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# Program Chair's Welcome

On behalf of the Scientific Program Committee I welcome you to Measuring Behavior 2005, the 5<sup>th</sup> International Conference on Methods and Techniques in Behavioral Research. I trust you arrived safe and sound and are eager to join the conference. After four very successful conferences in Utrecht, Groningen and Amsterdam, it is now Wageningen to set the stage for science and pleasure. You are invited to meet Wageningen, its University and Research Centers and explore and enjoy its lovely surroundings. But what is more important, you will be served a stimulating scientific program.

Throughout the years more and more scientists from all over the world discover this conference as the place to be for exchanging ideas on how to measure behavior. In fact, its growing popularity nearly got us in trouble this 5<sup>th</sup> conference, since we were getting overbooked and had to turn down many requests! One of the attractive aspects of this conference is that it creates the possibility for communication between scientists of very different backgrounds. From behavioral ecologists to psychiatrists, from physiologists to linguists. They are all intrigued with behavior one way or another, but their research questions, objects and applications may greatly differ. They may literally and figuratively speak very different languages but what binds them is a common goal: to enhance the accuracy, speed, ease and reliability of measuring, quantifying, analyzing or visualizing behavior. New methods, new techniques, new applications. As always the focus of Measuring Behavior is on 'how' and not on 'why' questions. This focus results in a very productive exchange and cross-fertilization of researchers from fields that would otherwise never meet.

As you can learn from the program, the conference includes a selection of events such as oral and poster presentations, special symposia, demonstrations, special interest group meetings, tutorials, and user meetings. Do not miss the special symposia where topics are grouped into a central theme. To name but a few: detecting abnormal human behavior, innovation in pain research, innovative use of the water maze and measuring olfaction in insects and mammals. Be eager to learn and visit the tutorials. They provide a valuable opportunity to instruct you in specific methods, techniques and equipment you can use in your behavioral research. There is a whole selection of topics such as measuring human and animal movement, remote physiological monitoring, data manipulation and management. Go deep and dive into a Special Interest Group (SIG) where participants focus on a specific methodological or technical topic and go for in-depth discussions.

Research centers in and around Wageningen invite you to visit their facilities during the scientific tours. There is a choice of eight different sites. This includes my own institute, the Netherlands Institute of Ecology (NIOO-KNAW), which has one of its centers, the Centre for Terrestrial Ecology, close to Wageningen. I look forward to showing you around.

There is so much to share and learn, it may become hard to drag you away to our informal social events, with good food and a relaxing atmosphere. But do not miss the opportunity to have dinner at the Rosendael Castle and to visit one of the Netherlands most beautiful nature conservation sites "De Blauwe Kamer". On behalf of the Scientific Program Committee and the Local Organizing Committee I wish you an immeasurably fun and interesting stay in Wageningen.

Louise E.M. Vet  
Chair, Scientific Program Committee

## Editors' Preface

This volume contains short papers of most presentations at Measuring Behavior 2005. It is the first time printed conference proceedings are published in addition to the program and abstracts books, and we are pleased with the positive response of presenters to our request to submit papers for the proceedings. We understand that for most authors, the ultimate destination of their research findings is an article in a peer-reviewed journal with a high impact factor. These conference proceedings do not attempt to compete with such journals. Instead, this book offers a complementary publication opportunity, in which authors can elaborate on technical details of their methods and instruments – including drawings, photographs and software screenshots – in a way not allowed by most research journals. A selection of the papers presented at the conference will be published as full papers in *Behavior Research Methods*, the journal that has been devoting a special issue to our conference since Measuring Behavior '98. In order to maintain a clear distinction between the short papers in the proceedings and the full papers in *Behavior Research Methods*, the length of short papers was limited to 4 pages for oral presentations and 2 pages for posters.

The response to this year's conference has been very positive in terms of submissions and participation. In line with the cross-disciplinary aims of the conference, we had a good balance between human and animal behavior topics, and between methodological and technical sessions. Eleven special symposia dealt with topics as diverse as measuring and analyzing facial expression, dominant-submissive behavior, pain, rodent behavior, dispersal, neurological disorders, learning and memory, meeting behavior, abnormal human behavior, zebra fish behavior, and olfaction. This year's special interest groups covered interesting new data acquisition and signal analysis techniques in the area of measurement of human motion, telemetric and behavioral monitoring, and Virtual Reality for skills training and performance measurement.

Last but not least we pride ourselves with the contribution of three very interesting keynote speakers. Jeffrey Cohn (Pittsburgh, USA) spoke about automated facial image analysis; Richard Morris (Edinburgh, UK), the inventor of the classical water maze task for rodents, addressed the move from spatial learning to episodic-like and semantic-like memory; and Sergio Velastin (Kingston upon Thames, UK) reviewed the state of the art in intelligent camera surveillance for the detection of abnormal human behavior. Dr Velastin's lecture received extensive coverage in Dutch radio programs and newspaper articles, illustrating the timely nature of the topic.

The organization of Measuring Behavior 2005 would not have been possible without the diligent efforts of a large number of volunteers and the staff members in the conference office. We thank in particular the members of the Scientific Program Committee, chaired by Louise Vet, for supporting us in reviewing abstracts and giving feedback to authors. All submissions were reviewed by at least three (for oral papers) or two (for posters and demonstrations) reviewers, none of whom are associated with the organizing company. This allowed us to keep the quality of the work presented at Measuring Behavior at a high level, and to avoid a conflict of interest. As editors we have done our best to assist authors with textual refinement of their paper, keeping in mind the multidisciplinary readership of this volume.

Our colleagues of the Local Organizing Committee did an excellent job in making it all happen, before and during the conference. Together with the tour guides and the student volunteers, they deserve to be applauded. We are also grateful to our sponsors and exhibitors, who are listed at the end of this volume.

Invaluable support for the submission and review process and for preparing the proceedings has been provided by the conference secretariat and local organizing committee members Mechteld Ballintijn, Marjoleine Bessels, Cécile Bruisten, Yvonne Leander and Joeke van Santen.

Our final thanks go to all Measuring Behavior 2005 authors who have enabled us to put together a high-quality, diverse and exciting conference program and proceedings.

Lucas Noldus  
Fabrizio Grieco  
Leanne Loijens  
Patrick Zimmerman

Noldus Information Technology bv  
Wageningen

# The Measuring Behavior Conferences

Measuring Behavior is a unique event. The mission of the conference is to present innovations and share ideas and experiences about methods, techniques and tools for the study of human or animal behavior, independent of the species being studied. While most conferences focus on a specific domain, Measuring Behavior creates bridges between disciplines by bringing together people who may otherwise be unlikely to meet each other. At a Measuring Behavior meeting, you will find yourself among ethologists, behavioral ecologists, neuroscientists, developmental psychologists, human factors researchers, movement scientists, psychiatrists, usability testers and others! While the research questions and applications may be highly diverse, all delegates share an interest in methods, techniques and tools for studying behavior. Experience tells us that the focus on methodological and technical themes can lead to a very productive cross-fertilization between research fields.

The first meeting, Measuring Behavior '96, chaired by Berry Spruijt, was a spin-off from the European project "Automatic Recording and Analysis of Behavior". The plan to share the results of our project with colleagues quickly evolved into an international event. Organized by Noldus Information Technology and hosted by Utrecht University, Measuring Behavior '96 attracted 153 participants from 25 countries. The 2-day program included 70 presentations and 4 scientific tours. Menno Kruk wrote a report of the meeting, which was published in *Trends in Neurosciences* (vol. 20, pp. 187-189, 1997).

The second conference, Measuring Behavior '98, under chairmanship of Jaap Koolhaas, brought more than 275 delegates from 32 countries together at the campus of the University of Groningen. During three full conference days, there were 140 presentations grouped into 14 thematic symposia, well balanced between human and animal research. The program included ample time for posters and demonstrations of software or equipment by participants. We had 6 lab tours, 20 set-ups for ongoing technical training and two companies organized user meetings. A 'video digitization service' allowed delegates to take a look into the world of digital video, which was new for most at that time. Finally, some 20 companies exhibited scientific books, instruments and software. For the first time, selected presentations were published as full papers in the journal *Behavior Research Methods, Instruments & Computers*.

Measuring Behavior 2000 was held in Nijmegen and hosted by Alexander Cools, attracting over 300 delegates from around the world, who attended more than 160 oral and poster presentations. New to the program at Measuring Behavior 2000 were special interest groups and workshops. Once again, different companies exhibited a wide range of research instruments and software, and delegates could visit different labs on scientific tours, receive ongoing technical training and attend user meetings organized by Noldus Information Technology.

We saw a further increase in attendance when Measuring Behavior 2002 was held at the Vrije Universiteit Amsterdam with Gerrit van der Veer serving as program chair. 325 delegates from 37 countries took part in a busy program, which included - for the first time - tutorials, short courses taught by expert instructors. Twelve of these were organized and received high ratings.

And now you find yourself at the fifth Measuring Behavior conference in Wageningen. Measuring Behavior 2005 is special in several ways. First of all, we have a record number of participants, as many as 450! The interest shown in the conference has been truly worldwide, with participants traveling to the Netherlands from 40 different countries. We also have a record number of presentations, with almost 300 oral papers, posters and demonstrations. This includes 3 renowned keynote speakers, 11 well-prepared symposia and 5 special interest groups. The conference publications have been expanded with a Conference CD and printed Conference Proceedings, containing short papers of most presentations. Finally, for Noldus Information Technology as the organizing company, this conference marks the opening of our new building, where we proudly receive all delegates for the welcome reception.

The local organizing committee has done its best to prepare an optimal mix of scientific, technical, social and culinary ingredients. We hope that you find Measuring Behavior 2005 a rewarding experience and wish you a pleasant stay in Wageningen.

Lucas P.J.J. Noldus  
Chair, Local Organizing Committee  
Managing Director, Noldus Information Technology bv

# Acknowledgements

## Scientific Program Committee

- Louise Vet (Program Chair), Netherlands Institute of Ecology (NIOO-KNAW), Nieuwersluis, and Laboratory of Entomology, Wageningen University, Wageningen, The Netherlands
- Ring Carde, Department of Entomology, University of California, Riverside, CA, U.S.A.
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- Eco de Geus, Department of Biological Psychology, Vrije Universiteit, Amsterdam, The Netherlands
- Stan Gielen, Department of Medical Physics and Biophysics, Radboud University Nijmegen, Nijmegen, The Netherlands
- Ilan Golani, Department of Zoology, Tel Aviv University, Tel Aviv, Israel
- Jaap Harlaar, Department of Rehabilitation, Vrije Universiteit Medical Center, Amsterdam, The Netherlands
- Frans van der Helm, Man-Machine Systems & Control group, Department of Mechanical Engineering, Delft University of Technology, Delft, The Netherlands
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- Hans van Trijp, Marketing and Consumer Behavior Group, Social Sciences Group, Wageningen University, Wageningen, The Netherlands and Unilever Research, Vlaardingen, The Netherlands
- Jonathan Vaughan, Department of Psychology, Hamilton College, Clinton, NY, U.S.A.
- Gerrit van der Veer, Department of Information Management and Software Engineering, Vrije Universiteit, Amsterdam, The Netherlands
- Peter Wittenburg, Max Planck Institute for Psycholinguistics, Nijmegen, The Netherlands

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- Mechteld Ballintijn (website, logistics, general communication, program and abstracts books)
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- Melvin de Bruijn
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# Conference Publications

Measuring Behavior 2005 resulted in the following conference publications:

## Program book

The program book contains a complete overview of all conference events, schedules of sessions and presentations, social events, information about exhibitors, conference organization, and practical information.

## Abstracts book

The abstracts book contains the summaries of all 296 conference presentations, listed alphabetically by first author.

## Conference CD

Bundled with the abstracts book is the conference CD. This disk, published for the first time for Measuring Behavior 2005, contains 220 papers presented at the conference. A search function is provided, allowing you to find papers by author, co-author, title or keyword. Several papers on the conference CD are accompanied by digital video clips.

## Printed proceedings

This book, published for the first time in conjunction with Measuring Behavior 2005, contains 230 papers presented at the conference, including those that reached the editors after the conference and did not make it to the conference CD. Because most journals allow little space for detailed descriptions and illustrations of methods, tools, and setups, the Measuring Behavior proceedings offer a unique collection of papers with a methodological and technical emphasis. All papers focus on the details of methods and techniques, rather than a discussion of scientific hypotheses, data and results. This makes it a unique volume for your professional library.

## Special issue of *Behavior Research Methods*

The conference organization has an agreement with *Behavior Research Methods* (BRM), a peer-reviewed journal (ISSN 0743-3808) published by the American Psychonomic Society ([www.psychonomic.org](http://www.psychonomic.org)). This journal (previously called *Behavior Research Methods, Instruments & Computers*) devotes a special issue to the Measuring Behavior conference. Copies of the special issues can be obtained directly from Psychonomic Society Publications at [www.psychonomic.org/brmic/special.htm](http://www.psychonomic.org/brmic/special.htm). The links below take you to the table of contents of the special issues that have been published so far:

- Measuring Behavior '98: [www.noldus.com/events/mb98/brmic.htm](http://www.noldus.com/events/mb98/brmic.htm)
- Measuring Behavior 2000: [www.noldus.com/events/mb2000/brmic.html](http://www.noldus.com/events/mb2000/brmic.html)
- Measuring Behavior 2002: [www.noldus.com/events/mb2002/brmic.html](http://www.noldus.com/events/mb2002/brmic.html)

## Conference website

After each conference, the Measuring Behavior website is converted into an archival site, with abstracts of all presentations, which remain accessible. The websites of past conferences form a valuable resource on methods and techniques for behavioral research. These are links to the websites of all five conferences:

- Measuring Behavior '96: [www.noldus.com/events/mb96/mb96.htm](http://www.noldus.com/events/mb96/mb96.htm)
- Measuring Behavior '98: [www.noldus.com/events/mb98/mb98.htm](http://www.noldus.com/events/mb98/mb98.htm)
- Measuring Behavior 2000: [www.noldus.com/events/mb2000/](http://www.noldus.com/events/mb2000/)
- Measuring Behavior 2002: [www.noldus.com/events/mb2002/](http://www.noldus.com/events/mb2002/)
- Measuring Behavior 2005: [www.noldus.com/events/mb2005/](http://www.noldus.com/events/mb2005/)

## Keynote speaker

# Richard Morris

*Division of Neuroscience, Edinburgh University, Edinburgh, United Kingdom*

## About the speaker

Richard Morris is an elected Fellow of the Royal Society of Edinburgh, of the Royal Society in London, and the Academy of Medical Sciences. He was recently elected to the American Academy of Arts and Science. He has won several awards for his research, notably the Zotterman Medal of the Swedish Physiological Society in 1999 given at the Nobel Forum in Stockholm. He is also active in aspects of science administration, including a period as Chair of the British Neuroscience Association, and current membership of the Advisory Boards of a Research Centre in Tokyo, a Max Planck Institute in Munich and of the Picower Center for Learning and Memory at M.I.T. in Cambridge, USA.



## Moving on from spatial learning to episodic-like and semantic-like memory

Spatial learning - in T-mazes, radial-mazes and the water maze - has long been a popular choice amongst behavioral neuroscientists interested in the neurobiology of learning and memory. Drawing largely on studies using the water maze, I shall describe classical findings that have emerged over the past 20 years, ranging from standardized spatial reference memory protocols through to delayed-matching-to-place and other procedures expressly designed to address specific theoretical issues relating to hippocampal function, the role of synaptic plasticity in memory, and animal models of neurodegenerative disease. A key theme is that the water maze, no less than types of apparatus, is merely an apparatus; it is important to maintain a sharp distinction between the water maze as an apparatus and the water maze as a set of distinct training protocols. The value and pitfalls of different procedures will be highlighted.

Increasingly, students of the neurobiology of learning and memory are interested in investigating wider issues, notably how spatial and contextual memory can provide a framework for remembering events. My research group has recently developed a new paired-associate paradigm for investigating episodic-like and semantic-like memory in animals. We call the apparatus the 'Event Arena' and within it, rats are trained to find a specific flavor of food in a particular location. Learning may take place over 1-trial (episodic-like), or over several trials and days (semantic-like). The characteristics of the two styles of training will be described, together with illustrations of the flexibility of this new appetitive paradigm for investigating a wider range of issues, such as the puzzle of system-level memory consolidation. Certain implications of the apparatus for the development of new automated systems for tracking animals and for automatically recognizing their behavioral actions will also be discussed.

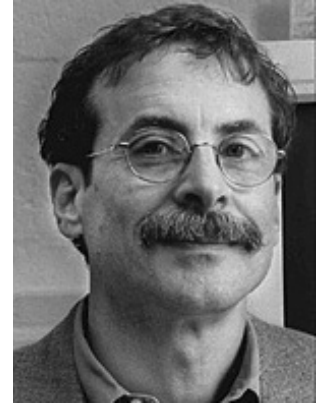
## Keynote speaker

# Jeffrey F. Cohn

*Division of Psychology, University of Pittsburgh, Pittsburgh, PA, U.S.A.  
and the Robotics Institute, Carnegie Mellon University, Pittsburgh, PA, U.S.A.*

## About the speaker

Jeffrey Cohn is Professor of Psychology at the University of Pittsburgh and Adjunct Faculty at the Robotics Institute, Carnegie Mellon University. He earned his PhD in Clinical Psychology from the University of Massachusetts in Amherst and completed Clinical Internship at the University of Maryland Medical Center. For the past 20 years, he has conducted investigations in the theory and science of emotion, depression, and nonverbal communication. He has co-lead interdisciplinary and inter-institutional efforts to develop advanced methods of automatic analysis of facial expression and prosody and applied these tools to research in human emotion, communication, biomedicine, biometrics, and human-computer interaction. He has published over 90 papers on these topics. His research has been supported by grants from the National Institute of Mental Health, the National Institute of Child Health and Human Development, the National Science Foundation, the Office of Naval Research, and the Defense Advanced Research Projects Agency.



## Automatic facial image analysis

Facial expression is one of the most powerful, natural, and immediate means for human beings to communicate their emotions and intentions. The face can express emotion sooner than people verbalize or even realize their feelings. To make optimal use of the information afforded by facial expression, reliable, valid and efficient methods of measurement are critical.

Two major advances toward this goal were the human-observer-based Facial Action Coding System (FACS) and facial electromyography (EMG). The third major advance - automatic facial image analysis - combines the best features of FACS and EMG: Comprehensive description of facial expression (FACS) and quantitative, automatic measurement, which was previously possible only by using invasive facial sensors (EMG).

This talk reviews previous approaches to facial measurement (FACS and facial EMG) and the advances in automatic facial image analysis they inform. It reviews technical and conceptual challenges that lay ahead and the approaches of leading investigators. It describes capabilities and limitations of current systems, applications in emotion and social interaction, and what we have already learned using these new systems about the configuration and timing of facial expression.



## Keynote speaker

# Sergio Velastin

*Digital Imaging Research Centre, School of Computing & Information Systems,  
Kingston University, Kingston-upon-Thames, United Kingdom*

## About the speaker

Dr. Sergio A Velastin obtained his doctoral degree from the University of Manchester (UK) for research on vision systems for pedestrian and road-traffic analysis. He then worked in industrial R&D and project management before joining the Dept. of Electronic Engineering in Kings College London (University of London). In 2001, he and his team joined the Digital Imaging Research Centre in Kingston University, attracted by its size and growing reputation in the field, where he is currently a Reader. He was Technical Coordinator of the EU-funded project PRISMATICA working on the integration of technology (networking, video/audio processing, wireless transmission) and human-based processes for improving personal security in public transport systems. His research interests include computer vision for pedestrian monitoring and personal security as well as distributed visual surveillance systems. Dr. Velastin is a member of the IEE, IEEE and the British Machine Vision Association (BMVA).



