Instituto Juan March
de Estudios e Investigaciones

37 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Cellular and Molecular Mechanisms in Behaviour

Organized by

M. Heisenberg and A. Ferrús

D. L. Alkon
A. Artola
H. Breer
M. A. Castro-Alamancos
J. Cudeiro
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E. B. Keverne
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The lectures summarized in this publication were presented by their authors at a workshop held on the 27th of February through the 1st of March, 1995, at the Instituto Juan March.

Depósito legal: M. 12.657/1995
I. S. B. N.: 84-7919-537-1
Impresión: Ediciones Peninsular. Tomelloso, 27. 28026 Madrid.

INDEX

PAGE

Alberto Ferrús: Introduction..... 7

FIRST SESSION. Chairperson: Alberto Ferrús..... 11

Heinz Breer: Olfactory reception: The molecular basis of signal recognition and transduction..... 13

Eric B. Keverne: Olfactory learning and memory..... 14

Nicolas Franceschini: The eye of the fly: A user friendly processor for studying the neural basis of visuomotor control..... 16

Karl Georg Götz: Classification and selection of visual targets in *Drosophila*..... 18

SECOND SESSION. Chairperson: Martin Heisenberg..... 21

Martin Heisenberg: How flies recognize patterns..... 23

Tim Tully: Genetic dissection of memory..... 24

Alcino J. Silva: Gene targeting and the biology of learning and memory..... 25

THREE LECTURES OPEN TO THE PUBLIC:

Eric Kandel: Genes, synapses and long-term memory..... 27

Wolf Singer: Neuronal mechanisms of perception..... 31

Daniel L. Alkon: A non-Hebbian synaptic transformation for memory storage in the hippocampus..... 33

THIRD SESSION. Chairperson: Daniel L. Alkon..... 35

Alain Artola: Relationship between long-term depression and long-term potentiation of excitatory synaptic transmission in neocortex and hippocampus..... 37

Daniel L. Alkon: A role for a specific G-protein that regulates protein synthesis in memory storage and Alzheimer's disease..... 39

PAGE

Catharine Rankin: Behavioral and cellular analysis of habituation in *C. elegans*..... 41

Eric Kandel: Molecular mechanisms contributing to synaptic plasticity (without abstract)

Maurice Moulins: Modulation and specification of oscillatory motor circuits in lobsters..... 43

FOURTH SESSION. Chairperson: Wolf Singer..... 45

Javier Cudeiro: On the role of nitric oxide in the processing of information in the visual system..... 47

Wolf Singer: Development and organization of representations in the mammalian cerebral cortex..... 48

Steven P.R. Rose: The transition between short- and long-term memory: Role of synaptic adhesion molecules..... 50

Henning Scheich: Learning-induced plasticity of auditory cortex..... 51

José M. Delgado-García: Short-term and long-term changes of mammalian central neurons during attentive states, motor learning and regeneration..... 52

FIFTH SESSION: Chairperson: Alberto Ferrús..... 55

Manuel A. Castro-Alamancos: Behaviorally induced neural plasticity in the cerebral and cerebellar cortices..... 57

Russell D. Fernald: Social control of gene expression..... 58

William A. Phillips: The contextual guidance of learning and processing as a basis for cortical computation..... 59

Alberto Ferrús: Increasing the number of synapses in *Drosophila*..... 60

Martin Heisenberg: Concluding remarks..... 61

PAGE

POSTERS..... 65

Esther Alcorta: Contribution of maxillary palp information to the olfactory behavior in *Drosophila melanogaster*..... 67

Konstantin Anokhin: Involvement of gene expression in the olfactory bulb in formation of odor recognition memory in rats..... 68

Herwig Baier: Modules for molecules? Topographical organization of the zebrafish olfactory system..... 69

Alessandro Cellerino: The physiological action of BDNF on retinal ganglion cells: an unsolved puzzle..... 70

Matthew Cobb: Genetic and behavioural measures of olfactory coding in a simple system..... 71

Dámaso Crespo: Effect of chronic intake of cytidine-5'-diphosphate-choline (CDP-choline) on behavioral and neuromorphologic features of the aged mice..... 72

Miguel Escudero: The nitric oxide is implicated in the generation of eye movements..... 73

Carlos Gómez: Cortical potentials associated to visual stimuli, manual responses and saccadic eye movements during gap and non gap paradigms..... 74

Agnès Gruart: Kinematic analyses of the blinking behavior.. 75

Luis de Lecea: Molecular dissection of long-term potentiation by subtractive hybridization..... 76

Manuel Martínez Padrón: Patch-Clamp recordings from single synaptic boutons in *Drosophila* neuromuscular junctions..... 77

Brian McCabe: Cellular and molecular analysis of the olfactory jump reflex in *Drosophila*..... 78

Elisa Mengual: Distribution of nitric oxide synthase positive neurons and neuropil in the ventral striatum and ventral pallidum of the cat..... 79

Hendrik Rosenboom: Calcium metabolism of honeybee Kenyon cells. A combined patch-clamp and fluometric approach..... 80

PAGE

Dmitri A. Rusakov: Expression and topography of N-CAM isoforms in chick forebrain following training..... 81

Sean T. Sweeney: Targeted expression of tetanus toxin in *Drosophila* specifically eliminates synaptic transmission and causes behavioural defects..... 82

LIST OF INVITED SPEAKERS..... 83

LIST OF PARTICIPANTS..... 89

INTRODUCTION

A. Ferrús

INTRODUCTION

A. Ferrús

Neuroscience has entered a new era. Several behaviours, learning and memory in particular, have begun to be understood in terms of the underlying cellular and molecular mechanism. The progress along this reductionist approach has been parallel by similar advancements in the physiology of ensembles of cellular networks. It is quite fortunate that scientists working from both ends have begun to interact and to find common grounds for discussion and, consequently, to create novel interpretations. This workshop represents one such fortunate occasion where neuroscientists with diverse fields of specialization have met because of their common basic interest to understand behaviour.

Behaviour is the final manifestation of the biology of an organism. Quite properly, the biological meaning of all cellular processes lead to this final function. Also, it is becoming apparent how behaviour is indeed a major force in evolution. This workshop has served to review the existing data that support the previous statements. The coming years will witness major developments of the basic findings now discovered. Animals with specific changes in the molecular constituents of their synapses will be generated and the behavioural consequences will be studied with a level of resolution never reached before.

The integrative nature of behaviour requires a proper mixture of technical expertises towards a single goal. However, it is becoming apparent that the genetic foundations need to be studied in a much greater depth in order to unravel the governing rules of behaviour. The functional organization of the genome is causaly linked to physiology and, thus, to behaviour. In turn, behaviour modifies the genetic activity closing the ever moving wheel of biology and tracing the path of evolution.

Three days and a tight programme of presentations and discussions have served to evaluate our views on these subjects and, most certainly, will modify our future behaviour.

FIRST SESSION

Chairperson: A. Ferrús

Olfactory reception: the molecular basis of signal recognition and transduction

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The sense of smell allows to continously monitor the chemical environment for small volatile molecules and plays a central role in driving basic patterns of behavior in most animals including identification and evaluation of territory, food and reproductive partners as well as prey and predators. The primary process of odor perception, i.e. recognition and transduction of olfactory stimuli are mediated by the chemosensory olfactory neurons. The specificity of the system is supposed to be based on the interaction of distinct odorous molecules with specific receptor proteins in the membrane of a subset of cells. Interaction of odorants with suitable receptors activates transduction pathways that modulate the excitability of the sensory neurons; these transmembrane signalling mechanisms, which include second messenger cascades, provide a considerable amplification of the initial signal, the basis for the sensitivity of the system. The identification of molecular elements comprising the olfactory signalling machinery revealed that each step of the chemo-electrical transduction process in olfactory neurons appears to be an adaptation of a general principle in membrane signalling; i.e. the task of the olfactory system in detecting and discriminating myriads of extraneous chemicals is not based on de novo paradigms but rather is accommplished by widely conserved molecular devices, such as G-protein coupled receptors, second messenger cascades and ion channels. Thus, the convergent results of recent experimental studies employing modern biological techniques provide some new insight into the mechanisms underlying the flow of information from odor molecules to the neuronal activity of sensory olfactory cells.

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OLFACTORY LEARNING AND MEMORY

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Memory permits the acquisition, retention and retrieval of different kinds of information, providing a reserve of experience from which animals may take advantage. The question has recently arisen as to whether memory is a unitary event explained by a single set of general principles, or whether multiple systems underlie the diverse phenomena of memory. Neuropsychological evidence suggests that the mammalian brain possesses a number of different and potentially independent memory systems with different anatomical dispositions in the brain. Our interest concerns an olfactory (pheromone) recognition memory in mice located at the first relay in the sensory processing network[1]. It is acquired with one trial learning contingent on noradrenaline activation at mating[2], and lasts for several weeks[3]. Neurally the mechanism is simplistic, involving synaptic changes at dendrodendritic synapses in the accessory olfactory bulb[4]. As a result, males made familiar by mating are recognised by the female thereby mitigating pregnancy block. Such a memory function is of significant biological importance to the female, for without it, she would rarely become pregnant.

To illustrate the conservation of neural mechanisms, the very same parameters have been shown to be active in other species and other contexts. Mother infant recognition in both sheep and mice likewise have critical periods dependent on noradrenergic activation, which is triggered by stimulation of the female's reproductive tract (parturition)[5]. Failure to make this olfactory recognition results in cannibalism in mice, and the loss of selectivity in ewes allowing unrelated lambs to suckle.

The synaptic circuitry of the first olfactory relay is relatively simple. The mitral cells form reciprocal dendro-dendritic synapses with granule cells, the main class of interneuron. Granule cell synapses are depolarised by excitatory amino acid release from the mitral cells, and in turn provide a feedback inhibition to the mitral cells via GABA release. Since glutamate mediates the transmission from mitral to granule cells, we have investigated the effects of selective (NMDA) and non-selective excitatory amino acid antagonists on the recognition memory, formed by female mice to male pheromones. These studies together with GABA-ergic antagonists in mice, and in vivo micro-dialysis studies in sheep have demonstrated changes in synaptic plasticity at the mitral to granule cell synapse[6]. The findings illustrate how a

relatively primitive trilaminar structure has the capacity for synaptic changes rendering the processing of biologically significant odours different on subsequent exposures.

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THE EYE OF THE FLY: A USER FRIENDLY PROCESSOR FOR STUDYING THE NEURAL BASIS OF VISUOMOTOR CONTROL

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Because of the crystalline organization of its neurones, the compound eye of the fly can be used to analyse in great detail how a message received from the physical world is processed by a natural neural network. Here the higher diptera (flies) offer unique advantages as material for research because:

* their visual capacities are highly evolved and yet can be easily quantified
* the mechanisms at work can be analysed using genetic dissection in *Drosophila*
* the same identifiable visual neurones can be pinpointed in several different individuals
* photoreceptor cells can be identified, analysed, and stimulated individually using real time techniques on living, intact animals.

The fly with its panoramic compound eye is therefore an invaluable model system. We are using many different angles of approach with a view to strippIng this particular sensory device down to its elementary microcircuits and components, and thus discovering the cellular logic behind the neural network in question. We are focussing on the one hand on the actual wiring diagram, and on the other hand on the working principles which govern the neural microcircuits and enable the animal's eye to extract information about shape, movement, distance, colour ... from the physical world. These *ad hoc* microcircuits are all controlled by a mosaic of 50.000 photoreceptor cells which were analysed not only with electrophysiological techniques but also with novel methods we have devised, such as *in vivo* microspectrophotometry and *in vivo* microspectrofluorimetry (2). In providing us with unique means of studying single neurones in their natural environment, with a resolution comparable to that attainable with cell cultures, these methods recently lead us to the discovery of phenomena of general interest in Neuroscience: neuronal "photo-degeneration" and neuronal "photo-permeabilization", which have interesting potential applications in neuroanatomy, neurophysiology and neurosurgery (6,7).

The optical methods we have devised for analysing receptor cells *in vivo* (1) can now be used to stimulate single, identified cells while simultaneously recording the activity of various neurones in the visual chain by means of microelectrodes. For instance, in order to call up the microcircuit responsible for motion detection and examine how this proper "sequence discriminator" works, we recorded from the identifiable neurone H1 while stimulating two neighbouring photoreceptor cells sequentially, so as to simulate a micro-movement in the animal visual field (3). The optical instrument we have constructed for this purpose is a kind of microscope-telescope, the main lens of which is quite simply one of the eye's facets (diameter 25 µm; focal length 50 µm). With this instrument, two micro-spots with a diameter of 1µm can be projected onto two preselected receptor cells located 3,5 µm apart within the focal plane of the facet under investigation. The results of this "apparent motion" experiment carried out at the single cell level is that H1 responds with an increase in spike frequency when the two receptors are presented with a sequence that mimics motion in the "preferred" direction, and with a decrease in spike frequency for the opposite sequence (4). A series of experiments with microstimulation of single photoreceptor cells have helped to elucidate the "hidden logic" governing an animal motion detector and to specify its kinetic properties and nonlinearities (8).

