentrated on comparison of time budgets related to housing conditions. One of the tools to find individuals that show aberrant time budgets is correspondence analysis [2]. In addition to this work enrichment experiments are done to ameliorate the keeping conditions of animals. Single case analysis is used to evaluate enrichment statistically. Examples of the use of this technique are:

- single case analysis and meta-analysis of the effect of food deprivation on crowing in the rooster [3],
- single case analysis of environmental enrichment in an Amur tiger, in which the amount of stereotypies was drastically reduced ($P < 0.001$), and
- single case analysis and meta-analysis of feeding enrichment in a group of three orangutans [4].

As an example, the research on orangutans will be explained here in some more detail. Time budgets of orangutans (*Pongo pygmaeus pygmaeus*) show large amounts of spare time. Feeding enrichment is one of the means to increase appetitive activity related to feeding. In a randomized design we presented three conditions available to the orangutans (A = feed dispenser; a log with holes filled with peanut butter, B = raisins hidden under the straw substrate, and C = control, the normal condition). During one month on 14 different days these conditions were randomly presented to the orangutans at the beginning of the day. Behavior was measured for 5 hours after releasing the animals in their enclosure. The time budgets of the animals were compared between the three conditions by Single Case Randomization Tests [1]. Single case analysis shows significant increases in activities on an individual level ($P < 0.05$). In conclusion, the comparison of time budgets of animals between zoos with different keeping conditions gives indications of welfare of individual animals related to housing and keeping conditions. Carefully planned experiments, statistical tests at the level of the individual and combining results of these tests in

meta-analysis provide sophisticated methods for evaluation of environmental enrichment in zoos with only one or few subjects.

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Measuring behavior: integrating behavior and physiology

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Understanding the physiology of behavior has been hampered for a long time by the technical limitations to analyze behavior in great detail and to measure physiological processes in freely behaving animals and human beings. However, recent developments in technology have created fascinating possibilities for the advancement of research in the field of behavioral physiology. This introductory lecture will illustrate some of this progress on the basis of our own research on the behavioral physiology of social behavior in rats and mice.

Rats and mice live in rather complex social structures in which individuals differ in their social relations to other group members. The social structure is maintained by agonistic behavior. Studies in wild populations of mice indicate that the level of agonistic behavior is bimodally distributed over the individual group members. Extensive behavioral research indicates that the distribution of agonistic behavior reflects a more general individual differentiation in coping style. Aggressive males adopt a proactive coping style in a wide variety of challenging situations, whereas the non aggressive males in the population appear to adopt a reactive coping style. Our research focuses on this individual differentiation in coping style because it reflects not only a differentiation in vulnerability to stress mediated diseases, but also a differentiation in central nervous and neuroendocrine mechanisms.

The methodology used and the need for further methodological developments will be illustrated briefly in three lines of our research. The first line of research tests the hypothesis that a fundamental behavioral difference between the two coping styles concerns the degree in which behavior is self-controlled or guided by or depends on environmental stimuli. This is studied by a detailed transition analysis of a social interaction between a resident and the intruder in its home cage, using frame-by-frame analysis of video recordings. A second line of research concerns the question to what extent the social structure of a group of male rats, its stability and the development of stress pathology depend on the composition of the group in terms of coping styles. This study uses video tracking methods to monitor the social structure and radiotelemetry to measure physiological parameters in group-housed rats. A third line of research aims at understanding the long term (weeks and months) behavioral and physiological consequences of a single social defeat experience. In view of the duration of the effects, these studies require various automated recording techniques for behavior and physiology.

The three lines of research have in common the need for advanced in vivo brain imaging techniques with a sufficient resolution in space and time. Moreover, the advancement of automated recording techniques of behavior and physiology generates an exponential increase in the amount of data. Efficient handling, reduction and integration of these large data sets will be a major challenge for the near future.

Telemetry: a new method to measure blood pressure in freely moving mice

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Measurements of blood pressure (BP) by radio-telemetry have been described for many animals but not for mice. We describe the use of a commercially available telemetry and data acquisition system to record the systolic, diastolic and mean BP as well as heart rate (HR) and respiratory rate (RR) of freely moving mice. By using implantable telemetry, measurements from freely moving animals are more efficient, reliable and less labor-intensive than the measurement techniques described in the literature thus far.

Experiences with the use of a new BP implant for monitoring mice will be presented, including a surgical procedure which has been found to be successful for implantation of the device as well as special considerations and necessary precautions, like using an operation room with an operation microscope and using completely sterilized equipment for performing surgery on mice. Three mice were successfully operated upon. After surgery, recovery of the animals was indicated by an increase in weight after an initial decrease and by changes in their behavior such as building a nest of the available paper towels. Animals appeared lively throughout the study and no behavioral changes were observed compared to the mice without transmitters. Two days after the operation the daily recording was started and the lowest values in HR (300-400 beats/mean), diastolic (70-80 mmHg), systolic (102-112 mmHg) and mean BP (86-96 mmHg) were found while the mice were in sleep in their home cages. Stressful situations such as hand restraint of the animals or placement of the mice in other cages increased the HR to a maximum of 750-800 beats per mean (bpm), the diastolic pressure to 105-120 mmHg, the systolic pressure to 140-155 mmHg and the mean BP to 123-138 mmHg, respectively. Circadian rhythms in HR and BP (low during the light period: 07:00 to 19:00 h, and high during the dark period: 19:00 to 07:00 h) could be established in the same mice, however no circadian rhythms in RR could be found. In conclusion, it appears to be possible to implant a transmitter to monitor BP, HR, RR and activity by telemetry in freely moving mice. The most important advantage is the possibility for the direct and accurate measurement of chronic effects of cardioactive compounds, without the added stress which results from conventional measurement techniques.

Assessing behavioural effects of neuroleptic drugs in rhesus monkeys

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We present the results of a behavioural analysis of the effects of the neuroleptic drugs clozapine and risperidone in rhesus monkeys. The subjects for the study were social colonies of 3-5 year old adult rhesus monkey (*Macaca mulatta*) weighing 4-6 kg. Each colony comprised eight monkeys (one male and seven females). Only active healthy animals were accepted for the study. Each colony was housed in a 20' x 12' x 8' cage. The monkeys were kept under controlled temperature conditions ($22\pm 5^{\circ}\text{C}$), humidity and ventilation (with 100% fresh air) and light (12 hour light/dark cycle) to maintain their breeding and physiological rhythm. The light intensity was about 300 lux. A balanced diet was provided in the morning and evening and water was available *ad libitum* through an automatic watering system. Animals were allowed 4-6 weeks time to stabilise their behaviour in the new surroundings. To reduce the stress induced by handling and dosing, the animals were handled by a single experimenter. During the period of habituation, monkeys were caught and removed from the cage at least once a week and administered saline intramuscularly or orally.

Behavioural observations took place prior to (base-line control) and after drug or vehicle treatment. A checklist of social and solitary behavioural responses [1] modified from the parameters described by Schlemmer and Davis [2] was used. The behaviour was observed on a video monitor placed in an adjacent room with the help of two strategically placed 180° rotating video cameras, provided with zoom lens, fixed in the behaviour chamber. Behavioural responses were video taped for analysis and archiving. On any one day, only one animal from the group was injected with the drug and the behaviour was observed as described below. The other animals of the group were injected with the drug by rotation and score of normal behaviour compared with the behaviour after treatment. A minimum interval of 10 days was maintained between two doses of the drug to the same monkey in order to washout the effect of previous dose. Each monkey was observed in rotation for 1 minute at every 10 minutes for 2 hours initially as well as after drug or vehicle treatment. The scores of each behaviour from the twelve 1-minute intervals were summed up for individual animals and that represented the day's score for that monkey. The monkeys were also observed at 4, 6, 8 and 24 hours post treatment for any significant alteration in behaviour. Statistical analysis was performed for all behavioural data by using Mann-Whitney U test.

Clozapine (5-20 mg/kg, po) produced a decrease in social and solitary behaviour. In the highest dose (20 mg/kg, po) it produced a marked decrease in all social and solitary behaviours and catalepsy along with continuous salivation. Risperidone (0.25-0.2 mg/kg, po) showed a decrease in social and solitary behaviour. The highest dose (0.2 mg/kg, po) produced marked sedation only. It may be concluded on the basis of above findings that the profile of behavioural effects of risperidone is similar to that of clozapine in certain parameters but it has no extrapyramidal side effects and does not produce hypersalivation.

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Visual behavior towards a still face at birth

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Previous research showed that newborns aged 3-4 days are interested in observing a human face; that is evident from the fact that they look longer at their mother's face than at a broadly similar stranger's face [1, 3]. Moreover, at the same age, newborns show more interest in a schematic face than in scrambled control stimuli [2]. Therefore we may hypothesize that newborns get activated by a still face and consistently look at it before habituation starts. However, as the present research will show, this was not the case when newborns were observed few hours after birth.

Method

20 healthy newborns, sex balanced, not yet fed, aged 4-7 hours were considered. When in spontaneous awakening state, they were shown, in alternate order, two stimuli: a square with black frame and a still face made out of plastic. Each stimulus was presented for 720 seconds with an inter-stimulus interval of 180 seconds, at a distance of 25 cm from newborn's face. The looking behavior was videotaped and micro-analyzed using the Noldus Video Tape Analysis system and The Observer software. The four dependent variables were: eye opening (= gazing at + gazing out), gazing at, gazing out and facing at.

Results

Concordance for gazing at/out gives an average Cohen kappa of 0.82; concordance for facing at/out is $K = 0.91$. A four-factor ANOVA (2 sex x 2 order of presentation x 2 stimulus x 4 interval) with stimulus and interval as repeated measures was performed on each of the four dependent variables. Sex, order of presentation, interval and their interactions were not found to have significant effects on the four dependent variables. Stimulus effects on the four dependent variables are shown in Table 1. The stimulus has a small main effect ($p < .08$) and a significant interaction with interval on eye opening ($p < .05$).

Table 1. Effect of the stimulus (square or still face) on the duration of four behaviors. Duration (mean \pm SD) is expressed in seconds.

	Square	Still face	F	p
Eye opening		235.8 \pm 177.5	3.5 (16, 1)	0.08
Gazing at	146.7 \pm 124.3	43.3 \pm 45.7	18.8 (16, 1)	<0.001
Gazing out	41.4 \pm 46.5	192.5 \pm 145.7	33.7 (16, 1)	<0.001
Facing at	440.8 \pm 209.8	288.1 \pm 190.2	24.0 (16, 1)	<0.001

Figure 1 shows how newborns habituate (decrease of eye opening) to the square but not to the still face. Moreover stimulus has significant effects (without interactions with sex, order of presentation and interval) on gazing at, gazing out and facing at. Both stimuli show a gazing at vs. gazing out significant difference (t paired; still face: "at" < "out", $p < .001$; square: "at" > "out", $p < .001$). Nineteen newborns (out of 20) when looking towards the still face look "out" more than "at"; the opposite is true for the square. Furthermore, Table 2 shows that 18 out of 20 newborns change the gazing modality when the stimulus changes.

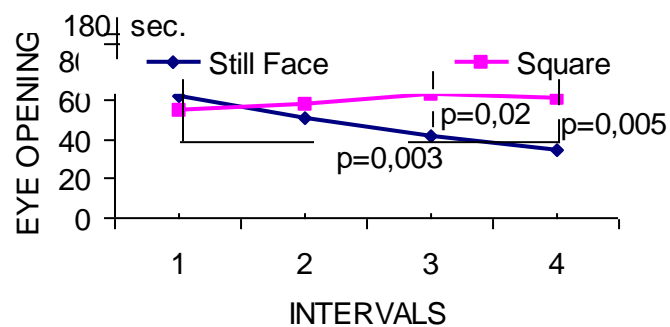


Figure 1. Effect of stimulus and interval on eye opening.

Table 2. Distribution of the 20 subjects according to two categories of Gazing at / Gazing out ratio and according to the stimulus. McNemar Chi-square = 16.1 (df=1; p<0.0001; 2-tailed).

Square	Still Face	
	Gaze at < Gaze out	Gaze at > Gaze out
Gaze at > Gaze out	18/20	1/20
Gaze at < Gaze out	1/20	0/20

Conclusions

Newborns at 4-7 hours of life are activated from the still face (720 s of exposition) but at the same time they show a consistent visual avoidance towards it. The same subjects are activated by the square and at the same time they consistently look at it. A first explanation is that communicating faces seen by neonates before 4-7 hours of life determine expectations of vocalization and/or movements associated with the faces themselves; the still face frustrates such expectations and, as consequence, is avoided [4]. The square does not frustrate any expectation and, as a consequence, is not avoided.

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CRISMAS - a new system for computer-assisted semen analysis

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Introduction

Assessment of sperm motility by manual semen analysis is a subject of great inter-observer variation. Therefore systems for computer-assisted semen analysis (CASA) have been developed in order to obtain a more objective assessment of sperm motility. By CASA several parameters of velocity and linearity, defined by the World Health Organization, are calculated. Until now, however, existing CASA-systems have not fulfilled previous expectations as better predictors of male fertility than conventional semen analysis. One major problem is that the CASA-systems cannot always discriminate between immotile spermatozoa and other static cells in the semen, which leads to incorrect estimations of sperm concentration and motility. We have developed a new CASA-system called "CRISMAS". The main purpose of introducing a new CASA-system is to limit the error rate by optimizing the image quality and implementing tail detection. An 'edit function' allows the user to play back the image sequence and correct errors by deleting and connecting tracks. As part of a validation of CRISMAS we have made a pilot study which compares CRISMAS with a well known and widely used CASA-system.

Aim of study

To compare the outcome of two CASA-systems: CRISMAS and the Hamilton-Thorne Motility Analyzer - Integrated Visual Optics System (HTMA-IVOS) (version 10.7k).

Materials & Methods

Semen samples were collected from 20 men. The samples were diluted in a phosphate buffer, placed in Makler chambers and analyzed simultaneously with CRISMAS and HTMA-IVOS. The results were examined by the Spearman rank correlation coefficient (r_s) and the Wilcoxon matched pairs test.

Results

The measurements of the two systems were generally very well correlated, but at different levels, which often resulted in significant P-values in the Wilcoxon test. The calculated sperm concentrations were significantly correlated ($r_s = 0.86$, $P = 0.0001$). However, CRISMAS generally measured concentrations that were lower than HTMA-IVOS, but differed less from conventional sperm concentration than HTMA-IVOS did (median differences = 6.7×10^6 and 35.5×10^6 , respectively). The proportions of progressive and non-progressive spermatozoa also showed good correlations, although the absolute values differed slightly ($P < 0.05$). The velocity parameters were clearly correlated, but CRISMAS generally measured lower velocities than HTM-IVOS.

Discussion

We have developed a new CASA-system, CRISMAS, and compared it with the well known HTMA-IVOS system. CRISMAS measured more accurate sperm concentrations than HTMA-IVOS, which is probably partly due to the use of the 'edit function' of CRISMAS, but not of HTMA-IVOS. CRISMAS measured a lower proportion of immotile spermatozoa and lower sperm velocities than HTMA-IVOS. This can be explained by the differences in the settings and definitions of the two systems.

Conclusion

This pilot study has proven a new CASA-system, CRISMAS, a reliable alternative to existing systems. Furthermore, the study clearly illustrates the need for a standardization of the settings and algorithms of CASA-systems in order to avoid variation between the measurements of different systems.

The trimodal brain: integrating auditory neuroscience, brain imaging and the study of behavioral disorders

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One of the greatest challenges to students of the neurological correlates of behavior in humans is interpreting the dramatic degree of difference observed between individuals. Such differences characterize virtually all sophisticated measures, from behavioral assessments of psychophysical skills, to noninvasive imaging of neuroanatomical and neurophysiological correlates of behavior. Yet this rich diversity of detail is often either ignored, or considered experimental noise and actively suppressed through use of statistical methods which emphasize group rather than individual results.

Our research is based on the assumption that accounting for the 'repertoire' of individual patterns is a prerequisite for understanding the variety of ways in which human brain organization underlies behavior. In this presentation, we will describe the design and use of the 'auditory-system cross-section battery', a relatively inexpensive noninvasive test battery which makes it possible to describe a representative 'neurological fingerprint' for each individual. Just as with forensic uses of real finger prints, the dependent variables yielded by the battery offer not only means of uniquely specifying each individual, but also an objective basis for classifying individuals into groups, to support formulations of universal principles of brain/behavior relations. Such classification may not only be important in and of itself, but also crucial as a background for understanding individual differences observed in experiments based on neuroimaging. The minimal battery consists of a combination of behavioral and physiological measures. Behavioral tests include: (1) an audiometric assessment, and (2) a set of measures of fine motor control. Physiological tests include: (1) otoacoustic emissions (OAEs), (2) the Repeated Evoked Potentials version of the Auditory Brainstem Response (REPs/ABR), and (3) quantitative electroencephalography (qEEG) collected under resting conditions. Physiological protocols assess right/left and afferent/efferent relations characterizing each level of the system. When possible, the minimal battery can be supplemented by MRI studies to document the anatomical integrity of cerebral structures, and quantify volume asymmetries of specific regions such as perisylvian cortex, and/or functional imaging to corroborate the electrophysiological findings on asymmetries.

The 'bottom-up' approach represented by the design of this battery derives from the conviction that a focus on the range of individual expressions may suggest

principles of brain/behavior classification that could be derived in no other way. One example is our Trimodal Model of brain organization, which proposes a new formulation of functional asymmetries, and posits a continuum of individual differences regarding access to functional-asymmetry skills, dependent on prenatal hormonal conditions, and trimodally distributed in the larger population. The resulting three major variants of human brain are considered to be adaptive for life in pre-industrial human and proto-human societies. Their features have implications for explaining many types of individual psychological and physiological differences including special skills such as athletic ability and musical aptitude, the etiology and nature of psychiatric and developmental disorders, and differences in response to and recovery from acquired injury. Examples of applications of the auditory-system cross-section battery for studying aspects of the Trimodal Model will be presented, to illustrate use of the battery as well as the potential utility of the Trimodal Model for understanding individual differences in human brain/behavior relations.

Video tracking: improved methods for identification of animals with color markers

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Video tracking is an established technique to follow a single animal in an experimental setup. With basic image processing it is possible to find the object in an image and determine its spatial position. Identification is not necessary because there is only one animal. Automatic tracking of multiple animals, however, requires a different approach. Tracking multiple individually identified animals can be achieved by marking the animals. One technique that is used for tracking two animals uses the difference in *apparent size* to identify the animals (available in EthoVision® Pro). In practice, this technique can only be used reliably with two animals. In order to identify and track more than two animals, a technique known as *color tracking* can be used: animals are marked with different colors that enable individual recognition by the tracking system (available in EthoVision® Color-Pro).