## Intelligent CCTV surveillance: Advances and limitations

The installation of closed circuit television (CCTV) cameras in urban environments is now commonplace and well-known. The UK leads the world with an estimated 4 million public cameras installed (20% of the world's deployment). Public attitudes to these systems reflect the balance needed between two conflicting requirements: (a) Concerns over invasion of privacy and fears of authoritarian control of the population, and (b) Welcoming the increased safety in public spaces and reductions in crime and antisocial behavior. Recent events and what appears to be the effectiveness of the CCTV infrastructure in assisting law enforcers to understand how the events took place and the people involved, seem to have tipped the balance towards (b), at least momentarily. What we should not forget however, is that such events are thankfully very rare but that there is a cumulative significant effect of mundane daily events that we need to deal with. For example, it has been estimated that what in the UK is called "antisocial behavior" costs the country around € 5000 million a year. A single London Borough (municipality) spends annually around € 1 million to remove graffiti, 44% of women feel unsafe at bus stops at night, a single bus company in a major city is known to have replaced 8,000 windows in one year and a study showed that in a single day in the UK there were around 66,000 reports of nuisance or loutish behavior. At the same time, there are reports that when CCTV has been installed there has been a 35% reduction in crime over 5 years. Indeed, crime in general is decreasing while uncivil behavior is on the increase. It turns out that one of the major limitations of conventional CCTV systems is the impracticality of deploying sufficient number of people to be in front of television screens observing largely uneventful video. As long as this is the case, CCTV will tend to remain a reactive tool. The inability of being truly pro-active, producing timely alarms and eventually being able to prevent incidents, is what ultimately limits these systems. As a preamble to the main associated symposium, this talk will illustrate some of the efforts that the research and industrial communities are making towards realizing automated means of detecting video events involving human activity. It will show the kind of progress made but also the current limitations of this technology.



# Automatic facial expression analysis and synthesis

M. Pantic

*Electrical Engineering, Mathematics and Computer Science, Delft University of Technology, Delft, The Netherlands*

With an ever-increasing role of computers and other digital devices in our society, one of the main foci of the research in Artificial Intelligence is on Impacts of Emerging Human-Machine Systems. A related, crucial issue is that of Human-Machine Interaction (HMI). A long-term goal in HMI research is to approach the naturalness of human-human interaction. This means integrating “natural” means that humans employ to interact with each other into the HMI designs. With this motivation, automatic speech recognition and synthesis have been the topics of research for decades. Recently, other human interactive modalities such as body and facial gestures have also gained interest as potential modes of HMI. The objective of this symposium is to review recent advances in automatic facial expression analysis and synthesis and their potential applicability to natural HMI. Facial expression is one of the most cogent, naturally preeminent means for human beings to communicate emotions, to clarify and stress what is said, to signal comprehension, disagreement, and intentions, in brief, to regulate interactions with the environment and other persons in the vicinity. Automated analyzers and synthesizers of facial expressions have, therefore, numerous applications in behavioral science, medicine, security, and HMI. They form the essence of automated tools for lip reading, bimodal speech analysis, videoconferencing, face and visual speech animation, virtual character animation, affective computing, etc. In the robotics area, the facial expression is also very important for natural/comfortable man-machine interaction. KISMET (AI Lab., MIT) and Face Robot (Science University of Tokyo) are good examples. Although several promising approaches have been reported in the literature in the last ten years, automatic detection, interpretation and synthesis of human facial expressions remain rather difficult to achieve. This Symposium on *Automatic facial expression analysis and synthesis* represents an internationally respected scientific forum for presenting and discussing different approaches to automatic facial expression detection, analysis, synthesis, and their applications to the field of HMI.

## **Towards a real-time and distributed system for face detection, pose estimation and face-related features**

J. Nesvadba, A. Hanjalic, P. Fonseca, B. Kroon, H. Celik and E. Hendriks

This paper discusses the challenges and possibilities of automatic detection and analysis of human faces in the scene. The evolution of storage capacity, computation power and connectivity in Consumer-Electronics(CE)-, in-vehicle-, medical-IT- and on-chip- networks allow the implementation of grid-computing-based real-time and distributed face-related analysis systems. A combination of facial-related analysis components – Service Units (SUs) – such as omni-directional face detection, pose estimation, face tracking and facial feature localization provide a necessary set of basic visual descriptors required

for advanced facial- and human-related feature analysis SUs, such as face recognition and facial-based mood interpretation. Smart reuse of the available computational resources across individual CE devices or in-vehicle- and medical-IT- networks in combination with descriptor databases facilitate the establishment of a powerful analytical system applicable for various domains and applications. The SUs for advance analysis of human faces developed at Philips Research Laboratories (NatLab) in cooperation with Delft University of Technology are described.

## **Learning spatio-temporal models of facial expressions**

M. Pantic, I. Patras and M.F. Valstar

This paper explains first the benefits of a robust facial expression analyzer for fields as diverse as psychology, medicine, security, education, and HMI. Then, it reports on two different methods aimed at automatic recognition of facial muscle activations (i.e., facial action units, AUs) from nearly frontal-view face video. The first proposed method constructs temporal templates from the input face video and applies a two-stage learning machine, combining a kNN algorithm and rule-based reasoning, to recognize shown individual AUs and AU combinations. The second method exploits particle filtering to track facial fiducial points such as the mouth and eyebrow corners in the input face video and applies temporal rules to recognize AUs and their temporal segments (i.e., onset, apex, offset) occurring alone or in combination in the input image sequence. Finally, a case-based reasoning system is discussed, which is capable of classifying facial expressions (given in terms of AUs) into the emotion categories learned from the user. The utilized case base is a dynamic, incrementally self-organizing event-content-addressable memory that allows fact retrieval and evaluation of encountered events based upon the user preferences and the generalizations formed from prior input.

## **The CMU/Pitt automated facial image analysis System**

T. Kanade and J.F. Cohn

Both the configuration and the timing of facial actions are important in emotion expression and recognition. To investigate the timing and configuration of facial actions, the CMU/Pitt Automated Facial Image Analysis (AFA) System has been developed. The latest version of the system is based on Active Appearance Models (AAMs). These are generative, parametric models and consist of a shape component and an appearance component. The shape component of is a triangulated mesh that moves like a face undergoing both rigid motion (head pose variation) and non-rigid motion (expression) in response to changes in the parameters. The appearance component of the AAM is an image of the face, which itself can vary under the control of the parameters. As the parameters are varied, the appearance varies so as to model effects such as the

emergence of furrows and wrinkles and the visibility of the teeth as the mouth opens. Two disadvantages of traditional AAMs are that they are 2D and rigid head motion and non-rigid facial motion are confounded in the shape model. To address these problems, we recently developed an extension to AAMs that augments the usual 2D shape model with a 3D shape model. This advance separates the 3D rigid motion of the head and 3D non-rigid facial expression into two disjoint sets of parameters and recovers the 3D shape of the face. This approach works well as long as out-of-plane head motion is small to moderate. As out-of-plane head motion becomes large, automatic recovery of 3D shape becomes increasingly difficult because of self-occlusion. To solve this problem, a single AAM is fitted to multiple images captured simultaneously from synchronized cameras.

### **Bimodal emotion recognition**

N. Sebe, E. Bakker, I. Cohen and T. Huang

Recent technological advances have enabled human users to interact with computers in ways previously unimaginable. Beyond the confines of the keyboard and mouse, new modalities for human-computer interaction such as voice, gesture, and force-feedback are emerging. Despite important advances, one necessary ingredient for natural interaction is still missing - emotions. Emotions play an important role in human-to-human communication and interaction, allowing people to express themselves beyond the verbal domain. The ability to understand human emotions is desirable for the computer in several applications. This paper describes the challenging problem of bimodal emotion recognition and advocates the use of probabilistic graphical models when fusing the different modalities. We test our audio-visual emotion recognition approach on 38 subjects with 11 HCI-related affect states. The experimental results show that the average person-dependent emotion recognition accuracy is greatly improved when both visual and audio information is used in classification.

### **A robust scheme for facial analysis and expression recognition**

S. Ioannou, M. Wallace, K. Karpouzis and S. Kollias

Facial analysis includes a number of processing steps. One of those is the extraction and tracking of the movement of facial components and facial fiducial points. Due to noise, illumination variations and low resolution capturing devices, the detection of facial feature points can be inaccurate. Hence, mechanisms are required that can automatically evaluate the quality of each computed mask, assigning a confidence level to it. The emotion recognition system can take advantage of each feature's confidence level when analyzing them. Exploitation of anthropometric knowledge in the form of a set of criteria, evaluating the relation of the extracted features, can form such a mechanism. This paper explains further how the

detected facial features can be used to extract the Feature Points considered in the definition of the Facial Animation Parameters (FAPs). Finally, we discuss techniques such as clustering and neurofuzzy methods that can transfer variations of the FAP variables into rules for recognition of the user's emotional state and then adapt these rules to specific user's characteristics.

### **Emotion and facial expressions in creating embodied agents**

T.D. Bui, D. Heylen, A. Nijholt and M. Poel

This paper describes the work done on Obie, an embodied conversational agent framework. An embodied conversational agent, or talking head, consists of various components. The authors have created a face model and a facial muscle model in such a way that realistic facial expressions can be produced in real-time on a standard PC. In particular, they have defined a face model that allows high quality and realistic facial expressions, which is still sufficiently simple in order to keep the animation real-time and is able to assist the muscle model to control the deformations. They have also implemented a muscle model that produces realistic deformation of the facial surface, handles multiple muscle interaction correctly and produces bulges and wrinkles in real-time. Besides this graphical part, the developed system accounts for the actions (dialogue) and emotions of the agent. Namely, the authors have implemented an emotion model and a mapping from emotions to facial expressions. For the animation, it is particularly important to deal with the problem of combining different facial movements temporally. In this respect, the dynamic aspects of facial movements and the combination of facial expressions in different channels that are responsible for different tasks received special attention.

### **Fast facial animation design for emotional virtual humans**

S. Garchery, A. Egges and N. Magnenat-Thalmann

Designing facial animation parameters according to a specific model can be time consuming. This paper presents a fast approach to design facial animations based on minimal information (only feature points). All facial deformations are automatically computed from MPEG-4 feature points. An extension of this approach that allows to personalize or to customize the deformations according to different characteristics and then with minimal manual interaction is also presented. Different prototypes of the facial animation system, available on different platforms, are described. How emotions and expression can be incorporated into the facial animation system is demonstrated as well. Finally, different approaches to emotions and personality are presented.

# The CMU/Pitt Automated Facial Image Analysis System

T. Kanade<sup>1</sup>, J.F. Cohn<sup>2</sup>

<sup>1</sup>The Robotics Institute, Carnegie Mellon University, Pittsburgh, USA

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## Abstract

To investigate the timing and configuration of facial actions, an interdisciplinary group of behavioral and computer scientists developed a computer-vision based approach, the CMU/Pitt Automated Facial Image Analysis (AFA) System. AFA has progressed through several versions and is capable of automatically recognizing facial action units and analyzing their timing in facial behavior. The latest version is based on Active Appearance Models (AAMs). These are generative, parametric models and consist of a shape component and an appearance component. The shape component is a triangulated mesh that deforms in response to changes in the parameters corresponding to a face undergoing both rigid motion (head pose variation) and non-rigid motion (expression). The appearance component of the AAM is an image of the face, which itself can vary under the control of the parameters. As the parameters are varied, the appearance varies so as to model effects such as the emergence of furrows and wrinkles and the visibility of the teeth as the mouth opens. The system extracts and separates head orientation, 3D shape deformation, and appearance change of the face, which are then input to a facial action recognizer. AFA has demonstrated concurrent validity with human-observer based facial expression recognition and both human-observer and EMG based analysis of timing.

## Keywords

Automatic facial image analysis, facial expression, timing.

## 1 Introduction

People are highly sensitive to the timing of facial actions (Edwards, 1998). For example, slower facial actions, within limits, appear more genuine [18, 22], as do those that are more synchronous in their movement [14].

The timing of facial actions has been difficult to study in

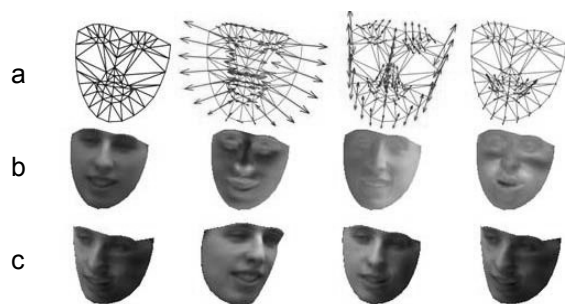


Figure 1. (a) The mean face shape (left) and 1<sup>st</sup> 3 shape modes. (b) The mean appearance (left) and 1<sup>st</sup> 3 appearance modes. (c) Four example faces generated with the AAM in (a) and (b).

part because manual measurement of timing is relatively coarse and labor intensive [1]. Until recently, the only automatic method of measuring facial action has been facial electromyography (EMG). Facial EMG effectively quantifies the timing of covert muscle action [11], but the need to attach sensors to the face restricts its use to controlled settings, and may in itself affect facial behavior [2]. For more natural and less obtrusive

measurements of facial actions (both timing and configurations), an interdisciplinary group of behavioral and computer scientists developed a computer-vision based approach, the CMU/Pitt Automated Facial Image Analysis (AFA) System. AFA is capable of unobtrusively recognizing facial action units and analyzing their timing in facial behavior.

## 2 CMU/Pitt Automated Facial Image Analysis

The latest version of the CMU/Pitt Automated Facial Image Analysis (AFA) system is based on Active Appearance Models (AAMs) [10, 20]. These are generative, parametric models that consist of a shape component and an appearance component. The shape component is a triangulated mesh that deforms in response to changes in the parameters corresponding to a face undergoing both rigid motion (head pose variation) and non-rigid motion (expression). The appearance component is an image of the face, which itself can vary under the control of the parameters. As the parameters are varied, the appearance varies so as to model effects such as the emergence of furrows and wrinkles and the visibility of the teeth as the mouth opens.

The complete AAM in which shape and appearance components are combined can generate faces undergoing a wide range of expression changes. For example, Figure 1(c) contains face images generated using the AAM in Figure 5(a-b) by setting appropriate model parameters. The key technique that allows an AAM to be used for facial analysis is a fitting algorithm. Given an input image of the face, a fitting algorithm searches for the model parameters that best match the input image.

### 2.1 Recovery of 3D head motion and shape

Traditional AAMs are actually "2D" in the sense that rigid head motion and non-rigid facial motion are confounded in the 2D-mesh shape model [20]. Thus, a 2D-based AAM does not handle larger head motions. To address these problems, we use an extension to AAMs that augments the usual 2D mesh model with an actual 3D shape model, thus separately and explicitly modeling the 3D rigid motion of the head and 3D non-rigid facial expression into two disjoint sets of parameters [25]. This advancement allows us to extract and separate the pose, 3D shape deformation, and appearance change of the face. We refer this approach as a 2D+3D AAM because it estimates both 2D and 3D parameters.



Figure 2. An example of fitting a 2D+3D AAM to a single image. Shown are the initial fit, intermediate result, and final result after the algorithm has converged. The 3D shape estimate (white) is projected onto the original image and also from two novel viewpoints (top right). Estimates of 3D pose (top left) and 2D shape (blue dots) are displayed as well. Estimated head orientation (pitch, roll, and yaw) is shown in the top left-hand corner of each image. From [25]

Figure 2 shows an example of fitting a 2D+3D AAM to a single image. The first image shows the initial estimate of 2D and 3D shape parameters. The former appear as blue dots, and the latter as a white mesh. The fitting process is iterative. The final result is shown in the last figure.

This same approach can be applied to an image sequence with change in pose (rigid head motion) and expression (non-rigid head motion). This is illustrated in Figure 3. A 2D+3D AAM was fit to each frame in an image sequence. The face mesh tracked the rigid and non-rigid motion of the face. By separating rigid and non-rigid motion, the timing of facial actions, such as smiling, is not confounded by rigid head motion.



Figure 3. An example of tracking an expressive face in a video by fitting the model successively to each frame. The results in 3 frames from a longer image sequence are shown here. As the model is fit to the image, the 3D shape (white mesh), 2D shape (blue dots), and the 3D pose are estimated. Estimated head orientation (pitch, roll, and yaw) is shown in the top left-hand corner of each image.

**2.2 Parameter estimation using multiple cameras** As out-of-plane head motion becomes large, automatic recovery of 3D shape becomes increasingly difficult because of self-occlusion. To solve this problem, we fit a single AAM to multiple images captured simultaneously from multiple cameras. An example of this approach is shown in Figure 4. The first row shows the initialization of the mesh at the first iteration. The initial mesh at first does not correspond to its correct position on the face, but converges to the right one. The bottom row shows the result after the algorithm converges and the correct result is obtained.

### 3 Capabilities and Limitations

This version of AFA is capable of fully automatic initialization and recovery of 3D face shape and appearance. By using an individualized 3D face model, it more accurately extracts change in facial features than previous versions [3]. Processing speed exceeds video frame rate (30 Hz) and long image sequences can be processed. The system is not limited to offline use. It can be used in real-time applications, such as gaze estimation while driving [16, 17].

The system in its current state has some limitations. One, before use with video of a new person, it must first

be trained on images of the person's face. This training may require hand labeling 10 to 20 or more images and is time consuming. Efforts are underway to make training more efficient. Two, facial features extraction is based on a set of 68 points, only a subset of which is informative of facial expression. Many of the points are for head tracking and feature registration. Increasing the number of feature points may be informative for facial feature analysis. A final limitation is the need for multiple synchronized video streams when out-of-plane head motion becomes moderate to large. A possible solution is to integrate the 3D cylindrical head tracker that had been used in the previous version [26] with the 2D+3D AAM-based head tracking.

## 4 Use of AFA to Investigate the Configuration and Timing of Facial Actions

### 4.1 Configuration of facial actions

We have used this and previous versions of AFA to recognize facial action units, make comparisons with criterion measures of facial dynamics, and investigate the timing of facial behavior and head motion. In directed facial action tasks, AFA has shown high agreement with manual FACS coding for approximately 20 action units.[8, 19, 23, 24] The action units recognized include most of those that have been a focus in the literature on facial expression and emotion [13]. In the more challenging case of spontaneous facial behavior with non-frontal pose, out-of-plane head motion, and occlusion from glasses or facial jewelry, AFA achieved 98% agreement with manual FACS [12] coding of blinks



Figure 4. An example of multi-view 3D AAM fitting. Each image is overlaid with the corresponding 2D shape for that image in blue dots. The head pose is displayed in the top left of each image as pitch, roll, and yaw. The single 3D shape is displayed in the top right of the center image. This 3D shape is also overlaid in each image as a white mesh. From [15].

[7] and 89% accuracy for eyebrow raising and lowering [4].

