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IN CONFIDENCE

SCIENTIFIC RESEARCH GRANT REPORT FORM

A COPY OF THIS FORM AND GUIDANCE NOTES IS ALSO AVAILABLE ON THE WWW @ http://www.mrc.ac.uk

This form should be completed in typescript or black ink. The submission of a Scientific Report is regarded by the Council as an essential requirement of their Grant Schemes. Parts 1-5 should correspond with the information contained in the award letter. Any changes must be clearly identified and explained in the text of the report.
Send this form and your detailed report (see Section 19) with (two) copies of each to reach the MRC within 3 months of the end of the research grant. Failure to do so will result in financial penalties. This report may be used as part of a Progress report or when requesting a Renewal or Extension. Investigators are asked to restrict their comments to the spaces provided on this form.

1. GRANT NUMBER:	2. GRANT PERIOD:	3. TYPE OF REPORT:
951497M1 N G TYPE OF GRANT:	START DATE: 01 01 96 31 12 98 END DATE: ____/____/____	PROGRESS RENEWAL EXTENSION FINAL Final

4. INVESTIGATOR(S): Dr Alex M. Thomson and Dr James Deuchars

INSTITUTION(S) / AUTHORITY

Department of Physiology
Royal Free Hospital School of Medicine
(now Royal Free and University College Medical School
University College London)
Rowland Hill Street
London NW3 2PF

DEPARTMENT WHERE WORK DONE

5. TITLE OF INVESTIGATION:

Modulation of morphologically defined inhibitory synapses by benzodiazepines, barbiturates and steroids.

6. OBJECTIVES OF THE RESEARCH:
List the main objectives as stated in the original proposal in order of priority

PROMPT
Scientific aims & any plans for the application / exploitation as stated when funding originally sought.

To determine whether differences in the pharmacology of $GABA_A$ receptor mediated synaptic events in cortical regions are correlated with: i) the class of presynaptic interneurone involved, ii) the location of the inhibitory terminals on the postsynaptic target cell, and/or iii) the class of postsynaptic target cell. To identify the morphologically definable synapses that are most sensitive to each of three major groups of therapeutically interesting drugs that act by augmenting GABA actions. Finally to indicate whether all inhibitory synapses received by a given class of postsynaptic cell, or all those supplied by a given class of presynaptic interneurone, display the same pharmacology and therefore utilize the same combinations of receptor subunits.

7. ACHIEVEMENTS OF THE RESEARCH:
Describe the extent to which the objectives of the research have been achieved and relate the significance of the scientific advances/insights achieved to recent work in the field.

Identify important results and relate to general developments of the field. Please explain any changes in objectives during the study.

Pentobarbitone enhanced all IPSPs studied more than did benzodiazepines. Zn^{2+} depressed some (bistratified cell IPSPs > basket cell IPSPs), including some that were enhanced by diazepam. Thus some dendritic inputs are mediated by both diazepam-sensitive and Zn^{2+}-sensitive receptors, ie. possibly by both γ-containing and γ-lacking receptors. Zolpidem, a selective benzodiazepine-1 (BZ_1, α_1 subunit) agonist rapidly enhanced most classical CA1 basket cell IPSPs. Putative chandelier, wide arbour basket and bistratified cell IPSPs were enhanced less in the first 30 minutes. The $BZ_{1/2}$ agonist diazepam further enhanced wide arbour basket and chandelier cell IPSPs after equilibration with zolpidem > basket cell IPSPs. BZ_1 (α_1) receptors might therefore localize to somatic synapses, with BZ_2 ($\alpha_{2/3}$) receptors at dendritic sites and axon initial segments. A recent study of mice with point-mutated BZ-insensitive α_1 subunits shows that hypnotic, but not anti-epileptic effects of BZ agonists are α_1 dependent (Mohler), correlating well with our findings of differential BZ_1 distribution. Surprisingly, benzodiazepines generated a biphasic enhancement of IPSPs. After the initial enhancement, IPSPs were stable for 20-30minutes when a second, sharp increase occurred. Studies of the mechanisms underlying the time course of action of these clinically important drugs have just begun. Preliminary studies indicate that this second increase is also mediated via BZ-sensitive receptors. Preliminary studies with Etomidate (a $\beta_{2/3}$ subunit selective anaesthetic) indicate a wide range of sensitivities.

8. PROGRESS OF THE RESEARCH:
(i) Outline the methodology used in the research.

Examples (design) tissues/cells, techniques/ approaches, measurements/outcomes.