During the past year we have been working on improvement of the algorithms to achieve more accurate and robust tracking results. In particular, improvements have been made on the identification of the colors (a pattern classification problem) and extension of the user interface to give the user more control over the process of color assignment and tracking. Apart from enhanced color tracking software, this work has resulted in valuable knowledge on lighting techniques and other important variables in the color tracking process: what colors can be used, what are optimal initial settings on the frame grabber, etc.

The result of this work is a prototype of a new version of EthoVision Color-Pro, able to adequately identify and track at least eight animals. The new program will be demonstrated during the conference.

Automatic recognition of behavioral patterns of rats using video imaging and statistical classification

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Automatic behavior recognition has been the topic of joint research by Noldus Information Technology and the Rudolf Magnus Institute for several years. The spin-off of this work is the EthoVision[®] video tracking, motion analysis and behavior recognition system. Until now, this system is mainly used as an instrument to measure the behavior of animals on the basis of movement (using path-related parameters). To increase the capabilities of EthoVision to measure subtle behaviors of rodents, we are currently focussing our research on the automatic recognition of behaviors which are not related to movement. This approach is based on shape analysis. Examples of standard image features that describe the shape of an object are surface area, circularity, perimeter, moments, etc. In the cases of rodents, however, these standard object shape features do not provide enough information for automatic behavior recognition. Therefore we have developed new algorithms for the extraction of *model-based features*. Since our first report on this work [2] we have improved the detection of these features and have extended the range to include the following: X,Y coordinate of the head point (snout), tail base (point where the tail is attached to the abdomen), corrected center of gravity and several additional features derived from these three points. These new features together with the features describing the shape of the object are being used for automated behavior recognition.

Automatic scoring of behaviors can be seen as a form of pattern classification, the process which assigns classes to signals that are derived from objects using a sensory system. In pattern classification there are, in general, two approaches used for classification. One is the use of a statistical classifier, the other is the use of neural networks. At Noldus Information Technology we are investigating the statistical approach, while our project partners at the Rudolf Magnus Institute study the use of neural networks [1]. For statistical pattern classification we use a Bayes classifier. This classification method is based on the following two prerequisites: (1) the damage involved when an object is classified incorrectly can be quantified as *cost*, and (2) the expectation of the cost (known as *risk*) is acceptable as optimization criterion. Before actual classification of the data, the classifier has to be trained. During training, information about the distribution of the features for the different classes is calculated. The data used for training consists of the features describing the object plus the behaviors as scored by a

human observer. Once the classifier is trained it is ready to use for classifying new data.

It is expected that especially standard behavioral tests such as the elevated plus maze and open field experiments can be further automated with this kind of technology. Examples of some typical behaviors that may be recognized automatically are head dip, head raise, stretched attend, rearing, grooming and crouching.

The research is still in progress; the results obtained so far will be presented at the conference. This work is carried out with financial support from Eureka project no. EU88011.

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Computerized monitoring of activity and spatial behavior is important in different learning paradigms

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In order to analyze the activity and spatial behavior of mice during learning and retention in different learning paradigms such as fear conditioning and object discrimination, it is useful to apply automatic recording systems. We will demonstrate this with two examples.

Fear conditioning

Fear conditioning is an emotional and stressful one-trial learning paradigm which has been extensively used to study the mechanism of learning and memory and the involved brain structures in rat and mice. Traditionally the degree of learning in this paradigm was evaluated by only one subjectively measured behavioral parameter, freezing, which is defined as the lack of movement besides respiration and heart beat. We have introduced two automatically and objectively measured parameters, activity and exploration area during training and retention, which permit a more detailed analysis of the degree of learning in mice. Activity and exploration area are measured by a photobeam device which is positioned around the box in which the animal is tested.

Object discrimination

The object discrimination test is a non-spatial, non-emotional and non-stressful one-trial learning paradigm. The animal has to distinguish between a familiar and novel object. Such evaluation has been traditionally performed by measuring frequency and duration of exploring the objects, i.e. of directing the nose toward one of the objects in a distance of less than 2.5 cm. Object-recognition memory was defined as the percentage of time spent exploring the novel object as a function of the total time spent exploring both objects. We have included automatically and objectively measured spatial parameters to have a simpler and more detailed analysis of the discriminative ability of the animals.

With a computerized video system the distance of the animals to the objects is recorded during the test sessions and analyzed for the following parameters:

- % time spent in the different distances to the familiar or novel object,
- % time spent close to the novel object as a function of the total time spent close to both objects,
- frequency of visits to enter the area close to the object, and

- latency to visit the familiar or novel object.

Both examples show that automatic recording of activity and spatial parameters significantly contribute to the detailed analysis of the behavior of the animals.

Measuring locomotion behavior through gait analysis based on accelerometry

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Gait is a bare necessity for performing most of the activities of daily living (ADL). Through gait a person is in control of getting from one place to another at any place or time, therefore good quality gait is significant for a feeling of good health and subjective quality of life. An impairment of the locomotor system can result in disability or even handicap, but many therapeutic methods exist to overcome impairment caused by trauma, disease, or wear off. Tools and methods for the evaluation of impairment such as X-ray photography are integrated in clinical routine, however, in the evaluation process little objective information is collected about improvement at the level of performance in normal daily activities. To date a method that is practical though sufficiently sensitive is missing. New methods of gait analysis based on accelerometry [1, 2] may accommodate the demand for objective gait data, as well as provide a cost-effective means for collecting and reporting such data. The aim of this paper is to discuss accelerometry compared to other gait analysis methods with regard to considerations for clinical implementation. We regarded position analysis (e.g. Vicon[®], MacReflex[®]), force plates (e.g. Kistler), walkway (e.g. GAITRite[®]), goniometers (e.g. Penny & Giles), pressure sensors under the feet (e.g. Infotronics) and body-fixed accelerometers (e.g. DynaPort[®]). Of this range of gait analysis methods we rated the specificity, clinical validity, responsivity and ease of use. Reliability is regarded a prerequisite for all. We rated specificity on the basis of detail and precision, clinical validity on how real and acceptable the measured samples of gait are, responsivity on the extent to which the measurements can support in assessment of clinical questions, and ease of use on how quick, simple and affordable measurement is. The authors' ratings are presented in table 1.

Table 1. Types of gait analysis related to criteria for clinical implementation. Reliability is regarded a prerequisite for all. Legend: KM = kinematics, KN = kinetics, BR = body region, MV= measurement volume, IN = interference, AW = awareness, DI = disorder, IM = impairment, DA = disability, PO = portability, DA = data, CO = cost. The criteria are rated by the authors: o = not good, + = moderate, ++ = good.

Gait analysis Type	Criteria for clinical implementation											
	Specificity			Clinical validity			Responsivity			Ease of use		
	KM	KN	BR	MV	IN	AW	DI	IM	DA	PO	DA	CO
Position analysis	++	+	++	+	o	o	++	+	o	+	o	o
Force plates	o	++	o	o	+	o	++	+	o	o	o	o
Walkway	+	o	o	+	+	o	+	+	o	+	++	++
Goniometry	+	o	+	+	+	+	+	+	o	++	+	+
Foot pressure	+	+	o	++	+	+	+	+	o	++	+	+
Accelerometry	+	o	++	++	++	++	+	++	+	++	++	++

It is concluded that position analysis is the most elaborate and specific method for gait analysis, however gait is constrained and only a small number of strides can be assessed. Also, the cost and effort required to establish and operate a gait lab based is high. Though specificity is to be elaborated, accelerometry excels in clinical validity and ease of use. It offers very responsive measures of gait in almost any natural situation with minimal movement constraints. Unsupervised collection of measurement data during ADL supplies objective information as well as its dynamics over time. We conclude that for broad implementation in clinical practice accelerometry has large potential as it offers a clinically valid, easy and affordable method for gait analysis.

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The relationship between stress, stereotypic behavior and physiological state in farmed mink

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Stereotypic behavior in mink is an animal welfare problem in the sense that it concerns animal protection groups and the public opinion. Stereotypies probably also signifies a lack of possibilities to express some behavioral wish or need in the animal. However, it is, at this point still not determined whether stereotypies, per se, are detrimental to the welfare of the animals performing them. Work done on mink and other animals suggests that high stereotyping animals are physiologically "calm", e.g. low in cortisol, whereas the opposite is the case for low stereotyping animals. This suggests that stereotypies could be an active coping strategy, a way to lower the anxiety or stress perceived in the situation that the animal finds itself in. It should be kept in mind, however, that other factors, such as a relatively higher metabolic rate, could explain the low cortisol levels in very active/stereotyping animals. Although it is possible to breed a low stereotypy line of mink, it seems undesirable to breed a mink which may look calm, but may suffer from high physiological stress levels.

In order to investigate whether the converse relationship between stereotypic activity and physiological state also exists in mink, the following experiment was set up. Six female mink, selected for being high stereotyping (HS) and six selected for low stereotyping levels (LS) were implanted with a radio transmitter, continuously transmitting heart rate and body temperature data, while housed in standard mink cages. Cortisol was measured on 24h urine samples and the mink's behavior was recorded with a time-lapse VCR. The resulting video tapes were analyzed using a custom-made program encoding behavior on a Husky handheld computer for later analysis on a PC.

After an initial period of base line measurements, the mink were confined in standard traps for an hour every day for nine days. During this period the measured physiological variables related to stress declined for both groups, suggesting that habituation had occurred. Furthermore, the HS group was more reactive (measured on cortisol) than the LS group.

CARDIA, a program package for combined cardiovascular/behavioral data acquisition and analysis in animals

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CARDIA's recording modules record instantaneous heart rate and/or systolic and diastolic blood pressures on a beat-to-beat basis for over 40.000 beats, together with up to seven channels of behavioral protocol. On-line display provides direct feedback to the experimenter. These modules, running under MS-DOS, perform reliably even on the lab's out-of-date XT- and AT-compatible computers. Recording module version 4 is designed for use with PhysioTel (Data Sciences International) implantable transmitters for blood pressure or combined ECG/body temperature measurement and requires an AT-compatible. It presents an oscilloscope-type display of the live signal, in addition to the time plot of heart rate, blood pressures or temperature, and behavior. It connects directly to the output of the receiver via its serial (COM) port and does not require additional hardware. Behavior can be protocollated via the keyboard. Presently, the built-in detection algorithms for systolic and diastolic pressure points and the ECG R-wave are specifically tuned to the rat. The previous version 3 employed a 10 kHz timer plug-in card, an A/D converter card in case an analog blood pressure transducer was used, and/or an external R-wave trigger circuit if an ECG was available, and an external protocollator unit (all hardware was built by our Electronics department). This version accommodated any animal species.

CARDIA's analysis module (version 3) offers flexible display options and calculates a number of cardiovascular statistics, per behavioral channel and pooled over all channels. Measures of heart rate variability include long-term variability (standard deviation of instantaneous heart rate and inter-beat intervals) as well as short-term variability (root-mean-square of successive differences). The analysis module includes an editing facility for data files. It can export data and analysis results in plain text or spreadsheet-readable formats. Hard copies of screen displays can be made on a Star-compatible matrix printer or an HP-compatible laser or ink jet printer, either during analysis or in batch mode.

Real-time pattern detection versus standard sequential and time series analysis

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A multivariate time pattern type corresponding to a large variety of everyday behavior and social interaction patterns is proposed. The definition and detection of this type of pattern considers the order of pattern components, the time intervals between them and the real-time location of each pattern component within each pattern occurrence. --- The difficulty of seeing (perceiving) many such patterns directly, even under relatively optimal conditions, is illustrated as well as the difficulty of detecting such patterns with standard sequential and time series analysis methods.

A computational detection procedure and software (Theme) has been developed for the detection of this kind of (hierarchical/syntactical) temporal behavior patterns (T-patterns). This heuristic search procedure is based directly on elementary probability theory. It searches for a particular statistical relationship between all pairs of time point series, in a given data set, where each series represents the occurrence times of a behavioral event type. Based on information obtained through this search the procedure constructs/detects patterns of increasing complexity. As the same underlying behavior pattern can thus be detected in many partial and redundant ways, which can easily lead to combinatorial explosion, the procedure also involves competition and selection of patterns such that only the most complete or longest patterns survive. The method has now been tested for robustness through simulation (statistical experiments) and the essential results are presented.

Illustrative examples of patterns detected in various kinds of behavior some of which was coded with The Observer[®] are presented (see Figure 1 for an example). Applications of this particular pattern detection approach in the analysis of social and therapeutic interactions in autistic and handicapped children are cited [1, 2, 3, 5, 6, 7, 8] but application in the analysis of football and boxing is in progress [4] as well as in combined behavioral and physiological studies of sleep.

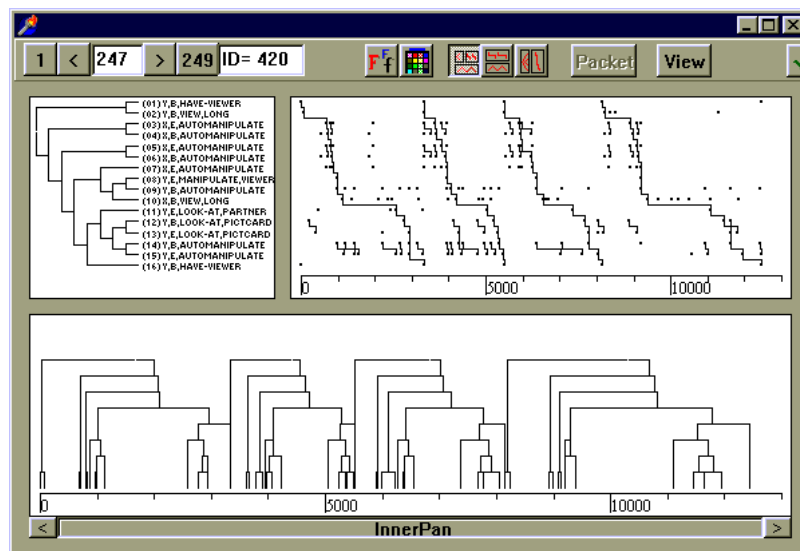


Figure 1. This screenshot shows a social interaction pattern detected with Theme in a dyad between two five-year-old girls (X and Y) playing together with a single picture VIEWER for 13.5 min. Only one of them could VIEW each PICTURE CARD at the same time. An "event type" here means some actor's beginning (B) or ending (E) of some behavior. For example, "X,B,LOOK-AT,PARTNER" (see figure) is an event type meaning: "actor X begins looking at her partner". Nearly 100 different event types occurred in this dyad. Theme discovered that the 16 event types (leaves) of this pattern occurred four times in a similar temporal configuration. The top left box shows its (recursive) hierarchical structure as a pattern of event types or of other patterns of the same kind and reflects Theme's bottom-up gradual detection method. The top right box: (1/15 s time scale) shows the occurrence time points for each of the 16 event types and the way they get connected. The four occurrences of the pattern (tree) are shown below with leaves 1 to 16 ordered by their occurrence times within the pattern, i.e. from left to right. Note that the last pattern occurrence is somewhat longer (slower) and then the children stopped playing. (LONG means > 3 sec. AUTOMANIPULATE means "fiddle with something without watching it".)

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Walking balance study using a triaxial accelerometer

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Introduction

A measurement system based on a triaxial accelerometer and a portable data logger has been developed and four performance parameters have resulted from experimental testing [3]. These are sensitive to balance-related changes introduced in the gait pattern. They are: correlation between the signals of all the possible pairs of steps in a walking sequence (aI), area under the power spectrum curve having first subtracted the regular part of the signal (aII), variation in stride duration (aIII), and conservation of mechanical energy (aIV) [4]. Parameter aI detects step signals that deviate from the normal morphology by being either too regular or too irregular; aII is related to the neuromuscular effort to maintain balance; aIII detects cadence irregularities and aIV detects inefficiencies in gait which are sometimes related to imbalance. Quantitative balance measurements can be useful in monitoring illness, or the effectiveness of therapy. They can also be a research tool in biomechanics, human factors or behaviour studies. The evaluation of balance can be made under different cognitive conditions, by introducing dual tasks to help discern between apparently normal and truly normal balance [1].

Methods

A triaxial accelerometer was placed on the subject's back at the approximate height of the centre of mass. The sensor was attached to the body with a motorcyclist belt and levelled with respect to gravity. Data were recorded on a data logger worn around the neck. Nine normal subjects between 23 and 27 years of age volunteered. Besides walking with their usual gait, they were asked to walk with two balance challenging patterns: along a thin line -- reducing the base of support -- and walking with locked knees -- introducing a mechanical inefficiency and a greater difficulty to recover after a physical disturbance. All walks were also performed under dual task conditions for which the subjects were asked to subtract the number four, six or seven successively starting at 600 or 700. Twelve sets of data were gathered from each subject corresponding to two 35 m walking sequences under each of the six test conditions.

Results

Statistical tests showed the performance parameters can distinguish in a unique way between the different gait patterns tested. Statistical results pertaining to dual tasking are mostly not significant, as would be expected of normal individuals [2].

Discussion

Used together, the performance parameters can be useful in the evaluation of walking balance. The system is portable making long term measurements and measurements outside of the laboratory possible. Each subject acts as his or her own control. The instrumentation is inexpensive. The present test is easy to administer and easy to interpret.

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Influence of CM-46 on the behavior of rats in the open field

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In our experiments we used the new anxiolytic belonging to the merkaptobenzimidazol group (synthesized under the supervision of prof. S.B. Seredenin in the Institute of Pharmacology of Russian Academy of Medical Sciences) which does not possess side effects typical of benzodiazepin. We investigated the effect of CM-46 administered intraperitoneally to white male Wistar rats at the dose of 5 mg/kg of body weight diluted in 0.2 ml distilled water in "unescapable" stress situation (open field consisting of 25 squares 0.2 x 0.2 m). The observation was held for three minutes during three days: 24 hours before, 30 minutes after and 24 hours after CM-46 administration. We measured motor activity: vertical and horizontal (the number of squares passed in the central area and peripheral zone, the number of passes across the central area); time of freezing, grooming and orientation - searching activity (scenting the air in the corners of the open field). In comparison to control groups (1. without any treatment and 2. which received intraperitoneally 0.2 ml of distilled water), CM-46, 30 minutes after its administration shortened the time of the orientation - searching activity ($p < 0.05$). CM-46 at the dose of 5mg/kg did not change in a significant way the motor activity of Wistar rats in the open field test, however it, decreased the orientation - searching activity.