### 4.2 Comparison with facial EMG measurements

To evaluate the temporal precision of AFA, we compared it with facial EMG. Facial EMG is considered a gold standard for measurement of facial muscle activity. AFA measurement of lip-corner displacement and *Zygomaticus major* EMG were compared [5, 21]. Facial EMG was recorded while subjects watched a film clip of a comedy routine intended to elicit spontaneous smiles (AU 12 in FACS). We analyzed an 11-second interval beginning 1-second prior to the punch line of a joke and continuing for 10 seconds. This interval was intended to capture the onset, peak, and offset of each smile. Smile onsets were highly correlated ( $r > 0.90$ ),

with EMG onset preceding AFA onset by about a quarter second. (See Figure 5 for an example). The amount of lip corner motion was also in agreement in 72% of cases with distinct EMG onset, where the amount was quantified in EMG by its amplitude, and in AFA by the visual displacement  $\Delta d = \sqrt{\Delta x^2 + \Delta y^2}$ . Note that EMG can detect occult changes in muscle activation which may not result in visible motion (AU12) that AFA can detect, so we consider this a good agreement. Relating physiological measurement (i.e., EMG) and visible behavior (lip motion) at this level of precision has not been possible using manual coding, which lacks sufficient temporal resolution [1].

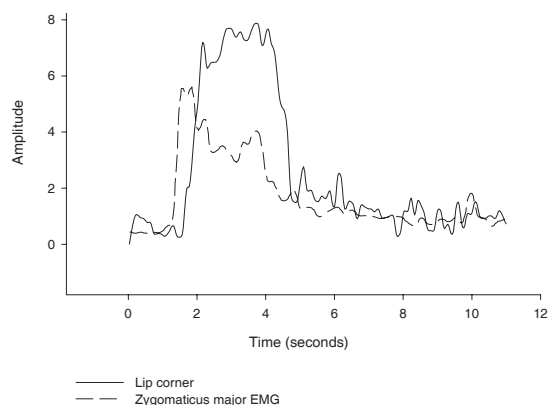


Figure 5. Relation between zygomatic major EMG and lip-corner displacement in a spontaneous smile (AU 12).

#### 4.3 Dynamics of interpersonal behavior

In work with Daniel Messinger at the University of Miami, we have used AFA to track changes in facial expression of mothers and infants during face-to-face

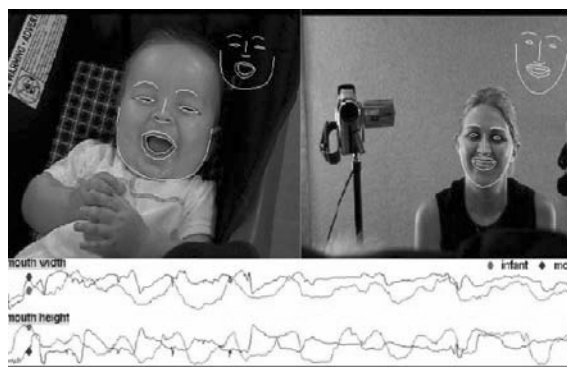


Figure 6. Intra- and interpersonal coordination of lip-corner displacement (smile intensity) and mouth opening in mother and infant.

interaction. Figure 6 shows an example from one of two mother-infant dyads time series for lip-corner displacement, a measure of smile intensity, and mouth opening. These and related measurements enable us to more rigorously test parent-infant bidirectional influence and synchrony than was previously possible [6], and provide new capability to investigate features of emotion regulation [9].

#### 5. Conclusions

The CMU/Pitt Automated Facial Image Analysis System has progressed through several versions. The current version uses generative, parametric shape and appearance models and estimates both 2D and 3D parameters. For large out-of-plane head motion, a multi-camera version may be used. The system is able to extract and separate

head orientation, 3D shape deformation, and appearance change of the face. Previous versions have demonstrated concurrent validity for action unit recognition in both deliberate and spontaneous facial behavior with out-of-plane head motion and occlusion. The current version is more robust to long image sequences, has greater ability to represent the 3D structure of the face, and shows concurrent validity with facial EMG for the timing of facial actions. Unlike facial EMG, it is unobtrusive and has high specificity for observable facial action.

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# Defining and measuring dominant-submissive behavior

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Neuroscientists make every effort to automate behavioral tests to minimize subjective judgment and manual labor, and to maximize repeatability of the results between laboratories while increasing the throughput of experimental endpoints. In recent years, these efforts have accelerated due to the development of many inbred or transgenic mice strains that needed systematic behavioral phenotyping in order to find the functions delineated to strain differences or specific genes. At the same time, the development of *in vitro* biochemical high-throughput drug screening tests highlighted similar needs in behavioral neurosciences where the intact organism is a subject of study.

This symposium focuses on dominant-subordinate behavior across species. Social hierarchy is common in many animal phyla including fish, reptiles, birds and mammals. This extends to different species of a phylum. For example, within mammals this behavior is found among mice, rats, dogs, and most primates as well as humans. The premise of this symposium is that dominance and subordination observed in humans can be related to mania and depression and that important elements of both mania and depression can be modeled in animals based on observation of dominant and submissive behavior exhibited under well-defined conditions.

Human dominant-subordinate social behavior can be viewed as abnormal (e.g., active non-participant 'manic' and passive non-participant 'sad') or normal social behavior (active participant 'warm' and passive participant 'shy'). The interaction of subjects under these four conditions and their subsequent behavior on a dyadic game will be described in terms of the behavioral mechanisms of social adaptation. Investigators have used various endpoints to measure dominance in animals. This includes observation of hierarchy in groups of animals (two or more) by denoting their communication through different body postures, competition for priority of access to different resources using distinct types of scoring, and social defeat based on animal territorial instinct. These distinct approaches to measure dominance and submissiveness will be discussed by different speakers during the symposium, with the focus on the criteria used to define dominance and submissiveness and efforts to automate measurements.

Just as affective disorders are mood illnesses with two opposite poles, melancholia (depression) and mania that are expressed to different degrees in affected individuals, dominance and submissiveness are also two contrasting behavioral poles distributed as a continuum along an axis with less or more dominant or submissive animals. The importance of the selection process for these behavioral extremes will be underlined as a necessary factor for successful modeling of mania by dominance and depression by submissiveness.

## **Divergent patterns of social behavior result in rejection and reduced social reinforcement**

A.J. Bond and W.S. Tse

Social skills are important for integration into society. These skills can be defined as the ability to acquire and use behaviors necessary for effective and satisfying interpersonal functioning and include both non-verbal and verbal elements. Substantial evidence has found that impaired social functioning is correlated with current depression. Two psychosocial models of depression, Coyne's social interaction model, and Lewinsohn's social skill model, have been proposed to explain the mechanism of depression on social integration. In general, both models agree that depression weakens social skills, resulting in reduced social support. To elucidate these mechanisms, non-verbal and verbal behaviors involved in interpersonal interaction can be studied. Untreated depressed patients are less active in engaging in social interaction, as indicated by paucity of speech and increased response latency and they participate less, as indicated by lack of eye contact in interpersonal exchanges. This behavior can lead to rejection by others. Much less work has examined the consequences of manic behavior on social integration. Therefore these two behaviors, depression which can be viewed as passive or submissive and mania which can be viewed as active or dominant were modeled in the laboratory. A standard behavioral measurement protocol in which interpersonal interaction style can be classified was constructed and resulted in 4 different styles. Two of the roles portrayed abnormal social behavior, active non-participant 'manic' and passive non-participant 'sad', and two portrayed normal social behavior, active participant 'warm' and passive participant 'shy'. The interaction of subjects with a confederate acting these 4 roles and their subsequent behavior on a dyadic game was recorded. Subjects were more likely to reject confederates in the manic or sad roles. This was shown by both non-verbal behavior and verbal report. They also behaved more punitively on the dyadic game. Thus displaying depressed or manic behavior not only results in social rejection but also in the reduction of social reinforcement.

## **Ethological analysis of rodent behaviour: elucidation of the behavioural effects of psychotropic drugs**

P.J. Mitchell and P.H. Redfern

A wide diversity of animal models has been used to examine psychotropic drug activity. In recent years antidepressant drug research has focused on the search for new therapies with a rapid onset of action. It follows that, to be relevant, animal models must have the ability to measure the time course of drug-induced changes in behavior. Highly sophisticated animal models have been developed which yield a positive behavioral response to prolonged, chronic, drug treatment.

Two 'ethologically-relevant' animal models, the resident-intruder and social hierarchy paradigms, have been

especially useful in elucidating the behavioral effects of antidepressant drugs. In the resident-intruder paradigm, male Wistar resident rats are housed in isolation for a minimum of 3 days before being exposed to an unfamiliar conspecific intruder. During the ensuing social encounter, control resident rats exhibit a wide range of non-social, social and conflict-related (i.e. agonistic) behaviors which are quantified during subsequent ethological analysis. In the social hierarchy model male Wistar rats are housed in triads. All group members are routinely involved in intense levels of social and agonistic behavior at the onset of the dark phase of the light:dark cycle. Ethological analysis of such behavior (where the 'winner' and 'loser' of each social encounter is identified) reveals the relative social position of each group member (the most successful group member during these encounters indicates the dominant animal).

Together these models of rodent social and agonistic behavior have demonstrated that chronic treatment with antidepressant drugs (irrespective of their acute pharmacological activity) increases rodent aggressive behavior which, in turn, results in increased hierarchical status in closed social groups. Furthermore, the increased rodent aggression is most likely a behavioral manifestation of increased assertive behavior and arguably reflects similar changes in human behavior (including the externalization of emotions) expressed during the recovery from depressive illness.

#### **Monitoring the effects of social defeat in mice by automated observation in the home-cage and observer-based scoring during a social-interest test**

J.E. van der Harst, M. Lubbers, M. Eijkhoudt and B.M. Spruijt

Social defeat in mice has been applied in several different paradigms and for several different reasons. However, as a model for depression it has only been studied on a few occasions. Mostly a long period of daily defeats is applied of which the effects are investigated immediately afterwards. Therefore, little is known about the time of onset and development of the effects of this chronic social stress. This study aimed to investigate the effects of a particular defeat-paradigm in mice during the long-term social-stress period of 20 days. For this, home cage behavior was automatically and continuously recorded during both the light and the dark phase in specially designed cages (PhenoTyper®, Noldus Information Technology bv, The Netherlands). Several parameters that may reveal depressive-like symptoms such as altered locomotor activity and sleep/wake cycle were analyzed.

It became apparent that social defeat resulted in several acute effects (thus, at day 1) on activity, velocity and use of the shelter that were not all persistent over time. Other measures, such as a decreased frequency of movement and increased time spent in the shelter during the first part of the dark-phase seemed to become more persistent over time indicating chronic stress effects, possibly related to the development of depressive-like symptoms.

To further validate the applied defeat-paradigm as a model for depression, a so-called Partition-test was conducted at the end of the long-term defeat-period. During this test the defeated mouse was confronted with another unfamiliar mouse at the other side of a perforated partition-wall. This test is used in several depression-studies to investigate anxiety-related behavior and social interest. Using

observer-based scoring (The Observer®, Noldus Information Technology bv, The Netherlands), 2 classes were investigated in this test: zone and behavior.

It appeared that defeated animals displayed a significant decrease in social interest, activity and exploration and an increased alertness.

The results are discussed in the light of the success of the applied social-stress paradigm as a model for depression in mice and the development and onset of the symptoms.

#### **The impact of continuous variation in heritable personalities on the social structure in the great tit (*Parus major*)**

P.J. Drent

Great tits of both sexes show continuous variation in consistent phenotypically individual differences in exploration of a standard new environment (a gradual variation from fast to slow explorer). Although the absolute values of repeated tests varied with the year cycle, the inter-individual differences persist across time. This 'exploration score' is phenotypically correlated with many other behavioral traits related to coping with (environmental) challenges (e.g. boldness, risk-taking, aggressiveness, routine-formation, foraging patterns). Bi-directional selection and crossings experiments using a cross fostering design with guest-pairs show that these different behavioral traits are strongly genetically correlated. This all indicates a more general behavioral syndrome or coping strategy within the life history of the species, comparable with the variation in human personality.

Hand-reared and wild birds were used in an array of experiments to study the impact of these personalities on the composition, structure and hierarchy in winter flocks with a scrounger producer character. The dominant-submission interactions between the members of a group were standard scored on and around a feeding table. Males dominated females. Males with territorial status and for females mating with a territorial male have the highest position in the rank. In the hierarchy of territorial males the nearby the territory the higher the position in rank and within that: faster explorer dominated slower ones. In mixed groups of age and status classes the time of presence and the personality determined the rank between non-territorial males whereby in contrast to territorial birds long present slow explores dominated fast ones. This is caused by impact of actions of (old) territorial males on the non territorial juveniles that is different for the different personalities.

#### **Reduction of dominant or submissive behavior as models for antimanic or antidepressant drug testing: technical considerations**

E. Malatynska, A. Pinhasov, J. Crooke and D.E. Brenneman

Using observer-based scoring we have previously shown that dominant behavior measured in a food competition test, can serve as a model of mania and submissive behavior as a model of depression. These two models are based on a selection of animal pairs where one animal shows the behavioral trait of dominance and another submissiveness. Three criteria have to be achieved to assign dominant or submissive status to the animal. First, there has to be a significant difference between the average daily drinking scores of both animals in a pair.

Second, the dominant animal score has to be at least 25% greater than the submissive animal's score. Third, there must not be any 'reversals' during the pair selection week, where the putative submissive rat out-scores its dominant partner on isolated occasions. Twenty-five to thirty-three percent of the initial animal pairs achieve these criteria. The importance of the application of these criteria to the selection process as a reflection of the experimental outcomes will be discussed in this presentation.

Recently we have used automatic scoring of the time spent by rats in the feeder zone done by a multiple subject video-tracking system. We have noticed a similar reduction of rat submissive behavior after treatment with antidepressants, imipramine or fluoxetine as we have done previously with observer-based scoring. It is possible to

observe four pairs of rats during each five-minute experimental session (one set). A duplicate parallel set enables the immediate switch to the observation of the next four pairs. The multiple video-tracking system increases the capacity of antidepressant drug testing and reduces the variability between observations.

The onset time of the drugs in the two models was delayed. The mechanism of delayed therapeutic activity of antimanic and antidepressant drugs is not known and animal models reflecting this clinical feature will enable progress in this area. The application of the reduction of submissive behavior model to such studies is also discussed.

# Reduction of Dominant or Submissive Behavior as Models for Antimanic or Antidepressant Drug Testing: Technical Considerations.

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## Abstract

Using observer-based scoring we, have previously shown that dominant behavior measured in a food competition test can serve as a model of mania and submissive behavior as a model of depression. These two models are based on a selection of animal pairs where one animal develops the behavioral trait of dominance and another submissiveness after repeated interactions. The importance of the application of the three criteria to the selection process was reflected in the experimental results and discussed in this presentation. In automatic scoring the time spent by rats in the feeder zone is done by a multiple subject video-tracking system. We have shown that controls maintain established behavioral relation in both systems and that a reduction of rat submissive behavior after treatment with antidepressant, fluoxetine was similar to that measured previously with observer-based scoring. It is possible to score four pairs of rats during each five-minute experimental session (one set) by video tracking. The multiple video-tracking systems increase the capacity of antidepressant drug testing and reduce the variability between observations.

## Keywords

Dominance, submissiveness, mania, depression, competition test.

## 1 Introduction

The Reduction of Submissive Behavior Model (RSBM) was developed as an animal model of depression based on previous work with the Clonidine-Reversal of Dominance Model (CRDM) [1-5]. Both models result from the hypothesis that submissive behavior in social animals is related to human depression. In the earlier CRDM, submissive behavior was induced by treatment with the  $\alpha_2$ -adrenergic receptor agonist clonidine. The induction of submissive behavior by clonidine could be reversed by treatment with a wide range of antidepressant drugs, while many non-antidepressants did not have this effect [1, 5]. Thus the model has good predictive validity. However, the principle weakness of the CRDM is a lack of construct validity. This is seen in the subacute nature of the effect of clonidine and antidepressants in the CRDM. The CRDM is only as good a model of depression as clonidine treatment is. The CRDM was subsequently abandoned in favor of the RSBM. The RSBM does not require pretreatment with drugs to show antidepressant effects and provides a better model of time-dependent phenomena associated with depression [6]. We have shown that the antidepressants, fluoxetine, imipramine, desipramine and maprotyline increased competitiveness of submissive rats while diazepam, naltrindole, and amphetamine did not. The Reduction of Dominant Behavior model (RDBM) also originated from the CRDM. Clonidine, which is used in the clinic to alleviate episodes of acute mania, reduced dominant behavior in the CRDM. This observation

prompted us to test the effects of other drugs used in the clinic to treat mania such as lithium, sodium valproate and carbamazepine. We have shown that all of these drugs reduced dominant behavior [7].

Dominance and submissiveness are two opposite poles of extreme behavior that otherwise are distributed as a gradual continuum along an axis with less and more dominant or less and more submissive animals. Less than half of animals studied in our tests form clear dominant-submissive relationships (DSR). The majority of animals form flexible relationships without dominant or submissive behavior. The process of animal selection is very important for the isolation of the relatively homogenous groups of dominant, submissive or neutral animals. In this paper we, have reviewed evolution of methods leading to our current formula defining behaviorally distinct groups.

## 2 Basic principles of the DSR method

### 2.1 Apparatus.

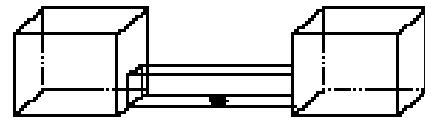


Figure 1. RSBM Apparatus. The RSBM apparatus consists of two plexiglass chambers connected by a passage having a small feeder dish with milk in the center from which only one rat can drink at a time.

A DSR is established and observed in a specially designed apparatus (figure 1) that was first constructed in the Institute of Psychiatry and Neurology (Warsaw, Poland) as a part of Dr. Malatynska's graduate work [1]. The apparatus is described in several publications [1-5]. This apparatus consists of two transparent plexiglass boxes (20 x 15 x 12 cm) connected with a narrow holloway (8 x 8 x 40 cm). A feeder is placed in the middle of a hallway. The small top opening is located in the hallway ceiling above the feeder allows for easy milk filling.

The basic apparatus is the same through all changes in the study techniques with the exception of couple minor modifications that facilitate the study. First, the tunnel now has narrow holes cut on both sides of the feeder for easy gate insertion on the end and removal on the beginning of experiment. In this way, paired rats have an equal starting position at the beginning of the experimental session. Second, the 10 ml beacker (about 2 cm diameter) feeder was replaced with specially constructed self-refilling feeder with smaller diameter (1 cm).

## 2.2 Animals.

Either Wistar or Sprague-Dawley rats weighting 140-200 g were used in these experiments. Animals were housed in groups of 4 (RSBM) or 5 (CRDM) rats per cage. Animals in competing pairs were always housed separately. They only met each workday during the 5-min testing period. At the end of the 5-min. period, the animals were separated to individual cages and given free access to food for one hour. Thus, the food restriction time was about 23 hours a day during weekdays. The animals were given free access to food from Friday afternoon following testing to Sunday afternoon when they were once again food-deprived. Rats showed normal weight gain while on this feeding schedule.

## 2.3 Procedure used in the CRDM

On the beginning of the experiment, rats were individually placed in the apparatus for 15 min during first two experimental days (on Thursday and on Friday) for habituation purposes. On Monday of the subsequent week, rats were randomly paired and their time spent on the feeder was recorded during the 5-min. daily session. A point was assigned for each 5-sec period when a rat was drinking milk. At the end of the week, pairs of rats that differ significantly ( $p < 0.05$  Student t-test) in their scores were judged to have established dominant-submissive relations. Using this method, about 80% of all pairs were selected as having dominant-submissive relations. In the following week (5 days), one group of dominant rats were treated with vehicle, a second group with clonidine and a third group with clonidine and an antidepressant or non-antidepressant drug. The percentage of dominant submissive pairs in these experiments was very high and many of them would not maintain this relationship through chronic experiments. When the protocol was changed to use the submissive rat as a model of depression that required chronic treatment, we had to extend the time for pair formation as is described in the next subsection.