In an attempt to meet the challenge of the sophisticated visuomotor control system of the fly, we have constructed artificial motion sensors, which put our modelling of the biological system severely to the test. These electro-optical circuits are hardware simulations of the signal processing steps discovered in the fly, including their kinetics and nonlinearities. This synthetic approach ended up with the construction of an autonomous mobile creature that uses a compound eye with an array of *local motion detectors* to avoid obstacles and steer itself through a cluttered, previously unexplored environment (5).

The compound eye of our mobile robot consists of about 100 ommatidia, with facet lenses, photoreceptor cells and optic fiber "rhabdoms". The first "optic ganglion" performs signal conditioning, the second one consists of a retinotopic array of about 100 smallfield *local motion*

detectors (L.M.D.'s) derived from their physiological counterparts. They drive a third "optic ganglion" which is devoted to obstacle avoidance. The latter ganglion also performs the major task of fusing the 100 signals from the L.M.D.'s with the 100 signals from an accessory, target-seeking eye. A single output signal results, which expresses (in Volts) the steering angle that the robot has to adopt to prevent collision on its way to the target.

Obstacle avoidance is based on a local map of the obstacles, expressed in polar coordinates. It is available at the end of each translatory step in a retinocentric frame of reference and immediately converted into a motor map that eventually initiates an eye saccade towards the newly determined heading direction. The collision-free paths of this cartoon-like sensorimotor system gives evidence that smart, visually-guided locomotion can be achieved without symbolic representation and logical reasoning about obstacles, without permanent world model, without a central processing unit, without signal digitization, without accurate and precisely matched visual sensors, without large memory resources. Computation taking place on-board this electro-mechanical creature essentially relies on *brainlike, parallel, analogue, continuous time, asynchronous* networks, with many additional features common to advanced animal visual systems, such as nonuniform retinal sampling, retinotopic projections of sensory maps, saccadic suppression, and "corollary discharges" that inhibit vision during eye rotations (9). All circuits are hardwired, yet adaptive behaviour is achieved in the sense that the robot immediately copes with novel environments without learning. The "surface mounted devices" (SMD) technology used throughout to construct the visual system has some ability to carry out complex signal processing in a relatively small volume.

In our attempt to link real-time vision with real-time action, specific emphasis was given to completing the sensory-motor control loop, with the constraint that the "art of signal processing" should remain similar to that of the nervous system, in spite of the great number of interconnects required by the parallel, analogue implementation. In this project, both the computer simulation and the construction phases provided us with deeper insights into the real problems common to any, living or synthetic vehicle, which makes use of vision to move forward in a complex, uncertain environment.

:

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From insect vision to robot vision
Phil-Trans-Roy SOZ B 337 283-294

Classification and selection of visual targets in *Drosophila*

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The affinity of *Drosophila* for visual objects in the environment depends not only on the apparent contrast, size and shape of an object but also on the object-related sensory context in space and time. A variety of experimental results supports this notion.

In a choice between promising visual landmarks *Drosophila* is likely to approach and explore one of the nearest targets. This minimizes the average distance covered between successive trials. Depth perception by stereopsis is limited to a range in the order of the body length. The long-range distance discrimination in a choice seems to be achieved by evaluation of the apparent slip-speed, or motion parallax, between figure and ground as seen by a moving fly: Near figures stand out by their motion parallax. Remote figures appear to be embedded in the background and do not elicit lasting curiosity in *Drosophila*[1]. Local interaction between figure-specific and ground-specific signals seems to account for the preference of near figures[2].

Almost nothing is known about the nervous correlates of the perception and discrimination of visual targets in *Drosophila*. A few results have been derived from the motor system: At least three pairs of flight control muscles respond to displacements of the retinal images of figure and ground. Each pair contributes to fixation and

tracking of a figure, and to stabilization of course and altitude with respect to the ground. Two pairs support a rigid strategy of 'instructional' fixation. The third pair engages in a flexible strategy of 'operant' fixation suitable to cope with artificially inverted displacements. Only 'instructional' fixation is found in the corresponding muscles of the mutant *'small optic lobes'* [3;4].

To investigate choices between different figures in a flight simulator, a flying *Drosophila* is held in fixed position and orientation at the center of a cylindrical panorama. The intended turns are recorded and control the rotation of the panorama about its vertical axis. By appropriate training the fly learns to avoid a course towards patterns that have been previously associated with noxious heat shocks[5]. In similar experiments, *Drosophila* was successfully trained to avoid a course towards one of two figures that has been associated with the repellent odor benzaldehyde[6]. Previous exposure to benzaldehyde in the larval state and presumably even in the embryonic state seems to increase the tolerance towards this odor in the adult fly. A valid explanation of this effect requires further control experiments. So far, we can exclude substantial inhibiting effects of the repellent on both perception and discrimination of the figures used in the paradigm.

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SECOND SESSION

Chairperson: M. Heisenberg

How Flies Recognize Patterns

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Tethered *Drosophila melanogaster* flies in the flight simulator can be conditioned to avoid certain flight orientations relative to visual patterns that had been combined with heat or exafferent pattern oscillation (pattern avoidance conditioning). In this situation flies first learn to switch the temperature by their changes in flight orientation and subsequently associate no-heat with certain visual patterns. Conditioned pattern preferences are still observed after 24 hours. Only the safe patterns are remembered whereas the heat-associated patterns seem to be forgotten instantly.

In the same apparatus without deliberate reinforcement flies in a choice between a novel and a familiar pattern prefer the novel one (novelty choice). Familiarity is apparent already after a one-minute exposure and lasts for more than 5 min. Novelty choice is not dependent upon operant training in the flight simulator.

Restricting the visual field to two opposing 90°-windows reduces the learning performance in both paradigms. For pattern avoidance conditioning with the windows positioned laterally, a significant learning index is obtained indicating that pattern recognition is not a purely foveal task. With the windows in front and back no pattern memory is detected. Curiously, this relation is the inverse for novelty choice.

In both paradigms patterns presented during a training phase have to be recognized in a subsequent test. Recognition is abolished if in the test, patterns are displayed at a different height, in a different size, rotated or with inverted contrast. No special invariance mechanisms are observed. A template matching process is sufficient to explain the data. Small invariances can be explained by a graded similarity function. The experiments are best described by a function which is the ratio of the area of maximal overlap to the area of the actual image. No analysis of shape is required for this type of visual pattern memory.

Mutants are used to study the two paradigms at the structural (cellular) and molecular level.

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Genetic Dissection Of Memory

T. Tully, M. Del Vecchio and J.C.P. Yin
Cold Spring Harbor Laboratory

Behavioral, genetic and pharmacological analyses of memory formation after Pavlovian olfactory learning have revealed four functionally distinct phases-- short-term memory (STM), middle-term memory (MTM), anesthesia-resistant memory (ARM) and long-term memory (LTM). Memory processing appears sequential through STM and MTM and then branches into two parallel pathways represented by ARM and LTM. Both ARM and LTM show properties of consolidated memory but are genetically distinct components of memory. ARM is not disrupted by cold-shock anesthesia or the protein synthesis inhibitor, cycloheximide (CXM), and is disrupted by the single-gene mutation *radish*. LTM is CXM-sensitive but is not disrupted by the *radish* mutation.

We have shown a specific block of LTM formation in transgenic flies carrying an inducible repressor isoform of *dCREB2*, a *Drosophila* homolog of vertebrate the cAMP-responsive transcription factor. These results demonstrate for the first time that regulation of gene expression underlies the formation of long-lasting memory in behaving animals. More recently, we have produced an enhancement of LTM formation in transgenic flies carrying an inducible activator isoform of *dCREB2*. These opposing effects suggest that CREB acts as a "molecular switch" for LTM. A model is proposed.

Gene Targeting and the biology of learning and memory.

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The key difficulty in the study of learning and memory is the **integration** of behavioral, anatomical, and physiological information into a theory unrestrained by disciplinary boundaries. Thus, physiologists struggle with studies of specific mechanisms in behaving animals, and behaviorists face the daunting task of uncovering the biological processes underlying the acquisition, processing and storage of information.

Novel genetic techniques now provide the opportunity to study the impact of the loss of specific genes in the biochemistry, electrophysiology, anatomy and behavior of mice[1]. Thus, our laboratory derives mice mutant for genes expressed in key forebrain structures, such as the hippocampus and neocortex, and then analyses the physiology and behavior of these mice. The results of these studies suggest that this approach might uncover the cellular processes underlying learning and memory formation in mammals. The ease with which new mutants are generated, with overlapping but importantly different properties, indicates that models integrating the multidisciplinary analysis of the mutant mice will be readily testable[2]. This is crucial because the greater the complexity of a problem the easier it should be to test candidate solutions. Below, I will summarize key features of the phenotypes of mutants studied in our laboratory.

I- The αCaMKII can both promote and limit neurotransmitter release.

Previously, we found that the αCaMKII is required for long-term potentiation in the CA1 region of the hippocampus[3]. Recently, we have confirmed that this kinase also has a crucial role in pre-synaptic plasticity[4]. With field and whole-cell studies we have shown that paired-pulse facilitation is blunted in the CA1 region of mice heterozygous for a targeted mutation of αCaMKII, confirming earlier results with field recordings of αCaMKII homozygotes, and indicating that this kinase can promote neurotransmitter release. Unexpectedly, field and whole-cell recordings of post-tetanic potentiation show that the synaptic responses of mutants are larger than those of controls, showing that the αCaMKII can also inhibit transmitter release immediately after tetanic stimulation. Thus, αCaMKII has the capacity to either potentiate or depress excitatory synaptic transmission depending on the pattern of presynaptic activation [4].

II- Impaired learning in mice with abnormal short-term plasticity.

The loss of the αCaMKII in mice homozygous for a targeted disruption of this kinase leads to a deficit in LTP in the hippocampus (CA1), and to abnormalities in pre-synaptic plasticity [5]. Heterozygotes show similar pre-synaptic abnormalities, but no LTP deficit in CA1. To determine the impact of deficits of short-term plasticity in learning and memory we tested these mutants in the water maze tasks. In the visible-platform test (hippocampal-independent) the performance of the heterozygotes was indistinguishable from that of controls. However, in the hidden-platform test (hippocampal-dependent) the heterozygotes were impaired after 3 days of training, In contrast to homozygotes, the heterozygotes learn to find the platform with an additional 2 days of training.

To extend these findings, we tested mice on another task known to require hippocampal function: contextual fear conditioning. After a single trial, the controls, but not the heterozygotes, were able to show contextual fear conditioning (40+5% and 6+4% respectively). However, the heterozygotes did show conditioning to a discrete CS (tone; 43%±5), which is known to be hippocampal independent. Similarly to the water maze, the heterozygotes showed significant contextual conditioning (43+4%) with extended training (5 trials); homozygotes did not. Our results show that despite impaired short-term plasticity, heterozygotes have normal LTP (CA1), and suggests that their deficits in hippocampal short-term plasticity could underlie the behavioral impairments detected in hippocampal-dependent tasks. To further address this possibility we looked at another mutant (mice lacking Synapsin I) with normal CA1 LTP but abnormal short-term plasticity (increased PPF [6]). The results were consistent with our model since synapsin I mice only learned as well as controls after intensive training (contextual conditioning task).

II- The cAMP Responsive Element Binding Protein (CREB) is required for LTP and for long-term memory.

CREB is a factor that mediates transcriptional responses to changes in the intracellular concentration of cAMP and Calcium [7]. Interestingly, phosphorylation of CREB at serine 133 increases dramatically its ability to promote transcription of CRE containing genes. CaMKII and PKA both phosphorylate CREB at this site. Since studies in our laboratory had involved CaMKII in learning, and in the induction of long-term potentiation (LTP) in the hippocampus we determined whether CREB is involved in the maintenance of LTP and in memory consolidation. CREB has also been implicated in the activation of protein synthesis required for long-term facilitation [8], a cellular model of memory in Aplysia. Our studies with fear conditioning and with the water maze show that mice with a targeted disruption of the α and δ isoforms of

CREB are profoundly deficient in long-term memory. In contrast, short-term memory, lasting between 30 and 60 minutes, is normal. Consistent with models claiming a role for long-term potentiation (LTP) in memory, LTP in hippocampal slices from CREB mutants decayed to baseline 90 minutes after tetanic stimulation. However, paired-pulse facilitation and post-tetanic potentiation are normal. These results implicate CREB-dependent transcription in mammalian long-term memory [9].