Cat odor avoidance: an animal model of anxiety

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Work over the past two decades has shown that rodents will exhibit strong defensive behavior in the presence of predatory odors [1, 2]. We have developed a new 'cat odor avoidance' apparatus with which to study this behavior in detail [3]. Briefly, rats are placed in chambers which comprise a rectangular arena with perspex walls. At one end of the chamber is a small wooden box termed the 'hide box'. On the front wall of the hide box is a small hole that allows just enough space for a rat (but not a cat) to enter the box. On the opposite wall to the hide box is placed a piece of a cat collar that has been worn by a domestic cat for a period of at least two weeks. Data for time spent hiding from and approaching the cat collar are collected automatically using photobeam detectors feeding a Macintosh computer running Workbench Mac software [4]. Rats show a robust avoidance response to the worn cat collar spending approximately 80% of their time in the hide box. This compares with only 20% hiding in rats that are exposed to a 'virgin' cat collar that has not been worn by a cat.

This response has been documented in rats of different strains including Lewis, Wistar and Hooded Wistar. The avoidance response is completely reversed by administration of the a low dose (0.375 mg/kg) of the anxiolytic drug midazolam. In contrast, the avoidance response is exaggerated in rats given anxiogenic drugs such as the cannabinoid agonist CP 55,940. The avoidance response may be age-dependent in as much as our recent work suggests that aged rats (120 days old) show a loss of avoidance relative to juvenile rats (30-60 days old). With repeated exposure to the odor, the avoidance behavior gradually dissipates. Work continues to determine whether the anxiety displayed by rats in the presence of cat odor maps onto human anxiety disorders such as specific phobia, panic disorder or generalized anxiety.

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Long term consequences of social conflict in rats: telemetric measurement of heart rate, body temperature and locomotor activity

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Social conflicts in rats induce acute autonomic nervous and neuroendocrine alterations which may be considered as a classical adaptive stress response. Besides acute and short-lasting effects, aggressive interactions sometimes result in behavioral and physiological changes that last up to weeks after the actual encounter, for instance, a suppression of explorative behavior and a reduction of the daily rhythm amplitude of activity and body temperature. Yet, these long-lasting consequences of a social conflict vary considerably between individuals.

In the present study we tried to correlate this variation to quantitative behavioral aspects of the interaction. Male wildtype rats were provided with telemetry transmitters for recording heart rate, body temperature and locomotor activity (TA11CTA-F40, Data Sciences, St. Paul, MN, USA). Heart rate and temperature were sampled for 10 sec every 10 min. Locomotor activity was recorded continuously and stored at 10-min intervals. For each individual, the daily amplitude of the rhythms of heart rate, temperature and activity were calculated as the difference between average 12h dark and 12h light values, i.e. values for circadian resting and activity phases. To assess the effects of social interactions, the daily amplitude after the tests were compared with the baseline amplitude before the interactions. The animals were confronted with a young male in their own territory for 10 consecutive days. The experimental animals systematically attacked and submitted the intruder and were clearly dominant in all the fights. These dominant-subordinate confrontations did not have any long-lasting effects on the daily rhythms of heart rate, temperature and activity. Seemingly a conflict does not have long-lasting consequences for the animal that is the aggressor and controls the fight.

Six days after the last of these dominant-subordinate interactions, the experimental animals were themselves introduced in the cage of another aggressive and pre-trained male. This confrontation between two highly aggressive animals resulted in fierce fighting and caused a strong decrease in the daily rhythm amplitude in the experimental animals. The effect lasted on average 2 weeks, however, with considerable variation between individuals. Some animals hardly showed any change while in others the rhythms only normalized after 3 weeks. The long term effects of the encounter did not seem to depend on the intensity of the conflict in terms amount of aggression received. The changes in rhythm amplitude did not correlate with the number of attacks received from the cage owner. Contrary to

this, the change in amplitude showed a clear negative correlation with the aggression of the experimental animals themselves. Animals fighting back and counter attacking the cage owner subsequently had less decrease in rhythm amplitude. In line with this, there was a negative correlation between the total time spent fighting and the subsequent decrease in rhythm amplitude.

The results support the idea that the long-term consequences of a social conflict do not depend so much on the intensity of the fight but, rather, on the controllability and outcome of the encounter.

Behavioral/neurophysiological test systems in marmoset monkeys

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The marmoset monkey is a small primate. This primate is used for developing certain disease models, e.g. Parkinson's disease, experimental autoimmune encephalitis and anxiety. To monitor the disease activity objectively and to have a quantitative read-out system for the effect of drug treatment, TNO Pharma has developed several read-out systems that have proven very useful in this respect:

1. A motor activity test (the bungalow test) has been developed that registers the free movement of the animal in a closed system existing of four compartments each of which is interconnected with three other compartments. The time an animal remains in one compartment and the number of compartment changes are automatically registered.
2. A hand-eye coordination test has been developed to register the learned behavior and motor activity. The learned behavior is the animal learns to grasp a sweet through one of two shutters in a panel that opens after a light signal has appeared above the shutter. A robot transports the sweet reward behind the shutter with different speed.
3. The startle reflex test (like in the rat) uses the pressure of the front and hind legs when the animal is exposed to a frightening event (a sound). If the startle stimulus is given within a period of fear, for instance when the animal is conditioned to show fear, startle response is clearly enhanced.
4. Neurophysiological parameters: Visual Evoked Responses and EEG. These were recorded using telemetric approach.

The application of those read-out systems in monitoring the development of symptoms of Parkinson's disease, experimental autoimmune encephalo-myelitis and anxiety will be shown.

The determination of body posture reveals behavioural lateralisation in zebrafish

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Cerebral cognitive lateralisation was once thought to be confined to humans. During the last 20 years it was shown that rats, chicks and other animals also exhibit cognitive lateralisation that is best displayed by their asymmetric perceptual processing of various classes of stimuli. If fish showed behavioural lateralisation, asymmetrical functioning could be an inherited feature of the vertebrate brain. We observed the body postures of zebrafish in the course of their approach to novel and familiar stimuli in a long runway. All trials were videotaped and analyzed later. A computer generated cursor was placed over the image of the fish on the video screen and the angle of vision in relation to the object was determined. Analysis was confined to the periods when the fish was within 25 cm or less of the end of the swimway at which the test object. Since fish have no mobile neck the position of the body also defines the position of the eyes that in turn define the viewing angle of the retina. Five such measurements were taken per 1 second. In the analysis postures were grouped into bins of 10 degrees. For the analysis of viewing corresponding right and left sectors were compared.

In most trials we found that body positions between 0-20 degrees and 170-180 degrees were the most common which suggests that these body positions allow particularly high acuity vision. Strange objects were viewed at first exposure chiefly with the right frontal field (at 0-20 degrees); so was a complex and unfamiliar scene made up of familiar components. In the second trial using the same stimulus or scene, left frontal viewing tended to be used. A familiar heterospecific fish was viewed left frontally.

We suppose that similarly to other vertebrates (e.g. chick) right eye is used when it is necessary to inhibit premature response and left is used when it is necessary to keep an eye on a familiar scene.

The design and performance of an implantable device for monitoring blood pressure, heart rate, and movement activity from conscious freely moving laboratory mice

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The impetus to develop a new miniature implantable telemetry product (figs. 1 and 2) and the challenges of development will be presented within the context of new growing market needs and new emerging industrial capabilities. Descriptions of challenges in the development process and the resulting solutions will be presented. The transitions from concept to invention to prototype to product will be discussed. Data and anecdotal experiences from early laboratory work and initial validations will be presented.

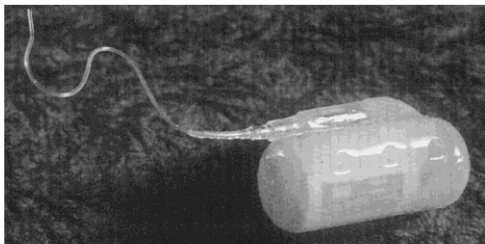


Figure 1. PA-C20 mouse blood pressure implant.

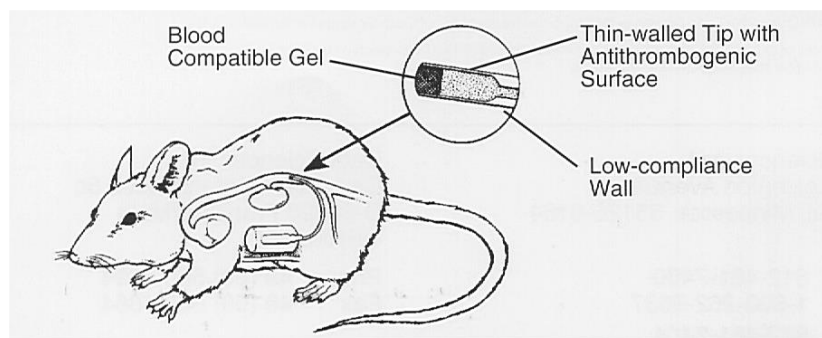


Figure 2. The PA-C20 is typically placed in the peritoneal cavity with the sensing catheter inserted into the lower abdominal aorta.

**Measurement of work load in realistic work situations:
an integrated approach on the basis of physiological and behavioral
measurements**

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Several studies have shown that the relation between task performance in normal daily work and task performance on simple laboratory tasks is not a simple one. The same holds for the physiological effects of long lasting work, i.e. several hours or a complete working day. Several reasons can be put forward for these differences, ranging from measurement restrictions in the field to qualitative and quantitative differences in task performance. This latter problem can be related to the way complex, composed tasks are executed as compared to simple laboratory tasks. It becomes clear that task performance has to be studied and modeled on more than one level: e.g. the strategic, tactical and executive level.

In order to increase the knowledge in this field a laboratory setup has been made in which a broad range of realistic work situations can be simulated, including cooperation between a number of colleagues [1]. The laboratory supports physiological and behavioral measurements and its analysis methods. In the present contribution attention will be paid to the goals of the system, the experimental setup and implementation of two initial task paradigms which represent both sides of the mentioned task spectrum. The first is a system for simulation of a calamity scenario in which a small team of people is cooperating in order to control the simulated calamity. The second example concerns the so-called 'synwork' task in which a number of (e.g. four) laboratory tasks (including aspects of memory load, focused and divided attention, mental arithmetic, etc.) have to be performed simultaneously. With this setup quality of multiple task performance will be studied in relation to changes in cardiovascular state during several hours of work. Both aspects will be shown in a demonstration during the conference [2].

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A test of lumped occurrence of patterns in sequences

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Many behaviors such as birdsong or play often are described and analyzed as sequences of discrete patterns which do not overlap in time. The most commonly used technique in analyzing such sequences is the application of a Markov chain model and analysis of first (or higher order) transition rates between patterns. Using this model requires that the sequence is stationary, i.e. that the probability of a specific pattern is, if at all, influenced only by the specific quality of the immediately preceding pattern(s) and does not show a general or temporary increase or decrease due to other factors. However, often probabilities of behaviors are not stationary, i.e. they change temporarily or in longer terms within sequences. Many species of the bird family Turdidae, for instance, tend to repeat a song type within the next few songs after it occurred once [1]. If such a tendency to repeat patterns within short intervals does not lead to an increased rate of immediate repetitions or recurrence after a specific interval, it may not be detected by analyzing transition rates. Thus, as an alternative approach for analyzing sequences with regard to a possible lumped occurrence of patterns of the same type, I suggest to analyze the distribution of intervals between those patterns. When patterns of the same type occur lumped within a sequence, the distribution of intervals between them should be biased towards shorter intervals.

For this purpose I developed a procedure which estimates the distribution of intervals between patterns of the same type, as expected under the assumption that the sequence is a random sequence. This procedure is based on a randomization technique. A visual comparison of this expected distribution and the distribution obtained from the original sequence allows a first decision whether or not intervals between patterns of the same type in the original sequence are biased towards shorter intervals. To test if this bias is significant I developed a second procedure, which is again based on a randomization technique. This procedure estimates whether or not the frequency of intervals being shorter than a certain value is significantly larger than expected under the null hypothesis. I tested several different sequences using the described procedure and found that it allowed to detect a lumped occurrence of patterns of the same type also in cases where common procedures such as the runs test or tests based on chi-square failed. On the poster I will present the procedure in general, an example of its application, and discuss the number of permutations needed for a valid result.

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Intonation patterns in autistic and non-autistic children

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An impairment in communication is a marked characteristic of people with autism. When speech does develop, it often sounds 'different' from the speech of non-autistic people. However, little is known about what exactly distinguishes the speech of people with autism from the speech of other people. Speech has several parameters like intonation, duration and intensity. These parameters of speech can be measured and diagnosed by specialized equipment. The present study examined the differences between speech of autistic children and non-autistic children. Speech of autistic children is often described in the literature as 'monotonous' and 'flat'. We therefore hypothesize that intonation is deviant in people with autism.

To investigate the difference in the speech of autistic and non-autistic children, a number of utterances from both groups were compared. The frequency characteristics of intonation patterns were measured using speech analysing software. The parameter of frequency was chosen for study since developmentally it appears to stabilize first. The present investigation focuses on simple declarative subject-verb-object utterances, produced spontaneously under controlled conditions. Frequency measurements were obtained using a pitch meter and oscillomink tracing. In a separate experiment trained human listeners were asked to evaluate the intonation in the utterances produced by autistic and non-autistic children. Their task was to label utterances as deviant or not. The same utterances from both groups of children that were diagnosed by the speech analysing software were diagnosed by trained people. To make sure these people were not distracted by the meaning of the utterances themselves, the utterances were resynthesized keeping the original intonation, while converting meaningful sounds to nonsense syllables. During presentation of the stimulus the utterance was written on a computer-screen. In this way we were ensured that the listeners knew which utterance contour to expect, while not being distracted by other characteristics than intonation. The results of both experiments will be discussed.

Test methods for the analysis of sexual behavior in male rats

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Background

Many psychopharmacological drugs have sexual side effects. Thus: up to 75% of all patients treated with selective serotonin reuptake inhibitors (SSRIs) complain of reduced libido, reduced (or lack of) ability to reach orgasm etc. In males, the latter is associated with prolonged ejaculation latency.

Aim

The purpose of this study was to establish a model of male rat sexual behavior, in order to screen psychopharmacological drugs with regard to sexual side effects.

Methods

Sexually active male rats were treated with paroxetine (a SSRI) for 4 weeks, and their sexual behavior was quantified. Both precopulatory and copulatory behavioral parameters were measured once a week. All behavioral test sessions were recorded on video tapes and later scored using a simple event recorder program for PCs. The program was able to score number of events, latency times, and duration - all by pressing pre-assigned keyboard keys.

In the preference test the male rat could choose between proximity to a female rat in heat or one not in heat. This was done in a test chamber (50x50cm) with two side chambers attached. Side chambers contained incentive animals separated from the male test animal by grids. Video recording was done dorsally. Number of visits to each proximity zone was measured as well as total duration of visits in each zone. On this basis the time spent per visit in each zone and time ratios of preference were calculated.

Copulatory activity and efficiency of the males was measured as well. Male test animals were habituated to the test chamber (50x50cm) and then introduced to a female rat that had been brought in heat and tested for appropriate sexual behavior. Tests began with introduction of female rat and ended with first mount of the second copulatory series. Video recording was done laterally. The number of mounts and intromissions were measured as well as latency times for the first mount, first intromission and ejaculation of the first copulatory series and latency time for the first intromission of the second copulatory series. In addition to these data the duration of the post-ejaculatory interval (PEI) was calculated.

Discussion

The sexual behavior of the male rat is highly stereotyped, readily elicited in the laboratory, consistent in an individual over time, and sensitive to experimental intervention. At the same time it is a non-invasive model of emotionality using a naturally occurring behavior. This model has many advantages and could be used in a variety of ways.

Living together - feeding apart: how to measure individual food consumption in a social species

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In ethological as well as in physiological research it is of interest to measure the amount of food consumed by an individual. While this is an easy task in animals kept solitarily it often is problematic in species kept in groups. Here, individual food consumption typically can only be measured with some interference from the experimenter and thus disturbance of the animals. To investigate the Behaviour and the maternal investment of communally nursing house mice we developed an automatic device that allows to individually control and measure the food intake of female mice kept in groups. For this purpose we use an automatic identification system that triggers an individual's access to a feeding station. For identification we use subcutaneously implanted microchips (transponder) with a diameter of 2.1 mm and a length of 11 mm. An electromagnetic field at the entrance of the feeding station, tuned to the transponders' frequency, allows to discriminate between individuals according to their microchip. The feeding device consists of a movable hollow metal arm filled with commercial food pellets. In case of correct identification, the metal arm will be moved by a little servomotor in close contact to the lid of the cage, so that the animal can feed on the pellets as long as it remains in the feeding station. We not only use this device to quantify food consumption but also to individually limit the daily amount of food available.

This fairly simple method of identifying animals with passive transponders can also be used to control individual access to other resources like nesting sites or mating partners. With the help of several such reading devices it is also possible to register the movements of individuals in a seminatural enclosure.

Measuring behavior in virtual environments

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Observing human behavior

Structured observation of human behavior is a widely accepted method to gather information about how people perform a task or interact with each other. It results in time-based data suitable for quantitative analysis. Direct observation or computer-aided analysis of video recordings have become standard practice among researchers of human behavior. However, these methods fall short if one wishes to measure someone's behavior under circumstances that are not available 'live' or cannot be reproduced in front of a video camera. A good example is the interaction between an operator and a machine that has yet to be built. The designer wants to deliver a machine with an optimal 'user interface', but how can one evaluate its ergonomics if the machine does not yet exist? A technical drawing of the machine is usually too abstract for this purpose and is not easily interpreted by the future user. However, a usability test of the completed product, followed by redesign and adaptation, is a very expensive and unattractive option. The same applies to the construction of scale models, the construction of which is very time-consuming and costly.

Virtual Reality

As an alternative for physical mockups, most designers nowadays use 3D drawing programs to visualize their concepts. Abstract 2D construction drawings are thus converted into realistic images. Although such visualizations give a good impression of what the end product or environment will look like, they don't show if one will manage to use the product or navigate through the environment, because there is no *interaction* between the user and the visualization. The limitations of conventional 3D visualization tools can be overcome by Virtual Reality (VR) technology. With VR technology, abstract information about a design and possible alternatives can be turned into a virtual environment in which a person can navigate and interact with objects or other persons. Using VR simulation, virtual prototypes of designs can be built in a relatively short time. This enables one to discover design flaws and generate new ideas at an early stage. However, most VR systems have had limited use for ergonomics research because there is no record of where the user has been in the virtual world or what the user has seen and done.