## 2.4 Procedure used in early RSBM and RDBM

Similarly to previous experimental conditions animals were randomly assigned to same sex pairs. Behavioral testing was performed once a day for a 5-min period on weekdays. Testing was suspended on weekends but drug administrations were continued. The behavioral test consisted of placing each animal from a pair into opposite chambers of the apparatus after placing fresh milk in the feeder. The 5-minute period was divided into 5-second intervals. Animals observed drinking milk during each interval received one point. Drinking scores were tallied for each animal on each day of testing. Scores for the first five days (week 1) seldom showed a clear pattern and were not used. During the second week of testing, about half the pairs tested developed a pattern of behavior where one animal consistently out-scored the other. The second-week

data from each member of a pair were tested for a significant difference using the two-tailed t-test. The member of a pair having a significantly lower drinking score ( $p < 0.05$ ) was defined as submissive and his/her partner as dominant. Pairs showing this relationship were continued in the study while pairs not showing this difference were dropped from the study. Drug treatments started on Saturday after second week of testing. One

Table 1. Timetable for basic experimental unit

procedure	time	N° of animals	N° of animals selected	N° pairs with D/S relation
habituation	5 days	16		
selection	5 days	16	10 - 14	5 - 7
drug dosing	3-6 weeks	5 - 7		5 - 7

member of a dominant-submissive pair was treated with the drug (dominant for testing antimanic drugs or submissive for testing antidepressant drugs). The partner of a drug-treated animal was always injected with vehicle. In a control pairs, both animals were injected with vehicle. The phases of experiment and a number of animals used in each phase (in manual scoring) are listed in the table 1.

In this experimental setting, we still used point scores instead of time score and the only selection criterion was significant difference between paired animals during the second week of study. This changed in the subsequent studies

## 2.5 Selection criteria for DSR

In further experiments, we have simplified data gathering by direct measurement of time spent on the feeder by

Table 2. Pairs of rats selected according less stringent criteria

name score, sec	Rd1		Y1		Rd2		Y2		Rd4		Y4		Rd3		Y3		Rd1		Y1	
	Day 1	155	240	217	192	215	282	100	239	240	286									
Day 2	156	200	271	178	204	274	160	243	242	293										
Day 3	210	222	260	190	257	285	249	281	264	300										
Day 4																				
Day 5	185	187	266	249	271	286	252	269	256	300										
AVG	176.5	212.3	253.5	202.3	236.8	281.8	190.3	258.0	250.5	294.8										
SUM	706	849	1014	809	947	1127	761	1032	1002	1179										
P		0.09		0.05		0.07		0.2		0.001										
AVG DL		35.8		51.3		45.0		67.8		44.3										
SUM DL		143.0		205.0		180.0		271.0		177.0										
%		16.8		20.2		16.0		26.3		15.0										

paired animals without converting it to the point system. During the process of the experiments we, realized that control pairs did not hold stable relations as consistently as we have predicted from the beginning. Thus, we have designed experiments comparing less stringent to more stringent criteria for the selection of animal pairs with a dominant-submissive relationship. The scores of rat pairs selected with less stringent criteria are in the Table 2. Pairs of rats with scores presented in this table were borderline in statistical significance of difference in time spend on

Table 3. Pairs of rats selected according more stringent criteria

name score, sec	Rd1		Y1		Rd1		Y1		Rd2		Y2		Rd3		Y3		Rd2		Y2		Rd4		Y4		Rd3		Y3		
	Day 1	212	151	158	203	155	238	240	157	254	189	230	49	221	192														
Day 2	225	135	148	236	180	291	276	162	280	210	249	152	237	138															
Day 3	209	177	177	224	126	277	262	181	298	240	250	119	250	147															
Day 4	230	162	128	237	187	288	202	111	300	204	237	152	243	131															
Day 5	230	169	184	202	166	286	246	170	300	231	178	132	252	147															
AVG	221.2	158.8	159.0	220.4	162.8	276.0	245.2	156.2	286.4	214.8	228.8	120.8	240.6	151.0															
SUM	1106	794	795	1102	814	1380	1226	781	1432	1074	1144	604	1203	755															
P		0.0002		0.0015		0.0001		0.0009		0.0005		0.0022		0.0003															
AVG DL		62.4		61.4		113.2		89.0		71.6		108.0		89.6															
SUM DL		312.0		307.0		566.0		445.0		358.0		540.0		448.0															
%		28.2		27.9		41.0		36.3		33.3		89.4		59.3															

feeder by dominant and submissive rats. They were also well below the 40% value of difference between dominant and submissive rat in time spend on feeder as a percentage of dominant rat feeder time. In contrast, data presented in Table 3 are fulfilling these criteria. The results of this

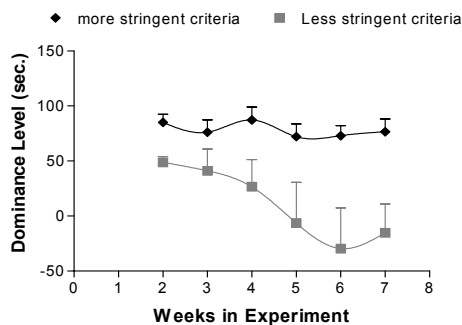


Figure 2. Shows stability of the dominance level in pairs of rats during seven weeks of experiment under application of selection criteria with different stringency (see Table 2 & 3). Pairs of rats selected using more stringent criteria (Table 3) are represented by black diamonds and pairs of rats selected using less stringent criteria (see Table 2) are depicted by gray squares.

experiment are presented in Figure 2. The dominance level in pairs of rats selected using less stringent criteria decreased while dominance level in pairs selected using more stringent criteria was stable for the experiment duration. Such condition would not obscure the effect of drug administered to test either RDBM or RSBM. The final criteria are summarized Table 4.

Table 4. Criteria used to select dominant, submissive and neutral animals

Characteristic of DL value *	Dominant – Submissive Pairs	Neutral Pairs
confidence level (two tail t-test)	P < 0.05	P > 0.6
% difference of higher scoring animal	at least 40%	up to 8%
Reversal of dominance **	no	yes

\*Difference in time spend on the feeder by paired animals  $DL = T_{A1} - T_{A2}$ ;  $T_{A1}$  time spend on feeder by animal #1  $T_{A2}$  time spend on feeder by animal #2.

\*\*Indicates reversals of daily success as expressed by longer and shorter time spent on the feeder by an animal from the pair (occasions when submissive animal outscores its partner) during first two weeks.

### 3 Endpoints and data analysis

Two endpoints were used in data analysis. First, the feeder time (FT) measured for each animal of a pair during a 5-min daily session. The units recorded were seconds. There was a maximum of 300 seconds of time on the feeder possible for one rat. Significant differences between time spent on the feeder by dominant and submissive rats were determined by ANOVA using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA) followed by a two-tailed t-test ( $P < 0.05$ ). Usually the weekly (5 days) average from daily feeding time was calculated. Second, we used dominance level values to measure the social relation between paired subjects. Dominance level (DL) =  $FTD - FTS$  where FTD was the feeder time of dominant rats and FTS was the feeder time of submissive rats. To enable comparisons between treatment groups, data were normalized to the initial (second experimental) week DL value. The normalization was conducted according to the formula below. The statistical significance of the difference in dominance level between the control group (pairs of rats were both

$$DL_{\text{week } n} (\%) = \frac{DL_{\text{week } n}}{DL_{\text{week } 2}}$$

dominant and submissive animals were treated with vehicle) and the treatment group (submissive rat was treated with drug and dominant rats with vehicle) was determined by ANOVA, followed by a t-test.

### 2.6 Automation of DSR observation and scoring and its validation in the RSBM

The basic testing apparatus did not change and was used as described in previous sections. The observation system was automated so, that during the 5-minute daily sessions, the time spent in the feeder zone by each rat was recorded by the video tracking software (PanLab, San Diego Instruments, CA) (Figure 3). The camera can distinguish rats by different colors. Thus, the rats' heads were colored for the purpose of video tracking, red in one cage and yellow in the other cage. The data were automatically

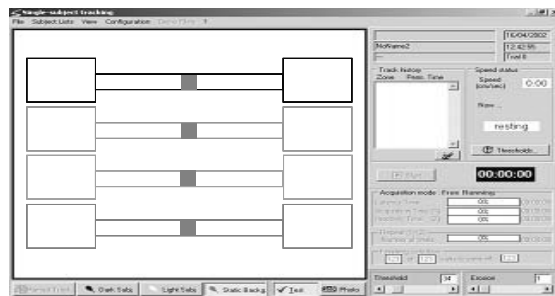


Figure 3. The computer display from the PanLab software (San Diego Instruments, CA) depicting the RSBM apparatus with defined zones. Solid squares represent the feeder areas. The time of the presence of a rat's head in this zone is recorded during each 5-minute experimental session.

saved into a text file and then pasted into an Excel extraction file through cell references. The basic apparatus was replicated for a second system. A total of four pairs of rats can be video tracked simultaneously on one table,



Figure 4. DSR test apparatus: the experimental set-up as viewed by the overhead video-camera. A unit of the apparatus consists of two plexiglass chambers connected by a passage having a small feeder dish with milk in the center from which only one rat can drink sweetened milk at a time. Four units are placed in parallel. The time spent at the feeder is scored for each of eight rats (four pairs) during one 5-minute experimental session.

using one camera (Figure 4). Similarly a second table with four apparatus and a camera were set in the same room. This enabled faster experimental manipulation with first trial rats were put away while the second trial was running, and the third trial rats were being put in place. The experimental procedure and data analysis were conducted

as described in the previous sections. We have compared stability of the controls (when both animals were injected by the vehicle for five weeks) and the effects of the SSRI inhibitor fluoxetine using the manual and automatic scoring methods. When both dominant and submissive rats were treated with sterile water for the period of five weeks, there was a statistically significant difference between FTD and FTS in these two behaviorally distinct groups of rats at each week (ANOVA followed by t-test). This was true for data generated by both manual and automatic scoring (Figure 5 A & B). The lines depicting submissive and dominant rat performance were parallel and there was no statistically significant difference in the performance of dominant or submissive rats between the selection week and the following five weeks of vehicle injections (Figure 5 A & B) in manually and automatically scored groups.

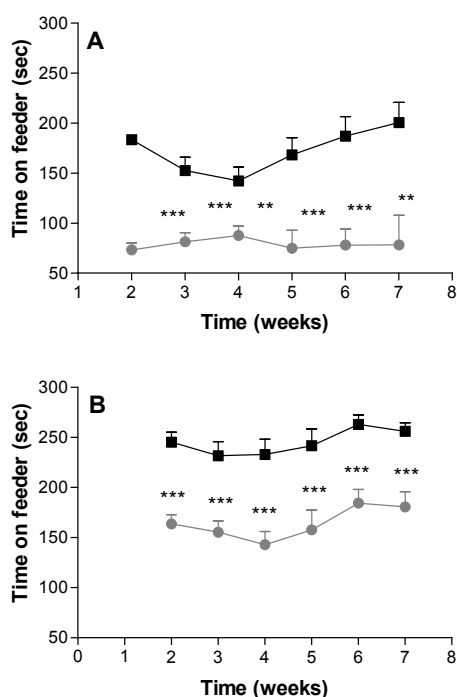


Figure 5. Stability and dynamics of dominant-submissive relations in pairs of rats during seven weeks study. (A) Data scored manually by observer. (B) Data collected by video-tracking system (San Diego Instruments, Inc., San Diego, CA). Dominant and submissive animals were treated with sterile water for the five weeks. The difference between dominant and submissive rats scores marked \*\* at  $P < 0.01$  and \*\*\* at  $P < 0.001$ .

The fluoxetine effect of increasing the competitiveness of submissive rats was similarly observed using manual and automatic scoring (Figure 6A&B).

#### 4 Summary and Conclusions

One of the most significant clinical findings of treatments for mania and depression concern their time course, both in the development of the disease and response to treatment. Submissive behavior develops gradually over time as depression develops in humans. We have shown in the RSBM and RDBM that antidepressant or antimanic drugs need to be administered chronically to submissive or dominant rats to alter submissive behavior or dominant behavior. Thus, the response of submissive animals to antidepressant treatment and dominant animals to

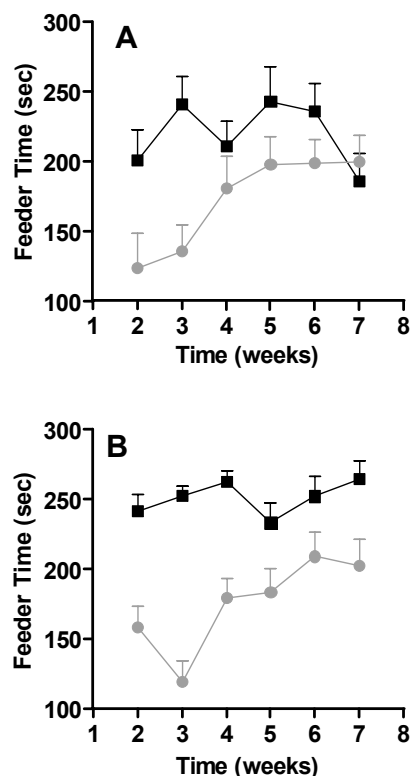


Figure 6. Effects of fluoxetine 10 mg/kg (A) Data scored manually by observer. (B) Data collected by video-tracking system (San Diego Instruments, Inc., San Diego, CA). Dominant animals (black squares) were treated with sterile water and submissive animals (grey circles) were treated with fluoxetine (10 mg/kg) for the five weeks.

antimanic drug treatment requires chronic administration as seen for human patients. With this similarity and the development of automatic version of the model, the described tests are ready for screening new medication candidates in search for one with faster onset of activity.

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# The automation of observing and analyzing rodent behavior: possibilities and limitations

B.M. Spruijt

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The increasing number of genetically modified animals to be characterized demands for the development of a reliable tool for observing and analyzing challenge-induced and baseline behavior. Since a functional interpretation of the gene effect on behavior is the ultimate goal of many phenotyping studies, an ethological rationale underlying testing is a logical consequence. So far batteries of already existing tests that have been applied involve two caveats:

- Behavior is induced in a novel environment and, thus, the limited observation time excludes assessment of habituation, baseline levels, rhythmicity, etc.
- Most tests have a specific focus on one motivational system, e.g. exploration, anxiety, etc., and a limited number of measurement parameters, whereas complex functions are the result of interacting motivational systems.

Recently, several systems have been developed equipped with hardware and software allowing observations over days. Since the animals are very precisely monitored by computers, behaviors have to be explicitly defined in terms of movement, position and form. Implementation, thus, requires redefining ethograms, which is an exciting process. Explicitly defining behavioral elements makes one aware of subjective influences in previously defined behaviors. The inevitable validation of data collected with new systems against data obtained with previously used well-known tasks can never be full proof as the latter tests have never been standardized themselves. Video imaging techniques also has disadvantages in that they do not cover neurological disturbances.

Apart from standardization and validation another issue emerges: the challenge resides not only in what can be measured, but how can large sets of data be reduced to a functional description of behavior and to what extent can relevant changes in complex behaviors of vast numbers of genetically modified animals be detected. For instance, the at first sight simple behavior "activity" consists of different components (speed, bouts, angularity, etc.) in combination with different spatial zones; modeling methods are required for an appropriate description and deduction of critical parameters, which then have to be subjected to statistical procedures such as principal component analysis and t-pattern analysis. In fact, a complex test requires complex statistics to have the full benefit of this approach. The fact that data can now be collected over days contributes significantly to the power of statistics.

Presently different systems are being developed these days and continuously extended in the near future with devices and sensors allowing the measurement of anxiety and conditioned place preference, conditioned place aversion, object recognition, etc. It is self-evident that the automated collection of data obtained in standardized and validated cages contributes to the reliability and reproducibility of such behavioral changes displayed by mutant mice. Different data sets illustrating and validating the applied techniques will be presented with a perspective on future:

possibilities and limitations. In this symposium different systems which are being used are highlighted: IntelliCage and PhenoLab. Secondly, attention is paid to video imaging and the data streams generated by such an automated system and possibilities for analysis.

## The 'mouse fitness centre': A standardized test system to assess motor function

S. S. Arndt

The non-automated multi-task testing apparatus mouse fitness centre (FC), allows testing a large number of animals within a short period of time. This apparatus is simple-to-do and enables the quick assessment of an important aspect of the general health of mice, namely motor (dys-)functions.

The system integrates eight tests that enable to detect motor dysfunctions:

- 1 The vertical pole.
- 2 Placing response/Footprint gangway.
- 3 Horizontal wire and -gimp.
- 4 Grip strength.
- 5 The inclined- and upside-down grid.

All motor tests should be performed in close succession in order to minimize testing-related stress in the mice. This is realized by installing all components of the FC on a so-called 'basis', which guides all necessary adaptations for performing the next test in a series. These adaptations are defined in a standard protocol that must be applied strictly. Working with 'standardized' animals (i.e. animals with similar motor abilities) increases the likelihood that results from different studies and laboratories are similar and that findings can be reproduced. To our knowledge no automated system exists covering the functionality of the FC. However, specific features of the FC probably can be automated.

The FC, as part of the assessment of the general health status, should proceed any further behavioral testing of mice (and other rodents). Pre-screening and detection of motor dysfunctions allows, for example, to select cognition tests that do not require the compromised motor function. Moreover, these data help to interpret results obtained by use of other tests and to put them into perspective.

## Automated behavioral analysis of mice using IntelliCage: Inter-laboratory comparisons and validation with exploratory behavior and spatial learning

H.-P. Lipp, O. Litvin, M. Galsworthy, D.L.

Vyssotski, A.L. Vyssotski, A.E. Rau, F. Neuhäusser-Wespy, H. Würbel, R. Nitsch and D.P. Wolfer

IntelliCage™ is a large home cage containing four complete operant conditioning units placed in the corners. A central computer continuously controls and monitors



activity and learning of up to 16 transponder-tagged mice per cage without human interference.

In a recent standardized multi-lab study, we noted identical strain rank order in the open-field, elevated Null-maze, water maze and object exploration, but comparison of absolute values revealed significant differences between laboratories, or strain-by-laboratory interactions. A subset of 46 female mice was also tested in 4 IntelliCages, located in different animal facilities of the University of Zurich (2 cages per site), each unit housing 11- 12 transponder-tagged mice from different strains. The system measured continually visits of drinking sides, development of place preference and activity parameters. IntelliCage revealed significant strain differences in initial exploratory activity and baseline activity during the first day. Most importantly, the behavioral scores of the strains as observed in both laboratories were statistically indistinguishable.

We then compared the first 10 min after introduction to IntelliCage with standard tests of exploration lasting about the same time span. The measure in IntelliCage was the number of visits to the yet unfamiliar test corners. It was positively correlated with visits to the center in the open-field. For the elevated Null-maze, the number of head dips correlated strongly with the number of corner visits in IntelliCage, while measures of anxiety such as time in the protected area did not correlate significantly. In the water maze (WM), we found a significant positive correlation between average escape latencies and place preference learning in IntelliCage. No correlations were found with WM probe trial scores. These findings indicate that either test measures the acquisition of spatial learning, but the WM testing took four man-weeks, while IntelliCage showed the same findings after 24 hours without presence of an experimenter.