III- The mutation of the NF1 GTPase activating protein (NF1-GAP) gene in mice affects behavior and synaptic transmission.

Recent biochemical studies in our laboratory show that the αCaMKII can phosphorylate the NF1 GAP (neurofibromin), a protein that when mutated causes the genetic disorder known as Neurofibramatosis type I (NF1). NF1 is an autosomal dominant genetic disorder affecting 1/3500 humans. The manifestations of this disorder are complex, but they have a clear impact on tumorinogenesis and on the function of the CNS: in 30-50% of children affected, the partial loss of the NF1 protein leads to cognitive deficits and learning disabilities (and sometimes seizures). The NF1 protein is the most abundant GAP in the brain, and it is known to be highly enriched in the cytoskeleton and in dendritic endoplasmic reticulum of neurons.

We have studied a mouse with a targeted disruption of the NF1 gene [10]. Similar to humans, the homozygous mutation is lethal in mice. Strikingly, the heterozygous mutant mice have subtle but significant "learning" deficits: Performance in the hidden-platform version of the Morris water maze is impaired, while performance of the mutant mice in the visible-platform version is indistinguishable from controls. We also generated mice with heterozygous mutations on NF1, and on either the NMDA receptor 1 (NMDAR1), or αCaMKII. In both cases the addition of the NF1 heterozygous mutation exacerbated significantly the "learning" deficits of the mutant mice. Additionally, electrophysiological analysis showed that the NF1 protein is involved in the modulation of synaptic function. These data demonstrate that the NF1 mutation affects brain function in mice, and they strongly suggest that the mutant mice are an important model to investigate the etiology of the neurological disorders associated with NF1. These studies suggest that this approach might be useful to model neurogenetic disorders.

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GENES, SYNAPSES AND LONG-TERM MEMORY

Cognitive psychological studies have shown that there are at least two distinct types of learning: learning about people, places, and things (explicit or declarative forms of learning), and learning motor skills and perceptual strategies (implicit or procedural forms of learning). These two forms of learning have been localized to different neural systems within the brain. Explicit learning requires regions within the temporal lobe of the cerebral cortex including the hippocampus, whereas implicit learning involves the specific sensory and motor systems recruited for the particular task (for review see Squire, 1992). As a result, implicit learning has been studied in a variety of simple reflex systems, including those of invertebrates such as *Aplysia, Hermissenda*, and *Drosophila*. By contrast, explicit forms of learning can best (and perhaps only) be studied in mammals.

In my talk I will consider the question: To what degree do thes two major learning processes share common molecular steps?

One clue to shared mechanisms comes from the study of memory storage, the retention of information acquired through learning. Memory has stages, and is commonly divided into two temporally distinct phases, short-term memory which lasts minutes to hours, and long-term memory which can last days, weeks, or even longer. Studies of both implicit and explicit learning indicate that a transient application during learning of inhibitors of mRNA and protein synthesis selectively block the induction of long-term memory without affecting short-term memory. By contrast, a similar application of inhibitor has no effect on the maintenance of long-term memory once it is established. These studies suggest that the switch to long-term memory requires the induction of genes and proteins not required for the short-term memory.

I will describe recent studies that have begun to identify some of the proteins involved in the switch from short- to long-term memory. Studies of sensitization of the gill- and siphon-withdrawal reflex in *Aplysia*, a simple form of implicit learning, have

revealed a representation of short- and long-term memory at the cellular level. Training leads to a strengthening of the connections between the sensory and motor neurons of this reflex. The short-term enhancement of synaptic strength occurs by means of post-translational modification of preexisting proteins mediated primarily by the presynaptic action of cAMP-dependent protein kinase (protein kinase A) but also involving protein kinase C, which results in an increase in transmitter release from the presynaptic terminals of the sensory neurons. The long-term process requires cAMP-mediated gene expression and new protein synthesis, which results in the growth of new synaptic connections. Studies by Yin, Quinn, Tulley and their colleagues have shown a similar dependence of long-term memory in *Drosophila* on cAMP-mediated gene expression.

Is there a similar representation of memory storage for explicit forms of learning in the mammalian brain? Memory storage for explicit forms of learning requires structures within the temporal lobe including the hippocampus. The hippocampus itself has three major (and a number of minor) synaptic relays in series. Input to the hippocampus comes from the neurons of the entorrhinal cortex by means of their axons, the *perforant pathway*, that synapse on the granule cells of the dentate gyrus. The granule cells in turn send their axons, the *mossy fiber pathway*, to synapse on the pyramidal cells of the CA3 region. Finally, the axons of the pyramidal cells in the CA3 regions, the *Schaffer collateral pathway*, terminate on the pyramidal cells of the CA1 region. Each of these pathways makes a direct monosynaptic connection on its target cells, and damage to a single pathway within the hippocampus is sufficient to produce memory disturbance in humans.

In 1973 neurons of the hippocampus were shown to have plastic capabilities of the kind that might be required for memory storage. Brief, high-frequency trains of action potentials in any one of these three neural pathways within the hippocampus produce an increase in synaptic strength in that pathway. The increase can last for hours in the anesthetized animal and for days and even weeks in the freely-moving animal. This activity-dependent increase in synaptic strength is called long-term potentiation (LTP).

These three pathways can be studied *ex vivo* in hippocampal slices, where all three pathways have been shown to use glutamate as their transmitter, and in all three pathways LTP looks quite similar. Nevertheless, the pathways use two different inductive mechanisms for triggering LTP, distinguishable both by the critical roles of different glutamate receptors in initiating LTP and by the pre- or postsynaptic locus of induction. In the medial perforant and the Schaffer collateral pathways LTP is initiated in the postsynaptic cell. Induction involves activation of the NMDA-type glutamate receptor and requires influx of Ca^{2+} into the postsynaptic cell through the NMDA receptor channel. By contrast, LTP in the mossy fiber pathway is induced presynaptically and requires neither activation of the NMDA receptor nor Ca^{2+} influx into the postsynaptic cell.

LTP has been studied extensively at the NMDA-dependent synapses, and particularly in the synapses of the Schaffer collateral pathway. Here, the Ca^{2+} influx triggered by activation of the NMDA receptor recruits several second-messenger kinases in the postsynaptic cell, including calcium/calmodulin kinase II, protein kinase C, and tyrosine kinases. Once induced, LTP in the Schaffer collateral pathway shows distinct phases. There is an early phase, lasting 1 to 3 hours, that does not require protein synthesis, and a later persistent phase that requires the activity of PKA as well as new protein and RNA synthesis.

Is the requirement for cAMP-mediated protein and RNA synthesis a general feature of LTP in the hippocampus? Does NMDA receptor-independent LTP in the mossy fiber pathway also have this requirement? In the mossy fiber system, LTP has both an early and a late phase and PKA contributes to both phases. We have found that the early phase is induced presynaptically and involves a mechanism that is protein synthesis-independent, whereas the late phase, whose locus of expression is as yet undetermined, requires protein and mRNA synthesis.

Thus, the early phase of LTP in the mossy fiber synapse, which involves PKA, is distinct mechanistically from the early phase of LTP at the Schaffer collateral synapses, which depends instead on the Ca^{2+}/calmodulin kinase, PKC, and tyrosine kinases. The

mechanisms for the early phase of LTP in the two pathways are distinguished not only by the kinases recruited for their expression but also by the locus of induction. Schaffer collateral LTP is induced postsynaptically and requires activation of the NMDA receptor channel and the subsequent influx of Ca^{2+}. By contrast, LTP in the mossy fiber system is induced presynaptically and does not require activation of NMDA receptors or Ca^{2+} influx into the postsynaptic cell.

Despite these differences in induction and expression of the early phase, the mechanisms for the late phase of LTP in these two synaptic pathways seem similar in outline, although they are likely to differ in detail. In both pathways, the late phase is dependent on new RNA and new protein synthesis, and in both cases, the cAMP-dependent kinase seems to be involved. In turn, these mechanisms for the late phase of LTP, thought to be important for long-term memory storage following explicit forms of learning, resemble those utilized in *Aplysia* and in *Drosophila* for storing behavioral long-term memory for implicit tasks. This convergence of findings suggests that even though implicit and explicit forms of learning are fundamentally different, they do not necessarily use different mechanisms for storing long-term memory. Rather, the two different classes of learning can, at least in certain cases, use a common class of molecular mechanism for converting a labile, short-term form into a stable, long-term form: the induction of genes by cAMP and PKA. As a corollary, these several findings suggest that, even though different forms of learning recruit activity in a variety of parallel and distributed neuronal pathways (each of which is likely to have a number of synaptic relays capable of giving rise to distinctive short-term processes), the molecular mechanisms utilized at these various sites for the storage of long-term memory may be quite restricted and conserved.

Neuronal Mechanisms of Perception

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The cerebral cortex, a 2 mm thin sheet of nerve cells and connections covering the cerebral hemispheres, is the latest invention of evolution and the substrate of the cognitive abilities and motor skills which distinguish humans from primates and lower mammals. Because the intrinsic organization of the neocortex is strikingly similar across areas devoted to different functions one has to infer that the computational operations performed by different cortical modules are similar and of such general nature that they can be exploited for functions as different as object recognition, orientation in space, motor control and language.

One primordial function appears to consist of the selective association of distributed input signals and the representation of the resulting constellations by neuronal responses. When viewing a complex visual scene, e.g., a very large number of neurons in many different cortical areas are activated simultaneously and the visual system has to resolve the task to identify those subsets of responses which are associated with a particular object. These selected responses then have to be bound together for further joint processing and it needs to be assured that they do not get confounded with the responses originating from other objects or the embedding background. One solution to this binding problem consists of the implementation of binding units which receive converging input from selected subsets of feature coding neurons and which respond only if the respective set of input neurons is conjointly activated. The responses of individual binding units would then signal the presence of the specific feature constellation characterizing a particular perceptual object. However, there are limits to this binding strategy as it seems unlikely that specific binding units can be implemented for the nearly infinite number of possible feature constellations that have to be coped with in perception.

A complementary solution to the binding problem is the dynamic association of selected neurons by creating functionally coherent cell assemblies. In that case all members of the assembly represent conjointly a particular constellation of features, or at higher levels, a whole perceptual object. This greatly economizes on the number of required cells because a particular cell can join at different times different assemblies and hence can participate in the representation of many different constellations of features. The problem here is how responses can be selected and

flexibly bound together into assemblies that are easily identifiable for further joint processing. A common strategy to select responses consists of increasing their saliency. This can be achieved in two ways. The selected cells could be made to discharge more frequently as this enhances their impact on target cells at the next processing stage due to temporal summation. Another way to increase the impact of a neuronal discharge is to make it coincident in time with discharges of other neurons provided these contact the same target cells, because simultaneously arriving inputs summate particularly effectively. The advantage of response selection by synchronization is that it permits to create different assemblies in rapid succession without running the risk that they get confounded. The reason is that unlike increased discharge rates coincident inputs can be detected without requiring temporal integration. The particular properties of cortical networks, their high degree of divergence and convergence, the low efficacy of individual excitatory synapses, relatively low firing rates and short integration intervals are favorable for response selection by synchronization.

Over the past few years experimental evidence has become available that cortical neurons are indeed capable of synchronizing their discharges with the required precision and flexibility. Predictions on specific relations between neuronal synchronization and stimulus configurations could be confirmed and cortico-cortical association fibres have been identified as substrate responsible for such feature specific response synchronization. Thus, it is likely that one of the prominent functions of neocortex, the representation of perceptual objects - which requires selection and binding of specific constellations of input signals - is achieved by dynamic selection and association of responses in parallel and distributed architectures by synchronization and coincidence detection.

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A non-Hebbian Synaptic Transformation for Memory Storage in the Hippocampus

Abstract. Human memory is characteristically associative or relational. Thus, we typically do not remember isolated bits of information, but the relations of those bits in time and space. Human memory is also characteristically extremely long-lasting, often persisting for many decades. Our strategy to uncover how relations are learned and stored for such durations by cellular and subcellular mechanisms began with a search for fundamental network architectures responsible for a simple learned relation such as Pavlovian conditioning. We then identified molecular and biophysical steps in memory storage that are conserved and, therefore, present in reduced nervous systems (e.g., that of a mollusc) as well as far more complex brains (e.g., those of mammals). Evidence for such conservation was obtained for:

(1) behavioral features of associative memory;

(2) synaptic transmission;

(3) ionic channels;

(4) second messengers;

(5) protein substrates; and

(6) regulation of gene transcription.

One example of a conserved change in molluscan and mammalian synapses during associative learning paradigms is a new phenomenon which we call Long-Term Transformation or LTT. To summarize, LTT of a GABAergic response from synaptic inhibition to excitation, can be induced in CA1 pyramidal cells.