Virtual Observer

To bridge the gap between VR modeling on the one hand and behavioral observation and analysis on the other hand, we have developed the “Virtual Observer”. This is a real-time 3D simulation of one or multiple objects, actors and actions within a simulated 3D environment on a computer. The environment can be displayed on a standard computer screen, the display of a VR helmet, or even a room-size 3D ‘cave’. The user interacts with the virtual world using a computer mouse, joystick or ‘data glove’. While the user is navigating through this environment, all sorts of events may occur: an alarm goes off, a light goes on, the user walks in a certain direction, enters a room, presses a button, grabs an object, etc. The Virtual Observer can log all these events together with the corresponding visual scene in a format suitable for subsequent replay and quantitative analysis. During a simulation, events are automatically recorded and written to a log file. Besides this, a digital video file can be generated with user-defined ‘camera viewpoint’ for playback and review of the simulation. The exact synchronization between the events in the log file and the corresponding video frames in the video file provides a direct link to the review and analysis options present in The Observer Video-Pro. One can calculate latency, frequency and duration of behaviors, analyze the sequential structure of the behavioral process, or graph events of interest in a time-event plot. In addition, search and review functions help the user to produce a data summary or a highlight compilation of the movie file which facilitates the presentation of findings to colleagues, test subjects or clients.

Applications

Virtual Observer is especially suited for observational studies in situations that are too expensive or time-consuming to be reproduced in the real world, or in situations that are potentially harmful for the persons involved. Examples of applications are work place design, simulation of handicaps, training operators on new machines, and assessing skills of job applicants.

Measuring work load in real working environments: the marriage between behavioral and psychophysiological data

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In the study of man at work several indices can be used to identify loading aspects of task performance. Some researchers emphasize the analysis of human behavior and production figures while others stress the measurement of (psycho)physiological data. In this presentation the necessity of an integration of both types of indices will be put forward and will be illustrated by the results of some field studies. These results indicate that the combined analysis of cardiovascular indices and observational data is useful in identifying mental loading aspects during task performance. Especially, changes in heart rate are sensitive to changes in workload. The integrated analysis seems a fruitful approach in the study of employees working in an office environment and could be used in usability tests.

The presentation during the conference will consist of a demonstration of the program CAMERA+, designed to integrate and analyze both observational and physiological data.

Studying foraging behavior of desert gerbils: an individual-based approach using thermal imaging

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Coexistence between two psammophilous, nocturnal gerbil species in the Negev is based on environmental heterogeneity and a trade-off between the dominance of *Gerbillus pyramidum* and the foraging efficiency of *Gerbillus allenbyi*. We studied the effect of changing population density on individual foraging behavior to understand mechanisms underlying population and community patterns. We used thermal imaging cameras to record gerbil foraging behavior in artificial resource patches placed in two 1 ha plots enclosed with rodent-proof fences. Seed harvest rate by both species was lower at high population densities, but total foraging time was not affected by density. This decline in per capita harvest rate was positively correlated with the increasing number of aggressive interactions among gerbils at high densities. Body mass change during the experiments varied greatly among individuals and was correlated with the individual's ability to monopolize resource patches. In 70% of our observations, males dominated resource patches and gained in body mass, while females lost body mass. Within sexes, small individuals gained relatively more mass than did large ones. This observed variability in relative mass gain and resource monopolization provides a quantitative behavioral perspective to the previously described population and community patterns such as temporal and spatial segregation.

Alcohol consumption in vervet monkeys: biological correlates and factor analysis of behavioral patterns

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We have previously reported that approximately 20% of socially-housed vervet monkeys (*C. aethiops* St Kitts) will voluntarily consume beverage alcohol to excess (>8 g/kg/day) and that nearly 80% of animals will drink smaller amounts of alcohol in the absence of behavioral or dietary coercion. In the present project, we probed the patterns of consumption in a population sample of over 600 animals tested under alternative conditions. For quantitative evaluation of alcohol consumption, animals are individually housed and tested in a two-bottle choice paradigm (10% ethanol in vehicle vs. vehicle alone), with variation of the duration of ethanol exposure, and/or the time of day of exposure, and/or the vehicle (tap water vs. sweetened tap water). The period of ethanol exposure ranges from one to four weeks for a given condition. Data reduction produced three measures for each condition: g ethanol consumed /kg body weight/day, proportion of fluid consumed as alcohol, and proportional change in alcohol consumed over the test period.

Using this repeated measure paradigm, alcohol consumption is remarkably stable in adult animals ($r > 0.85$) tested two or more times under standard conditions (tap water, 24 h access in week 1, 4 h access in week 2) at intervals of 6-12 months. There is substantial individual variability with respect to consumption of alcohol vs. sweet, ratio of alcohol to vehicle, preferred vehicle, time of day, etc. Patterns of alcohol consumption were extracted from a principle components analysis of over 2000 trials performed under standardized conditions, with gender, age and 3 dependent measures for each test condition entered into the equation. Four factors were extracted, corresponding to (1) abusive binge drinking, (2) heavy steady drinking, (3) moderate drinking, and (4) avoidance of drinking. Both abusive and heavy steady drinkers consume > 5 g/kg/day, but abusive drinkers drink rapidly to intoxication or coma, sometimes repeatedly within a single 24 h period. Abusive drinkers also exhibit a significantly greater alcohol:water preference ratio under scheduled access drinking and can be distinguished pharmacologically from heavy social drinkers. Moderate drinkers are more likely to be female, to prefer alcohol in sweetened fluid, and to drink little in the morning. The similarity of this solution to the human condition is striking.

More recently, we have explored the biochemical and pharmacological correlates which might distinguish abusive drinkers from heavy social drinkers. Heavy social drinkers, as a group, show exaggerated lymphocyte signal transduction, as compared to non-drinkers, while abusive drinkers show a suppressed pattern of signal transduction. Acute exposure to beverage alcohol elevates CSF levels of the serotonin metabolite 5-HIAA and the dopamine metabolite HVA in all individuals, but the magnitude of increase does not predict alcohol preference. Basal levels of 5-HIAA are not correlated with the quantity of alcohol consumed, but abusive drinkers typically display 5-HIAA levels in the lower 2 deciles of the population distribution. Naltrexone reduces alcohol consumption in heavy social drinkers, but is marginally effective in abusive drinkers. Although our own primary interest is to determine the genetic factors underlying these different patterns of alcohol consumption in vervet monkeys, the potential utility of this population to investigations of mechanisms of the gene-environment interactions in this and other addictive disorders is obvious.

Supported by the Medical Research Council of Canada and Behavioral Sciences Foundation, St. Kitts.

Continuous time Markov chain models: a useful tool in understanding behavioral patterns

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Most behavioral experiments, involving direct observations of sequences of events and the time at which they occur, require detailed time records of behavior. Statistical methods primarily designed to analyze the time structure of behavior give information about effects of the investigated factors on the time structure providing better insight into the behavioral processes. The continuous time Markov chain models are the simplest way to describe the time structure. The parameters of the model represent behavioral tendencies to switch between acts and to start and stop activities, and subsume the information of both the duration and the alternation of each act providing a complete summary of the behavioral pattern. The transition rate, $l(AB)$, from a behavior (A) to another (B) represents the chance per time unit that one behavior is followed by another, so it can be interpreted as the priority of the following behavior while the animal performs the preceding one. The termination rate of state A, $l(A)$, is the chance per unit of time that the item A is interrupted and hence any other item may be shown. The transition probability $P(AB)$ from A to B is the probability to switch from A to B. The relation between these parameters is: $P(AB)=l(AB)/l(A)$. The maximum likelihood estimators are: $P'(AB)=N(AB)/N(A)$, $l'(A)=N(A)/S(A)$, $l'(AB)=N(AB)/S(A)$ where $N(AB)$ denotes the number of observed transitions from A to B, $N(A)$ the number of bouts of A, and $S(A)$ the time spent in A. By analyzing the transition rates, which contain all the information concerning distributions of the sojourn times and the probabilities of alternations between acts, one can detect, for example, whether an increase in time spent on behavior is due to an increase in the tendency to start the behavior or to a decrease in the tendency to stop the behavior. Such distinctions, which are lost when analyzing only frequency and duration, are obviously important. These methods have been applied to assess the effect of a toxicant (low (L) and high (H) concentrations) on social-aggressive interaction in adult mice, undergoing a 20-min agonistic encounter with a standard opponent.

Behavior was videotaped, and scored using a keyboard event recorder system (The Observer). The scored items were: Attack (ATT), Chase (CHA), Aggressive grooming (AGR), Tail rattling (TRA), Offensive upright posture (OFF), Defensive upright posture (DEF), Submissive posture (SPO), Freezing (FRE), Escape (ESC) and Other (OTH). Non-parametric analysis of variance highlighted significantly higher duration and frequency of ATT in L- than in H-mice. The analysis of the

behavioral sequences showed that the concentration modifies also the behavioral patterns of ATT: H-mice less frequently showed both TRA and OFF after an episode of ATT. Also the transitions rates from ATT to both TRA and OFF were lower in the H-mice. Moreover, H-mice showed significantly lower probability and tendency to switch between CHA and ATT, and lower probabilities to switch both from DEF to OTH, and from OTH to ATT. As shown above, the analysis of sequence gives a detailed description of the behavior allowing us to better understand changes in behavioral processes due to treatment.

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SDIS-GSEQ 2.0: software for the analysis of behavior sequences

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SDIS-GSEQ 2.0 is a general-purpose software for representing, analyzing, recording, and plotting behavioral sequences, a kind of data commonly used in a variety of research fields, such as the study of interaction in clinical and educational settings, social behavior in animals, communication processes, children's play, and so on. All these phenomena have a prominent feature: the process under study unfolds in time and can be observed objectively and systematically. SDIS-GSEQ is an essential toolbox for performing sequential and synchronic analysis of sequences of concurrent and non-concurrent behavioral codes.

The SDIS data language [1, 2] provides a common framework for representing sequential data, and recognizes five different sequential data types: Events, States, Timed events, Intervals and Multi-events, the last four permitting code co-occurrence. This is especially useful for observational researchers, because standard statistical packages are not suited for analyzing the kind of sequences they obtain. The programs are designed for analyzing behavior sequences in a flexible and general way: data can be obtained by direct observation of dyads, groups, or even single individuals, either in experimental or non experimental settings, as SDIS permits representation of design variables.

A variety of analyses are possible with the GSEQ program: data can simply be described, obtaining measures like frequency, duration, rate, relative frequency, average duration, etc., for every behavioral code in the data. Lag sequential and co-occurrence analyses can be carried out by defining contingency tables for given and target codes, specifying lags, and requesting sequential statistics, like chi-square, adjusted residuals, or Yule's Q. Users have complete control over which codes are included in the sequential tables and how they must be analyzed. Moreover, they can request that results are provided for pooled combinations of design variables. GSEQ permits also several interesting data modifications, like RECODE and WINDOW, which create new codes that can be subsequently analyzed using sequential techniques. New, more global codes can be created from the existing ones using RECODE or LUMP, for example. In State, Timed event, Interval, and Multi-event data, the WINDOW command is specially useful for defining new codes linked to onsets and offsets of the existing ones; then, the new codes can be included in subsequent lag 0 (or co-occurrence) analyses in order to detect, for

example, possible sequential associations between a certain given code and some pre-post periods of some target code.

A first version of the programs was published by Bakeman & Quera [2]. Current version 2.0 includes a new data type (Multi-event), enhanced features in the SDIS language, more result exportation capabilities (into SPSS, BMDP, SAS, ILOG or Excel formats), and a new and enhanced user interface with a menu-driven command composer. The programs run in MS-DOS or as a DOS program under Windows 3.1 or Windows 95.

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P-SPACE: a program for simulating spatial behavior

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A computer program that simulates proxemic behavior in a group of individuals is presented. This software implements a migration model [1, 3] based in cellular automata theory. We make a distinction between a micro level of agent behavior and macro level with macro effects. Our objective is to describe and understand the principles of proxemic behavior, group interaction and social dynamics by investigating micro level features. Of course, empirical data will be necessary in the future to validate our model. Agents or individuals are located in a two-dimensional space or lattice, usually a rectangle-shaped one, their initial positions being either random or fixed. Our program uses integer units to represent both the time and space dimensions. Thus, every agent can move one space unit at each discrete time unit or iteration within its Moore neighborhood [2]. A set of valences for each pair of agents is specified per simulation. Valences are represented by desired (or ideal) distances in a non-symmetrical matrix. Positive and negative valences correspond to distances that are short or large relative to lattice size, respectively, while neutral valences are represented by non specification of an ideal distance. Agents move according to an optimization criterion, which consists in minimizing an objective function of the discrepancy between possible future distances (as computed from available positions in the agent's Moore neighborhood) and ideal distances among agents. At each iteration, agents will move to a cell in a current neighborhood for which the function is minimized. Depending on the specific values in the non-symmetrical matrix, different kinds of social dynamics or proxemic behavior can be obtained. Users can control the dynamics of the process by specifying either constant or variable valences. Valences can be made to vary as a function of time or as a function of proximity.

The P-SPACE program (Proxemic Space) was developed in C language and runs in MS-DOS. An external file is used to specify simulation parameters: Number of agents and objects, lattice dimension, initial agent positions, fixed object positions, number of iterations, valences, etc. The program generates graphical and ASCII output. The former permits visual inspection of proxemic dynamics and the latter allows numerical information to be read by standard statistical packages or spreadsheets.

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Integrated measurement and analysis of testosterone levels, dominance hierarchy and mate attraction behavior in captive male European starlings

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Most studies carried out on birds have shown that sexual as well as social behavioral repertoires in males are under strong control by gonadal secretions, most notably testosterone. The purpose of our research is to elucidate the effects of testosterone on the dominance hierarchy and mate attraction behavior in captive male European starlings (*Sturnus vulgaris*). For this purpose, we used the 'phenotypic engineering method'. This means that we create novel phenotypes by manipulating the testosterone concentrations of the birds and then we compare the behavior of altered and unaltered individuals.

In our study, we used two ways to manipulate the plasma concentrations of testosterone. On the one hand, we castrated starlings and on the other hand we experimentally elevated testosterone levels by giving males implants of silastic tubing, packed with testosterone. Castrated males, implanted males and control males were placed together in an outdoor aviary to measure dominance. We observed the birds and we recorded every interaction. To evoke aggressive interactions between the starlings we took away the food a day before observation to make the birds very hungry during the observation period. Based on these observations we determined a dominance matrix which shows for each individual the total number of victories and defeats. Then we divided the total number of winning interactions by the total number of interactions that an individual participated in, to calculate the dominance index. Using this dominance index, we could determine the dominance hierarchy and dominance positions.

Testing the assumptions with a two-way ANOVA, it became clear that implanted males were dominant over castrated and control males. After removing the implants, we again determined the dominance hierarchy. Now, males that had previously been implanted, no longer had a higher dominance position than castrated and control males. In contrast to control males, castrated males did not show mate attraction and song behavior in the presence of a prospecting female. However, these behaviors were reinstated after the castrated males were treated with testosterone implants. We found this by observing a group of castrated males and a group of control males. Every minute, we recorded which males were singing and which were occupying a nest box. After some time we treated half of the castrated starlings with testosterone implants and we remeasured song activity and nest box occupation. For each individual we measured the proportion of time a male sang or occupied a nest box as an indication for his song activity and the

time he spends in a nest box. Statistical analysis of our data was carried out using SAS.

Classification of rat behavior by a neural network

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The limited human capabilities to recognize and record behaviors and disagreement between observers on the delineation of behavioral categories have prompted the development of automated behavior registration methods. The use of image processing software and hardware to quantify the locomotion of animals in various experimental settings is becoming a standard method in biomedical research. In this study an attempt is made to qualify the on-going behavior of rats from digital images with the postures of the rats. At a certain conceptual level behavior can be considered as a continuous stream of postures while the transition from one posture to the next marks the onset of a new behavior.

Video recordings were made of 12 male Wistar rats placed on a black rectangular platform. Using the EthoVision[®] system, the recordings were converted to digital movies of 8 minutes duration each at a sampling rate of 5 frames per second. The behaviors were classified from the video recordings by two experienced observers according to the following ethogram: walk, sit, groom, rear, hunch, head dip, head raise, rotate and stretched attend. A third observer reviewed the digital movies frame-by-frame and classified the posture of the rats in each of the 28800 video frames. In figure 1, typical examples of some of the behavioral categories are displayed.

Various parameters describing the shape of the rat were calculated from the digital movies by a special-purpose image analysis program. These parameters were normalized and the data from 3 consecutive frames was used as input parameters for a neural network. We chose a neural network as a classification paradigm because of its ability to classify complex fuzzy data and because no presumptions have to be made about the underlying structure of the classes contained in the data. The data was split into a training set on which the neural network was trained and a validation set with which performance of the neural network was evaluated. A standard back propagation network with a momentum term and 120 hidden neurons was trained with the training set. After training, the network correctly classified 84.85% of the behaviors in the combined training and validation sets. This reliability was comparable to the inter-observer reliability between the two human observers.

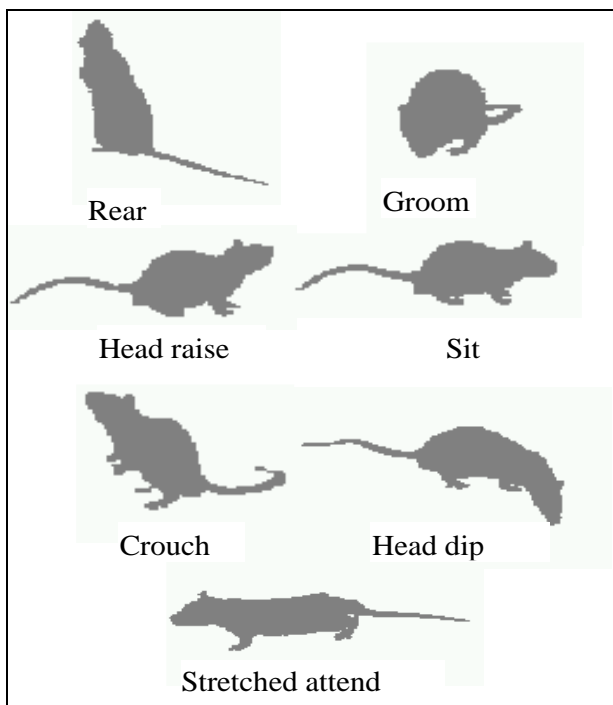


Figure 1. Typical examples of the postures corresponding to the behavioral classes used to train the neural network. Classes which consist of a number of consecutive postures, as walking and rotate, are not displayed.

The classification of rat behavior using image processing techniques and neural networks seems to offer an important additional tool potentially circumnavigating a large number of shortcomings of human observations. The application of this technique to a number of standard behavioral tests must be investigated further.

Financial support was received from Eureka project no. EU88011.