We conclude that optimal standardization and comparability is best achieved by the use of automated procedures, and that such automation measures the same behavioral dimensions as standard manual tests, yet with much less stress for animals and experimenters.

### **Monitoring animal behavior in the Smart Vivarium**

Serge J. Belongie

In the course of modern medical research, it is common for a research facility to house thousands of caged mice, rats, rabbits, and other mammals in rooms known as vivaria. In any experiment involving a group of animals it is necessary to perform environmental and physiological monitoring to determine the effects of the procedure and the health of the animals involved. Such monitoring is currently performed by human observers, and for practical reasons, only a small subset of cages can be inspected for limited amounts of time. In this talk, I will outline the computer vision and machine learning technology behind the Smart Vivarium, a system for automated, continuous animal behavior monitoring. The Smart Vivarium will serve as an invaluable tool for medical researchers as it will make better use of fewer animals. Early discovery of sick animals will prevent diseases from spreading, and in general will lead to more efficient caretaking of animals. Additionally, the proposed technology can serve as a powerful tool for monitoring sentinel cages in potential bioterrorism targets and chemical agent research facilities. The Smart Vivarium project is a California Institute for Telecommunications and Information Technology (Calit2) collaboration between the Jacobs School 's Computer

Science & Engineering and Bioengineering Departments and the UCSD Animal Care Program.

### **Multi-level analysis of mouse behaviour in a home cage environment using PhenoLab**

L. de Visser, R. van den Bos and B.M. Spruij

To contribute to the refinement of behavioral phenotyping methods for inbred and mutant mice, we developed a reliable tool for observing and analyzing behavior in a home cage-like environment (PhenoLab®, Noldus Information Technology, Wageningen, The Netherlands). Testing animals in their home cage environment holds several advantages; it allows for continuous observations over consecutive days and the evaluation of both challenge-induced and baseline behaviors. Home cage testing also minimizes human intervention (such as handling) and reduces interactions with other environmental factors not related to the behavioral test (such as animal transport). By carefully designing the home cage environment, different behavioral domains can be studied simultaneously, for example, by providing the animals with different stimuli and tasks (light, sound, novel objects, cognition tasks).

Here, data is presented of studies in inbred strains of mice (C57BL/6, DBA/2, C3H and 129S2/Sv) on locomotor activity and anxiety-related behavior in the PhenoLab system. First, strain differences in locomotor activity were dependent on the time of testing (novel vs. baseline conditions) due to differences in rate of adaptation to the environment. Second, Principal component analysis (PCA) revealed two major components within the domain of locomotor activity, which could be interpreted as 'general activity' (or: how active is the animal?) and 'way of moving' (or: when active, how is the animal moving through the cage?). Anxiety-related behavior was further studied by introducing an aversive light stimulus in the cage after an adaptation period of 4 days. The light stimulus illuminated the area around the feeding station, creating an approach-avoidance conflict for the animals.

The results of these experiments are discussed in the light of the possibilities and limitations of PhenoLab as a tool for the behavioral phenotyping of genetically modified mice.

### **Behavioral phenotyping: Getting more information from video tracking data**

W.W. Kuurman

Mice are used worldwide as a research animal or model for a large variety of biological processes. The search for mice with interesting or exceptional phenotypes is important, so that this research can be done effectively. To facilitate this search PhenoLab®, (Noldus Information Technology bv, The Netherlands) was developed. In this PhenoLab several mice are individually housed for a week in a home cage and tracked using an overhead camera and EthoVision® (Noldus Information Technology bv, The Netherlands). Based on contrast differences, X- and Y-coordinates of the center of gravity for the mouse are recorded a number of times each second. During the week the mouse shows various spontaneous and induced behaviors. The behavioral phenotype of the mouse needs to be deduced from the X- and Y-coordinates (integration of location and movement).

Video tracking data is often summarized in time bins and then mean of each time bin is used for statistical analysis.

For data which does not have a known unimodal distribution, however, mean and standard deviation ignore variation in the data. This variation can be utilized using all data available and mathematical modeling of each variable. Parameter estimates from these models can then be used as phenotypic information for each mouse tested. This approach yields high-resolution phenotypic information, so that detection of mice with interesting or exceptional phenotypes is more successful.

Some examples of this approach are velocity and distance to home cage wall data. For velocity a mathematical model was developed to distinguish low, intermediate, and high velocities. This model can be used to analyze locomotive activity in greater detail. For distance to home cage wall a mathematical model was developed to determine how often the mouse stays close to the wall, where the border is between being close and being away from the wall, and how these measures change over time. This model can be used to analyze anxiety and habituation in greater detail.

Additional models should be developed to utilize information collected by video tracking software. The future challenge is to integrate information obtained with this approach, so as to facilitate interpretation of the data.

### **The Erasmus Ladder: a new tool for the automated measurement of motor performance and motor learning in mice**

A. Cupido, S.P. Krygsman, C.M. Snoeck, J.A.Th. Bos, C.I. De Zeeuw and S.K.E. Koekkoek

Recent developments in the creation of mouse models for human diseases have called for tools that are able to quickly screen mutant mice for their deficits. In our department, we have developed two successful tools for detecting mouse mutants with cerebellar deficits: eye blink conditioning and VOR adaptation. However, for screening large amounts of mice these systems are too time consuming, as they require specialized and invasive surgery. Therefore we developed a new tool: The Erasmus Ladder.

The Erasmus Ladder is a horizontal ladder with two cages. The horizontal ladder has 25 rungs. The rungs are divided in a left side and a right side. All subdivisions of the rungs are equipped with pressure sensors. The rungs can be automatically protracted and retracted.

The mice are trained to walk with a constant speed from one cage to the other. We have developed software to analyze the walking pattern of the mice.

In this presentation we show quantitatively the level of the motor performance and motor learning of mice with cerebellar deficits.

The advantages of the Erasmus Ladder are fourfold. First, the experiments do not require surgery; second, the Erasmus Ladder will be completely computer controlled, thus the experiments are not time consuming; third, the Erasmus Ladder has a feedback control, which allows us to modify the position of rungs during ongoing locomotion; fourth, with a few adjustments it is also possible to screen for hippocampal and amygdaloidal deficits.

# Multi-level analysis of mouse behavior in a home cage environment using PhenoLab®

L. de Visser<sup>1,2</sup>, R. van den Bos<sup>1,2</sup>, W.W. Kuurman<sup>1</sup>, B.M. Spruijt<sup>1,2</sup>

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## Abstract

To contribute to the refinement of behavioral phenotyping methods for inbred and mutant mice, we developed an automated tool for observing and analyzing behavior in a home cage-like environment (PhenoLab®, Noldus Information Technology, Wageningen, The Netherlands). Testing animals in their home cage environment holds several advantages; it allows continuous observations over consecutive days and the evaluation of both challenge-induced and baseline behaviors. Home cage testing also minimizes human intervention (such as handling) and reduces interactions with other environmental factors not related to the behavioral test (such as animal transport). Here, data will be presented of studies in inbred strains of mice (C57BL/6 and DBA/2) on locomotor activity and anxiety-related behavior in the PhenoLab® system. We found that different aspects of activity behave differently over time. Anxiety-related behavior was studied by introducing an aversive light stimulus in the cage after an adaptation period of 4 days. The light stimulus illuminated the area around the feeding station, inducing pronounced avoidance behavior in the animals. Possibilities and limitations of PhenoLab® as a tool for the behavioral phenotyping of mice are discussed.

## Keywords

Automated home cage observations, mice, locomotor activity, anxiety.

## 1 Introduction

The continuous increase in mouse models for central nervous system functioning demand extensive behavioral assays to elucidate the impact of genetic alterations [2,4,7]. Current methods for behavioral phenotyping of mice often involve batteries of individual tests, each addressing different motivational systems, such as activity in novel environment, anxiety, spatial cognition, etc [1,3]. Although most tests are pharmacologically validated and relatively easy to perform, some limitations are evident. First, long term development of behavior as a response to stimuli (e.g. novelty or drug effects) and circadian processes are ignored due to the limited testing period. Second, information on the complex interactions between motivational systems is often difficult to acquire.

We developed a new tool to study mouse behavior in a home cage like environment [5]. This approach has several advantages; it allows continuous observations over days and the evaluation of both challenge-induced and baseline behavior. Furthermore, the confounding influence of stress caused by handling and transport is minimized.

In this paper we present data from two separate experiments to demonstrate some of the possibilities of automated home cage observations. The first experiment addresses time-courses of specific aspects of locomotor activity as mice gradually become habituated to the novel

home cage system. In the second experiment, an aversive stimulus is used to create an approach-avoidance conflict, which allows for the detection of parameters indicative of anxiety.

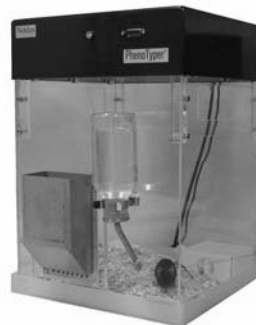
## 2 Methods

### 2.1 Animals

Female mice of the C57BL/6OlaHsd (n=6) and DBA/2OlaHsd (n=10) strain were used in the first experiment. Male mice of the C57BL/6OlaHsd strain (n=10) were used in the second experiment. All mice were purchased from Harlan (Horst, The Netherlands). Upon arrival at the animal facility, mice were either housed in pairs (females) or single (males) and allowed to acclimatize for two weeks under a reversed light/dark cycle (lights on: 19.00 hrs). All mice were provided with a shelter, tissue and paper shreds as enrichment. Humidity was kept at a constant level and room temperature was maintained at  $21.0 \pm 2.0$  °C. The Animal Ethical Committee of Utrecht University approved the experiments.

### 2.2 Automated home cage observations

Locomotor activity was automatically recorded with video tracking in specially designed home cages (PhenoLab®, Noldus Information Technology, Wageningen, The Netherlands, see Figure 1). Each PhenoLab® system consists of four cages, connected to a PC that runs EthoVision 3.0 (Noldus Information Technology) for videotracking. On top of each cage a unit is placed containing a digital infrared sensitive camera and infrared lights. This allows continuous recordings during both light and dark period of the day. The top unit further contains a bright white light stimulus that could be switched on automatically by programming EthoVision 3.0. This lightspot illuminates approximately one quarter of the cage with a light intensity of 1000 lux.



**Figure 1.** Single cage with top unit of the PhenoLab® system for automated home cage observations. Each cage contains a fixed water bottle, feeding station and shelter.

### 2.3 Experiment 1

For the first experiment, mice were introduced to the PhenoLab<sup>®</sup> cages and locomotor activity was recorded for six consecutive days. Cages were equipped with a fixed water bottle and feeding station, and shelter, tissue and paper shreds for enrichment. For details on parameter settings in EthoVision 3.0, see [6]). Parameters presented in the present paper are 'duration of movement' as a percentage of total observation time and 'velocity' in cm/s. Values for each parameters are calculated for each individual in 1-hour bins and subsequently averaged over 12 hours to differentiate between dark and light period of the day.

### 2.4 Experiment 2

In the second experiment, mice were introduced to the PhenoLab<sup>®</sup> cages following the procedure of experiment 1, but now the bright white light stimulus was programmed to switch on, on day 5 immediately at the onset of the dark period. The light spot was directed on the feeding station for three hours continuously. This created an approach-avoidance conflict for the animals. Again, locomotor activity was recorded for six consecutive days in total. Parameters analyzed for this paper were 'duration of movement' in seconds per hour and the 'time spent in feeding zone'. The feeding zone was defined as one body length around the feeding station. During the time the light spot was on, this zone was illuminated.

### 2.5 Statistical analysis

Statistical analysis was conducted using SPSS 10.0 for Windows. Repeated measures ANOVA were performed to test for overall effects of within-subjects factor 'day' and between-subjects factor 'strain' on duration of movement and velocity in experiment 1. *Posthoc* independent samples t-tests were used to compare strains on each experimental day. Levels of significance were assigned at  $p=0.05$ .

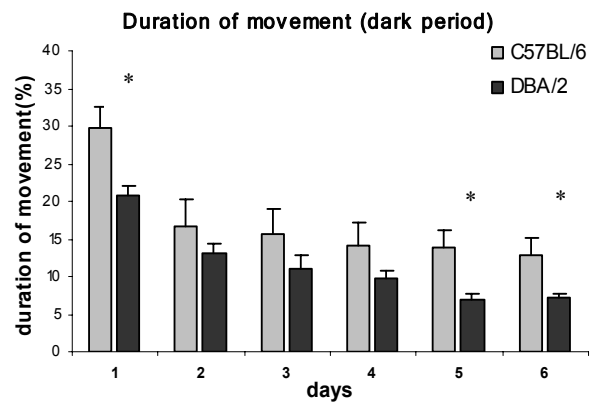
## 3 Results

### 3.1 Experiment 1

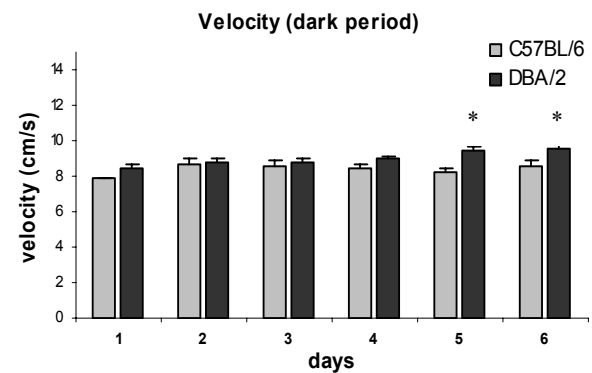
Results from experiment 1 are modified from the study described in [5]. Figure 2 presents the 'duration of movement' during the dark period of each experimental day. Overall, C57BL/6 mice showed higher levels of movement (repeated measures ANOVA between-subjects factor 'strain'  $F_{1,14}=6,663$ ;  $p=0.022$ ) compared to DBA/2 mice. *Posthoc* comparisons per day revealed significant higher duration of movement in C57BL/6 on day 1, 5 and 6. Duration of movement changed over time (repeated measures ANOVA within-subjects factor 'day'  $F_{5,70}=41,559$ ;  $p<0.001$ ) but this was independent of strain (repeated measures ANOVA 'day' x 'strain':  $F_{5,70}=1,227$ ;  $p=0.310$ ). In Figure 3 strain differences on the parameter 'velocity' are shown. Overall, DBA/2 mice moved with higher velocity compared to C57BL/6 (repeated measures ANOVA between-subjects factor 'strain':  $F_{1,14}=7,060$ ;  $p=0.019$ ). However, when tested per experimental day differences were only significant on day 5 and 6. Velocity changed significantly over time (repeated measures ANOVA within-subjects factor 'day':  $F_{5,70}=5,041$ ;  $p=0.008$ ) independent of strain (repeated measures ANOVA 'day' x 'strain':  $F_{5,70}=2,339$ ;  $p=0.100$ ).

### 3.2 Experiment 2

The mice showed a strong cyclic activity pattern with high levels of movement duration during the dark phase as compared to the light phase.



**Figure 2.** Duration of movement (as a percentage of time) during the dark period for each experimental day (1-6). Means + SEM per 12-hour period are used. \*  $p<0.05$  for differences between strains; independent sample t-test.



**Figure 3.** Velocity (in cm/s) during the dark period for each experimental day (1-6). Means + SEM per 12-hour period are used. \*  $p<0.05$  for differences between strains; independent sample t-test.

The light spot induced a small decrease in overall activity as reflected by lower duration of movement (Figure 5). Furthermore, there was a shift in activity towards the end of the dark phase on day 5, compared to day 4. 24 hours after the light spot (day 6), duration of movement was still lower than what would have been expected from day 4.

The light spot induced pronounced avoidance behavior in male C57BL/6 mice (see Figure 4). Time spent in the feeding zone decreased markedly during the time the light spot was switched on (day 5) compared to the day before (day 4). Some degree of habituation to the stimulus occurred, which was reflected by the increase in time spent in the feeding zone within the three hours the light spot was on. Notably, after the spot was switched off, there was a marked increase in the time spent in the feeding zone during the fourth hour of the dark phase. Values exceeded the duration in feeding zone during the fourth hour of the dark phase of day 4 and day 6.

24 hours after the light spot (day 6), time spent in feeding zone was still decreased compared to day 4. However, during the first three hours of day 6, time in the feeding zone increased faster compared to this time period on day 5.

## Time in feeding zone

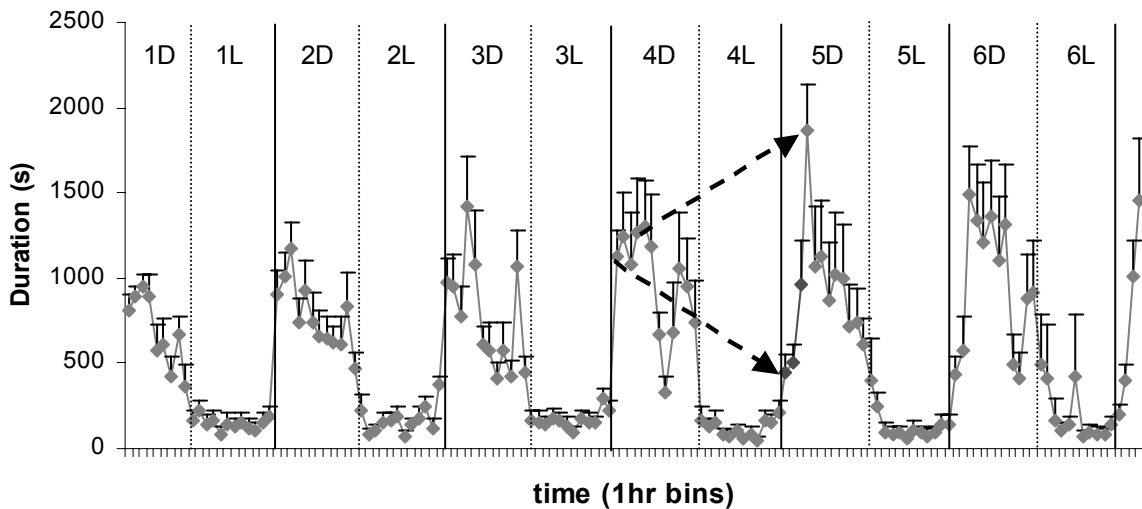


Figure 4. Time spent in feeding zone during six consecutive days. Means and SEM of 1hr bins are used. Days are indicated by numbers (1-6) and dark and light periods are distinguished by either D (dark period) or L (light period). Black data points on day 5 represent time window when light spot was switched on. Arrows indicate the main findings, see text for further explanation.