This observation extends to a mammalian brain structure, the rat hippocampus, a novel long-term synaptic modification recently observed in the visual-vestibular network of the mollusc *Hermissenda*. Pairing of pre- and post-synaptic excitation transformed i.p.s.p.'s elicited by basket cell stimulation into e.p.s.p.'s. This transformation was blocked by furosemide but was not eliminated by AP5 together with CNQX. Moreover, exogenous GABA, when paired with post-synaptic depolarization, also produced LTT. This transformation of inhibition into excitation, produced by 5-10 pairings, persisted for more than 60 minutes. Other experiments provide evidence that pairing-induced long-term potentiation (LTP) and LTT share common underlying mechanisms. Each is blocked by furosemide, a Cl^- pump blocker that eliminates soma but not dendritic $GABA_A$ elicited response. Both LTT and pairing-induced LTP were prevented by 1 μM anandamide. LTT differs from LTP in that the latter produces prolonged quantitative changes (e.g., enhanced e.p.s.p.'s) while the former produces a prolonged qualitative (and thus non-Hebbian) response change (i.e., conversion of i.p.s.p. into an e.p.s.p.).

LTT was initially observed with stimulus parameters chosen to simulate sensory stimuli that produced Pavlovian conditioning of living animals. The role of this persistent synaptic transformation, therefore, could provide a new behaviorally relevant context for pairing-induced LTP. Receptor-mediated anandamide responses include inhibition of adenylate cyclase, blocking N-type Ca2+ channel conductance, and enhancement of K+ conductance. Anandamide's inhibition of LTT and associatively-induced LTP is consistent with one or more of these receptor-mediated inhibitory effects. It might be speculated that a physiological role for anandamide in behavioral learning, therefore, is to inhibit storage of less relevant sensed information that might obscure storage of information that is at the focus of attention.

THIRD SESSION

Chairperson: D. L. Alkon

RELATIONSHIP BETWEEN LONG-TERM DEPRESSION AND LONG-TERM POTENTIATION OF EXCITATORY SYNAPTIC TRANSMISSION IN NEOCORTEX AND HIPPOCAMPUS

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The physiological substrate of memory is likely to involve persistent, use-dependent changes in synaptic efficacy. Since the discovery of long-term potentiation (LTP) in the hippocampus[1], use-dependent enhancement of synaptic strength has been observed in a large number of brain structures including neocortex. In the majority of cases, induction of LTP is input specific and associative; that is, modifications are restricted to the activated inputs, and synapses are potentiated only if there is near simultaneous coincidence of synaptic activation and adequate postsynaptic depolarization (for review see Ref. 2). More recently, evidence has been obtained that synaptic transmission can also undergo long-term depression (LTD). In contrast to LTP, LTD has been observed to occur either at inputs whose activation contributes to the induction of the modification ('homosynaptic' LTD) or at inputs that had been inactive during induction ('heterosynaptic' LTD) (for review see Ref. 3).

The finding that injection of Ca^{2+} chelators into the postsynaptic cell blocks LTP as well as LTD[2,3] strongly support a role for intracellular Ca^{2+} ($[Ca^{2+}]_i$) in the induction of both synaptic modifications. It has been proposed[4] that properties of the Ca^{2+} signal control the direction of change in synaptic strength: a small rise in $[Ca^{2+}]_i$ favors the induction of LTD while a larger one leads to LTP. This is supported by the notion that the induction of homosynaptic LTP requires a stronger postsynaptic depolarization than that of homosynaptic LTD. Indeed, results obtained, first in the visual cortex[5] and subsequently in other brain areas, have led to the notion of two voltage-dependent thresholds. Homosynaptic LTD is obtained if postsynaptic depolarization exceeds a critical level (Θ^-) but remains below a second threshold (Θ^+) whereas LTP is induced if Θ^+ is reached.

It is long known that raising extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) transiently from 2 to 4 mM for 10 min produces, after return to 2 mM and in the absence of synaptic activation, a marked LTP in all hippocampal areas[6,7]. The same exposure to elevated $[Ca^{2+}]_o$ initiates LTD in excitatory synapses located in layer II-III pyramidal cells of the rat visual cortex. This Ca^{2+}-induced LTD occludes homosynaptic (tetanus-induced) LTD. As Ca^{2+}-induced LTP in the hippocampus, Ca^{2+}-induced LTD in the neocortex is voltage-dependent. Comparison between the results obtained in hippocampus[8] and in neocortex shows that Ca^{2+}-induced LTP is obtained at more depolarized membrane potentials than Ca^{2+}-induced LTD. The two thresholds for Ca^{2+}-induced LTD and LTP resemble those for stimulation-induced LTD and LTP. These results suggest: 1/ that a transient increase in $[Ca^{2+}]_i$ is alone sufficient to elicit a synaptic modification and 2/ that whether, at any given instance, a synapse potentiates or depresses may depend in part on the spatial and temporal dynamics of changes in postsynaptic $[Ca^{2+}]_i$.

Several observations in hippocampus indicate that two different enzyme cascades initiate either LTD or LTP. Although a protein phosphatase cascade is involved in LTD[9, 10], it is thought that a cascade of protein kinases[2] may contribute to the induction of LTP. Thus the control of synaptic efficacy at excitatory synapses may be under the regulation of a complicated network of interacting and mutually regulatory signalling cascades, the functions of which are to control the phosphorylation state of critical substrate phosphoproteins. It remains to determine the critical substrate proteins contributing to the control of synaptic efficacy...

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A Role for a Specific G-protein That Regulates Protein Synthesis in Memory Storage and Alzheimer's Disease

Abstract. Recent observations of Alzheimer's disease (AD)-specific changes of K^+ channels and of receptor mediated, IP_3-induced intracellular Ca^{2+} release in human fibroblasts, and related K^+ channel changes in human olfactory neuroblasts together with some of our other observations, suggested that there may be key proteins (other than β-amyloid and tau) that are critical for AD, but also contribute to storage of associative memory. To identify such proteins and their physiological functions, we undertook to analyze a specific memory-associated protein, cp20, in fibroblasts from AD and control donors. cp20, a high-affinity substrate for PKC, shows specific differences of phosphorylation in neurons of molluscs and mammals that undergo associative learning. In response to temporally specific training stimuli in such paradigms as Pavlovian conditioning and spatial water maze learning, elevation of intracellular Ca^{2+} together with DAG and arachadonic acid cause translocation and thus activation of protein kinase C (PKC) and phosphorylation of cp20. This GTP-binding protein, which induces a number of memory-specific neuronal changes [e.g., K^+ current reduction, focusing of synaptic terminal branches], also regulates retrograde axonal transport and is a member of the ARF-protein family that has been implicated in the trafficking of particles between the Golgi and the endoplasmic reticulum. In addition, it has been recently found that cp20 activates DNA transcription in nerve cells, which correlates with our previous findings showing increased

mRNA synthesis in conditioned animals. Moreover, cp20 was found highly enriched in ribosomes and nuclei of both squid optic lobe and sea urchin oocytes. Taken together, these observations indicate that cp20 may be an important component of a signal transduction pathway that includes regulation of gene transcription and is essential in memory storage processes. We report here that cp20 is consistently and markedly reduced in the fibroblasts of both AD patients and non-affected close relatives of AD patients, but not in aged-matched controls who are not members of families with hereditary AD. Incubation of normal fibroblasts with low concentrations of soluble β-amyloid reproduced the AD phenotypes for cp20.

These findings extend our previous observations showing that cellular steps (K^+ channel regulation, Ca^{2+} release) in memory storage are altered in AD. Since cp20 is an extremely potent regulator of K^+ channels, its absence or reduction in AD could have some relationship to the previously observed differences of K^+ channels for AD fibroblasts and olfactory neuroblasts. The previously demonstrated regulation by cp20 of retrograde axonal transport, as well as its sequential homology with the ARF protein Sar1p (involved in vesicle trafficking), suggest that its absence could also influence the predisposition to and/or development of the proteinaceous plaques and neurofibrillary tangles that characterize AD pathology in the human brain. These pathological processes, like cp20, directly or indirectly involve vesicle trafficking and, possibly, alterations of microtubule-associated proteins. Phosphorylation of tau (a potentially pathological event) by mitogen-activated protein (MAP) kinase, can be promoted by APP (amyloid precursor protein, the protein from which β-amyloid originates) and prevented by inhibition of ras proteins. The ras involvement in this process is intriguing, since ras and cp20 share functional properties and also some degree of homology.

Behavioral and Cellular Analysis of Habituation in *C. elegans*

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The development of *C. elegans* as a system for the study of learning an memory offers a number of unique opportunities for answering questions concerning the cellular mechanisms of learning and memory. These opportunities are the result of the worms simplicity and the large amount of biological research that is has focused on *C. elegans*. *C. elegans* is a self-fertilizing hermaphroditic nematode that has been the target of intensive developmental, anatomical and genetic analysis. The complete cell lineage is available for each of the 959 somatic cells (Sulston et.al., 1986; Sulston et.al., 1988). The nervous system of *C. elegans* consists of 302 identified neurons that have been mapped at the electron microscope level, with all morphologically distinct electrical and chemical synaptic zones recorded (White et.al., 1986; White et.al, 1988). In addition *C. elegans* has been the target of genetic analysis; investigators from around the world have combined their findings to map over 95% of the genome (summarized in Coulson et.al., 1986 and Hodgkin et.al, 1988). Furthermore, a large number of mutant strains have been isolated and the molecular basis of the many of the various mutations determined. Since *C. elegans* can survive freezing (at temperatures around -80°C) these mutant strains can be collected and stored indefinitely; for a review of much of the research on *C. elegans* and a listing of available mutant strains see W. Wood, 1988).

In our experiments we have focused on a simple reflex behavior, a withdrawal response to vibratory stimulation that we have called the tap withdrawal reflex. This reflex shows the forms of non-associative learning of habituation, dishabituation and sensitization. In addition we have demonstrated that the tap withdrawal reflex shows long-term memory for habituation training for at least 24 hours, a considerable fraction of the worms 14 day lifespan (Rankin, et. al., 1990). We have made considerable progress in understanding the behavioral characteristics of habituation in the tap withdrawal reflex and the neural circuit underlying it.

In the research reported here I will focus on our work on habituation which Groves and Thompson (1970) defined as the decrease in response magnitude that results from repeated stimulation. We have shown that, like many other organisms, *C. elegans* shows greater habituation and faster spontaneous recovery to short ISIs than to long ISIs (Rankin and Broster, 1992) and that the ISI is a more important component in determining recovery than are the number of stimuli received and the amount of habituation present. In a second series of experiments (Broster and Rankin, 1994) we investigated the effect of changing ISI on habituation and recovery from habituation. We found that habituation is more rapid at fixed intervals than at variable ones for both long and short ISIs. When we changed the ISI from long to short or from short to long halfway through training we found that recovery was at the normal rate for the ICI delivered second. We also found that long ISIs delivered first influenced the rate of habituation of the following short ISIs but that short ISIs delivered first did not appear to impact the following long ISI. All of our experiments

point to the importance of interstimulus interval in determining features of habituation. This has led us to hypothesize that habituation may be mediated by a number of different cellular mechanisms, that are differentially recruited by long and short ISIs. Thus some of the cellular processes mediating habituation at a long ISI may be different from some of the processes mediating habituation at short ISIs.

In addition to our behavioral research we have also made considerable progress in our understanding of the neural circuit underlying the tap withdrawal response through the use of laser ablation of identified neurons. We have tested the roles of more than 35 different identified neurons and combinations of neurons (in more than 900 worms) in response to a mechanical tap to the dish and have shown that the tap withdrawal circuit consists of 3 head-touch cells (ALML, ALMR, and AVM), 2 tail-touch cells (PLML and PLMR), and 5 pairs interneurons (AVAs, AVBs, AVDs, PVCs, and PVDs) and 1 putative stretch receptor (DVA). These neurons make up two competing circuits that are activated when a tap is delivered: the head touch circuit that produces reversals (swimming backwards), and the tail touch circuit that produces accelerations (swimming forwards). Each of the two circuits has an input to the final observed behaviour, and plays a role in the plasticity observed in habituation. Studies of each circuit independently (by ablating the opposing sensory cells) have shown that the head touch circuit habituates more quickly than the tail touch circuit, and the competition between the two circuits produces more rapid habituation than we would see with either circuit alone. No single neuron plays a major role in habituation, rather it appears that the decrements take place at varying rates over a number of synapses in the circuits.

Through our studies of habituation and our analysis of the neural circuit underlying it we have extended our understanding of the process of habituation as well as our understanding of how neurons in a circuit work together to produce behavior.

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MODULATION AND SPECIFICATION OF OSCILLATORY MOTOR CIRCUITS IN LOBSTERS.