A comparison of various analysis parameters of locomotion patterns of rats in an open field

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Over the years numerous automated registration methods have been developed to quantify locomotory behavior of rats in open fields. These include infrared beam detectors, capacitance plates, balance systems and video systems. The X,Y-coordinates representing the location of the animal at a certain time point have been used to calculate various parameters to quantify and qualify the locomotory pattern. The parameters quantifying the pattern of movement must meet a number of criteria. They must be independent of various aspect of the experimental setup: sampling frequency, spatial resolution of the recording device, shape of the arena, size of animal relative to the arena.

Various parameters obtained by the registration systems described above, have been reported on in literature. These parameters include distance moved, time in zones of the open field, speed of movement and parameters describing the heading of the animal. More complex parameters include fractal dimension, parallelism index [1], temporal and spatial scaling [5], gamma parameter based on a discrete Markov model [4] and various methods to determine the 'home base' of an animal [2, 3]. These movement parameters can distinguish the effects of various physiological and behavioral manipulations. Generally these kind of parameters are validated with a number of drugs which modify locomotion, such as amphetamine. The analysis of the open field test is performed in different ways and with different parameters. Some of the conflicting results in the literature could be accounted for by these differences. The effect of these parameters on locomotion in an open field was assessed through a meta-analysis of data from a large number of experiments conducted over the last years at our laboratory. The drugs used in the experiments include NMDA, morphine and PTZ.

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Graze: a program to analyse recordings of the jaw movements of ruminants

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“Graze” is a user-friendly, Microsoft Windows 95/NT program for analysing jaw movement recordings taken with the “IGER Behaviour Recorder” [2, 3]. The program displays a plot of the jaw movement amplitude (vertical axis) against time (horizontal axis). Various scales for the horizontal (time) axis can be selected, allowing the user to zoom in and out of the recording. Individual jaw movements can be automatically identified using amplitude and frequency criteria specified by the user. Bouts of jaw movements can then be analysed, and are automatically identified as either grazing or ruminating. If, when processing a bout, the program fails to recognise any jaw movement patterns associated with eating and ruminating, the bout is marked as undetermined. Identified jaw movements and bouts of behaviour are superimposed on the jaw data display. This allows the user to check that the program has correctly identified both jaw movements and bout behaviours. Jaw movement identification criteria can then be changed, if required, to improve the accuracy of automatic identification. Individual bouts of behaviour can also be edited by the user, either to correct any mistakes in the automatic interpretation, or to remove any bouts which are not of interest to the user e.g. because they are outside the experimental measurement period. Behaviours other than eating and ruminating (such as 'drinking' or 'eating supplements') can also be identified and marked by the user. The bout information can be saved in a separate Bout Mark file. This file can then be read back into 'Graze' should the user need to re-analyse a file subsequently. Finally, the user can perform a 'bout analysis', which discriminates between bites and chews during eating, and generates a 'Results' file. This file summarises information for each bout of behaviour, and can be saved as either a text file or as a 'CSV' ('comma separated values') file that can be read directly into a spreadsheet (e.g. Microsoft Excel).

The identification algorithm [4] was developed for and has been validated for use with cattle jaw movement recordings [1]. Initial results suggest that the cattle analysis algorithm can also be used with sheep recordings, although this has yet to be fully validated.

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Measurement of behavioral sleep signs in honeybees with a custom designed video interface

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At present the honeybee is one of the very few invertebrates in which sleep-like behavior, similar in many respects to mammalian sleep, is known to exist [1, 2]. We now describe a new video interface which allows behavioral investigations of the microstructure of sleep in honeybees. The method permits contactless measurements of position and motion of parts of the body and the appendages with high accuracy and high temporal resolution (max. 50 measurements per s).

The video interface was developed in collaboration with the Department of Electrical Engineering and Information Technology of our university¹. The device scans contours by insertion of 4 measuring lines (2 horizontal and 2 vertical lines) into a video image. Starting at a certain point of the video image, the measuring line stops at a point which has a previously defined contrast with the background. The positions of the measuring lines on the video image as well as their starting points and the contrast thresholds of the contours can be chosen freely. During each half-frame, the lengths of the 4 measuring lines are determined. The measured values are available in analog (voltage) as well as in digital form at the corresponding output. It is possible to transfer either the coordinates of the starting points of the measuring lines or the measured values (lengths of the measuring lines; for each measuring line the range is between 0 and 255 pixels) to a computer. For both the display of the coordinates and the data logging we have developed suitable software. The video interface can be used during an experiment (on-line operation) as well as for later evaluation of video tapes (off-line operation).

For data processing and the subsequent time-series analysis with appropriate mathematical procedures we have developed a whole software package which allows treatment of the raw data in manifold ways. For example, one of these programs categorizes antennal motility, whereas another program determines the start and finish of ventilatory episodes. The video interface has already been used successfully for the analysis of a large number of video tapes. These video tapes were obtained during investigations of the nightly behavior of honeybees [3, 4, 5, 6].

¹ Our thanks to Prof. W. Zschunke and his team.

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Analysis of behavioral rhythms in free ranging animals

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The storage telemetry system ETHOSYS has been applied to ruminant and non-ruminant species in close to natural conditions. It was designed for automatic recording of different behaviors, such as motor activity and feeding [3]. Species-specific activity patterns became visible. They show a high number of short activity peaks in roe deer, a lower number of peaks in red deer and mouflon and very long peaks in Przewalski horses. These patterns can be explained by the different nutritional strategies of the species. In red deer, the ultradian rhythms differ seasonally reflecting the variable feeding strategy of an intermediate feeding type species [2].

In Przewalski horses the frequencies of the ultradian rhythms are nearly unchanged in course of the year. However, Przewalski horses change the daily level of activity and feeding throughout the year, depending on the feeding conditions. A stable ultradian structure of behavior became obvious especially for ruminants. A more unstable, adaptive time pattern was found in Przewalski horses [1]. Activity as a multi-purpose behavior was generally more variable than feeding which, in most cases, had a clearly rhythmic and harmonic structure. A high percentage of activity periods harmonic to the 24-hour-rhythm seems to be characteristic for a healthy and undisturbed individual. In several occasions, external disturbances or social stress resulted in a substantial decrease of this percentage. A measure was developed based on the power spectral analysis to quantify the functional frequency order of circadian and ultradian rhythms. It could be used to determine the influence of hunting and shooting, low social state and human activity.

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Animal alternatives in teaching and research

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The concept of alternatives includes not only replacement, but also reduction and refinement of the current practice in animal experimentation. Despite the fact that legislation requires the consideration of alternatives for use of animals in teaching and research, the implementation of existing alternatives is often seriously hampered. This seems to be due to two important factors. First, many alternatives do not properly fulfill the educational and research objectives. Secondly, both teachers and researchers often do not have a positive attitude towards alternatives. With these two factors in mind, the Department of Animal Physiology has been working on the introduction and use of alternatives for many years. One example of our approach, which strongly contributes to attitude building, will be presented.

In the curriculum of biomedical students, we make a careful transition from replacement in the first and second year towards refinement and reduction in the later phases of specialization. Replacement of animal experiments during practical courses is achieved by using computer-assisted interactive video disc programs. We currently have teaching programs on heart physiology, muscle physiology, blood-pressure regulation and embryology. The basic principle of these programs is the true replacement of experiments. This means that the student has to select the proper research question and the accompanying experimental design. The student is then faced with the fact that surgery has to be performed before the experiment, anesthesia, recovery, the condition of the animal before the experiment starts, etc. Moreover, after the particular experiment is shown on the computer screen, the student has to analyze the recordings himself and feed the results and conclusions into the computer before he can continue with the next question. Questionnaires on the efficacy of such alternatives show that they fulfill most of the educational objectives as adequately as a live experiment.

However, interactive video programs are insufficient to replace practice skills and hands-on experience with living animals. These skills become increasingly important for those students who want to specialize as biomedical researcher. These students should also become familiar with the possibilities for reduction and refinement. One of our ways to achieve these objectives is by using radiotelemetry techniques allowing continuous recording of heart rate, blood pressure, body temperature and activity in freely moving rats for a period of half a year. The use of this refined technique allows not only practical experience with animal experimentation on certain physiological principles with a limited number of

animals, but gives the student at the same time direct insight into the stress-physiological consequences of his actions. Such a gradual build-up from replacement to refinement in the course of the specialization of biomedical research students should form the basis of a new generation of researchers with a proper attitude towards alternatives.

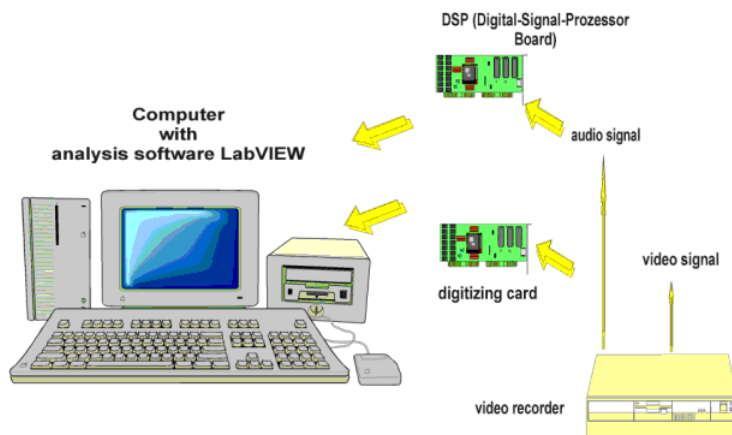
A sound analysis system based on LabVIEW® used to characterise domestic pig (*Sus scrofa*) vocalisations

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A sound analysis system based on the program development environment LabVIEW is presented and its capacities are demonstrated using suckling grunts and stress calls of domestic pigs (*Sus scrofa*) as examples.

LabVIEW represents a versatile tool for the development of sound analysis software including sound processing and sound statistics. Particularly, the increasing need for flexible numerical processing of different acoustic parameters can be considered. LabVIEW offers a wide range of opportunities both for conventional processing and for the development of new applications in bioacoustical analysis. The graphical object oriented language allows programming without special experience because it uses terminology, icons, and theories familiar to scientists and engineers. LabVIEW is available for Macintosh, Sun, HP-UX, Windows 95, Windows NT and Windows 3.1. It can also be used as a teaching tool to gain technical knowledge on signal analysis and signal processing.



Paralleled by behavioural observations, the bioacoustical studies of animals at various levels including the incorporation of techniques that are used in human

speech recognition or in technical acoustics are able to reveal the specific meaning of vocalisations. The video signal of the behaviour and the audio signal of the call are fed in parallel to the analysis system and displayed online on the computer screen. The analysis tools for data acquisition and data pre-processing allow the precise separation of the decisive parts of the animal vocalisation for detailed investigation.

For more information please refer to web page
<http://www.fbn-dummerstorf.de/fb5/schoen/bioacous.htm#Bioacoustic>.

Comparing inbred mouse strains: integration of behavioral and neurochemical assessments

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Comparing different inbred strains is a common neurogenetic approach to identify gene-dependent behavioral as well as neural variables. Such comparisons require detailed knowledge about the behavioral and physiological characteristics of such strains. Here, we present data which address the research methodology with which (a) differences between mouse strains can be detected and quantified at the behavioral level, and (b) can be related to neural variables. These data are based on a complex and integrative analysis of male and female adult mice from the C57Bl/6 and Balb/c strain. Behavioral testing included open-field and plus-maze behaviour, inhibitory avoidance, and several tests of motor coordination (i.e. rotarod, grip- and pole-test). Furthermore, lesions with MPTP were performed, a neurotoxin which is used to destroy the nigrostriatal pathway and which serves as an experimental model for Parkinson's disease. In addition to the behavioral analyses, the contents of dopamine, norepinephrine, and serotonin as well as their metabolites were determined in the neostriatum and ventral striatum using a post mortem analysis with HPLC.

Our analyses provide a number of indications for strain- and sex-dependent differences. Behaviorally, C57Bl/6 mice were more active in the open-field than Balb/c; C57Bl/6 females showed the highest levels of activity, but also the strongest habituation. Further quantitative and qualitative behavioral strain differences were observed in plus-maze behaviour, inhibitory avoidance, rotarod, and the grip- and pole-test. The occurrence of such strain differences were found to be affected by environmental factors, such as the lighting conditions. The behavioral differences were paralleled by neurochemical differences, especially of dopamine activity in the neostriatum. Treatment with MPTP proved to reduce locomotor activity and movement coordination in both strains, leading to different patterns of recovery. Here, open-field behavior and the pole-test proved to serve as sensitive indicators. Neurochemically, MPTP caused depletions of neostriatal dopamine levels in both strains and sexes; this effect was more pronounced in C57Bl/6 mice.

Our findings confirm the critical role of dopamine for locomotor and exploratory behavior. Regarding behavioral analysis, they indicate the need to carefully control environmental variables, such as light. Furthermore, they provide an extended set of data on two common inbred strains. These informations can, for instance, be used to choose appropriate strains, behavioral measurements and testing conditions in future neurogenetic models.

Video tracking of social behaviors and telemetered ECG measurements in colony housed rats

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In mixed-sex rat groups housed in semi-natural conditions, a clear hierarchical organization is usually present, with one dominant male, socially active subdominant males and subordinate animals. The present paper reports preliminary data on the relationship between social rank in wild-type rats housed in colony conditions and cardiac sympatho/vagal balance in baseline and challenging conditions. Social structure was determined using advanced image analysis techniques (EthoVision®, Noldus Information Technology b.v.) and cardiac electrical activity recorded by means of a radiotelemetry system (transmitters manufactured by Data Sciences International and receivers by the Department of Electronic Engineering, Biological Center, University of Groningen).

Four colonies were studied, each composed of 4 males and 4 females housed in large cages (120x150x200 cm). Experimental males (n=16) were provided with a colored perspex cap on top of the skull for automated imaging recording and with an intraperitoneal transmitter for ECG recording. After 2 weeks of individual housing following surgery, animals were grouped and kept in colony conditions for 2 weeks. 15-min baseline ECGs were recorded both in individual housing conditions and after grouping. At the end of the grouping period, each male was also exposed to a final 15-min defeat test obtained using a standard resident-intruder paradigm, immediately followed by additional 15-min psychosocial challenge. One day after, animals were sacrificed and the brain, heart, adrenals, thymus, and seminal vesicles removed for weight determination and/or morphological analysis. Additional males (n=6) paired with a female were used as controls. Quantification of inter-male behavioral interactions in the colony were mainly based on the parameters *speed moving towards* and *speed moving from*, obtained converting X-Y positions of each individual male (an option provided by EthoVision system). These parameters describe the spatial movements of each individual in pairwise interactions with other simultaneously tracked animals. At the end of data analysis, a *net relative movement* of each male can be obtained for each of its three pairwise interactions with the other male members of the colony and a *dominance score* established by averaging these three values. A negative sign of this parameter indicates that the animal is moving relatively more away from the others, a positive one indicates more approaching-like behaviors. Quantification of sympatho/vagal balance was obtained by means of simple time-domain parameters such as average R-R interval (RR), standard deviation of RR (SD), coefficient of

variance (SD/RR), root-mean-square of successive RR differences (r-MSSD), together with the incidence of different cardiac arrhythmias such as ventricular and supraventricular extrasystoles and II degree atrio-ventricular blocks.

Male rats housed in the colony had higher organ/body weight ratios as compared to controls, significantly for the right and left ventricles of the heart ($p < 0.05$) and tendentially for the adrenals ($p = 0.06$). Moreover, the weight of the right ventricle was positively correlated with the dominance score ($p < 0.01$). The body weight of the animals were positively correlated with the dominance scores, both when before grouping and after grouping body weight values were considered ($p < 0.01$). We also found a tendency toward a negative correlation between dominance scores and SD and SD/RR values during the final defeat test: the higher the social position, the lower the variability of RR ($p = 0.07$). Finally, a significant positive correlation was found between the overall number of ventricular extrasystoles observed in each animal and its dominance score ($p < 0.01$).

In conclusion, high social rank in rat colonies (as estimated by means of an automated video tracking system) was found to be correlated with larger body mass, signs of cardiac hypertrophy, sympathetic dominance during an acute social stress test and higher vulnerability to ventricular arrhythmias.

A method for measuring the social behaviour of individuals in a group: an example with sheep

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Introduction

Sheep, like most large herbivores, live in groups with some form of social organization, in which the distances maintained between members of a group may reflect relationships between individuals, as well as the cohesion of the group as a whole. From the measurement of nearest neighbor distances, evidence for associations between pairs of animals has been found in a diversity of domestic breeds of sheep [1]. We present a statistical method which can also detect individual differences in general "sociability", or the tendency to be close to others.

Data collection

Four groups of 10 female Scottish Blackface sheep grazed in separate 0.4 ha plots. Each group of sheep was observed for a 2-h period on 16 days spread over a 12-week period. During these 2-h observation periods, the identity of each sheep's nearest neighbor was recorded at either 5 or 10 minute intervals.

Statistical analysis

For each group of sheep 16 matrices were constructed. In each matrix the rows contained the proportion of observations in which each sheep was observed to be every other sheep's nearest neighbor on one of the observation days. A single matrix was then obtained by summing the 16 matrices and the relative frequencies with which individual sheep were any other sheep's nearest neighbor obtained by calculating the column totals. The data was then examined by comparing the variance of the single summed matrix and the variance of the column totals, with the variances of the same values derived from 1000 simulations. In each simulation for any day sheep numbers were randomized, but the nearest neighbor counts remained as observed on each day. This process allowed us to test whether differences between individuals in the frequency with which they were nearest neighbors were consistent across days. The calculations required for the matrix analysis were programmed using the statistical package Genstat [2].

Results

The single summed matrix and column totals for group 1 is shown in Table 1. Similar tables were constructed for the other groups. Within groups 1, 3 and 4 there were significant differences between individuals in the frequency with which

they were any other sheep's nearest neighbor ($P < 0.001$). In group 1, for example, sheep 1 had a much higher value than sheep 4 in the column totals (Table 1), indicating a greater degree of sociability. Associations could be identified between 4 pairs of sheep in groups 1, 2 and 4 ($P < 0.001$) and between 3 pairs of sheep in group 3 ($P < 0.01$).