## Duration of movement

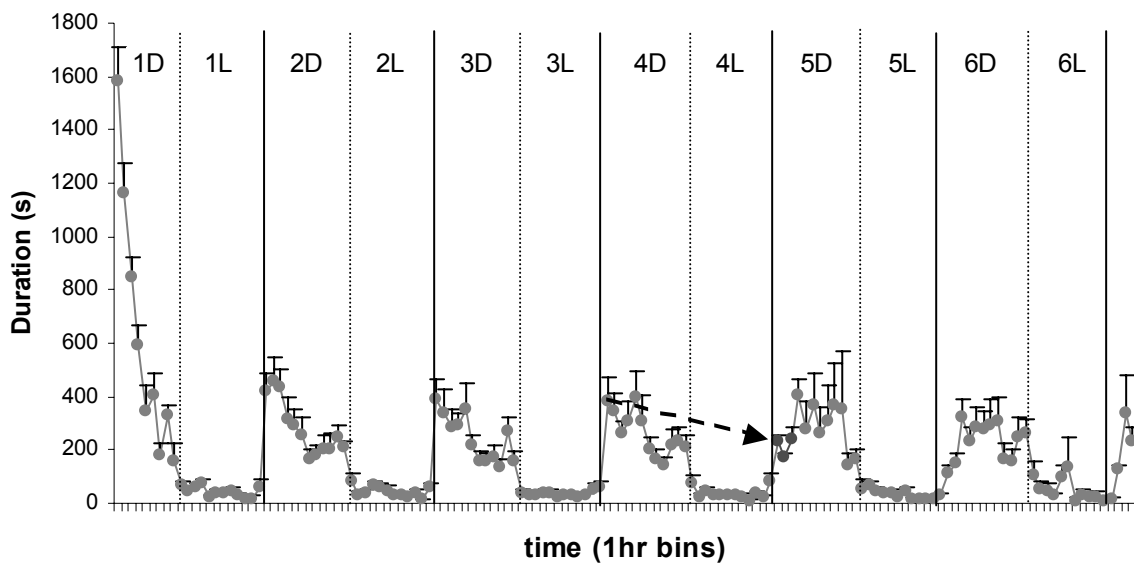


Figure 5. Duration of movement during six consecutive days. Means and SEM of 1hr bins are used. Days are indicated by numbers (1-6) and dark and light periods are distinguished by either D (dark period) or L (light period). Black data points on day 5 represent time window when light spot was switched on. Arrow indicates the main finding, see text for further explanation.

## 4 Discussion

Automated home cage observations offer several possibilities for the behavioral characterization of mice. Novelty induced exploration can be distinguished from activity in a familiar environment using a single test. It appeared that some aspects of locomotor activity, such as duration of movement, are more dependent on familiarity with the environment than others, such as velocity. Furthermore, by presenting an aversive stimulus when the animals were habituated to the environment, we were able to create an approach-avoidance conflict. This allowed evaluation of possible anxiety-related behavior, while avoiding the confounding influences of novelty, handling

and transport. Without these external stress factors it is possible to distinguish “state” from “trait” characteristics. Currently, experiments are being performed with the anxiolytic drug diazepam to find further evidence for the use of the aversive light spot as an anxiety test.

However, limitations of the presented method are in the time-consuming process of data handling and analysis. The vast amounts of data generated by the system demand sophisticated data handling software and statistical tests. Moreover, a functional interpretation of this computer output in terms of ethologically relevant profiles is a challenge in itself. To facilitate this, statistical tools for data reduction and clustering, such as Principal

Components Analysis, are adopted [6]. With these tools, the interrelation of parameters can be studied to reveal underlying motivational systems. Pharmacological experiments can further prove the predictive validity of parameters that showed differential potential between e.g. inbred mice.

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# Tracking individual insect flights with harmonic radar

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## Abstract

A harmonic radar system for tracking individual flying insects at low altitude has now been used successfully on bumblebees, honeybees, butterflies and moths. A small transponder is attached to the dorsal thorax of an insect, and this is detected by the radar over a range of about 900m (horizontally). The radar has provided novel insights into how individuals search for food resources in complex landscapes. It has also enabled us to answer questions about the navigational abilities of these insects whilst in flight.

To illustrate the power of this technique, examples are given of how honeybees, bumblebees and butterflies explore the landscape during their first flights, and how they exploit the landscape for food resources.

## Keywords

Harmonic radar, bee behavior, tracking individuals, insect flight.

## 1 Introduction

Tracking insect movements over space and time presents a great challenge, because insects are generally small, often very numerous, frequently winged and can travel extremely long distances. Direct methods of studying insect movement involve either characterizing the actual path of the insect's movement, or performing mark-recapture experiments. Movement paths have usually been characterized either by eye or by video. The insect's position at different intervals can be recorded digitally, on audio tape or with numbered flag markers. Although this is the most accurate and precise way of recording insect movement, it is time-consuming and can only be done on a relatively small spatial scale. Mascanzoni & Wallin<sup>5</sup> were the first to use a detection system, based on the harmonic radar principle (originally developed for finding avalanche victims) to locate ground-dwelling beetles. It has subsequently been used on other carabids<sup>3,4,16,17</sup>, and on butterflies<sup>14</sup> and caddis flies (Briers *et al.* unpublished data). The insect is tagged with a diode and aerial of 3-5cm length which, when illuminated with microwaves, radiates a signal at the second harmonic frequency of the transmitted one. The microwaves are emitted by portable detection equipment, used to locate the tagged insect, and a marker is placed in the ground where the insect is detected. After a time gap (e.g. 15 mins<sup>16</sup>), another search is made and another positional fix is taken. Although described as a "radar", this system gives no range information and is, rather, a portable *direction-finding* device: alone it cannot produce geometrically accurate maps. It is useful for slow-moving, or stationary objects, but is not suited to tracking "real time" trajectories, or use with fast-moving insects.

The scanning harmonic radar system described in this paper, developed by Professor Joe Riley and Alan Smith, is currently the only technique which enables accurate

tracking of the complete flight paths of individual, large, low-flying insects over hundreds of metres<sup>7,11</sup>.

## 2 Methods

### 2.1 Harmonic radar system

The harmonic radar is a 3.2 cm wavelength, 25 kW peak power, azimuthally scanning, dual frequency system. A transponder, consisting of a vertical dipole aerial (16mm long) with a small Schottky diode and inductive loop in the centre, is carefully attached to the dorsal surface of the insect (Figure 1.). The transponder weighs approximately 12mg. It captures some of the energy in the radar transmissions and re-radiates part of it at double the transmitted frequency. This returned signal is easily distinguished from even strong echoes reflected by ground features. Since the illuminating radar delivers the energy to operate the transponder, no "on-board" battery is required and extreme miniaturization is therefore possible.



**Figure 1.** *Bombus terrestris* worker with harmonic radar transponder, feeding on oilseed rape (*Brassica napus*) flowers

The transponder can be detected within a circle of radius 900m centered on the radar, in unobstructed flat terrain and can be detected from ground level to about 6m altitude. The two radar antennae (Figure 2.) rotate in azimuth at 20 revolutions per minute, so position fixes (precision  $\pm 3$  m) from the transponder are received approximately every 3 seconds. These are digitized to provide a temporally and spatially explicit, or geometrically accurate, track of the insect's flight path.



**Figure 2.** Scanning harmonic radar equipment

For individual insects, the direction, distance and straightness of flight can be measured, and even destination if it is within range. The insect's course control and navigational performance can be investigated by

examining flight speed and direction, and the relationship with wind speed and direction.

## 2.2 Flight performance of insects with transponders

The flight performance of honeybees and bumblebees is not affected to a significant degree by the transponders. For example, bumblebees were found to collect similar quantities of nectar and pollen with and without the transponder in place<sup>6</sup>, although foraging trip duration can be longer for bees with transponders. This is likely to be due to the transponder affecting how the bees manipulate certain flowers. Calculations indicated that the increased drag imposed by the transponder was negligible compared to the drag from the bee's own body<sup>7</sup>. Also, honeybees equipped with transponders have been tracked doing path integration on the way to a feeder and to the hive. The distances and directions of the vectors that displaced bees fly is exactly what other studies predict<sup>12,13</sup>.

Experiments in a flight room showed that small tortoiseshell (*Aglais urticae*) and peacock (*Inachis io*) butterflies are not hindered by the presence of the transponder and continue to fly and feed as frequently as they do without the transponder in place<sup>1</sup>.

## 2.3 Constraints of the system

The constraints of the system include the size of the transponder. Lighter versions (~3mg), have been used on moths<sup>8</sup> but they have not proved to be robust for use in the field on strong-flying insects such as bees. Individual insects with transponders cannot be distinguished from each other, so only one or two can be tracked at a time. A relatively flat landscape, devoid of tall vegetation, is required to utilize the maximum spatial range of the radar. The system can not be used in forests, tree-filled landscapes or hilly areas, and altitudinal range of the present system limits the variety of insects that can be studied. The radar equipment is large and, so far, has not been mobile during studies, although that would increase its horizontal range.

## 2.4 Studies of insects

The technique has been used to investigate flight behavior of bumblebees<sup>6,9,10</sup>, honeybees<sup>2,12,13</sup>, moths<sup>8</sup> and butterflies<sup>1</sup>. Details of the individual experiments are available in those papers. Some of the main findings are summarized here.

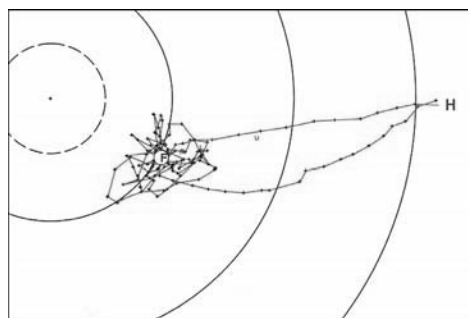
## 3 Results

### 3.1 Bee behaviour

In the first study of bumblebee foraging flight, 65 bees were tracked flying to and from their colony over a 700 m range (Fig. 2 in Osborne *et al.* 1999<sup>6</sup>). The results showed that bumblebees do not necessarily forage close to their nest. They fly along fast, straight flight paths and show route constancy between trips. They also actively compensated for wind speed and direction (Fig. 3 in Riley *et al.* 1999<sup>9</sup>).

The most recent harmonic radar research has provided detailed insights into honeybee navigation mechanisms (Figure 3.). Honeybees were tracked to provide a convincing proof of Von Frisch's hypothesis of the role of the waggle dance in bee communication<sup>13,15</sup>. It was also found that trained honeybees use an "automatic pilot"

mode, making vector flights and tending to disregard landscape cues<sup>12</sup> when they were displaced from a feeder with which they were familiar. Naïve honeybees have also been tracked on their first flights away from their colony. They were observed to make characteristic orientation flights that were very different from those of experienced foragers<sup>2</sup>. A subsequent study of bumblebee orientation flights using the radar showed that the orientation flights of bumblebees, before they start to forage, are more wide-ranging than those of honeybees and involve sampling of different forage sources. Bumblebee search strategies may differ from those of honeybees because they cannot obtain information on forage location from nestmates within the colony, as honeybees can through the waggle dance communication.



**Figure 3.** Harmonic radar track (red) of honeybee leaving hive H, searching for feeder at F (with which they had become familiar, but which had been removed for test), and returning to hive. Radar rings are 100m apart.

### 3.2 Butterfly behaviour

For the first time, the flight paths of five butterfly species were successfully tracked using harmonic radar within an agricultural landscape<sup>1</sup>. Until now, butterfly mobility has been predominantly studied using visual observations and mark-release-recapture experiments. For small tortoiseshell (*Aglais urticae*) and peacock (*Inachis io*) butterflies, two main styles of track were identified; (A) fast linear flight and (B) slower non-linear flights involving a period of foraging and/or looped sections of flight. The results provide tentative support for non-random dispersal and a perceptual range of 100 – 200m for these species and demonstrated that the harmonic radar methodology will be of significant value for future investigation of butterfly mobility and dispersal.



**Figure 4.** Small tortoiseshell (*Aglais urticae*) butterfly with radar transponder

## 4 Conclusions

This harmonic radar technique has thus proved invaluable for investigating the navigation and foraging behavior of bees and butterflies. It is most suitable for studying the flight patterns of large insects, flying at low altitudes over a range of hundreds of meters in relatively open



landscapes. To date, honeybees and bumblebees have been most frequently studied. Butterflies or moths, particularly those grassland species thought to be localized in their movement, are also an ideal choice. Other hymenopterans, such as large solitary bees or wasps, would also be suitable, and it would be interesting to try the technique on Coleoptera, Orthoptera or Odonata; orders containing large, sturdy insects capable of carrying the transponder.

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# Quantifying bradykinesia in neurological patients

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## Abstract

Bradykinesia is one of the four symptoms of Parkinson's disease and is characterized by slowness and irregularities during fast voluntary movements. Bradykinesia is also seen in other disorders like complex regional pain syndrome. Normally bradykinesia is visually scored by a physician, who uses this sign as an indication for a lack of neuromuscular control. This score is subjective and has a small resolution. Goal of the project is to develop a method that objectively scores bradykinesia. Subjects were requested to perform a finger tap task under a high speed camera. Markers were attached to the top of the thumb and index finger. Two parameters were selected to represent the slowness of the movement: median frequency (MF) and mean absolute acceleration (MA). Patients with bradykinesia had a lower value for both MF and MA. It is concluded that movement slowness can be objectively quantified, showing the relevance of the method.

## Keywords

Bradykinesia, Parkinson's Disease, Complex Regional Pain Syndrome (CRPS).

## 1 Introduction

Patients with neurological disorders, like Parkinson's disease, often show bradykinesia. Bradykinesia is demonstrated by slowness and irregularities during fast voluntary movements. Parkinson's disease (PD) is a neurodegenerative disorder characterized by: tremor, bradykinesia, rigidity, and impaired postural reflexes. PD is seen predominantly in elderly people. Slowness of voluntary movements is also seen in patients suffering complex regional pain syndrome. Complex regional pain syndrome (CRPS) is a painful condition that typically follows an injury to a limb, which can be minimal or severe (sprain/strain, fracture, contusion/crush injury), although in a number of patients no trauma can be identified. The syndrome manifests with variable combinations of pain, differences in skin color and temperature, edema and sweating (Paice, 1995; Ribbers et al., 1995). The syndrome may spread to other extremities. In addition to the sensory and autonomic signs and symptoms, patients may present or subsequently develop movement disorders (Van Hilten et al., 2001).

Physicians score bradykinesia as an indication for lack of neuromuscular control. Normally bradykinesia is visually scored by a physician. Patients are requested to perform a finger tap task, i.e. open and close the thumb and index finger as fast as possible, while the performance is scored, grading from 0 (normal) to 4 (can barely perform). This visual score is subjective, physician dependent and a clearly has small resolution. Goal of this project is to develop a device that records bradykinesia and a method that objectively and accurately scores bradykinesia.

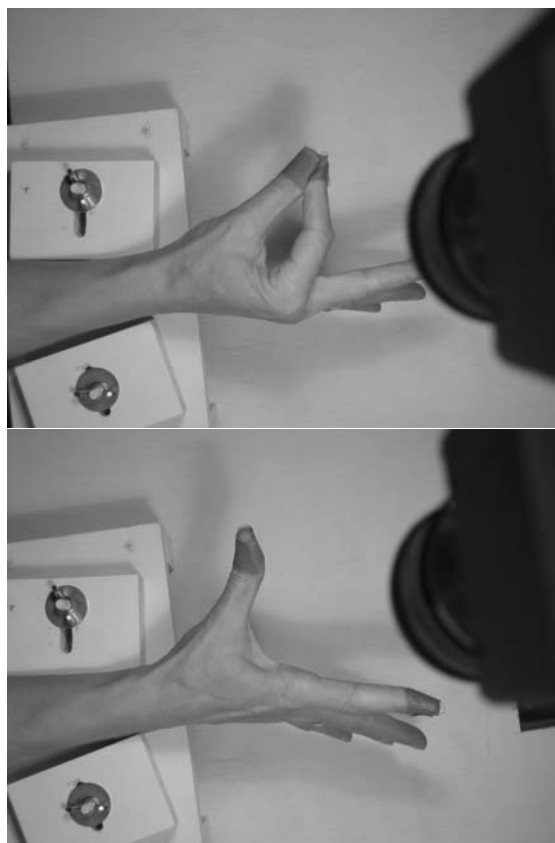
## 2 Materials and methods

### 2.1 Subjects

Eighty four subjects participated in this study. The subjects consists of patients suffering PD (n=14) or CRPS (n=14) and healthy control subjects (n=46). The patients are recruited at the Leiden University Medical Center. The healthy control subjects had no known history of neurological dysfunction and are recruited among partners of the participating patients and among hospital staff. All subjects gave informed consent prior to the experimental procedures.

### 2.2 Apparatus

The equipment consists of a high speed digital camera (60 fps) and an arm support. The lower arm is supported such that the hand and fingers can freely move. The camera is positioned above the hand, such that movement of the fingers can be recorded, see Figure 1. The arm and camera support assures that the distance between the camera and



**Figure 1:** Top view of the experimental setup. The upper plot shows the hand of a subject in 'closed hand' position and the lower plot shows the subject in 'open hand' position. At the right of the pictures the high speed digital camera can be seen. The subject's lower arm is supported to prevent movement of the lower arm and to keep the hand at a fixed distance of the camera.

	Control	PD	CRPS	significance	Post hoc (Tukey)			significance with correction
					PD / controls	CRPS / controls	PD / CRPS	
<b>Number of hands</b>	n=86 L=45; R=41	n=25 L=11; R=14	n=22 L=11; R=11	-	-	-	-	-
<b>Age [years]</b>	45,1 ± 16,6	65,0 ± 12,3	29,6 ± 9,6	<0,001	-	-	-	-
<b>Gender [M/F]</b>	M=23; F=31	M=6; F=8	M=0; F=14	<0,001	-	-	-	-
<b>MF [Hz]</b>	3,56 ± 0,94	2,25 ± 0,90	2,06 ± 1,05	<0,001	<0,001	<0,001	0,674	<0,001
<b>MA [m/s<sup>2</sup>]</b>	21,3 ± 7,36	9,76 ± 5,05	10,9 ± 8,07	<0,001	<0,001	<0,001	0,956	<0,001
<b>Trend MA [m/s<sup>3</sup>]</b>	-0,59 ± 0,47	-0,25 ± 0,43	-0,23 ± 0,29	<0,001	0,002	0,003	0,952	<0,001

**Table 1:** Summarizing the results of this study. The values after the ± denote the standard deviations. In the last column are the values after taking age and gender into account in the analysis.

the hand are fixed. The camera is connected to a PC through FireWire and the digital recordings are directly stored on hard disk as AVI files.

### 2.3 Procedures

The subjects sat on a chair in front of the equipment and were required to lay their lower arm onto the arm support. The subjects were instructed to close and open the thumb and index finger as wide and as fast as possible, see Figure 1. Markers of colored tape (Leukotape®) were attached to the top of the thumb (green) and index finger (blue). One trial lasts for 15 seconds, and both hands are recorded once. The procedure including instruction took less than five minutes per subject.

### 2.4 Data processing

For each subject two recordings are made of 15 seconds at 60 fps, resulting in 900 frames per trial. With special software (EthoVision®, Noldus IT) the centers of the markers were tracked with time. As the distance of the hand with respect to the camera was fixed the spatial orientation of the markers could be calculated. This data were exported and used for further analysis in Matlab (The Mathworks).

First the distance between markers was calculated. Two parameters were extracted from the recordings to represent the slowness of the movement: median frequency and mean absolute acceleration. The median frequency (MF) is obtained by Fourier transforming the signal and calculating the auto spectral density. The median frequency is defined as the frequency at which the power in the auto spectral density is equally divided. The mean absolute acceleration (MA) was calculated by double differentiating the distance and taking the mean of the absolute values. Furthermore the trend of the MA is calculated by taking the MA between 2.5-5.5 s and at 9.5-12.5 s. This parameter is selected to investigate possible 'fatigue' during a trial.

### 2.5 Statistical analysis

All parameters were statistically analyzed with SPSS. Differences between controls and patients are tested with an ANOVA. Differences between groups (CRPS, PD, and controls) were analyzed with a post-hoc test (Tukey).