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It is now widely accepted that the ability of central pattern generator (CPG) networks to produce rhythmic motor behavior derives both from the synaptic interactions between constituent neurones **and** their intrinsic membrane properties. Moreover, these synaptic and cellular properties are not invariant, but are subject to neuromodulatory influences that by modifying the bioelectrical behavior of individual neurones and/or the strength of their synapses, are able to adapt the output of a given motor circuit to the changing needs of the animal. Despite this ability to produce different functional configurations, the assumption remains of a CPG as a predefined assemblage of interconnected neurones that is dedicated to a particular behavioral task and functionally distinguishable from other neural circuits responsible for other behaviors. However, our recent studies on the stomatogastric nervous system (STNS) of Crustacea has begun to question this concept of the CPG as a discrete structural and functional entity within the central nervous system.

When the STNS of the lobster *Homarus* is isolated from the animal, it continues spontaneously to produce 4 fictive motor programmes (oesophageal, cardiac sac, gastric and pyloric rhythms) responsible for the different regional behaviours of the foregut. These independent patterns are generated by separate small networks comprised of different neurones and which operate at different frequencies. However, recently[1] we have shown that a single neurone, hitherto considered to be an integral member of the pyloric network, can at times leave the pyloric pattern and become active with the cardiac sac network. This switching of a neurone from one network to another is ensured by a specific modulatory induced alteration in the intrinsic membrane properties of the element itself. More recently[2][3], we have found that subject to the endogenous rhythmic discharge of a pair of identified interneurones, certain elements otherwise participating as integral members of these four STNS networks are reconfigured into a single new functional circuit responsible for swallowing-like behaviour. This dynamic circuit construction, which arises from a functional breakdown of the preexisting STNS networks, is achieved by diverse synaptic influences of the two interneurones, involving conventional synaptic excitation or inhibition of specific target neurones, and longlasting enhancement or suppression of their regenerative bursting properties.

On this basis, therefore the selection of the motor programme responsible for a particular rhythmic behaviour requires that the underlying network must first be specified from a pool of neurones of disparate origin. In a wider context, moreover, this forces us to reconsider our thinking about the unit construction of the CNS; if our building block is the neural network, then we must be aware that a functional circuit may exist only in a particular behavioral situation dictated by modulatory influences.

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FOURTH SESSION

Chairperson: W. Singer

ON THE ROLE OF NITRIC OXIDE IN THE PROCESSING OF INFORMATION IN THE VISUAL SYSTEM

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The visual cortex and the visual thalamus contain neurons or neuronal elements that may release one of a variety of putative neurotransmitters including excitatory aminoacids, the inhibitory aminoacid GABA and several neuromodulatory substances such as serotonin, histamine, norepinephrine or acetylcholine. Traditionally, excitatory and inhibitory aminoacids have been proposed to be utilized by the "specific" neuronal networks that perform the computations associated with neuronal sensory processing, while neuromodulators are utilized by the "modulatory" network systems that are thought to control the overall excitability and pattern of activity generated in thalamocortical systems in a state-dependent manner. One of such putative neuromodulators, acetylcholine, presents a widespread localization both in the visual cortex and in the dorsal lateral geniculate nucleus (dLGN), and it has been implicated with shifts in attention and increased arousal (see Singer, 1977; Fibiger, 1982). In the visual cortex, acetylcholine is found in terminals of fibers arising in the basal forebrain, and in the dLGN in fibers arising in the parabrachial region of the brainstem. Interestingly, recent evidence has shown that nitric oxide synthase (NOS), the enzyme responsible for the production of nitric oxide (NO), is also localized within cholinergic terminals (Bickford et al.,1993,1994). This raises the possibility that NO may act as a novel neuromodulatory substance in the visual system.

We have recently presented preliminary evidence using iontophoretic application of putative inhibitors of NOS that, within the cat LGN, NO acts to enhance visual responses, specifically and selectively enhancing NMDA mediated excitation (Cudeiro et al., 1994a,b). In order to examine the mechanisms of this effect more fully, we have derived dose-response curves for excitatory aminoacids and for the non-aminoacid excitant ACh in the presence and absence of the competitive inhibitor of NOS, N^G-nitro-L-Arginine (L-NOArg). The NO donor nitroprusside was also tested. We also present here preliminary data, suggesting that NO plays a similar role in the cat visual cortex. Experiments were carried out on adult cats anesthetized with a mixture of N_2O (70%), O_2 (30%) and halothane (0.1-5%) and paralyzed with gallamine (10mg/kg/h). Seven barrelled micropipettes were used for recording single-unit activity and the iontophoretic application of drugs, both, in the dLGN and in the primary visual cortex.

Iontophoretic application of inhibitors of NOS resulted both in significant decreases in visual responses, without change in response selectivity, and decreased responses to exogenous application of NMDA. These effects were reversed by co-application of the natural substrate for NOS, L-arginine, but not the biologically inactive isomer, D-arginine. Nitroprusside, a NO donor, but not L-arginine, was able to increase markedly both spontaneous activity and the responsiveness to NMDA application. Responses of cells in animals without retinal, cortical and parabrachial input to the dLGN suggest a post-synaptic site of action of NO. This modulation of the gain of visual signals transmitted to the cortex suggests a completely novel pathway for NO regulation of function, as yet described only in primary sensory thalamus of the mammalian CNS. The effects seen in the visual cortex also indicate that NO contributes to visual processing at this level.

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DEVELOPMENT AND ORGANIZATION OF REPRESENTATIONS IN THE MAMMALIAN CEREBRAL CORTEX

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One of the goals of neurobiological research is to understand how the brain constructs representations of its environment. Knowing the neuronal code of such representations is a prerequisite for any reductionistic explanation of cognitive functions such as perception, memory and learning. At present two hypotheses are pursued: One assumes that perceptual objects are represented by the responses of highly selective, object-specific neurons which are located at the top of hierarchically structured processing systems. The other favours the view that representations are distributed and consist of assemblies of cooperatively interacting neurons. A key feature of assembly coding is that individual neurons can participate at different times in different assemblies which greatly economizes the number of neurones required for the formation of different representations. This, however, requires a versatile mechanism of response selection which allows to associate in a highly flexible way subsets of distributed neuronal responses for further joint processing. Here, it will be proposed that synchronization of responses could serve as mechanism for the dynamic selection and binding of responses because it raises with great precision and without requiring time consuming temporal integration the saliency of responses containing synchronized epochs. Experiments will be reviewed which have been designed to test predictions derived from the synchronization hypothesis. It will be shown that feature selective neurons in the visual cortex can synchronize their discharges if activated by the outlines of the same visual object and that synchronization probability reflects some of the established Gestalt criteria for perceptual grouping. Evidence is further provided, that this synchronization is achieved at least in part by cortico-cortical association projections. The architecture of these connections is shaped during postnatal development by an experience dependent process. Experiments with strabismic animals suggest that cortico-cortical connections are selected according to a correlation rule and that modifications of these connections are reflected by altered synchrónization probabilities. Data will also be reviewed from strabismic animals which have developed amblyopia. Correlation analysis of multi-electrode recordings suggest that the amblyopic deficit is associated with a reduced ability of cortical neurones to synchronize their responses. Finally, evidence will be provided that the cortical connections mediating response synchronization remain susceptible to use dependent modifications of synaptic efficacy in the mature visual cortex: they can undergo long-term potentiation and depression. It is proposed that these modifications serve the experience dependent generation of new assemblies such as is required for perceptual learning. It is proposed that these results are compatible with the hypothesis that temporal relations between distributed neuronal responses play an important role in cortical processing.

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THE TRANSITION BETWEEN SHORT- AND LONG-TERM MEMORY: ROLE OF SYNAPTIC ADHESION MOLECULES

Abstract of lecture at Instituto Juan March

Madrid, February 28th 1995

(Symposium on Cellular and Molecular Mechanisms in Behaviour)

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Long-term memory is supposed to depend on modulation of neuronal circuitry via changes in synaptic connectivity. Both in behavioural models of learning and in long-term potentiation, early synaptic transients involve a time-dependent cascade of processes including glutamate release, upregulation of NMDA receptors, synthesis of the putative retrograde messengers NO and arachidonic acid, PKC-dependent phosphorylation of membrane proteins and intracellular calcium signalling. There have been proposals that long-term memory might simply require the permanent activation of enzymes involved in this sequence, such as CAMIIkinase. However, there is good evidence that if lasting storage is to be achieved, structural modification of synapses must occur, and that this is accomplished via gene activation (mediated by transcription factors and immediate early genes) and the de novo synthesis of a family of glycoproteins, which, inserted into pre- and post-synaptic synaptic membranes, serve to stabilise synapses in a new configuration.

We have studied both early and late phases of the cascade in a "natural learning model" involving a one trial passive avoidance task in the day-old chick. I will present evidence for the involvement of glycoprotein synthesis in the late phase, occurring some 5.5-8hr posttraining. The glycoproteins involved include the neural cell adhesion molecules NCAM and L1, and antibodies to either, or IgG or fibronectin fragments which bind homophilically to their extracellular domains, administered 5.5 hr postraining, will result in amnesia 24 or 48 hr later. A similar timewindow has been observed following passive avoidance training in rats, and there is also evidence for the role of cell adhesion molecules in the maintenance of LTP. Furthermore, weak learning,which is not normally retained beyond 6hr, can be potentiated by injections of corticosterone given around the time of training, and this dose of corticosterone enhances subsequent glycoprotein synthesis. Both the memory and the enhanced glycoprotein synthesis are blocked by steroid antagonists. Evidence that the effect of a number of so-called nootropic drugs is mediated by way of steroid hormones leads to a general model of long-term memory formation and points to novel possible directions in which pharamacological manipulation of memory may be attempted.

Learning-induced plasticity of auditory cortex

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The described investigations aim at elucidating the role of auditory cortex in acoustic avoidance conditioning and in this way at a central issue of the learning and memory problem namely the architecture of the sensory memory. Among at least seven fields in gerbil auditory cortex the primary field (AI) and the anterior auditory field (AAF) showed prominent tonotopic organization with parallel dorsoventral iso-frequency contours (Scheich et al. 1993, Thomas et al. 1993). Aversive tone conditioning paradigms reshaped frequency receptive fields of single units in AI in a specific way and also changed the spatial representation of tones in fluoro-2-deoxyglucose experiments (FDG) (Scheich et al. 1994). This suggests that spectral features as well as aspects of behavioural meaning of sounds may be represented in AI through learning.

As one aspect of plasticity antibodies against the immediate early gene product c-Fos identify the spatial distribution of neurons in auditory cortex which presumably change metabolism as a result of stimulation with novel auditory signals. Less than 3 min training with a novel tone led to tonotopic columnar expression of c-Fos in AI. Longer stimulation led to spreading of c-Fos expression across auditory cortex while habituation with the same tone prevented c-Fos expression.

The search for transmitters which mediate this gene activation is greatly aided by microdialysis through chronically implanted probes in auditory cortex. So far, metabolites of dopamine and serotonine transmission were found to reflect specific aspects of auditory avoidance conditioning in a shuttle box. Notably dopamine seems to reflect the forming of a behaviorally relevant association.

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SHORT-TERM AND LONG-TERM CHANGES OF MAMMALIAN CENTRAL NEURONS DURING ATTENTIVE STATES, MOTOR LEARNING AND REGENERATION

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The behavior of most animals seems to be the result of the highly complex action of neural circuits able to generate the necessary and sufficient commands signals. The research work of a neuroscientist is (or should be) oriented to the understanding of the transfer function from neural activity to overt behavior. Even simple and stereotyped motor acts, as reflex responses, are, first, generated in defined neural structures and, second, susceptible of being modified in their gains (output motor commands/input sensory signals) according to, or as a function of, external circumstances - namely, they are able to learn.

For the most current point of view, the above-mentioned behavioral changes are supposed to be embodied in the molecular architecture of composing neurons during a process that indicates the plasticity of the involved neural system. When dealing with more complex neural networks (i.e., more complex behaviors) we are obliged to accept a wider range of variability, i.e., plasticity. A slightly opposed view is that the plasticity of a neural system is -properly speaking- its physiology. This proposition means that research efforts should be directed to define the functional limits of a given motor ability (i.e., the nictitating membrane/eyelid reflex response) and the molecular and cellular bases of the supposed range of variability. In my opinion, the development of those neural properties that are commonly claims as higher functions (complex leaning tasks, declarative memory) is the result of the redundancy, structural undefinition and multiple use (S.G. Gould dixit) of different neural structures (nucleus, layers and circuits), but not of well established (i.e., functionally fixed) sensory-motor integrated networks. By definition, plasticity decreases and evolutionary processes tend to stabilize when a function is perfectly defined and adapted.