Table 1. Matrix for group 1, showing summed proportions of observations in which each sheep was the nearest neighbor (columns) of every other sheep (rows), with column totals. Values considered to identify associated pairs are indicated thus*.

nn	Sheep identification number									
	1	2	3	4	5	6	7	8	9	10
1		1.58	1.96	0.63	1.79	1.79	2.00	1.17	3.08*	2.00
2	2.08		0.58	1.87	1.29	1.25	2.71	3.12	2.00	1.08
3	2.04	0.63		0.88	1.62	1.00	1.54	0.88	0.67	6.75*
4	1.17	2.88	1.04		1.50	4.29*	1.54	1.37	1.50	0.71
5	1.63	1.58	2.13	2.00		2.17	1.33	2.04	1.50	1.62
6	1.75	1.04	0.96	3.33*	2.50		1.25	1.25	2.96	0.96
7	2.00	2.37	1.37	1.12	1.17	1.37		3.54*	1.25	1.79
8	2.00	2.71	1.42	0.96	1.62	1.46	3.75*		0.96	1.12
9	3.92*	2.12	1.17	1.08	1.50	3.08	1.00	1.04		1.08
10	2.50	0.67	7.00*	0.38	1.83	0.71	1.58	0.54	0.79	
Total	19.09	15.58	17.63	12.25	14.82	17.12	16.70	14.95	14.71	17.11

Conclusions

This method can detect differences in the frequency with which individuals are found close to others in a group, in addition to identifying associations between pairs. It may therefore provide a useful tool for describing the social behaviour of animals in groups.

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Redesigning a teleshop application using task analysis and experimental behavior of older users

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Designing for older users is designing for all. A teleshop system was designed for all ages. Existing teleshop systems were evaluated in three experiments with users of different age and with different levels of computer experience. Subjects had to perform the same shopping task in all experiments: ordering a list of groceries and making some changes afterwards. Measured was the time to perform the task, the number of mistakes and the amount of help from the experimenter. Older users had more problems than younger users and needed more help. Users with more computer experience performed better. All teleshop systems were analyzed using the GOMS (Goal, Operator, Method, Selection Rules) method of task analysis. A more detailed analysis of the task gives information which bottlenecks for which users exist. For example: older users need more time than younger users to learn a new task. The task analysis shows that typical computer language is frequently used. This is difficult for inexperienced users. Furthermore, the system is inconsistent, i.e. different methods and operators have to be used for the same sub-task. The first problem is reduced by using common world knowledge. The second problem by designing an interface with more consistency.

A new teleshop system was designed based on the outcomes of the three experiments and the task analysis of the systems. The new interface applied to existing knowledge of real shopping. Normal guidelines for designing interfaces were followed. The teleshop system runs on a normal PC with a mouse as input device. To start the shopping task users have to walk through the door of the mall using the mouse and thereby using their mental model of real shopping. To evaluate the new system it was compared to an existing CD-I teleshop system with the same structure. Three groups of users, ranging from 40 to 80 years had to perform the standard task on one of the systems. Each group was matched on computer experience and level of intelligence. The results showed that the new system was very user-friendly and all groups performed more or less well. The existing system gave more trouble to subjects with less computer experience and a lower level of intelligence. Some failures remained however in the new system. Changing the groceries list was a new task and this caused trouble. When subjects were reminded of their mental model of real-life shopping they could solve the problem. On the other hand mistakes were made where the system did not completely match the real world. In order to create a more realistic system, users

where shown graphical representations of the products. The researcher had the possibility to choose between a graphical or a textual representation.

Within the new system a component was built to register all the mouse movements and mouse clicks. With this information and a new version of the system, which was specially developed for this purpose, it was possible to replay an entire session on the PC. With the combination of both the replay program and a program that registers all events (including the mouse movements and mouse clicks) the researcher could easily evaluate specific problems in task performance.

A demonstration of the new system will be given at the conference, including a demonstration of the event replay program.

Automatic behavior recognition: what do we want to recognize and how do we measure it?

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Behavior has often been described as the ultimate output of the brain; in neuroscience biological functioning is described in terms of behavior. Since behavior is relevant for every day life of humans, everybody is - if not an expert - autodidactic in this field. For parameters (for instance physiological) for which we have no specialized cortical fields to interpret information we develop special techniques, equipment and algorithms to translate the measurements into terms of biological relevance. For behavior we use our primate brain even in case of other species. Therefore, behavioral definitions often reflect the interpretation and the presumed intention of the behaving individuals and lack the objectivity of other parameters for which special tools have been developed. Despite the efforts of ethologists and behaviorists to introduce clearly defined objective procedures to score and analyze behavior, shortcomings in terms of subjective descriptions and registrations are still present. The distinction between behavior and the contraction of a particular muscle is also vague. As our perception gains sensitivity due to the use of technology, the definition and interpretation of animal activity becomes more complicated. Another limiting factor for behavioral studies is the design of the experimental circumstances during which behavior is observed. Often those circumstances are chosen in such a way that the activity of the animal answers only a particular question. Some tests have been developed to measure predominantly anxiety, cognition, etc. It is tempting to use a one-parameter test which yields clear answers. This has resulted in the registration of numbers of visits to a particular area, time needed to reach a target, distances of simple movements, positions, etc. Moreover, measuring such parameters is easily automated [6, 7].

However, originally behavior is more recognized by changes in shape. Our sensory abilities are better suited to detect changes in shape than quantitative measures such as speed, distances, precise positions, etc.; the combination of both aspects of animal activity should yield the optimal ethogram. Therefore, and to avoid the inter-observer unreliability associated with human observation, an instrument (EthoVision[®]) was developed which makes use of image features describing locomotion and posture as well as the expertise obtained so far in choosing the relevant sets of muscle movements which are known as functional behaviors [2, 4, 5]. EthoVision has the possibility to derive a number of features from series of sequentially digitized images which provide information to discriminate between

various behavioral elements. Subsequently, pattern recognition techniques are applied to allow an objective description of functional behaviors based on the features extracted from the image sequences and manually registered behavioral states. We use two approaches for classification: statistical classifiers and neural networks. The statistical approach is described by Buma *et al.* [1]. In this paper we focus on the use of neural networks.

Neural networks have the ability to process fuzzy data and their output can be modified depending on the input used as examples, a training set of data. We have explored the possibility of a three layer network. The input consisted of features derived from digitized images of the rat's posture, position, speed, center of gravity, etc. The network was trained by images which had been scored frame-by-frame by experienced observers. Parameters essential for the performance are: nature of the input, i.e. digitized images of one or two camera positions, the number of features extracted from those images, the nature of the network, the reliability of the input, the incorporation of temporal relationships between elements, the architecture of the network. The level of resolution of such a tool does not depend on imaging parameters; sound but also physiological measures can be used as input as well. Especially non-invasively collected parameters such as body temperature may yield additional information about the state of the animal essential for an enhanced distinction of otherwise indistinguishable behaviors. In a pilot study a back propagation network which was trained with the features of repeated frames of digitized images, the reliability in scoring 7 behaviors was superior compared to human observers [3]. Our first results with such a network will be presented and the perspectives will be discussed.

This research is financially supported by Eureka project no. EU88011.

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Telemetric heart rate measurements in freely moving mice

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Mice are a useful genetic model for the molecular investigation of learning and memory. For the study of cognitive function of mice, Pavlovian fear conditioning experiments were performed in our laboratory. A single auditory fear conditioning trial was sufficient for the establishment of emotional long-term memory. The presentation of the conditioned auditory stimulus triggered significant changes in the defensive behavior of mice. The objective of our study was to investigate whether heart rate (HR) effects can be used as specific index of fear in freely moving mice. The method used for HR measurements determined the HR change: bradycardia was described for restrained rats, whereas tachycardia was reported for freely moving rats. Our results demonstrated that HR was a useful autonomic parameter indicating associative learning following a one-trial auditory fear conditioning of inbred mice [2].

ECG transmitters (Data Sciences TA10EA-F20) were implanted intraperitoneally [1] during a 15-25 min surgery of deeply anesthetized mice of a weight of 18-23 g. A suture tab on the transmitter body allowed to fix it to the abdominal muscles. Two recording electrodes extending from the transmitter body were guided through the abdominal muscles and placed subcutaneously. One was directed toward the right front leg, the other toward the left hind leg. Special care was taken to place the recording electrodes in such a way that no pressure was exerted onto the skin. In the few cases of wound infection, an antibiotic treatment was provided via the drinking water. After surgery, the body weight was controlled on a daily basis to monitor recovery. Behavioral experiments were performed 14-21 d after surgery, when the mice had regained pre-surgery weight.

HR was measured in the home cage of freely moving mice implanted with an ECG transmitter. For ECG recordings the home cage of a mouse was placed onto the receiver board (Data Sciences RLA1020) which was connected to an analog ECG adaptor (Data Sciences Option R08). ECG signals were recorded digitally with the help of an analog-digital converter (ADInstruments MacLab 4s) and stored on a Macintosh computer. An oscilloscope served as additional signal monitor. Depending on the position of the recording electrodes, at least the R and S waves of the ECG signal could be detected. HR was calculated offline from the R-R intervals of the ECG complex (ADInstruments MacLab Chart 3.5/s). The HR change and HR variability served as autonomic parameters [2]. Precise information on all steps from ECG surgery to equipment and costs of ECG

telemetry will be provided. Advantages and disadvantages of the method will be discussed.

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Assessment of analgesia and side-effects in an animal pain model

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Drug-induced side effects are frequently the limiting factor for achieving clinical analgesia. The purpose of this study was to provide a simple method for evaluating drug effects on both pain-specific and general motor activity in the same set of animals, thus allowing concomitant assessment of analgesic effects and side-effects. We also provide the outline for a computerized video analysis system which facilitates the analysis in terms of throughput and objectivity. The video analysis system is not commercially available, but some of the parameters may be obtained by other video tracking systems on the market.

Pain was induced in mice by subcutaneous injection of dilute formalin in a hind paw. Behavior was scored by using our video analysis system based on sequential digital image analysis. Orientation towards the injected paw was considered pain-specific behavior whereas locomotion, rearing and immobility was scored in order to quantify general behavior. In addition to inducing pain-specific behavior, formalin by itself reduced rearing and to a lesser extent locomotion and immobility. Morphine (3.1-25 $\mu\text{mol}/\text{kg}$) produced dose-related analgesia, increased locomotion and reduced immobility with slight separation between analgesic and motor effects. Higher doses caused a pronounced reduction in motor activity. Amphetamine in doses that had little effect on general behavior (0.78-3.1 $\mu\text{mol}/\text{kg}$) reduced the pain-related behavior while higher doses caused a marked motor activation. Diazepam (1.6-6.25 $\mu\text{mol}/\text{kg}$) reduced both pain-related and general behavior indiscriminately. The results are in agreement with the clinical experience that both morphine and amphetamine are analgesic compounds associated with side effects while diazepam has no direct analgesic properties.

We conclude that quantification of simple behaviors may predict some of the side effects of putative analgesics although the existence of species-specific drug-effects should be taken into account.

The electric-foot defensive behavior model as a method to discriminate individual coping strategy in the rats

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Backgrounds

We designed the electric-foot defensive behavior model to discriminate individual behavioral strategy of the rats in response to stressful experiences. As generally accepted now, individuals can display two coping strategies in aversive conditions: the active and the passive one [1, 2, 4, 6, 7, 8]. The active aversive strategy was determined as attempts to escape aversive stimulation ("coping with" behavior). Contrary, passive aversive strategy was described as a long-lasting freezing duration and "refuse" to cope with source of aversive stimulation. To study different aspects of response to stress in this approach, some experimental paradigms were developed, such as the shock-probe burying test and the conditioned defensive burying test [5, 9]. In these paradigms rats display a behavior strategy (active or passive) which is due to the experimental procedure but not to their "personality" [1]. Differently to that, we would like to propose a model which allows to discriminate diverse individual coping strategies of rats in response to the biologically identical stressful exposure.

Apparatus

In our model a cage (48 x 24 x 22 cm) with electrified grid floor is used. The electrical scheme includes direct and alternating (50 Hz) current, and provides electrical stimulation of the animal in any possible dispositions in the cage. The power of stimulation (in mA) is regulated by an amperometer.

Procedure

The first step of our test is the evaluation for each rat of the individual threshold of electric current intensity which is able to provoke its aversive response. Therefore we put the animal into the cage and 3 min later (when the rat stops to investigate a cage) we administrate electric current (0.5 mA) through the floor, which is augmented slowly until the intensity (0.8-1.4mA, for the majority of rats - 1.0 mA) when the rat flinches and shows running/jumping/rearing or intensive exploratory reaction (these behaviors were taken together to form "locomotor response"). This behavior is used as an indicator of active aversive strategy. If instead locomotor response rat display long-lasting freezing behavior, this is taken as a passive aversive strategy. In the current experiment rats are used after threshold evaluation at least 3 days later. We place a rat into the cage for 3 min, then we submit each animal to stimulation of intensity, which was found

individually for each rat in the first step. The duration of stimulation applied in rats with active aversive strategy was taken as a sum of latency of "locomotor response" + 2 s, but no longer than 30 s, and 30 s for rats with passive aversive strategy. Furthermore, we record the latency of first movement (time between start of stimulation and first movement of head, body or paws), latency of the locomotor response, and for 3 min after switching off a current, the duration of exploring, freezing and grooming behavior, number of rearings. From our data obtained in examination of Wistar rats (n=81) in described model, 86.67% of rats displayed active aversive strategy, and 13.33% showed the passive one. These data were found to correlate to those from available literature (Bohus B., 1995). Thus, the electric-foot defensive model can provide a discrimination of individual coping strategy of rats in aversive conditions.

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The eye movements characteristics in focal and ambient vision tasks in a pilot selection program

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The study was designated to evaluate the differences in eye movements characteristics and accuracy of performing dynamic visual tasks. The visual tasks consisted in controlling a position of a prisms picture. Pilots control the position and rotation of the prisms by two joysticks. The focal and ambient vision task was considered as a part of helicopter pilot selection program. The prisms is started peripherally in the left and right corners of the multi functional, collimating visualization systems. The focal vision loading task were accomplished by having the pilot respond to a series evenly spaced three number sequences. These sequences were presented centrally on the visual screen. The apparatus (a part of the helicopter simulator "Hyperion") was fitted with visual graphics system which provides multi colored, computer generated, real time interactive pictorial displays. The "Hyperion" screen measures approximately 40 deg visually and 180 deg horizontally. The summarized data of five consecutive trials were entered into statistical procedure and compared with eye movement tests. The subjects were the Air Force Academy candidates selected for the study - males aged 19-21. Thirty candidates were studied. The eye movements were measured by the ASL Eye Tracking System 4100H. The tests also includes profiles of pursuit and saccadic movements during observation of bright spot trajectory. The obtained results indicates differences between the process of pursuit and saccadic movements on the "Hyperion" simulator. The methodology of pilot's pursuit and saccadic movements performance may be utilized in the process of aircrew selection.

Analyzing behavior in the classroom

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Problem

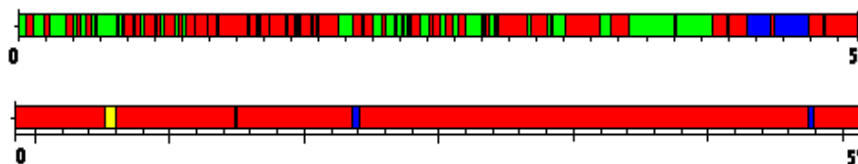
An innovative foreign language teaching model for children is being tested in several European countries [1, 2]. The model uses a narrative approach and in the classroom teacher and pupils act out theater scenes. L2 results showed that some teachers were more successful than others, although they had equal levels of interest and preparation. For understanding the problem, videos of teacher-pupils acting out were done. When we viewed the films it was clear that the successful teacher had something 'magic' which the other had not. The problem was to find a way of making explicit the feeling of magic the viewer got. In other words: is it possible to observe and classify magic?

Method

We used systematic observation whereby micro-categories of the teacher's behavior were grouped in several classes: gaze direction (gaze towards children, imaginary objects, classroom objects; fixed gaze on the other's face; closed eyes); verbal behavior (declare, narrate, praise, command, sing, pause); actions (mimic gestures, command gestures, instructional gestures, own body gestures, exortative gestures); smile (presence, absence, no coding); body position (standing, bowing down, to kennel, kneeling, no code). Video tapes were coded with The Observer Video-Pro (analog system). During continuous observation of one focal subject (the teacher), start and end of each behavior was recorded. Recordings lasted on average 30 minutes. Data were graphed as a time-event plot, followed by computation of the percentage of frequently occurring events, nested analysis and lag sequential analysis.

Results

Analysis showed that the magic teacher had a number of behaviors not present or infrequent in the other teacher's behavior. Typical of the magic teacher was the capacity of not interrupting the narrative behavior while the other teacher used to interrupt narration with other kinds of behaviors. As an example, the figure below shows that the 'magic' teacher alternates gazing at the imaginary objects with gazing at the children, while the other teacher alternates gazing at the children with gazing out. The table shows that the passage from narrative language to exortative language is not present in the magic teacher's behavior, while frequent in the other's.



Legend

- Gaze at pupils
- Gaze at imaginary objects
- Gaze out
- No code

Figure. Time-event plot of the gaze direction of 'magic' teacher B (upper bar) and teacher A (lower bar).

Table. Sequences in the verbal behavior of teacher A and B. The scheme clearly shows how teacher B passes from narration to pause and from pause to narration without interruption, while teacher A goes from narration to pause, next to exortative verbal behavior, back to pause and finally returns to narration.

	Teacher A	Teacher B ('magic' teacher)
Narration → pause	196	162
Pause → exorts	75	1
Exorts → pause	148	5
Pause → narration	135	163

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Automatic analysis of the behaviour of group-housed pigs by an image collection system

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It is often difficult to obtain behavioural data of animals by means of a machine vision system, because animals are not rigid, and interactions occur when group-housed. Since the paper-and-pencil method is hardly feasible and apt to misrecording, the value of an auto-recording system for group-housed animals is obvious [1]. We developed a conventional machine vision system to automatically extract meaningful behavioural data of group-housed pigs (3-4 pigs within a pen of 5 m²) from sequential images. A monochrome CCD camera with centre top view of the pen was connected to an image analysis system, which consisted of a PC, a Matrox Meteor frame grabber and a Matrox MGA graphic accelerator VGA card. User-friendly software, using an interface on a Windows platform, was developed by programming with Borland C++ 4.5 (Fig. 1). Main techniques adopted were image processing, logic reasoning and time-sequence-frame analysing. The behavioural categories were based on spatial and temporal information of the group activity. They covered the main behavioural patterns of group-housed pigs, like eating, sleeping, drinking, moving, and touching. Some sub-categories were also defined, like sleeping on side, and sleeping while touching. The accumulated time and frequency of each (sub)category can be calculated by the system for the whole observation period or at every grabbing interval. The observations can be videotaped and used by the system while the observer can make corrections. The processing of each frame takes 3 to 4 seconds, and the auto-recycling function makes the system capable to run for a sufficient long time. However, more pigs within the same observing area made the spatial-temporal analysis prone to mistakes since interactions between pigs were complex.