## 3 Results

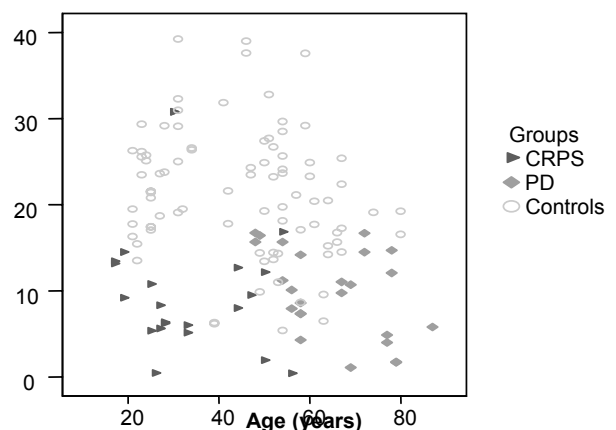
In some recording the markers could not be identified on all frames due to movements of the markers outside the

camera arena. These recording were not used for further analysis. All hands of the patients were visually inspected by a physician for bradykinesia. In this study only the affected hand(s) of the patients were used for analysis. In total 67 recording of the left hand were used for analysis (PD, n=11; CRPS, n=11; controls, n=45) and 66 recordings of the right hand (PD, n=14; CRPS, n=11; controls n=41).

The results are summarized in Table 1. It is noticed that a significant difference exists in age between the three patients groups. This was expected as PD primarily affects elderly and CRPS patients affects younger woman.

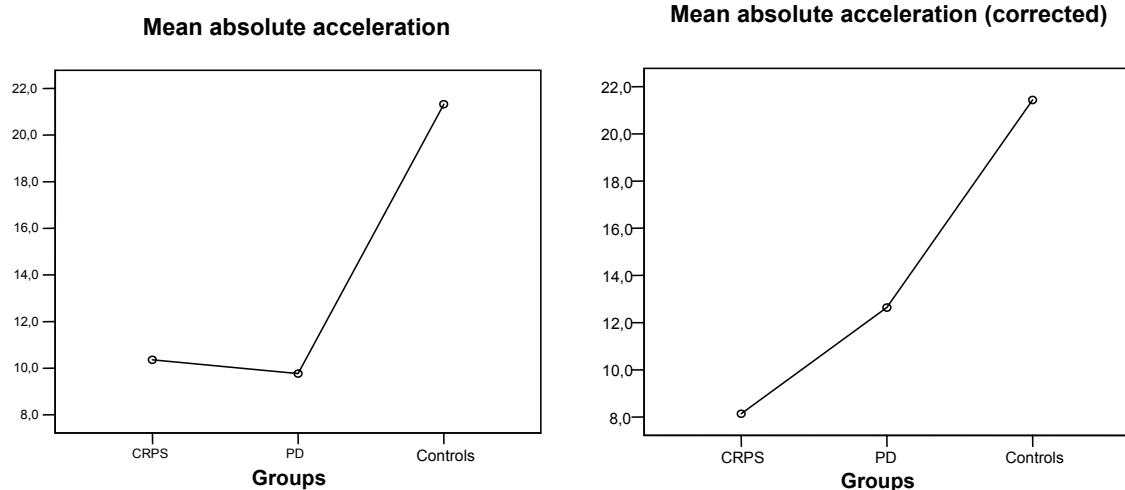
Patients had a significantly lower value for both MF and MA than controls. Post-hoc testing reveals that both patient groups differ from the controls, but no difference can be seen between the patients groups.

**Mean absolute acceleration (m/s<sup>2</sup>)**



**Figure 2:** The age of the subjects against the value for the mean absolute acceleration (MA). The control subjects are marked with the open circles, the PD patients are marked with the green squares, and the CRPS patients are marked with the blue triangles.

In Figure 2 is the MA plotted against the age for the two patients groups and the controls. It can be seen that the values for the MA are larger in the controls and the age of this group is spread evenly. Furthermore it can be seen that young controls have a higher score than older controls, i.e. the MA decreases with age. The PD patients score lower than the controls and are older. The CRPS



**Figure 3:** On the left are the values for the mean acceleration for the three group (CRPS, PD, and controls). On the right are the same parameters after age and gender have been taken into account.

patients are younger, and score even lower, than the PD patients.

Statistical testing reveals that age and gender have a significant effect on the parameters and should be taken into account. The results are corrected for age and gender by taking the age and gender as ‘co variable’ and ‘fixed factor’ into the ANOVA. In the left of Figure 3 it can be seen that a clear difference exists between controls and the patients. However the difference between the two patients groups is relatively small. After correction for age and gender the difference in the MA between CRPS and PD increases substantially. This effect is also seen in the MF parameter. By taking gender and age into account in the statistical analysis a significant difference exists between the three groups (CRPS, PD and controls); even between CRPS and PD.

The decrease in MA, i.e. the trend in MA, is in controls approximately twice as large as in patients. Differences are significant although with a higher p-value, compared to MA and MF. The trend in MA being twice as large is not surprising, as the MA itself is also twice as large.

## 4 Discussion

Bradykinesia, or movement slowness, is a common feature of many neurological disorders. Normally bradykinesia is visually scored by a physician. This study shows that bradykinesia can be scored by analyzing digital recordings of a finger tap task. Both the mean absolute acceleration (MA) and the median frequency (MF) show significant differences between patients and control. The method to record the finger movements takes only a few minutes. This study shows that the method can be useful as a diagnostic tool and future steps will be taken to develop this method towards a diagnostic tool.

### 4.1 Quantifying slowness in bradykinesia

In patients suffering bradykinesia, or movement slowness, both the values for mean absolute acceleration (MA) and the median frequency (MF) are significantly lower than in healthy control subjects. Although it should be noted that age and gender have an effect on both parameters and should be taken into account. With a larger dataset we will focus on the most differentiating parameter to describe movement slowness. MA is the primary candidate as acceleration depends on both the distance and speed of the

movements, and such is less sensitive to task variations (wide vs. fast). Finally, it is concluded that movement slowness can be objectively quantified, showing the relevance of the method.

### 4.2 Quantifying irregularities in bradykinesia

Bradykinesia is characterized by slowness and irregularities during fast voluntary movements. In this study parameters are sought, and found, that describe the slowness of the movement. However the second aspect of bradykinesia has got no attention: the irregularities of the movement. Especially Parkinsonian patients show a slow ‘saw tooth’ movement pattern. These irregularities seem an important feature of bradykinesia and in future studies we will try to parameterize these irregularities.

### 4.3 What causes bradykinesia?

Although a clear picture exists of how bradykinesia presents itself, little is known about its origin. Often a lack of neuromuscular control is mentioned. However many pathways exist between the planning of a movement in the CNS and the actual execution of the movement. This study only focused on quantifying the slowness of bradykinesia. Incorporation of the irregularities going with bradykinesia into the analysis may lead to a better classification of bradykinesia and such may contribute to a better understanding of the underlying pathophysiology.

*This study is part of TREND (Trauma RELATED Neuronal Dysfunction), a consortium that integrates research on epidemiology, assessment technology, pharmacotherapeutics, biomarkers and genetics on Complex Regional Pain Syndrome type 1. The consortium aims to develop concepts on disease mechanisms that occur in response to tissue injury, its assessment and treatment. TREND is supported by a government grant (BSIK03016).*

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# A new paradigm to analyze observational learning in rats

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## Abstract

A new paradigm of learning by observation was developed through an observational training in which rats observed companion rats performing different spatial tasks. Observer animals were separately housed in small cages suspended over a water maze tank. They repeatedly observed companion actor rats performing spatial tasks differing according to the experimental requirements. After the observational training, observer animals were or not surgically hemicerebellectomized. This surgical ablation was performed to block any further acquisition of new behavioral strategies during actual performance of swimming task. When cerebellar symptomatology stabilized, observer animals were actually tested in the Morris water maze task they had previously only observed. The observer rats displayed exploration abilities that closely matched the previously observed behaviors. The results obtained indicate that it is possible to learn complex behavioral strategies by observation using this new protocol. Furthermore, acquisition of the single facets that form the behavioral repertoire can be separately studied.

## Keywords

cerebellum; imitation; explorative strategies; Morris Water Maze.

## 1 Introduction

There is a wide psychological evidence that the actual execution of a task is not the only means to acquire an ability. In fact, even observation of actions provides an effective means of learning new skills. From an evolutionary point of view, the observation of motor acts performed by others and the comprehension of the meaning of these acts appear to be essential for humans as well as for animals, because they allow making inferences about others' behaviors [2, 3, 6]. Also, the observation of someone else performing an action is the first necessary step for imitating him and then acquiring the competencies to reproduce similar motor skills [7]. This highly adaptive strategy is such a powerful and widespread way of acquiring new behaviors from others (parents, teachers...), that it is recognized as a specific learning paradigm, described in the literature as learning by imitation [1]. As part of the broader phenomenon related to recognizing, intending and preparing a movement, the observational learning can be thus considered somehow related to motor physiology. However, its mechanisms are rather controversial and little is known on the neural structures involved.

By taking advantage of the specific role of the cerebellum in the procedural acquisition [12], we developed a paradigm of observational learning apt for rats, to analyze if they are able to learn by observation behaviors linked to environment exploration. It was observed that, in the presence of a cerebellar lesion, namely a hemicerebellectomy (HCb), it is impossible to learn any

new exploration strategy. In our observational learning paradigm, normal rats observed other conspecifics exploring a water maze to find the escape platform. After this observational training, "observer" rats underwent an HCb and were then tested in the Morris water maze (MWM).

As behavior to be learned we used the exploration of a new environment put into action in the water maze. It is an acquired behavior; it can be optimally learned through observational learning; it is a cerebellum-dependent behavior and thus freezable in the moment the cerebellar lesion occurs; it is based on sequenced explorative strategies that are used, separately or concurrently, to reach the escape solution with progressively shorter latencies and more direct navigational trajectories.

## 2 Materials

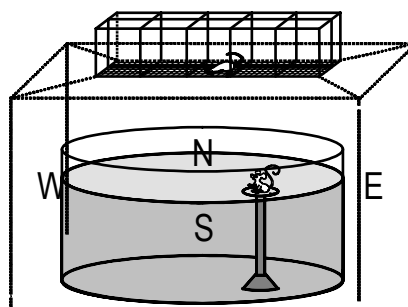
### 2.1 Animals

Adult Wistar rats (250-300g) were used. The animals were housed two to a cage with free access to food and water throughout the experiment and a standardized dark/light schedule (10/14 h). All efforts were made to minimize animal suffering and all procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, 1996).

### 2.2 Special equipment

For construction of the observation equipment:

Ten small wooden roofed cages (30 x 15 x 40 cm) with black inside walls were assembled together in two rows of five cages. The cage floors consisted of a metallic grid with open spaces of 1 x 1 cm, through which each rat observed the scene below. The cages leaned on a support located 60 cm over the water maze tank, where companion rats swam according to specific paradigms (Fig. 1).



**Figure 1.** Schematic diagram of the experimental setting used in the present observational learning paradigm.

For monitoring spatial performances in the MWM:

The paths taken by observer animals in the pool were monitored by a video camera (Sony CCD-IRIS Black and White) mounted on the ceiling. The resulting video signal was relayed to a monitor (Sony), that allowed both on- and off-line analyses, and to an image analyzer (EthoVision 3, Noldus Information Technology, Wageningen, The Netherlands). The x- and y- coordinates of the rat's position were sampled and stored on disk.

### 2.3 Detailed procedure

*Observational training.* The animals were housed separately in small cages suspended over the water maze tank. Through the cage floor they repeatedly observed companion rats performing spatial tasks that differed according to the experimental requirements. Each suspended animal observed 200 trials performed by conspecifics. During observational training, at each trial start observer animals were acoustically (radio music, whistle) and mechanically (slightly touching the animals inside the cages, moving the cage support, water sprays from the grid floor) stimulated to maintain a high level of arousal.

*MWM equipment.* A circular plastic pool (diameter 140 cm) with white inside walls was located in a normally equipped laboratory room, uniformly lighted by four neon lamps (40 W each) suspended from the ceiling (3 m). No effort was made to enhance (or vice versa to impoverish) extra-maze cues, which were held in constant spatial relations throughout the experiments. The pool was filled with water (24°C), 50 cm deep, made opaque by the addition of 2 liters of milk. A white steel escape platform (10 cm in diameter) was placed in the middle of one cardinal quadrant (NW, NE, SW, SE), 30 cm from the side walls; it was either submerged 2 cm below or elevated 2 cm above the water level. Each rat was gently released into the water from a wall point facing the center of the pool. The animal was allowed to swim around to find the platform.

*MWM protocols performed by "actor" animals and observed by "observer" animals.* The observer animals watched MWM trials performed by actor animals executing a specific explorative strategy, according to experimental requirements. The first group of animals observed 200 MWM sessions carried out by intact animals performing a basic MWM paradigm. The actor animals learned to reach the escape platform that was hidden in the NW pool quadrant in the first 16 trials, visible in the NE quadrant in the successive 8 trials, and again hidden in the NE quadrant in the final 16 trials. Another group of animals observed 200 MWM sessions performed by intact animals searching for (and not finding) the platform. The actor animals were put in the MWM tank without the platform for 60 sec. Thus, observer animals repeatedly observed animals that scanned the whole tank in search of an escape solution. Another group of animals observed 200 direct findings of the platform performed by well-trained intact animals that reached without hesitation a hidden platform whose position they had previously learned. At the end of the observational training, the observer animals were or not hemocerebellectomized, according to their specific protocol.

*Surgery.* A right hemocerebellectomy (HCb) was performed with an i. p. solution of ketamine (90 mg/kg) and xylazine (15 mg/kg). The dura was excised and the right cerebellar hemisphere and hemivermis were ablated by suction; care was taken not to lesion extracerebellar structures. The cavity was filled with sterile gel foam and

the wound edges were sutured. After recovery from anesthesia, the animals were housed two per cage.

*Motor assessment.* Testing was performed two weeks after the HCb, when no further change in cerebellar symptomatology was observed. The following aspects were taken into account: head and body tilts, position of hindlimbs, presence of ataxia, tremor, rearing behavior, falls to lesion side, wide-based locomotion, collapsing on the belly, pivoting, vestibular drop reactions and the ability to traverse a narrow path and to be suspended on a wire [9].

*MWM protocols performed by observer animals.* After the specific observational training, observer animals were tested in the MWM task according to a basic protocol. Each rat performed blocks of four trials, two blocks of trials per day. On reaching the platform, the rat was allowed to remain on it for 30 sec before being again placed in the water for the next trial. If a rat failed to locate the platform within 120 sec, it was guided there by the experimenter and allowed to stay on it for 30 sec. In the first four sessions (trials 1-16) the platform was hidden in the NW pool quadrant (Place I), in the successive two sessions (trials 17-24) the platform was visible in the NE quadrant (Cue phase) and in the final four sessions (trials 25-40) the platform was hidden in the NE quadrant (Place II) [10, 12].

*Recording of MWM performances.* To analyze the MWM performances, the parameters taken into account were successes in finding the platform, finding latencies and swimming trajectories. By considering spatial and temporal distribution of swimming trajectories, path length, swimming speed, percentage of time spent in inner or outer annuli, and headings (deviation between the rat's actual direction when leaving the edge of the tank and a straight line from the start location to the tank point containing the platform), exploration behavior was divided into four main categories: - circling, peripheral swimming at tank wall, without entering in the inner sectors of the arena; - extended searching, swimming in all pool quadrants, visiting the same areas more than once; - restricted searching, swimming in some pool quadrants, not visiting some tank areas at all; - direct finding, swimming towards the platform without any foraging around the pool.

*Histological controls.* After completion of behavioral testing, the HCbed animals were anaesthetized with Nembutal and perfused with saline followed by 10% buffered formalin. The extent of the cerebellar lesion was determined from Nissl-stained 40  $\mu$ m frozen sections. Surgical lesion of the cerebellar structures of the right side was considered appropriate if the right cerebellar hemisphere, the right hemivermis and the deep nuclei were ablated, while the left side of the cerebellum and all extracerebellar structures were completely spared. *Statistical Analysis.* Metric unit results of the different experimental groups were compared by using one-way or two-way "p x q" analyses of variance (ANOVAs) with repeated measures, eventually followed by multiple comparisons using Tukey's tests.

## 3 Results

The present protocol allows for different kinds of observational training, based on the observation of single explorative strategies, as well as on the observation of the entire explorative repertoire, to study their learning power in the acquisition of complex spatial behaviour. After the observational training, observer animals were tested in the

same MWM apparatus they had previously only observed. By comparing the frequency of actually using strategies that matched the previously observed strategies, it was possible to verify whether observational learning has taken place.

The explorative strategies of the observers were significantly influenced by the strategies the animals had previously observed. Observers of the entire repertoire of explorative strategies put into action by intact animals exhibited a competent exploration of the tank from the very first sessions, avoiding almost completely the repetitive circling in the pool peripheral areas characteristic of the initial steps of tank exploration. Of course, this efficient behaviour resulted in escape latencies significantly reduced in comparison to those of animals without observational training. Of course, as they explored the tank, the observer animals progressively learned the actually performed explorative strategies. Thus, the influence of the observational learning was detectable only in the first trials, when the learning based on actual performance was not yet present.

To analyse the influence of observational learning of single explorative strategies and single it out from the effects of learning through actual performance, it was thus necessary to block any further learning during the actual MWM performances. Of course, it was necessary that the animals maintained intact swimming abilities. Hemicerebellectomy fulfilled this double requirement. In fact, hemicerebellectomized animals are rather competent at swimming, and in water exhibit the same performances of controls in turning abilities, forepaw inhibition, and maintenance of nose above water level. Conversely, the cerebellar lesion blocks any further procedural acquisition, although it does not affect the procedural competencies learned before the cerebellar lesion. Thus, the spatial performances actually put into use by the observer animals, that underwent cerebellar lesion, had to have been learned before the cerebellar lesion and thus by observation.

As paradigm of observational learning, we used a group for which the observational training consisted of observing intact animals searching for (and not finding) the platform, called Os+H (Observation of searching behavior + Hemicerebellectomy) group. When actually tested in the MWM, this observationally trained group displayed performances biased by the previously observed performances. In fact, in spite of the cerebellar lesion, which, in the absence of pre-training, typically provokes repetitive circling at the pool periphery, they circled at the pool periphery only in the very first trials; then, throughout the testing, they persisted in swimming around the pool looking for the platform. Such searching behavior, although effective for searching (and then finding) the platform, was not an adaptively modifiable strategy, as indicated by the flattened slope of the latency time course and by the almost complete lack of evolution from one kind of exploration behavior to another.

Another paradigms of observational learning consisted of observing only direct finding of the platform, (group Of+H (Observation of finding behavior + Hemicerebellectomy)). These animals displayed long-lasting circling with a greatly reduced percentage of searching. Interestingly, they exhibited direct finding, as their second most frequent strategy. In fact, the animals either circled at the pool periphery or initially tried to detach from the pool walls towards the central sectors possibly containing the platform. If the escape solution failed, they immediately returned to

circle at the pool periphery. This pattern of behavior resulted in a very high percentage of peripheral circling, paradoxically accompanied by a rather high percentage of direct finding of the platform.

## 4 Discussion

The paradigm of observational learning is made possible by the rats' natural curiosity about the surrounding environment. The advantage of the present procedure is that, during the observational training the animals are free to move without constraint. Therefore, since they are unstressed, the observer animals are interested in the surrounding environment and in the actors' behavior. Furthermore, the suspension of the observers in single cages prevents social interactions that could distract them. Furthermore, the possibility of simultaneously training ten subjects on one hand speeds up experimental procedures and, on the other minimizes experimental variability linked to environmental factors.