Several examples will be introduced during the presentation that will cover the whole scope of the proposed title:

i) Plasticity and/or variability embodied for 100.000.000 years in reflex responses, as the vestibulo-ocular reflex.

ii) Very short-term changes (30-50 milliseconds) observed in the interaction between mossy and climbing fibers at cerebellar cortex and nuclei levels during attentive states and orienting movements.

iii) Degree of adaptability (in term of months) of adult mammal motoneurons to new motor tasks when obliged to regenerate in a foreign muscle, and compensatory mechanisms activated in synergic motor systems by the surgery.

iv) True learning (in term of days) as, for example, the change in the functional properties of brain stem circuits during the classical conditioning of the nictitating membrane/eyelid response. And,

v) Restorative properties of the central nervous system of adult mammals after axotomy or removal of target muscles or motor neurons.

As a central hypothesis of this presentation, it will be proposed that plastic phenomena responsible of the acquisition of new motor skills (described in **iv**) are similar to those observed during regeneration and reorganization of neural circuits following neural damage

(described in v). In this sense, restorative, compensatory and adaptive responses of the central nervous system after the lesion of the neural tissue represents the obliged use of neural mechanisms available for changes in motor behavior when environmental changes made them necessary.

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FIFTH SESSION

Chairperson: A. Ferrús

Behaviorally Induced Neural Plasticity in the Cerebral and Cerebellar Cortices

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Recent data indicates that different CNS structures (i.e. cerebral cortex, cerebellum...) are able to adapt to environmental changes and that these adaptations may be at the basis of information storage and processing. One plausible hypothesis which inspires current research is that changes in synaptic efficacy underlie normal learning and memory processes in which these structures may be involved. The basic experimental evidence from which these ideas have emerged in the cerebral cortex are studies showing reorganizational processes occurring in this structure after peripheral manipulations (i.e. nerve lesions, amputations..). More recent evidence indicates that similar plastic changes may mediate processes such as functional recovery after brain damage, the acquisition of motor abilities, sensory discrimination abilities and other experience dependent processes which occur throughout the life of the individual. The characteristic of these reorganizations occurring in the cerebral cortex is its distributed nature. In contrast, adaptations in response to experience involving simpler forms of learning (i.e. associate motor learning) which are typically not cortically mediated and involve other structures (i.e. cerebellum) may have a more localized representation. Similar mechanisms involving changes in synaptic efficacy (i.e. LTP, LTD) may be shared by both, the distributed and the localized acquired representations. The involvement of specific peptides in basic forms of learning mediated by the cerebellum, has recently been suggested and this evidence links both the behavioral processes (i.e. associative motor learning) and the proposed synaptic mechanism (i.e. LTD). In the cerebral cortex no link yet exists between the cortically mediated behaviors and changes in synaptic strength. Changes in synaptic strength have recently been described in the adult cerebral cortex and interestingly seem to show among other characteristics area dependent properties which may have important functional implications. In conclusion, distributed or localized adaptative processes are the mirror of our experiences throughout life and the investigation of the mechanisms responsible for these processes is providing insight on how the brain acquires, processes and stores information.

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Social Control of Gene Expression

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Survival requires that animals modify their behavior in response to changing physical and social environments. Some responses, such as fighting or fleeing, are immediate and can be understood or at least described in terms of their proximate physiological causes. Other responses may result in long term changes in animals including tissue growth (or loss), modified responsiveness to signalling molecules, or other alterations in the regulation of physiological systems. We have been studying an African cichlid fish where the connection between physiology and behavior can be made visible and the consequence of social success can be traced directly to changes in the brain, both in the short and long term. In this species, territorial males inhibit the sexual maturation of nonterritorial males during development, and the continued presence of territorial animals prevents sexual reproduction by nonterritorial males. Moreover, after a male becomes sexually mature and controls a territory, if he loses a territorial battle, he reverts to nonterritorial status and his gonads to regress rapidly. These changes are mediated by cells in the hypothalamus which contain gonadotropin releasing hormone (GnRH). The size of these cells and consequently the amount of GnRH they contain change in response to altered social status in the male. That is, in males which become dominant, GnRH containing cells hypertrophy and conversely, in males which become nonterritorial the same cells shrink. How does the recognition of changed social status result in cell specific changes in size and ultimately in cell specific changes in gene expression? We have recently cloned and sequenced genes which code for GnRH in this fish, and are studying their regulation in response to the social signals used to control social dominance.

The contextual guidance of learning and processing as a basis for cortical computation

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To be presented to the Workshop on Cellular and Molecular Mechanisms in Behaviour, Madrid, 27 February - 1 March 1995

In most connectionist theories of neural computation local processors treat all of their inputs in essentially the same way, and their synaptic inputs adapt through either supervised or unsupervised learning. The hypothetical local processors described in this talk have two distinct classes of input: receptive field (RF) input that provides the primary drive, and contextual field (CF) input that modulates the effects of the primary drive. A transfer function will be described through which the contextual predictions can guide processing so as to emphasize coherent patterns of activity, but without becoming confused with the information that is transmitted about the RF input. Long-range horizontal collaterals between cortical pyramidal cells provide a prima facie example of CF inputs. A possible role for these inputs is to emphasize information that is relevant to the context within which it occurs. Information theory will be used to make this goal precise, and thus to derive learning rules for the RF and CF connections. The rules derived have definite similarities to a form of synaptic plasticity shown to occur in slices of adult rat visual cortex. Simulations of multi-stream multi-stage nets built from such processors show that they can discover the relevant RF variables concurrently with and because of discovering the predictive relations between them. Anatomical, physiological, and neuropsychological evidence that contextual guidance and coherent population coding may contribute to computation in the mammalian cerebral cortex will be briefly outlined.

Increasing the number of synapses in Drosophila

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Learning is a faculty dependent upon modification of synaptic efficacy. The Hebbian model of learning postulates changes in the number of active synapses as the cellular substratum for learning. We have made a utilitarian use of a Drosophila mutant to increase the number of synaptic contacts in order to study potential changes in learning performance.

The mutation *gigas (gig)* causes an increment of cell size of about three times in differentiating cells. This increment in cell size results in a corresponding increment of synapses in the case of neurons. Although the mutant is lethal at the end of the third larval instar, we can analyze mosaics in which only the presynaptic neuron is mutant while the rest of the nervous system is normal. Based on these mosaics, we have found that the increment in synapse number is an autonomous property of the presynaptic cell which imposes upon the normal postsynaptic counterpart an increment of about three times the normal number of contacts. We have characterized the morphological features of this type of mosaics in the case of the photoreceptors by serial section reconstruction at the EM. Also, mechano and chemoreceptors have been studied by means of HRP retrograde tracings.

Each sensory modality of mosaics have been analyzed in behavioural tests of phototaxis, cleaning reflex and odour discrimination and sensitivity. We find behavioural changes in these responses coincident with the changes in synapse number and connectivity of these sensory neurons. The behavioural test evidence that the additional synapses are functional and their input is integrated in the CNS evoking a coherent, albeit unusual, response. Current experiments attempt to measure the threshold for olfactory conditioning and its retention time.

In addition, *gig* represents a convenient tool to register the electrophysiological activity of synaptic boutons directly. Voltage and patch-clamp recordings have been obtained from these motorneuron terminals and a number of new ionic conductances have been found in a particular type of boutons known as type III in the larval muscles.

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CONCLUDING REMARKS

M. Heisenberg

Concluding remarks

M. Heisenberg

"*Nothing makes sense in the Neurosciences except in the light of behaviour.*" This simple truism follows directly from the famous statement of T. Dobzhansky: "*Nothing makes sense in Biology except in the light of Evolution.*" Emphasis on behaviour in a scientific landscape in which a new glutamate receptor is valued more than a new behavioural paradigm, had been one of the guidelines in setting up the workshop. During the meeting behaviour did emerge as a common denominator of the diverse topics presented and was a recurrent theme in the intense discussions. It was apparent, however, that in the various projects behavioural research played very different roles. In some studies it was used as a kind of Lackmus paper to indicate the relevance of physiological or biochemical processes in the nervous system; in others it was analyzed to a degree that would allow deductions about and comparison with, the underlying neuronal machinery. In still other projects the ecological context of a particular behaviour provided the decisive cues for assessing the types of mechanisms underlying it. Finally, in studies of human perception behaviour dwindled to a loose framework within which to search for the internal constraints of the neuronal system.

Yet, it was not just the behavioural research that made the meeting so special. For much of this century, or at the latest since the discovery of single-unit recording it had been the uncontested conceptual base of functional neurobiology that behaviour was to be explained by the properties of neurons and neuronal circuits in the CNS. This era now is over. Molecular genetics has successfully undermined this belief. Nearly every presentation during these three days vividly documented that mechanistic analysis of behaviour requires both cellular and molecular levels. The excitement pervading the workshop, I believe, came from the solid and broad demonstration that behavioural mechanisms need to (and can) be understood in terms of molecular networks of which the cellular network is a part.

No doubt, molecular genetics has been a major driving force in this quiet revolution. In most studies presented molecular genetic tools were used in one way or an other, for instance for generating functionally active substances, for assessing the molecular diversity of the neural tissue, for identifying cells, tracing neuronal circuits and

manipulating synapses. In many laboratories transgenic animals, so far mainly flies and mice have entered the scene. More than that, molecular genetics is revealing in a quantitative manner to which astonishing degree phylogeny unifies the Neurosciences. We can learn from a synapse in a snail about synapses in the hippocampus. Similar processes underlie learning and memory in molluscs, flies and mammals. Homologous genes control eye development in flies and man. The basic organization of brain and behaviour may be common to all animals.

It is too early to tell whether we are witnessing a renaissance of the behavioural sciences or whether the interest in the molecules of the brain will still prevail for some time. In any case, I believe, the workshop has publicised and advanced an important branch of the brain sciences: the integrative analysis of molecular, cellular and behavioral functions. Moreover, it has reviewed outstanding research and, most significantly, it has been fun.

POSTERS

Contribution of maxillary palp information to the olfactory behavior in *Drosophila melanogaster*

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Maxillary palps have been proposed as secondary olfactory organs, after the antennae, in *Drosophila melanogaster* . Our study tries to quantify the relative importance of both organs as olfactory information mediators. Dose-response curves for three odorants: ethyl acetate, propionaldehyde and benzaldehyde were carried out for comparing olfaction in either complete animals or flies surgically deprived of antennae. Antennaless flies tested in a Y-maze behavioral assay showed indifferent, attractant and repellent responses depending on concentration, similarly as normal flies do. However, they clearly displayed less sensitivity than normal flies. The range of concentrations they were able to perceive was correlated to antennal sensitivity approximately by a factor 1:10 for ethyl acetate and benzaldehyde, and between 1:10 and 1:100 at high concentrations of propionaldehyde.

A complementary experiment was performed to test changes in olfactory behavior produced by removing maxillary palps in the presence of antennae. At high concentrations of odorant, responses to ethyl acetate and propionaldehyde experienced small changes when both palps were removed. Results are compatible with a summation model of all olfactory information reaching the brain either through antennae or palps. This summation must take place before brain integration of the olfactory information since maxillary palp information quantitatively affects the atractant-repellent decission.

INVOLVEMENT OF GENE EXPRESSION IN THE OLFACTORY BULB IN FORMATION OF ODOR RECOGNITION MEMORY IN RATS

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Olfactory memory plays an important role in the behaviour of newborn rats. The aim of this work was to study the involvement of the first relay structure of the olfactory system, the olfactory bulb, in the formation of the odor recognition memory in young rats and to investigate the role of gene expression in these processes. Rat pups of 10 days old were trained to find a lactating dam in a Y-maze using sunflower oil as an olfactory cue. Testing of olfactory memory took place in an odor preference test 1-24 hours later. Single training session of 8 trials was sufficient to establish a strong and selective preference of pups for the conditioned odor. Analysis of *c-fos* mRNA levels in the olfactory bulb after learning has demonstrated a marked elevation of this immediate early gene expression. Induction of *c-fos* in the olfactory bulb occured in all experimental conditions whenever a novel odor was presented to a pup. This increase of *c-fos* mRNA did not depend on the positive reinforcement or the success of learning. In order to inhibit the observed odor-induced gene expression a protein synthesis inhibitor cyclohexemide (CXM) was injected in the olfactory bulb at different times prior or after training. When injected 30 min before training CXM suppressed in a dose-dependent manner the odor-recognition memory tested 24 hours later. At the same time it did not disrupt learning of the olfactory task and its retrieval from 30 min to up to 9 hours after training. CXM was effective in inducing amnesia only when injected in a short time window within one hour after the end of training - the period which corresponds to stimulus-induced synthesis of immediate-early gene encoded proteins.

These results suggest that the olfactory bulb is engaged in the storage of the long-term odor recognition memory in rats and that consolidation of this memory is critically dependent on odor-induced gene expression in the olfactory bulb.