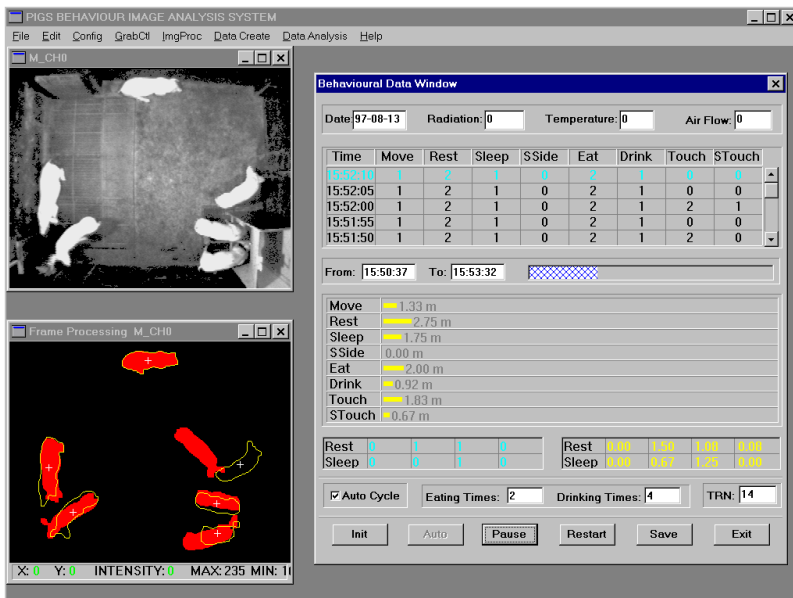


Figure 1. Image of the user interface. The upper table represents the consecutive interim behavioural events (last situation in light blue). The lower table represents the accumulative data in minutes.

We can conclude that automatic data collection of group-housed pigs using top view images is feasible for applied ethology research. The present system was validated in an experiment on the influence of radiation on pigs [2]. By adjusting the algorithms according to the species-specific behavioural patterns, this system can also analyse the behaviour of other group-housed animals, like calves. But, for the behavioural analysis of fast moving group-housed animals, like rats, the processing speed is essential. A good quality image with high contrast is a must for reducing the image pre-processing time and ensuring good image segmentation. One efficient way to obtain high contrast image is to apply pre-filtering according to spectral reflectance of the animal. Using colour image will make it possible to distinguish individuals by using colour marks and can greatly improve the data accuracy, but the quantity of signals and the cost of the system will be increased.

L. Tang and R. Zheng are financially supported by Flemish Educational Ministry; F. Mulken and S. Godrie by the IWT (Flanders). R. Geers is a research director of the FWO (Flanders).

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Measurement of neuromuscular performance in rats: a comparison of test methods

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Background

Measurements of neuromuscular performance in laboratory animal models play an important role in pharmacological evaluations and other biomedical research protocols. A variety of techniques employed in behavioral studies provide a valuable tool for assessing the safety and effectiveness of the regimen.

Aim

The present study was designed to compare the use of a grip-strength meter with a rotametric device in assessing the effect of Pyridostigmine (PYR), a cholinesterase inhibitor that may exert a deleterious effect on the skeletal muscle, on neuromuscular performance in rats.

Methods

Male Sprague-Dawley rats (250 g) were pretreated with normal saline or PYR (7 mg/kg body weight. i.p.) daily for 3 days and subjected to the following test at 0, 16 or 40 h later: (a) Grip-strength test: Fore limb grip strength was measured using a grip strength meter. The animal was placed on the electronic digital force gauge that measures the peak force exerted on it by the action of the animal. The animal was drawn along a straight line leading away from the sensor. The animal released at some point and the maximum force attained will be stored on the display. The highest reading (in Newton) of three successive trials was taken from each animal. (b) Coordination study: a rotametric device was used to examine the ability of the animal to co-ordinate while being placed on a rotating rod. The average reading (in seconds) of two successive trials was taken from each animal.

Results

Saline pre-treated rats was associated with grip-strength score of 8.3 ± 0.3^a , 8.2 ± 0.7 and 7.7 ± 0.9 , and rotametric score of 114 ± 4^b , 117 ± 3 and >120 at 0, 16 and 40 h, respectively. In contrast, the use of PYR resulted in grip-strength score of 6.3 ± 0.3^c (a vs. c: $p < 0.05$), 6.2 ± 1.1 , and 7.3 ± 0.9 , and rotametric score of 94 ± 8^d (b vs. d: $p < 0.05$), 83 ± 17 and 87 ± 19 at 0, 16 and 40 h, respectively.

Discussion

The short-term deleterious effect of PYR on the neuromuscular performance in rats was evidenced with the grip-strength and rotametric tests. There was a good correlation between grip-strength and rotametric scores, suggesting that both methods can be employed to determine the neuromuscular performance in laboratory animal models.

Studying the neurochemistry of behaviour: neglected factors in behavioural testing

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As most neurons communicate neurochemically, the study of extracellular neurotransmitter content is essential in order to understand the relationships between brain processes and behaviour. The introduction of in-vivo brain microdialysis and the development of sensitive neurochemical assays has provided means to locally study neurotransmitter levels in the brain of freely moving animals. Thereby it has become possible to monitor neurochemical activity in relation to specific behavioural tasks or presentation of significant stimuli. On the neurochemical side, methodological aspects of the microdialysis technique have frequently been investigated, whereas methodological aspects of behavioural testing have often been neglected. Here, we present results that are part of a series of studies designed to investigate cerebral cholinergic activity during basic behavioural testing procedures.

In experiment 1, we monitored acetylcholine (ACh) levels in the hippocampus, frontal cortex and nucleus accumbens of rats that were taken out of their home cage and exposed to an open field, in comparison to animals that were returned to their home cage. The respective procedure was repeated on the next day. Behaviour (rearing, locomotion, grooming and quiescence) was videotaped and analyzed subsequently with the help of a semi-automated computer system. Our results show, for one, that open field exposure and handling can lead to cholinergic activation in various brain regions with site-specific and experience-dependent differences in the pattern of activation. On the other hand the results underline, that a simple interaction like handling, can increase ACh in all three forebrain regions. Interestingly, in the frontal cortex, the cholinergic increase in the "home cage"-group was even bigger than that observed in open field exposed animals. This indicates that not only handling but also post-handling experience (e.g. "unsafe" open field vs. "safe" home cage) contribute to ACh release. Since handling is part of many neurobehavioural procedures, handling induced changes can interact with the imposed independent variables under investigation, such as pharmacological manipulations, and should be considered in the interpretation of any experiment employing handling of subjects.

In experiment 2, ACh levels were monitored in the hippocampus, frontal cortex and nucleus accumbens in two groups of rats that were exposed to an aversive stimulus in their home cage (115dB/1600Hz tone followed by 60sec 0.3mA foot

shock). In one group of animals, the home cage was situated in an environment with constant background noise (70dB/200-6300Hz), whereas in the other group no background noise was provided. Both groups showed an unconditioned behavioural response to the shock; however, an increase in ACh to the footshock was observed only in that group that was kept in the environment without background noise. Thus, situational factors, like background noise, which is often used during behavioural testing to mask other irregular noise, can block the neurochemical - but not behavioural - reaction to an aversive stimulus. In summary we conclude, that cortical, hippocampal and accumbal cholinergic activity is highly sensitive to "simple" behaviourally relevant stimuli and that this neurochemical activation is critically dependent on situational factors and experience.

The electrical penetration graph (EPG): recording of penetration events by piercing mouthparts of insects

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The electrical penetration graph (EPG) is used to study the interactions between parasitic insects with piercing mouthparts and their host organism. So far, plant feeding aphids (Homoptera) have been used mainly. Insects are attached to a thin (20 μm gold wire) electrode and a second (copper) electrode is inserted into a potted plant or into the soil [1]. The insect should have a low electrical conductivity with its feet to the plant. As soon as the mouthparts (stylets) penetrate, the primary circuit is completed and voltage changes can be recorded. About 10 different EPG waveforms have been distinguished on the basis of amplitude, frequency, voltage level (intra/extracellular), and electrical origin (resistance/electromotive force). Waveforms have been correlated experimentally with the insect's activities (saliva excretion, ingestion, mechanical stylet propagation) and stylet tip positions in the plant tissues (mesophyll, xylem, phloem). Also, intra- and extracellular positions can be distinguished as the stylets acts as self-penetrating microelectrodes which do not destroy the living plant cells they puncture. Host plant selection, phloem finding and phloem feeding are studied with this method. EPGs show many details that are not detected by any other method. Recently EPGs were able to elucidate the crucial events causing plant virus transmission (acquisition and inoculation) by specific aphid vectors [2]. Insect as well as plant physiological topics are studied.

The EPG uses a DC amplifier, 0-65 Hz, and an input resistor of 1 Gohm (10^9 ohm), equal to the average resistance of the insect/plant combination. This allows recording of most electrical resistance fluctuations caused by the insect's penetration activities, the resistance (R) component, and also, it is high enough to record biopotentials generated in the system, the electromotive force - EMF) component. An adjustable voltage source enables to compensate for any offset potentials in the primary circuit. Signals are digitized and recorded on a computer hard disk (and CD-ROM finally). For analysis they are displayed on screen where relevant parameters are retrieved for further data processing. The system is presently used for fundamental and applied studies in about 40 universities, research institutes, and industrial labs world wide. The number of insects and arachnids (mites and ticks) is increasing. EPG recording will be demonstrated (of 4 insects simultaneously) with a poster representing the main measuring principles and some (TEM) micrographs of stylet positions in plant tissues.

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Using biotelemetry for integrated measurement of behavior and physiology in laboratory animals

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Hyperthermia, tachycardia and hypertension are essential components of the stress response in humans and animals. A series of projects will delineate how the analysis of the acute and long-term responses to stress may benefit from concurrently recorded behavioral and physiological variables. The heart rate (HR), blood pressure and core temperature (Tc) of rats were measured by radiotelemetry for up to 6 months and the behavior of the resident and the intruder rats was evaluated from video records taken during the social confrontations. A drug-induced lessening of the stress could in this way be characterized by a change in the response profile that was composed of the animal's resting, locomotor, exploratory, defensive behavior, the ultrasonic vocalizations and its reactive or anticipatory autonomic reactivity. The dose-dependent anxiolytic or anxiogenic drug effects on the behavior of the rat intruding in the home cage of the resident were coordinated with the intruder's autonomic stress responses. The second set of data will compare autonomic and behavioral changes induced by self-administration of cocaine. Using operant conditioning procedures rats were trained to self-administer cocaine via the intra-venous route. Repeated, limited access to cocaine was found to synchronize HR and Tc rhythms in anticipation of these self-administration sessions that were scheduled daily at the same time, whereas prolonged unlimited access disrupted biological rhythms.

The analysis of individual differences in responses to stress stimuli profits of a comprehensive behavioral analysis supplemented by the concurrent evaluation of autonomic changes. Combined with a repeated measurement design this research strategy reduces the number of animals needed to make a valid statement about treatment effects.

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Heuristic approach for prediction of behavioral data

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Introduction

The aim of the study is to propose a new heuristic approach for the prediction of the behavior of living systems and to prove that the method is valid in the case of human locomotion.

The heuristic approach

The Volterra-Wiener representation makes it possible to obtain the input/output relation in case of two input parameters x_1 , x_2 and one output parameter y , using the homogeneous polynomial given by Eq. 1.

$$(1) y = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_3 x_1 x_2 + \alpha_4 x_1^2 + \alpha_5 x_2^2$$

Unfortunately in case of 10 input parameters the number of coefficients in Eq.1 is 200000. It is not possible to carry out such a large number of experiments. The proposed heuristic method is very similar to the mass animal selection which involves: (a) selection of one male and one female animal group (analogous to establish *hypotheses*); (b) breeding and receiving the first propagation (analogous to obtaining *the first generation of solutions*); (c) selection of the “best” newborn animals (analogous to select *the best solutions*); (d) next breeding and receiving the next new propagation (analogous to obtain *the second generation of solutions*) up to the moment when the properties of the newborn animals begin to degenerate (analogous to stop the computing).

$$(2) y = F(x_1, x_2, \dots, x_n)$$

$$(3) y_1 = f(x_1, x_2); y_2 = f(x_1, x_3); \dots; y_m = f(x_{n-1}, x_n)$$

Suppose y is given by Eq. 2 named “global description” and the experimental values form the data matrix **A**. We replace Eq. 2 with a series of “partial descriptions” given by the Eqs. 3. For each Eq. 3 we generate the mean value \bar{y}_l ($l=1,2,\dots,m$) using only Eq. 1. Using the statistical criterion F-test we compare \bar{y}_l with the known mean value \bar{y} and we select *the best solutions* that form the *first generation of solutions*. Using the same algorithm we generate the next “partial description” and we obtain the *second generation of solutions*. Generation by generation

the criterion F-test decreases. The procedure stops when the F-test value begins to increase.

Results of the prediction of human locomotion

The biomechanical goal is to predict the vertical components of the ground reactions, both for the right and the left leg, using only a set of 9 selected individual body parameters: x_1 -body height; x_2 -ground-*iliospinale*; x_3 - ground-*tibiale*; x_4 - *tibiale-sphyrion*; x_5 -*distancia cristarum*; x_6 -*conjugata externa*; x_7 - hip circumference; x_8 - knee circumference; x_9 - body weight.. Using force-plates the ground reactions of 25 individuals are registered, the first 7 harmonics are calculated and the 9 body parameters are measured. Every harmonic for every person is obtained using the heuristic approach. Finally, using the inverse Fourier transformation we obtain the predicted ground reactions for every person as functions of the time.

Conclusion

Five *generations of solutions* are enough to obtain with good convergence a sufficiently small F-test value. The proposed new heuristic approach constitutes a valid outline for diagnostic purposes and gives predicted results that are in very good agreement with the experimental data.

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Observing ergonomics: The Observer Video-Pro in RSI research

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Repetitive Strain Injury (RSI) gives rise to a high level of absenteeism and, to an increasing extent, ends in full incapacity for work. Making frequent repetitive movements, particularly of wrist, elbow, and shoulder joints, for a protracted period of time, is one of the main causes of RSI. The Department of Ergonomics of the University of Lisbon has been called upon to investigate RSI symptoms of employees working in a large car-assembly plant.

The ergonomists have opted in favor of detailed systematic observation of employees' tasks with The Observer Video-Pro (figure 1), using a timing resolution of 0.04 seconds. Although these tasks only last for an average of 11 seconds, the number of movements of the wrist, elbow and shoulder joints can easily run into hundreds, particularly when the employee has to manipulate pliers or other tools. This kind of detailed behavioral observation makes systematic video analysis an extraordinarily appropriate method of research.

Analysis results revealed repetitive movements, occurring at a high frequency, not only during the task as a whole, but particularly during movements at which a considerable amount of force had to be applied. In addition, the frequency of transitions between several wrist positions, turned out to be extremely high. These results enabled the researchers to identify those parts of the tasks that most urgently call for ergonomic enhancements.

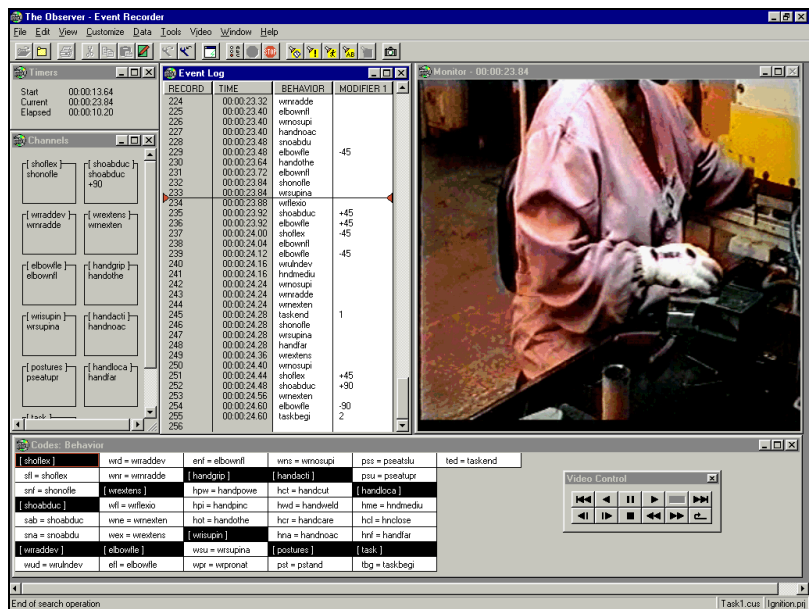


Figure 1. The application window of The Observer Video-Pro, showing part of a detailed data file associated with its corresponding video file.

Measuring driver behavior

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When asked about mental workload most people think about difficult tasks or conditions in which many tasks have to be performed simultaneously, or have to be performed in limited time. There is nothing wrong with this assertion, in particular the word 'difficulty' reflects mental workload very well. However, I will argue (as others have) that not only increased task demands, but also reduced operator capacity affect mental workload. The interaction between the task and the operator (driver) determine mental workload. So, not only increased task demands (e.g., the addition of a task, such as using a route navigation device), but also decreased driver capacity (e.g., as a result of experience or more temporary factors such as fatigue or illness) increase mental workload.

Techniques to measure mental workload are generally classified into three categories: performance (behavior), self reports and physiology. Measures from all three categories have been used in traffic research and an overview of use and usefulness in different experiments will be given. The measurement of task performance, self reports and measuring physiology will be illustrated by examples from experimental studies performed in an instrumented vehicle and studies performed in an advanced driving simulator. The primary task of car driving is to maintain longitudinal and lateral vehicle control. Primary task performance is reflected in the amount of 'weaving' and in headway control. Secondary tasks are sometimes added to assess mental workload, while self reports reflect the subjective experience of mental workload. In addition to this, measurement of the drivers' physiology, in particular heart rate, can indicate when mental workload is relatively low or high. The use of the so-called heart rate profiles and special 'applied' measures, such as mirror looking behavior, will receive specific attention.

Method for identifying behavior states among free-ranging bottlenose dolphins, *Tursiops truncatus*

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We present an observational method for identifying behavior states exhibited by free-ranging bottlenose dolphins. First is a description of the method, which is based on interpreting surface-visible dolphin behavior on eleven criteria: formation, dispersion, orientation, predictability of next surface location, tempo, synchrony, surface: submergence ratio, cohesion, group movement, homogeneity and behavioral events. We describe each criterion and explain how it can be applied in the field.

Second is a qualitative description of 18 dolphin behavior states that were identified using the criteria. These data were collected during 114 h of direct observation of bottlenose dolphins on 80 boat surveys of two sites, Cape Lookout, North Carolina, USA and Bahia de la Paz, Baja California Sur, Mexico, June 1992-August 1993. We present these data in tabled form to illustrate how individual dolphin behavior states are characterized by unique combinations of the criteria listed above.