The described observational learning protocol allows for acquisition of spatial strategies by observation. The observational training shapes the successive actual performances in the MWM task, improving the exploration of the arena in the search for the platform.

Paradigms of actually practiced learning, based on trial and error, have heavy limits to allow analyzing single behavioral steps, since the physical experience itself activates their sequential organization, making it almost impossible to single out their acquisition [5, 7]. Conversely, in the present protocol, by exploiting the potential offered by the observation of each single behavioral step in combination with the block of learning elicited by the cerebellar lesion [8, 11], behavioral steps can be singularly acquired. Thus, one very attractive feature of this new protocol is that the numerous facets of a complex performance can be singly analyzed. For example, this protocol can be not only used to analyze the capacity to learn single exploration strategies by observation but also to study whether it is possible to acquire localizatory knowledge about specific goal positions by observation.

Considering the results as a whole, it seems that the newly developed protocol of learning by observation was successful in shaping the exploration strategies according to the kind of observational training [4].

Besides these aspects, the described protocol has the potential to study whether it is possible to bias actual MWM performances through the observation of "wrong" exploration behavior, as the repeated observation of animals circling in the peripheral pool regions [4]. This approach can allow investigating the effects of negative models on different learning paradigms.

Of course, the present protocol may be also used to test the capacity to learn by observation in contexts and experimental settings that are completely different from the MWM task. Many behavioral tasks can be learned by observation, and by means of this protocol it is possible to fractionate their organization and study their sequential components. Radial maze, plus-maze, and open field with spatial or object change are behavioral tests that can be studied using this new approach.



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# Intelligent CCTV Surveillance: advances and limitations

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## Abstract

This paper outlines the motivation and requirements for CCTV surveillance particularly in public spaces and on how practical computer vision systems are beginning to be proposed to support the human monitoring task. The explosion of CCTV puts increasing demands on the sophistication required of such systems. We highlight here what has been achieved and the current limitations of such systems.

## Keywords

Closed-circuit television, surveillance, image processing, security, people tracking, crowd-monitoring.

## 1 Introduction

The installation of closed circuit television (CCTV) cameras in urban environments is now commonplace and well-known. Public attitudes to these systems reflect the balance needed between two conflicting requirements:

- a) concerns over invasion of privacy and fears of authoritarian control of the population
- b) welcoming the increased safety in public spaces and reductions in crime and antisocial behavior.

Fears in category (a) are not surprising, since within living memory, there have been countries governed by regimes with a tendency to the oppressive monitoring and control of their citizens, and the continuous tracking of actual or imagined 'dissidents'.

Support for category (b) arises from public concerns over both real and imagined risks of urban crime and terrorism. Controlling anti-social behavior and protection against terrorist threats is generally perceived to have a high priority, making intrusive monitoring relatively acceptable and encouraging the installation of advanced surveillance systems. Recent uses of the phrase 'homeland security', and plans for technologically-advanced personal ID cards have made this a topical issue. There is an assumption, founded on practice, that CCTV systems make a contribution to public security and safety, but the monitoring task is resource intensive and will benefit from some degree of automation.

## 2 The evolution of CCTV surveillance

CCTV based surveillance has developed from simple systems comprising a camera connected directly to a viewing screen with an observer in a control room, watching for incidents of crime or vandalism or searching for targeted individuals, to complex multi-camera systems spread over large geographical areas [15]. Advanced digital recording and playback techniques can be provided, with searching capabilities, and suitable for audit and to present observed results as evidence in legal proceedings. It is common to find manual camera control (pan, tilt and zoom) as an aid to track events or objects of particular types. As the number of cameras in each system increased, they first exceeded the number of monitoring screens and then exceeded the capability of the observing teams to

watch events effectively, as their attention span is inevitably limited [17]. For example, even if we assume that at best a person could monitor, say, 10 video sources simultaneously, major cities like London and Paris have or plan to have more than 10,000 cameras in their underground train systems, thus requiring at least 1,000 people per working shift. A British newspaper reported in 2004 that over four million CCTV cameras were deployed in the UK [6]. There are significant pressures on managers to provide cover for such CCTV monitoring facilities at the possible expense of having staff on the ground that can interact directly with people and improve the feeling of personal security.

Although the word surveillance sometimes carries negative undertones, we use it in the context of what is called a "capable guardian" as an approach to dissuade people from criminal or antisocial activities and to reassure citizens that they are protected and that public spaces are properly managed. In this context, what is most important is to pick up early indications of *potentially dangerous* situations that might affect safety or security so as to alert a human being who can then take control, assess the situation and deploy resources as necessary.

Human action is rarely decided on the basis of visual information alone, but to be able to cope with the vast and largely uneventful amounts of visual data it is highly desirable to automate the monitoring task through computer vision systems. Detection thresholds usually need to be biased in favor of false positives, since these can be quickly recognized and disregarded by a human observer, whereas missing real incidents is a serious deficiency. This is best done on-line to prevent and manage but also off-line to find evidence (searching large amounts of recorded video is never a trivial task). These are what we call Intelligent CCTV Surveillance systems.

In this paper we outline some representative work in the field of computer-assisted visual surveillance of human activity especially in public places which are subject to significant volumes and complexity of activity. Space limitations prevent us from mentioning all key work or from going into much detail (please see [4], on which this paper is partly based). This is better addressed by the accompanying papers that form part of a special symposium on "Automatic detection of abnormal human behavior using video processing" in this conference.

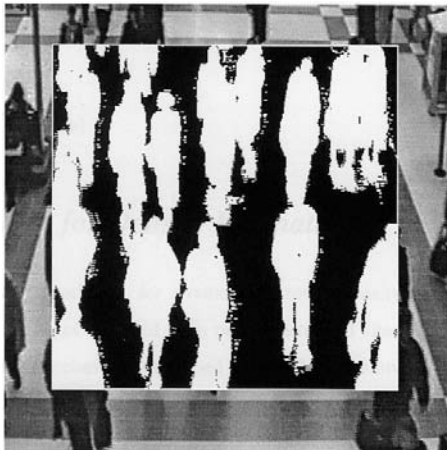
It is key to learn from what humans do in monitoring environments such as Control Rooms [12] where attention is focused on special (unusual) situations which are known (to an experienced operator) to be associated with the need to intervene. These situations are likely to be the *result* of unusual *behavior*. This subtle difference between behavior and situation is important to understand how reliable computer systems could be designed to guide human monitoring but not necessarily to replace it. A typical scenario is that of pick-pocketing where in most cases the behavior itself might be very difficult to see, but its situational effects (e.g. blockage in an escalator) is known to the trained eye to be correlated to a behavioral pattern.

Fairly successful means of detecting this type of situation even in cluttered conditions operate at the pixel (or groups of pixels) level without attempting to *understand* behavior itself and we show some examples of this in section 3. In section 4 we outline work aimed at tracking people as a means of identifying anomalous situations.

### 3 Crowd Monitoring

#### 3.1 Pixel-level processing

Early uses of visual surveillance included the estimation of global properties of urban crowds such as density and flow [3]. Applications have been found in densely-populated urban spaces such as city rail-stations, shopping malls and airport departure lounges. Data can also be gathered to assist architects in the planning of urban environments. The techniques do not attempt to identify individual pedestrians in a crowd – rather, the crowd is monitored as a generalized entity, and ‘average’ properties sought. Some analogies with fluid behavior and with the behavior of charged particles in an electric field may be observed. For example, if the area of an image occupied by pedestrians can be identified, then the ratio of ‘crowd area’ to ‘background area’ provides a rough estimate of crowd density (*Figure 1*). Compensation is needed for the different apparent size of objects at various distances. Recent work has shown this can be measured automatically [11]. Variation of lighting levels and directions presents many problems. In traditional surveillance with sparse spatial and temporal activity (e.g. sterile zones), this can be overcome using methods based on statistical models of such variation, provided that most of the time what is seen by the camera is background. Foremost in this category are the Gaussian-mixture models proposed in [13].



*Figure 1. Pedestrian extraction from crowd-scene [18].*

Difficulties still exist for crowded conditions, especially when people and objects might persist in the field of view for very long periods of time. Good results have been shown by methods that combine luminance statistics with image motion estimation (similar to the encoding of video material), an example of which is given in *Figure 2* [16]. In addition to improving background estimation, motion (or its absence) is an important clue for the detection of unusual situations.



*Figure 2. Extracted motion-vector field – arrows show direction of motion.*

#### 3.2 Detecting unusual situations

Once a robust way has been established to measure motion properties, to separate foreground from background, and relating them to the geometry of the scene, then the detection of spatial and/or temporal variation of these image properties can be correlated to situations that might merit the attention of an operator. For example, by monitoring density, it is possible to set thresholds above which safety might be endangered due to overcrowding or congestion (*Figure 3*). This is important because entry to city-centre rail stations may have to be periodically closed during rush-hours in order to keep crowd density on platforms at a safe level.



*Figure 3. Automatic identification of congestion (dense non-moving area), denoted by array of white dots.*

Detection of individuals who linger in one place for long periods while surrounding crowds are moving, or of individuals moving in a different direction from the majority, may be a potential indication of planned or actual criminal behavior (*Figure 4*). This detection might be appropriate to spot people considering committing suicide on the railways, known to linger at the end of the platforms for a long time before jumping in front of a train (the cost of which, financially and emotionally, is very high). A similar technical challenge exists for the detection of abandoned packages which are responsible for daily public transport disruption (*Figure 5*).



**Figure 4.** Maintaining detection of lingering people during occlusion (person on left hand side by the wall poster).



**Figure 5.** Detection of stationary object (a suitcase to the right of the central pillar).

As far as public safety is concerned, a typical situation is that of people who willingly or not walk too close to the edge of a platform (**Figure 6**). This is an instance of a general class of detection of *intrusion* (trespassing into a forbidden area). Note that the computer vision system does not need to *know* that what is being detected is people, but that just in a given context the presence of an area of image foreground of a given minimum size is an indication of a situation of interest. We hope that through this example the reader gets an appreciation of the power but also of the limitation of current computer vision systems (in this example, it is not possible to infer the detailed behavior of the intruder, let alone their intention).



**Figure 6.** Detection of a person too close to the edge.

#### 4 Individual Tracking

*Tracking* is generally understood as the process of first localizing each person in an image and then following the progress of each person from one image to another and (ideally) from one camera to another. Note that we use the term *localization* to make a distinction with *identification*

that implies obtaining the actual identity of a person through some biometric process (such as face recognition or gait). This process of tracking is currently only robust enough for relatively sparse human traffic (and mainly restricted to people walking). It is an important field of work because many researchers base leading edge attempts to interpret complex or subtle behavior on assumptions of accurate tracking.

Having obtained a classification of image pixels into background, foreground and moving, the foreground parts are segmented (grouped) into distinct objects, normally represented by an enclosing rectangle or “blob”. The tracking process then consists on using kinematic constraints and sometimes *appearance* measurements (size, color) to match corresponding blobs from one image to the next. This is referred to as the *correspondence problem*. We then end up with blobs that have been labeled with identifiers that uniquely associate an object (or person) in a given video sequence. A typical example [7] is shown in **Figure 7**.



**Figure 7.** Pedestrians marked by blobs (approaches marked by □ and departures by ⊥).

The tracking data is then used to correlate unusual positional and velocity temporal patterns (“dynamics”) with unusual behavior [10]. Typical examples include detection of lingering, movement from one car to another in a car park, taking an uncommon path. Difficulties naturally arise from occlusion. If the object disappears behind an obstacle, it is possible to use its velocity to estimate the place and time of its emergence assuming no change in its velocity (more sophisticated systems might use acceleration data too). Commonly, a Kalman filter is used to provide better estimates of future trajectories of objects which move into an occluded zone [8, 14]. However, it is possible to imagine many situations where this is unreliable – for example two people being tracked may disappear behind an obstacle, and while there may meet, hold a conversation, then split up and depart in opposite directions. To automatically determine which one is which from the image sequences, following their re-emergence, is still not at all easy [9].

Until recently, detection of these situations was effectively wired-in (pre-programmed). In environments that can be constrained, this is a reasonable approach that has shown to be appropriate in other fields (such as industrial inspection) and that could lead to successful implementations e.g. for home or office surveillance. However, for large systems deployed in open public places it is recognized that it is crucial to have systems that adapt and learn. For example, based on tracked positions a system can self-measure typical entry and exit points and common paths between them so that an observation that is outside what has been thus learnt, can be flagged (**Figure 8**). A different approach to behavior-related detection is described in [5], using goal-directed

models to have a system separate between interesting and non-interesting behavior.

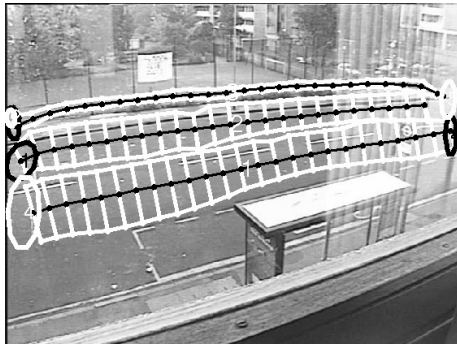


Figure 8. Example of learnt entries, exits and gates [1].

## 5 Conclusions

It is hoped that we have given here an indication of the current state of Intelligent CCTV Surveillance. The progress in computing and telematics (wireless LANs, mobile phones) all contribute to the continued deployment of more complex and advanced CCTV surveillance systems, which is likely to become increasingly unobtrusive as cameras decrease in size, and likely to be integrated with other sensors (audio, thermal, etc.).

The key current limitation of these surveillance systems is that they have to rely on human observation. Automatic recognition of behavior patterns is beginning to be demonstrated outside research laboratories and it will improve, so that it will become easier to detect and predict both legitimate and illegal activity. However, there is still a long way to go before these systems have capabilities approaching those of humans. Automatic analysis of gestures and posture [2] is key to identifying rich behavior in person-to-person interaction and requires significant advances for applicability in public places.

As a final related observation, clearly there is no assurance that these systems will always be used responsibly and only by those with the public interest and safety in mind. Misuse by official agencies and adoption by criminal elements in society may happen if there are insufficient safeguards. Just as the invention of the word processor has not yet resulted in the paperless office, the development of improved CCTV surveillance systems is not likely to lead to the crime-free city centre.

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# Chemosensory recognition among mice

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## Abstract

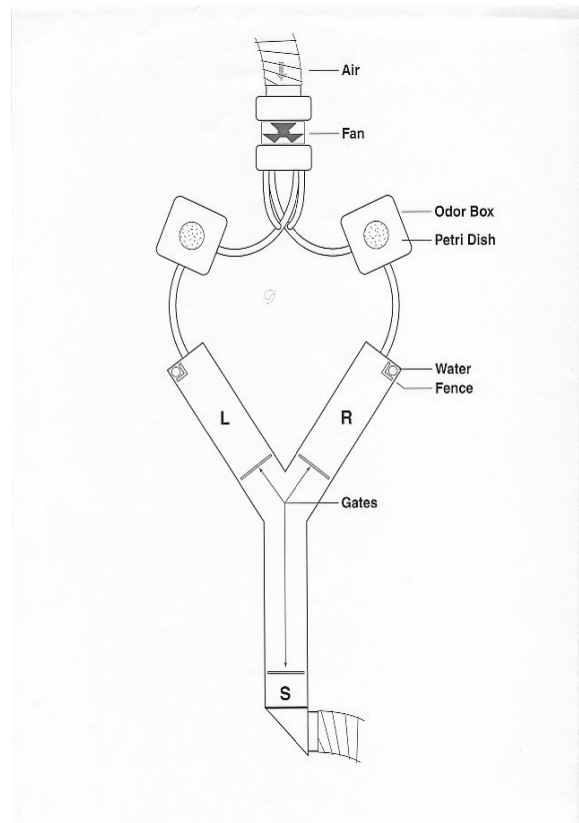
Body odor plays a prominent role in regulating social, sexual and endocrine responses of many species and specialized structures have evolved to produce and detect odorous signals. The major histocompatibility complex of genes (MHC) imparts to each mouse an individual odor, called an odor type, which reflects its MHC genotype. A prime experimental method we have used for identifying MHC odor types is a specially designed Y maze in which mice are trained, by water deprivation and reward, to distinguish odors from MHC dissimilar mice or their urines. An alternative or supplement to the Y maze is an automated olfactometer, a computer-programmed and fully automated apparatus in which a mouse or rat is trained for odor type distinctions, again by water deprivation and reward. In this presentation advantages and cautions associated with these methods are discussed.

## Introduction

Body odor plays a prominent role in regulating social, sexual and endocrine responses of many species and specialized structures have evolved to produce and detect odorous signals. Individual recognition, often communicated through genetically-determined body odors, may be critical in mate choice, incest avoidance, parental care, and other inter-individual interactions. We have found that the same set of genes that code for individual identity in the immune system (i.e. those genes that insure rejection of foreign tissue and organ transplants) also provide an animal with a unique odor. These genes, termed Major Histocompatibility Complex (MHC) genes, form a linked set in all vertebrates and they include the most polymorphic of any known genes. They provide each animal with what we have termed an MHC odortype as has been demonstrated by our group and others in studies with mice, rats and humans.

## Methods

A prime experimental method we have used to investigate MHC odortypes is the specially designed Y-maze (Fig.1). The purpose is to determine if mice can discriminate between body odors of 2 mice that differ only by genetic variation in specific MHC genes. If they can, this is proof that these genes modulate body odors. For training and testing in the Y-maze, gates are raised and lowered in timed sequence of up to 48 consecutive trials, the paired urine samples being changed for each trial. Reward for correct response is a drop of water, the trainee mouse having been deprived of water for 23 hr. Each mouse is first trained to discriminate between urine donors of two unrelated mouse strains, that differ in many genes throughout the genomes. Next, mice differing only at the MHC loci serve as odors stimuli. If the trained mouse is able to discriminate between these mice then interspersed unrewarded trials (about one out of every four) are included to familiarize the mice with occasional absence of reward following a correct response. The trained mice perform on the unrewarded trials with the same accuracy



**Figure 1.** Y-maze. Air drawn by a fan through a tube whose inlet is near the input vent supplying the laboratory is conducted through the left and right odor boxes. Each odor box has a hinged lid to admit a Petri-dish containing urine, the odor source. The air currents then pass to the left (L) and right (R) arms of the maze, which have hinged transparent lids. Each arm of the maze is fitted with a plastic tube perforated at the bottom to make one drop of water available. Each water tube is guarded by a fence that is raised only if the mouse enters the arm scented by the odor concordant with its training. Each arm of the maze is fitted with a gate that is lowered once the mouse has entered. If the choice is discordant, the fence is not raised, and the mouse is returned to the starting compartment (S). If the choice is concordant, the fence is raised to give access to the drop of water. The time interval in the starting compartment is set at 30 seconds to allow for changing the Petri-dishes in the odor boxes and for replacing the drop of water (if indicated); after this, on a timed signal, all three gates are raised to commence the next trial. Left-right placing is decided by a series for random numbers suited to the sample size. The time taken for trained mouse to make a choice is 2 or 3 seconds; the choice is made without pause, or after sniffing at the entrance to the arms, or sometimes with brief retracing from one arm to the other.

as on rewarded trials. Training and testing continue as described above but samples from interspersed unrewarded (generalization) trials are now supplied from new panels of H-2 types.

An alternative or supplement to the Y maze is the automated olfactometer, a computer-programmed and fully automated apparatus in which the rat is trained for odortype distinctions, again by water deprivation and reward. In this presentation advantages and cautions associated with these methods are discussed.

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