MODULES FOR MOLECULES? TOPOGRAPHICAL ORGANIZATION OF THE ZEBRAFISH OLFACTORY SYSTEM

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It is largely unknown how odor responses are spatially represented in the vertebrate olfactory system. By a combination of axon tracing and in situ hybridization techniques, we are testing the possibility that olfactory sensory neurons expressing the same odorant receptor molecule project to a common glomerulus in the olfactory bulb of adult zebrafish, *Danio rerio*.

We injected one previously identified glomerulus with DiI to trace back olfactory neurons connected to it. These neurons are widely scattered over the sensory surface without apparent topographical order. However, the relative positions of retrogradely labeled neurons are not completely random (i. e., generated by a Poisson point process), as judged from measuring nearest-neighbor distances. Rather, a spacing mechanism is involved: Olfactory neurons projecting to the same glomerulus keep a minimum distance of roughly ten cell diameters from each other. This short-range ordering provides for a more regular distribution of sensory neurons that feed into one glomerular unit. It can be speculated that this projection mode maximizes the probability to detect different odorants.

Odorant receptor molecules in zebrafish belong to a large gene family of about 50 members and are homologous to their mammalian counterparts. In situ hybridizations with probes directed against receptor sequences reveal that individual receptors are expressed by a small fraction of sensory neurons, which are widely scattered over the olfactory epithelium. We are currently investigating whether the properties of their distribution resemble those of neurons backlabeled from a single glomerulus. As a preliminary result of this analysis, we find cases of in situ labeled neurons adjacent to each other, arguing against a simple one-to-one correspondence. In a more direct, but technically demanding approach, we attempt to double-label olfactory neurons by the two methods.

THE PHYSIOLOGICAL ACTION OF BDNF ON RETINAL GANGLION CELLS: AN UNSOLVED PUZZLE

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During normal development more than 50% of the retinal ganglion cells are eliminated, and essentially all of them if their connections with the central target are severed. Programmed neuronal death is a general phenomenon in the nervous system and is supposedly regulated by the availability of extracellular signals provided by the target tissues. In the peripheral nervous system the members of the neurotrophin family NGF, BDNF, NT-3 support the survival of peripheral neurons *in vitro* that would otherwise die. They do also regulate neuronal elimination during natural development. BDNF supports the survival of retinal ganglion cells *in vitro* [1], as well as *in vivo* [2] after axotomy. It is however unclear whether BDNF regulates the survival of retinal ganglion cells in the intact organism. This question is of particular interest since a role of neurotrophins in the control of programmed cellular death in the central nervous system has been never directly addressed.

As a first step the presence of BDNF receptors on retinal ganglion cells during the period of neuronal elimination was investigated by *in situ* hybridisation.

Retinal ganglion cells express the BDNF receptor, trkB, in E11 and E14 chick embryos as well as newborn (P0) mice. Interestingly P0 retinal ganglion cells express also the NGF receptor trkA and the NT3 receptor trkC.

The presence of BDNF in the target of retinal ganglion cells, the superior colliculus, was investigated by Northern blot. BDNF can be detected in the innervation field of retinal ganglion cells in the mouse, as previously observed in the chick [3].

We investigated the natural elimination of retinal ganglion in experimentals models where the endogenous levels of BDNF were manipulated. Quantitative analysis of electromicrographs showed that the number of axons in the optic nerve of BDNF K.O. mice does not differ from wild type controls. In addition treatment of chick embryos with BDNF does not reduce the number of naturally degenerating retinal ganglion cells.

One possible explanation for these relatively unexpected results might reside in a substantial difference between peripheral and central neurons. The regulation of retinal ganglion cells elimination could result from an interplay between several different signals as suggested by the presence of trkA and trkC in the retinal ganglion cell layer. According to this scenario other trophic factors would compensate for the lack of BDNF. Survival effects are observed only when all trophic support is interrupted, for example as a consequence of axotomy. This pathological situation does not correspond to the situation encountered in the intact organism. Under these circumstances neurons could become more sensitive to activation of cell surface receptors and therefore responsive to BDNF. BDNF would thus act pharmacologically on a receptor that is present on retinal ganglion for the control of developmental processes didtinct from apoptosis. What could be that physiological relevance of BDNF? During the period or retinal ganglion cell death the overall organisation of the retino-tectal system is laid out. An involvement of NGF in the plasticity of the visual cortex has been recently proven [4,5,6]. It is well possible that in the developing retino-fugal system BDNF could participate in the shaping of neural connections, for example by acting as a retrograde messenger for synapse stabilisation.

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Poster Abstract

Genetic and behavioural measures of olfactory coding in a simple system

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Drosophila melanogaster larvae show behavioural responses to a wide range of olfactory stimuli (alcohols, acetates, acids, aldehydes...). Most odours induce varying degrees of attraction, some induce repulsion whilst others induce no behavioural response. The larval olfactory organ contains only 21 neurons, so the range of odours which can be detected is probably relatively small. EMS mutagenesis revealed the existence of a gene on the right arm of chromosome III which, when mutated, induces a complete and specific anosmia in response to Nonanol, an alcohol which normally repulses *Drosophila* larvae. This mutation, *Indifferent*, leaves behavioural responses to eight other alcohols intact, suggesting that an element specific to the processing of Nonanol is affected by the mutation. The study of geographical strains revealed the existence of other genes controlling specific and total anosmias to heptanol, hexyl acetate and pentyl acetate. These genetic anosmias are unique in *Drosophila*: other known olfactory mutants tend to show reduced responses rather than genuine anosmias.

In order to detect whether larvae can distinguish between odours with similar structures, adaptation tests were carried out. Larve were pre-stimulated with doses of a given odour for varying periods of time (1-60 minutes) and then presented with a range of test odours. If the larvae no longer respond to the odour used during pre-stimulation, auto-adaptation has occurred, suggesting that some element of the processing pathway no longer responds. This effect is transitory, and behavioural responses return to normal after 30-60 minutes. If another the larvae no longer respond to another odour (cross-adaptation), this indicates that the two odours share some element(s) of the processing pathway. These experiments reveal substantial differences in the affinity of different odours for the olfactory pathway, and suggest that two or three separate olfactory pathways exist for the processing of alcohols. Some different chemical functions apparently share some or all elements of the processing pathway. These results are discussed in the light of different models of olfactory processing and the relationship between the number of neurons and the number of receptors.

EFFECT OF CHRONIC INTAKE OF CYTIDINE-5'-DIPHOSPHATE-CHOLINE (CDP-CHOLINE) ON BEHAVIORAL AND NEUROMORPHOLOGIC FEATURES OF THE AGED MICE.

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Integral brain activity leads to the appearance of motor behavior. Moreover, it is a well known fact that several environmental conditions, such as; dietary variations, alcohol consumption, and nervous system-stimulating drugs may lead to the arousal of both cognitive and motor disorders. In this way, the effects of chronic administration of CDP-choline (a phosphatidylcholine precursor which determines neuronal estructure and function) were studied in 24 months old mice (CFW).The drug was administered diluted in the drinking water (150 mg.Kg-1 per day). The treatment started when animals were 12 months old, and two groups were employed. The treated group and a second set of control animals that were drinking tap water. When both groups were 2 years old, their motor behavior was characterized. For the behavioral purpose the animals were placed for 2 hours in a translucent cage. After one hour in the cage, the motor activity was filmed for 1h with a video camera connectedtoa VCR. Several behavioral parameters were studied such as; basic body postures consisting of the position displayed by the mouse, without movement (standing, lying down, etc), posture-associated motor actions (head grooming, scratching, etc), and interaction with the environment (burrowing, feeding, smelling, etc.). The main levels of behavioral activity were significantly increased ($p<0.01$) in the CDP-choline treated group versus the control.

For the neuromorphologic study, the dentate gyrus (DG) was studied by light and electron microscopy (EM). After general tissue processing for EM, semithin sections were used for the morphometric analysis of several parameters (cellular and nuclear sizes). The results obtained demonstrate that there is a significant increase in cellular and nuclear sizes of the DG neurons in the treated group when compared with control animals ($p<0.05$). The ultrastructural examination of these neurons in the control group indicated the aggregation of tertiary lysosomes. Moreover, they were unexistent in the CDP-choline. Taken all together these results suggest that the chronic administration of CDP-choline enhances motor behavior in aged mice and an increased neuronal activity in the neurons of the DG as the neurohistologic study suggest.

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Supported by grant PM91-0170 from DGICYT.

THE NITRIC OXIDE IS IMPLICATED IN THE GENERATION OF EYE MOVEMENTS

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In the last few years it has been reported that nitric oxide (NO), a free radical gas, plays a physiological role in the regulation of: i) the immune response, as mediator of macrophage actions; ii) blood vessel dilatation, as endothelial-derived relaxing factor; and iii) neurotransmitter release in the nervous system, as a retrograde messenger. Although there are some interesting experiments associating NO with complex functions *in vitro*, very little is known about its role in the processing of information in physiological conditions. In order to look for a system in which NO could participate, we have analyzed the distribution of inmunohistochemical stained NO synthase neurons in the brainstem of the cat. As result of this study we have found a nicely distribution of immunoreactive neurons in the oculomotor system, concretely, a high density of labelled neurons in the prepositus hypoglossi nucleus (PHN) and scattered neurons in the vestibular and abducens nuclei. All this nuclei are implicated in the generation of horizontal conjugate eye movements. Given the highest staining of PHN neurons we concentrated on this nucleus in the alert cat. Cats were prepared for chronic recording of eye movements with the scleral search-coil technique and implanted with stimulating electrodes on the VIth nerve. The location of the PHN was made by taking the centre of the abducens nucleus (maximum antidromic field potential recorded by stimulating the VIth nerve) as a reference, displacing caudally the micropipete by 1-1.5 mm and recording the neuronal activity at this point. Injections of L-nitroarginine methyl ester (L-NAME), D-NAME, N-monomethil-L-arginine (L-NMMA) and nitroprusside (SNP), at concentrations of 80 mM, were delivered at the selected zone by pulses of air pressure. Injected volumes were carefully controlled and ranged between 0.1 and 0.5 µl. Blockers of the NO synthase, L-NAME and L-NMMA, induced nystagmic conjugated horizontally-directed eye movements whit almost linear slow phases directed contralaterally to the injected side, being the velocity of the slow eye displacement proportional to the employed doses. During sinusoidal vestibular stimulation in darkness there was a displacement of the eye velocity in the same direction than spontaneous eye movements without modification of the gain and phase of the vestibulo-ocular reflex. On the contrary, the D-NAME at the same concentration as L-NAME did not induce any effect. The donor of NO, SNP, induced a nystagmus whose slow phases were ipsilaterally directed to the injected side. Injections slightly deviated from the PHN produced little or any effect. Present results indicate that NO is implicated in the brainstem generation of eye movements, and the characteristics of the induced effects point out that the NO plays a role in the regulation of the vestibular input signals impinging on the PHN.

CORTICAL POTENTIALS ASSOCIATED TO VISUAL STIMULI, MANUAL RESPONSES AND SACCADIC EYE MOVEMENTS DURING GAP AND NON GAP PARADIGMS

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The presence of a gap between the offset of a central light and the switching on of a peripheral light produces a shift advancement in the latencies for both eyes and manual responses. An experiment was conducted on naive human subjects to measure the time benefits on finger reaction times produced by the offset of a central fixation point 200 msec before the appearance of a target stimulus in the periphery. Subjects produced a shift advancement of manual reaction times. Simultaneously, the event-related potentials were recorded. The gap paradigm induced offset visual evoked potentials and a frontal negativity, it also induced a higher P300 than the non-gap condition. A similar experiment was performed asking to the subjects to move the eyes to the peripheral target. The gap, as during manual responses, induced a shift advancement in the saccadic latencies and a frontal negativity. The results suggest that the gap promotes the speeding of the responses by a cortical priming, such cortical activation by the gap it is similar when ocular or manual responses are required.

KINEMATIC ANALYSES OF THE BLINKING BEHAVIOR. Agnès Gruart, Laboratorio de Neurociencia, Departamento de Fisiología y Biología Animal, Universidad de Sevilla.

Classical conditioning of the eyelid response to a tone or a light seems to be a suitable paradigm in the study of the neural basis underlying associative learning. Thus, the simultaneous recording of the electromyographic (EMG) activity of the orbicularis oculi muscle and the movement of the upper eyelid may give precise information on the kinematics of unconditioned and conditioned responses.