Third is quantitative support for the unique combinations of criteria that characterize individual dolphin behavior states. These data are drawn from 86 hours of direct observation of bottlenose dolphins during 52 land-based surveys of the San Diego County study area between May-Dec 1997. Each criterion, except behavior events, was partitioned into biologically meaningful, qualitative categories, e.g., the criterion dispersion was partitioned into three qualitative categories to quantify inter-animal distances (<2, 2-5, and >5 dolphin body-lengths). Instantaneous scan samples of qualitative categories were collected on group behavior at 2-minute intervals. All-occurrence data on 29 predetermined behavior events were collected during two-minute intervals. We present quantitative support for detailed qualitative descriptions of three dolphin behavior states on six criteria. An example of a detailed qualitative description is the following for Indeterminate Travel. Characteristic criteria include behavior events producing rhythmic forward progress at a slow to moderate tempo in the same headfirst orientation in a general direction with occasional meandering. Dolphins tend to remain close to the surface in a moderate to high surface: submergence ratio. Cohesion is moderate due to a tendency for some group members to incorporate other activities while or instead of traveling. The criteria formation,

dispersion, synchrony, as well as other activities, tended to be more variable. Formation tends toward staggered ranks; in larger groups, several subgroups travel in ranks aligned in long formation with respect to one another. Ranks in long formations can become independent ranks. Changes in formation affect dispersion; dolphins stay in the proximity of one another. Synchrony is partial, exhibited primarily among members of the same subgroups and rarely between subgroups. "Other activities" include intermittent episodes of affiliative socializing or incipient sexual exchange, indicated by leaps, double bows, a series of low intensity breaches, skims, brief exposure of various body parts at the surface or slow-paced rafting; one or more dolphins may surf.

The systematic evaluation of criteria typical of all dolphin states improves the measurement of free-ranging dolphin behavior by providing a clear rationale for associating specific surface patterns with specific dolphin behavior states.

A method to evaluate visceral nociception in conscious free moving rats using activity monitors

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A method is described to measure visceral nociception following implantation of remote balloon devices into the descending colons of rats. Male Sprague Dawley rats were fed 'Transgel' for 20-24 h. Rats were subsequently immobilized by inhalation of O₂:CO₂ for 40 s and a small deflated balloon device was introduced by the intrarectal route approximately 2 cm into the descending colon. The balloon was made by placing a 27x3 mm section of polyethylene transfer pipette inside a finger cot. This was pushed into a small cylindrical PTFE collar 7.5x7.5 mm and an 8 mm aluminum cap with rubber septum was then crimped over the end. The balloon was inflated by an injection of saline. Rats were given 15 min to fully recover. The effects of balloon inflation on spontaneous exploratory rearing was measured for 5 min using a photocell activity monitor. Normal rats without rectal balloons exhibited frequent rearing activity within the 5 min observation time (244±13 infra-red beam breaks, n=8). The presence of an inflated balloon in the descending colon of rats suppressed spontaneous rearing activity compared to control animals without balloons. Increased balloon inflation volumes significantly (p<0.005) reduced rearing activity (178±14, 1 ml; 151±11, 1.5 ml; 145±13, 2.0 ml; 90±10, 2.5 ml) demonstrating a stimulus response relationship.

This method has advantages over those previously described. It does not involve indwelling cannulae which may be pulled or chewed [2]. Invasive surgery is not required to implant electrodes to measure visceral or muscular activity [1]. The rats are conscious and unrestrained. Observation time is short and large numbers of animals can be monitored quickly. The methodology parallels clinical diagnostic measurements of visceral pain in humans [3] and the readout is objective. This method should allow mechanisms of visceral nociception to be assessed more easily *in vivo*.

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Methodology for usability tests

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Usability evaluation of software interfaces can take place at various stages in the design process. The character and type of the evaluation is dependent on the stage of development of the interface. In the beginning, qualitative methods can be very valuable in providing rich information (broad band), and recommendations for improvements in the design, in particular with regard to the conceptual model of the interface. In later stages, quantitative tests are useful to test certain (critical) details of the design and usage, or to collect more objective data concerning the effectivity and efficiency of the interaction with the software application. However, qualitative tests can also be applied in later stages. The 'usability goals' are most important in deciding upon the most appropriate procedure, along with considerations concerning resources.

In the WIT-Lab (Laboratory for Work and Interaction Technology), various types of research are conducted, depending on the usability goals and the settings. A few typical examples of usability tests will be discussed, including the measurement methods.

Using real-time neuroimaging techniques for the study of cognitive processes

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In cognitive neuroscience, neuroimaging methods are used in order to visualize brain activity associated with cognitive processes in time and space. Modern techniques like PET (Positron Emission Tomography) and fMRI (functional Magnetic Resonance Imaging) offer the possibility to accurately localize changes in regional cerebral blood flow. However, the temporal resolution of these techniques is rather poor, since changes in blood flow are much slower (in the order of several seconds) than the neural activity eliciting these changes. Another neuroimaging method is source analysis on the basis of event-related potentials (ERPs). ERPs are obtained by averaging epochs of the electroencephalogram (EEG) time-locked to the occurrence of concrete sensory, motor, or cognitive events. ERPs reflect that part of the brain activity that is specifically related to the processing of these events. With ERPs it is possible to study information processing in the brain with a high temporal resolution (ms or less). Because of this it is possible to use sophisticated experimental designs in which stimulus and/or task parameters are varied on a trial-by-trial basis and to search for subtle relations between task performance and ERP components. For these reasons, ERPs are a powerful tool for studying the organization and time course of elementary processes in selective attention, memory, language, motor control, etc.

However, the relationship between the scalp-recorded brain activity and the underlying neural activity is complex and not completely understood. A common approach is to attempt to infer the activated sources in the brain on the basis of multi-channel ERP recordings and inverse dipole modeling. However, in this approach several basic assumptions have to be made regarding the nature of the volume conductor (i.e. the model of the head) and the sources of electrical activity in the brain. In the spatio-temporal dipole model [2], for instance, it is assumed that a certain latency range of ERP activity is explained by a small number of equivalent dipoles, the locations and orientations of which remain fixed over time. In complex cognitive tasks, however, ERPs may reflect many overlapping source activities, complicating dipole modeling. I will discuss an approach in which topographical analysis and dipole modeling is restricted to the lateralized part of the brain activity associated with covert orienting of attention and preparation of eye movements. The approach rests on a double subtraction procedure in which ipsilateral ERPs are subtracted from contralateral ERPs and averaged over left and right motor responses (LRPs [1]; in this case: attention shifts and eye movements).

This approach was originally developed for ERP derivations from a single electrode pair (C3/C4), and now applied to multi-channel topographical analyses. Using this approach, all non-lateralized brain activity is removed from the analysis, and thus it is more likely that relatively simple dipole models will suffice. Indeed it was found that this procedure resulted in relatively simple topographies in which an occipital asymmetry (probably related to attentional orienting) preceded a frontal asymmetry (probably related to oculomotor preparation). Furthermore, these results were highly consistent over different individual subjects.

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Subtyping stereotypic behavior in children: the association between stereotypic behavior, mood, social context, and heart rate

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Stereotypies, or the repetition of an invariant sequence of behavior, are a component of normal behavior in humans. Abnormal high rates of stereotypic behavior can be found in a number of handicapped populations like mentally retarded, hearing impaired, blind and autistic people. So far there is no univocal (biological) explanation for these seeming meaningless behaviors. The homeostasis interpretation assumes that there is an optimum level of stimulation and that stereotypy compensates for under- or overstimulating environments. According to the operant condition, stereotypy is maintained by reinforcers. These reinforcers can be either positive, involving some particular sensory input or attention from others, or negative, involving escape or avoidance of aversive stimuli. An alternative explanation suggests that stereotypy is neurobiologically based and neither adaptive nor functional.

Stereotypic behaviors do not form a homogeneous class of behaviors but vary considerably in onset, timing, form, intensity, frequency, cyclicality, variability and sensitivity to environmental change. There are inter-individual differences in conditions eliciting stereotypies and intra-individual differences in the effect situational conditions have on various stereotypies. This suggests that there is unlikely to be a single explanation for stereotypies. We were interested whether we could prove that differences in emotional context of stereotypies lead to differences in form and duration. Secondly we wished to study whether there is a correlation between heart rate changes, emotional context and form of stereotypy. Finally we wanted to study the social context and consequences of stereotypies.

To investigate differences in form and duration, each occurrence of stereotypic behavior was recorded from videotape by means of The Observer (Noldus Information Technology). Mood and form were added as modifiers to the code for stereotypic behaviors. Heart rate was recorded with the Sport Tester PE 3000. One B.P.M. value was calculated every five seconds. Of every occurrence of stereotypic behavior, the heart rate values within one minute surrounding the onset of stereotypic behavior were studied. To evaluate the social context and consequences we detected temporal patterns with the software Theme. The software package developed by M.S. Magnusson [1] recognizes temporal behavior patterns based on consistencies in interval.

The results show that stereotypic behavior should no longer be treated as a homogeneous class of behaviors with one function. The phenomenological division of stereotypic behaviors into those associated with elation, distress and composure was sufficient to reveal significant differences in the mean duration and form of stereotypies and in the pattern of heart rate over time around the onset of stereotypic behavior. Analysis of temporal patterns proved a useful method for analyzing the social context and consequences of stereotypies.

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Relationships between brain activity and cognitive performance during overnight work: prediction of errors

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Introduction

Patterns of electrical activity of the brain of healthy human subjects (electroencephalogram, EEG) depend on a variety of factors, including various aspects of behaviour. These include the basic EEG type of the subject, level of alertness and the type of mental activity being carried out. The EEG, recorded at multiple sites over the scalp, also shows spatial variations, with different brain areas co-operating during mental activity. These relationships between alertness, mental activity and the EEG are examples of the links between EEG activity and behaviour. The purpose of the present study was to use the EEG to predict errors in performance brought about by fatigue during an overnight period of work. During this study, behaviour was manipulated, firstly, by inducing fatigue, and secondly, by requiring the subjects to perform different types of mental activity. The mental tasks involved activities which are included in the performance of complex tasks, namely memory, attention, co-ordination, and mathematical and spatial processing ability.

EEG recordings and cognitive tasks

Brain activity was recorded from 19 electrode sites over the scalp, using an elasticated electrode cap (ECI Electro-Cap) while subjects carried out a variety of cognitive performance tasks. The EEG signals were recorded by a polygraph machine (Medelec 1121 series) and digitised using a commercially available data acquisition system (DATS, Prosig Computer Consultants Ltd.). Tests of memory function, vigilance, target detection, psychomotor performance (target tracking), mathematical processing and visual rotation were included, and the spontaneous EEG during rest was also recorded. The experiment consisted of an overnight period of work lasting approximately 18 hours, including the period between 23:00 and 06:30, and was intended to produce fatigue. The tasks were each carried out on seven occasions, in sessions lasting approximately 70 minutes. Six healthy subjects participated in the study, and were trained to plateau level before commencing.

Analysis procedure

The EEG was analysed using the Fast Fourier transform, based on epochs of data lasting 2 seconds, which were then meaned over several minutes (typically three to seven minutes corresponding to the duration of the task) to obtain stable estimates of power spectra. Regression analyses were then used to predict error rates in task performance from the EEG. The EEG parameters used in the regression were based on groups of electrode sites determined using analysis of variance and multiple comparison procedures, and on variables derived from the EEG spectra using principal components analysis. The EEG variables covered the delta, theta, alpha and beta frequencies of the spectrum. These frequencies are known to alter during task performance and when alertness decreases.

Results

During the later sessions of the experiment, task performance was reduced to levels of 30 to 40% of those seen during the daytime when subjects were alert and well-rested. The EEG data showed high correlations with performance, based on the relationships between groups of EEG channels and the percentage of correct responses for individual tasks. The results indicated that the EEG can predict decrements in cognitive performance in terms of the percentage of correct responses to within 10 to 20% accuracy. These predictions were based on the average value of power from groups of EEG sites, involving areas of the brain concerned with intellectual function (frontal and pre-frontal sites), memory (temporal sites) and motor function (central sites), and also regions which normally show changes as alertness decreases (parietal and occipital sites).

Discussion

The present study has demonstrated the capability of brain activity to predict decrements in cognitive performance, including memory, vigilance and target tracking. As such, they provide a clear link between the EEG and behaviour, specifically performance at a variety of cognitive tasks, which has a practical application. The predictions were from data involving all subjects and tasks combined, using a model that allowed for sources of variation due to subjects and tasks. Given that individual variability exists between the subjects' EEGs and also between tasks, accuracy of the predictions was acceptable in the majority of cases with this group of subjects for the tasks in the schedule. These predictions, using EEG recordings from 19 sites on the scalp, improve upon those based on EEG activity at a single electrode, using individual subjects' EEGs [1], where only extreme degradations in performance (less than 25% correct) were able to be detected by the EEG. The findings were, however, based on relatively long segments of EEG data covering the duration of various performance tasks and typically lasting several minutes. The overall aim of the current research is to predict performance in a real-time situation in occupational settings so that loss of alertness and errors in performance may be identified and prevented. Clearly, this

will need to be carried out in a dynamic manner on a considerably shorter time scale, of the order of a few seconds. Approaches to the above issues will be discussed.

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Image analysis of swine postural behavior

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Postural behavior is an integral response of animals to their environmental stimuli. Huddling, nearly touching one another and spreading apart among group-housed animals are the qualitative stereotypes of postural patterns corresponding, respectively, to cold, comfortable and warm/hot sensation. It is these postural patterns that have been routinely used by animal caretakers to assess thermal comfort state of the animals and to adjust the environment or management accordingly. This human observation and manual adjustment approach, however, has two inherent pitfalls. First, it is impossible for the caretakers to attend the animals around the clock despite the circadian thermal needs of the animals. Secondly, the interpretation of optimal animal comfort behavior may vary considerably from one caretaker to another.

The goal of this project is to develop a machine vision system that automatically assesses thermal comfort state of swine and make according environmental adjustments. As the first step toward this goal, a study was conducted to examine the feasibility of classifying thermal comfort state of pigs by neural network (NN) analysis of their behavioral images. Specifically, pigs from 2 to 4 weeks of age were exposed in groups of 10 to cold, comfortable, and warm environments. Postural behaviors of the pigs were recorded and processed into binary images. Fourier coefficients (FC), moments (M), perimeter (P), area (A) and combination of M, P and A were evaluated as the feature representations of the processed behavioral images. Using these features as inputs, a 3-layer NN was established and then used to classify each of the postural images into the cold, comfortable or warm category. The combination of M, P and A as inputs to the NN model produced the best classification rate.

The results suggest that this approach has a good potential as a non-invasive assessment of the animal's thermal comfort and ultimately may provide a practical management tool for an enhanced animal well-being. Subsequent studies have been conducted to develop algorithms for automatic image segmentation of the pigs in a commercial pen setting and automatic selection of the eligible behavioral images (via motion detection). Work is also underway to quantify relationships between physiological responses of the pigs (i.e., thermograph) and their behavioral displays. This information is essential for objectively building the NN, particularly fuzzy-logic NN, and thus improving the performance of the NN.

The use of topographic numeric imaging tools and computerized video tracking systems to study the reproductive behavior in fish: towards a “field-lab” integrative approach

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In many fields centered on behavioral research, numeric imaging techniques now allow the conceptualization of tools and systems whose descriptive precision, quantification abilities open original and very promising prospects. By the computing power offered by these new techniques, ethologists have the possibility to quantify and take into account a large number of variables which usually cannot be measured by usual methods of direct observation. This parameterization then allows attempts of modeling and simulation of behavioral patterns of animals according to the environmental situations to which they are exposed. There are two principal options in numerical imagery. First, the digitization of analog images, a process allowing the detection and the recognition of characteristic objects compared to a image of reference without object. Second, the creation of digital images from databases, for instance topographic measurements.

In a first field approach (conducted while scuba diving), we exploited this second option, using computer tools (Surfer[®] 6 and GIS-Idrisi[®] 2 for Windows 95) to model the reproductive microhabitat in the nesting Mediterranean labrid fish *Symphodus ocellatus*. The software produces 3-D surface maps of sites. With this topographic numeric imaging, we can analyze the evolution of the spatial distribution of algae nests built by territorial males during the reproductive season. A high density of *Symphodus ocellatus* is found on a rocky substrate within 50 m of the shore. This area of 1247 m² (43 x 29 m) was chosen as study site. We measured depths (z) every meter along parallel transects (x, y co-ordinates) between -10 m and the surface with a diving computer (precision: 10 cm). These data allowed us to create detailed 3-D numerical images of the zone. The second step was to count and to locate nests daily and report them on the maps. The results show that the maximum nest density is found between 6 and 8 m depth and that this density varies in the horizontal plan according to the light exposition of substrate for nesting. A nest/day curve shows a rapid increase of nests number in the beginning of the season (May) and a slow decrease at the end of June (last nests).

If this method provided a good estimation of space and time utilization modes for territorial males' nesting behavior, the other pole of numerical imagery will allow to test in laboratory (aquariums) the parameters influencing the reproductive behavior

in territorial fishes. With the future acquisition of EthoVision[®] Color-Pro, a computerized video tracking system (detection by color tracking of markers attached to animals) we will quantify for each fish locomotor activity (distance traveled, swimming speed), number of visits and amount of time spent in a defined zone of the aquarium (territory) and social interactions (approach, avoidance) according to intensity of light, photo-period, water temperature, stream, structure and nature of substrate, density of congeners, etc.

Coupling the numeric imaging techniques (field and lab), we hope to build a statistical model to better understand the relations between fish and its reproductive microhabitat.

Measurement and analysis of vocal aspects of communicative interaction

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The poster contains descriptions of several procedures for analyzing vocal aspects of communicative interaction. The task of these procedures is to find deviations from individual phonetic and prosodic standards. No attempt has been made to develop a fully automatic system, but digital audio signal processing algorithms are used to support the human perception of vocal variations (e.g. by contrast enhancement or feature extraction). Results from these procedures for vocal analysis are compared with results from other measurement and analysis procedures [1, 2, 3]. The audio data on which the vocal analyses are based are recorded in parallel and on the same time scale as are the data on which the other procedures are based.

The audio demonstrations contain examples for two types of audio processing for facilitating the perception of vocal changes: sequencers enable the listener to compare different sections of the speech signal by reducing the temporal distance between them and filters enable him to concentrate on specific phonetic and prosodic features by emphasize or even isolate these features. The corresponding algorithms are implemented in the graphical data flow language of the Laboratory Virtual Instrument Engineering Workbench (LabVIEW).

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