Under general anesthesia, cats were implanted with a small (3 mm in diameter) five-turn coil into the center of both upper eyelids, near the lid margin and with bipolar stain-steel hook electrodes in both orbicularis oculi muscles. Animals were also implanted with a holding device for head stabilization during recording sessions. Our aim was to study the latency and topography of eyelid movements during the acquisition of (new) motor responses to conditioned stimuli (CS) differing in either sensory modality (tones, weak air puffs) or side of presentation with respect to the unconditioned stimulus (US). The US always consist of a strong air puff. This approach was expected to help to elucidate the primary site in which the new motor response is elaborated (according to its latency) and the differences with well-established reflex responses (according to their kinematics).

Eyelid responses to air puffs applied to the cornea consists of a short latency (9-16 ms), fast (up to 2,000 deg/s) downward movement that lasts for 25-30 ms, followed by late, small downward sags that are sometimes still evident after stimulus offset and that are also dependent in their latency and amplitude on air puff pressure. However, these sags occurred at a dominant frequency of $\approx$ 25 Hz, which was independent of stimulus parameters, suggesting that this frequency is a property of the neural circuit controlling reflex blinks.

The latency of the conditioned response (CR) is a function of the CS (tone or air puff) and of the side where the CS is presented (ipsi- or contralateral to the US). For the 4 conditioning paradigms used, the latency of the CR was always in the range of the corresponding reflex response and did not depend on CS-US interval. It is concluded that CRs are initiated in the same brainstem circuits that produce the unconditioned response (UR), i.e., the reflex response, according to CS sensory modality, duration, strength and presentation side. The CR appeared as a downward lid movement that radiates from its onset, in a done temporal proximity to the CS, toward the US, where it reached its maximum amplitude. The CR was formed by successive small downward sags similar in amplitude to the late components in reflex blinks. The number of the sags increased, and their duration and amplitude decreased with successive conditioning sessions until straight and dumped lid CRs were reached. At the same time and as the proximal cause of these motor changes, the orbicularis oculi muscle changed from a phasic EMG activity to a tonic firing that was maintained along the CS-US interval.

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MOLECULAR DISSECTION OF LONG-TERM POTENTIATION BY SUBTRACTIVE HYBRIDIZATION. L.de Lecea, J.R. Criado, S.J. Henriksen, J.G. Sutcliffe.
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Long-term potentiation (LTP) is a well studied paradigm of synaptic plasticity and has been proposed as a cellular model of memory. The long lasting changes in synapses that are linked to long-term potentiation require the activation of gene transcription and *de novo* protein synthesis. Genes that are specifically induced in such conditions may include those involved in neurotransmitter uptake and release, and genes required for the physical remodeling of synapses.

We have used a subtractive hybridization method to isolate genes that are induced at different time points after tetanic stimulation to produce LTP in the perforant path. After subtraction, 95 % of the labeled target cDNA was removed, which represents the population of mRNAs that are present in both stimulated and unstimulated tissues. We have identified genes corresponding to the three waves of gene expression that occur after synaptic stimulation. These include immediate early genes, such as c-fos and NGFIA, and metabolism-related proteins (1hr post-stimulus); modulators of signal transduction at 6 hours after tetanus (e.g.protein kinase A inhibitor PKIα); and proteins involved in synaptic remodeling such as membrane ectopetidases (24h post tetanus).

The characterization of these cDNA clones will allow us to further understand the molecular mechanisms underlying the maintenance of long-term potentiation and, in general, of synaptic plasticity.

Patch-Clamp recordings from single synaptic boutons in Drosophila neuromuscular junctions. M. Martínez Padrón and A. Ferrús.

The study of synaptic transmission and modulation at the neuromuscular junction has been severely hampered by the small size of the presynaptic terminals, which precludes electrophysiological recordings. We have made use of the gigas (*gig*) mutation to generate motor endings of a size amenable to electrode impalement. Identified neuromuscular junctions exhibit at least three types of synaptic boutons, termed I, II and III. Type I boutons in *gig* mutants can be as large as 15 µm in diameter, whereas type III boutons can reach up to 9 µm. Quantal analysis of synaptic transmission suggests that mutant junctions are undistinguishible from those of the wild type. We have succesfully recorded single channel activity from type III boutons in the inside-out configuration, as well as whole cell currents using the nystatin perforated-patch tecnique. Several types of potassium channels are found in the synaptic terminal, and their properties are presently under investigation.

CELLULAR AND MOLECULAR ANALYSIS OF THE OLFACTORY JUMP REFLEX IN *DROSOPHILA*.

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We have conducted a novel form of behavioural screen using GAL4 enhancer traps to express tetanus toxin light chain in a variety of patterns in the central and peripheral nervous systems of *Drosophila*. Tetanus toxin light chain is a nervous system specific toxin that inhibits neurotransmitter release from the neurons in which it is expressed[1].

When wild type *Drosophila* are exposed to certain noxious odours a reflex pathway is activated which results in leg extension and a 'jump response'[2]. We have screened for and isolated GAL4 lines which, when crossed to UAS-Tetanus, jump with abnormally low frequencies after exposure to the odour benzaldehyde. A number of these enhancer trap lines are particularily interesting in that they have GAL4 expression patterns that do not overlap with the known neural components of the light-off jump response[3].

A more in-depth behavioural analysis of these lines is currently taking place to ascertain whether the tetanus mediated block in the pathway is at the olfactory level or further downstream. We also wish to determine whether the neurons identified play a role in the habituation of the jump reflex. We intend to test this using the GAL4 system to manipulate the intracellular signalling pathways of these cells.

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DISTRIBUTION OF NITRIC OXIDE SYNTHASE POSITIVE NEURONS AND NEUROPIL IN THE VENTRAL STRIATUM AND VENTRAL PALLIDUM OF THE CAT. E. Mengual, S. Rivas and J.M. Giménez-Amaya. Departamento de Morfología, Facultad de Medicina, U.A.M., 28029 Madrid, SPAIN.

The nitric oxide has been described as a neuromodulator that, among a wide variety of functions, seems to be also implicated in learning and memory tasks. Several anatomical studies have reported that the NADPH-diaphorase histochemical reaction accounts for nitric oxide synthase activity (NOS) in the brain tissue. The purpose of this study was to describe in detail the distribution of NOS-positive neurons and neuropil in the ventral striatum of the cat by means of NADPH-diaphorase histochemistry. The ventral striatum, which includes not only the nucleus accumbens but also an extensive part of the olfactory tubercle as well as the most ventral portions of the nucleus caudate and putamen, has been widely implicated in motor behaviour and learning. Four animals were used in this study, and their brains were processed for NADPH-diaphorase histochemistry as well as for other histochemical techniques in adjacent coronal sections. The main findings of our study are as follows: 1) The NOS-positive neuropil was heterogeneously distributed in the different subdivisions of the ventral striatum. The most intensely stained regions displayed a circular contour and were located in the ventral portions of the nucleus accumbens, being continuous with the most ventral regions of the putamen and the most lateral portions of the olfactory tubercle. 2) Patches of very intensely stained neuropil were detected in the superficial dense cell layer of the olfactory tubercle, corresponding to the islands of Calleja. 3) The ventral pallidum was remarkably devoid of NOS activity. 4) The distribution and morphology of NOS-positive cells at rostral levels of the nucleus accumbens were very similar to those of NOS-containing cells in the dorsal striatum. 5) At more caudal levels these NOS-positive cells were mainly concentrated within the ventral heavily stained circular areas of the nucleus accumbens and ventral putamen. 6) These areas were intermingled with poorly stained zones showing very few NOS-positive cells, that were preferentially located in the periphery of these zones. The multicompartmental distribution of NOS activity in the ventral striatum of the cat might indicate different functional assignments of the distinct subdivisions within this part of the basal ganglia. Supported by FIS 93/0337.

Calcium metabolism of honeybee Kenyon cells
A combined patch-clamp and fluometric aproach

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Kenyon cells are the intrinsic neurons of the mushroom bodies, a neuropil of the insect brain that is thought to be involved in learning and memory processes. We work with dissociated Kenyon cells from mushroom bodies of the brain of the honeybee *Apis Melifera*. Our aim is to study the electric properties of the Kenyon cells with respect to their assumed role in learning and memory processes. For that we performed whole cell patch-clamp recordings on short term cultured Kenyon cells[1]. In a next step, modulation of the examined currents by neurotransmitters were investigated. Because calcium plays a key role in many second messenger pathways and affects several kinase systems that might be involved in neuromodulation, we started to measure changes of the intracellular calcium concentration by fluometric means. The measurements give insight into a complex interplay between the two possible calcium sources, the extracellular solution and internal stores.

As we know from the patch-clamp data, calcium can enter the cell through a voltage dependent calcium channel and a nicotinic type of ACh-receptor, that was shown to be highly conductive for calcium. Calcium from internal stores can be released by caffein application. There is good evidence for a calcium induced calcium release from the fluometric experiments; complex time functions of the calcium signal could be observed as oscillations, depending on the amount of calcium entering the cell.

To understand the electrical behaviour of a nerve cell and especially the changes in the input response function under the influence of transmitters as would be expected in learning processes, calcium fluometry seems to be a powerful tool to get insight into parts of the modulatory network that steers the function of the ion channels and therewith the electrical properties of the Kenyon cells.

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Expression and topography of N-CAM isoforms in chick forebrain following training

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Possible involvement of neural cell adhesion molecule (N-CAM) isoforms in mechanisms of the learning-related neuroplasticity was investigated using passive avoidance training in one-day-old chicks. A quantitative immunoelectron microscopy study was carried out in an area of the chick forebrain, *hypestriatum ventrale*, which exhibits profound morphological plasticity and biochemical changes following training [1,2].

An antibody against three major protein isoforms (120, 140, and 180 KDa) and one against polysialic asid (PSA), associated with N-CAM, were employed to label (separately) with immunogold the protein epitopes in post-embedded tissue. Preparations were taken from control birds and those 6 hours after training, the time window which corresponds to a training specific amnesia induced by an N-CAM injection [3].

Comprehensive morphometrical designs, icluding ANOVA and statistics of random processes, were used to analyze the densities and arrangements of immunogold particles in neuropil. The results show that: (1) a narrow sub-membrane region of nerve cells is 10-30 times more enriched in N-CAM isoforms, and has a two fold higher proportion of PSA-N-CAM, than the tissue (membrane plus non-membrane) as a whole; (2) there were no significant changes in levels of labelled N-CAM or PSA following training; (3) 375-400 nm regular arrays of PSA-N-CAM were revealed in membrane profiles in the control, but not the trained, group. These data suggest that (a) the training task may cause subtle re-arrangement, rather than changes in the expression level, of N-CAM isoforms; (b) learning-related changes in N-CAM isoform turnover can be attributed to a narrow sub-membrane region of the nerve cells involved [4].

A pilot experiment with double immunogold labelling in the control group of birds has shown that: (1) labelled major N-CAM isoforms and PSA are co-localized spatially at the sub-cellular level; (2) labelled PSA exhibits a profound spatial clustering (within a 50-150 nm range), in contrast to major N-CAM isoforms [5].

The data obtained provide the basis for further studies of the subtle relationship between learning-associated neural changes and subcellular re-distribution of protein isoforms related to cell adhesion.

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Targeted expression of tetanus toxin in *Drosophila* specifically eliminates synaptic transmission and causes behavioural defects

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Tetanus toxin cleaves the synaptic vesicle protein synaptobrevin, and the ensuing loss of neurotransmitter exocytosis has suggested a key role for synaptobrevin in this process. To further the study of synaptic function in a genetically tractable organism, and to generate a tool to disable communication between defined neurons for behavioural studies, we have expressed a gene encoding tetanus toxin light chain in Drosophila. Expression of toxin throughout development of the embryonic nervous system leads to elimination of neuromuscular synaptic transmission. No other developmental or morphological defects were detected. Correspondingly, *Drosophila* neuronal synaptobrevin, n-syb, but not the ubiquitously expressed syb protein, is cleaved by tetanus toxin when produced in an *in vitro* translation system. In some cases targeted expression of toxin is associated with behavioral defects. When tetanus toxin is expressed under control of one P-GAL4 line, a specific defect in the olfactory escape response is observed. We are currently constructing tetanus resistant forms of the n-syb gene to express concomitantly with the tetanus toxin light chain. Expression of a tetanus resistant n-syb would enable us to examine the possibility that there may be other cellular substrates for tetanus toxin in the nervous system of *Drosophila*.

List of Invited Speakers

Workshop on

CELLULAR AND MOLECULAR MECHANISMS IN BEHAVIOUR

List of Invited Speakers

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CELLULAR AND MOLECULAR MECHANISMS IN BEHAVIOUR

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Organizers: C. S. Goodman and F. Jiménez.

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Organizers: C. Nathan and A. Celada.

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Organizers: M. B. Mathews and M. Esteban.

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Organizers: M. McClelland and X. Estivill.

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The lectures summarized in this publication were presented by their authors at a workshop held on the 27th of February through the 1st of March, 1995, at the Instituto Juan March.

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