Dual intracellular recordings from slices of adult rat hippocampus.
IPSPs were elicited in single postsynaptic pyramidal cells by single interneurones and drugs (Zolpidem, Diazepam, Pentobarbitone, Flumazenil, Etomidate, Zn^{2+}) were applied in the bathing medium.
Biocytin-filling of recorded cell pairs, histological and recently immunofluorescent processing of filled cells and identification/reconstruction of presynaptic interneurones.

(ii) Was there any significant change in the research work or programme of work compared with the original proposal. YES/NO

If YES give reasons for changes. i.e. Did the research proceed as expected and on time? If NO give details. Were there any circumstances which aided or impeded the progress of the research? If YES, explain the steps you took to overcome them. Examples of problems could include difficulties in recruitment of staff, late delivery of equipment and malfunction of equipment.

The surprising biphasic nature of responses to benzodiazepines (previously not reported) required more detailed studies of the time course of zolpidem/diazepam effects than originally envisaged and extension of the project to include BZ receptor blockade by flumazenil. Etomidate was shown to be selective for specific receptor sub-types during the project. This drug was therefore included in our investigations.

9. FURTHER RESEARCH:

(i) Has the research led to further investigations or collaborations which have led to other applications to the MRC or others? YES/[redacted]

If YES, give details of the outcome. List grant applications giving dates and grant reference. Indicate value of any grants awarded. Give details of the outcome. List non - MRC grant applications, collaboration in EC research programmes and industrially supported work.

Applications :

MRC Programme Application: "Function of identified hippocampal interneurones; role, localization and modulation of $GABA_A$ receptors" (unsuccessful)

MRC Co-operative Group Component: "Function of identified hippocampal interneurones; role, localization and modulation of $GABA_A$ receptors" (submitted)

Novartis Pharma, continuing collaboration on glutamate and GABA mediated synaptic transmission, funded with a small project grant and a studentship.

Wellcome Trust: "Pre- and postsynaptic mechanisms contributing to pattern-dependent cortical circuit behaviour, combining dual and triple recordings, morphological reconstruction, ion channel pharmacology and compartmental modelling."
Ref. 052251 Jan 1998-Dec 2001, £147K. (Mr A. Peter Bannister).

10. RESOURCES AND PEOPLE:

i) Detail all grants/contracts/major increases funded by the Host Institution which arose through this research proposal.

Give title, funder, tenure and value.

ii) List the staff employed directly on the grant. List any other research fellows and research students associated with the research project and their sources of support.

Give the grade and period of working on the project for all staff. Indicate source of support for any research fellows and research students (include MRC and all sources of support). Also indicate the degrees for which students are enrolled.

1)	Dr J. Deuchars co-applicant	01.05.96 - 31.05.97
2)	Dr H. Pawelzik Postdoctoral RA	01.01.96 - 31.12.98
3)	Dr M. Ilia Postdoctoral RA	13.10.97 - 31.12.97
4)	[redacted] Technician	01.05.96 - 31.07.96
5)	[redacted] Postdoctoral RA	01.02.98 - 31.03.98

(iii) Describe what staff development and training has resulted from this project.

Detail any training or development benefits to staff employed on the grant including PhD awards that have arisen from the research.

1) Appointed to a lectureship (Leeds, Physiology) and awarded several new grants. Training in electrophysiology. Development of existing histological skills.
2) Training in dual intracellular recording, pharmacology and histology.
3) Training in cns physiology, histological processing etc.
4 and 5) Short term employment only modest training etc. provided.

11. COLLABORATION:

Did any other person or institution / organisation collaborate in the research.

Give details and describe the extent of all the collaborations anticipated in your proposal or which emerged during the collaboration (e.g. include grants from or formal collaborations with industry, exchanges of staff, materials or results arising from the research). Indicate the extent to which arrangements between the awardholders and collaborators have been realised and whether the Collaborators contribution differed from anticipated.

Dr D.C. West: On-going collaboration, development/implementation of analysis software.
[redacted] A wealth of invaluable advice and knowledge about $GABA_A$ receptor structure/function/pharmacology.
Dr A. Destexhe (Laval, Canada): Computational insight into the function of $GABA_B$ receptors (paper in press).
[redacted] Reciprocal visits the 2 labs invaluable in assessing the histology and in developing immunocytochemical techniques.
[redacted] Supply of antibodies for immunocytochemistry
Novartis Pharma: Supply of selective receptor antagonists and future collaboration for localization of $GABA_B$ receptors planned.

All extremely valuable, helpful collaborative ventures.

12. FACILITIES:
Give details of any facility used for this research. NA.

Name of Facility	Time Allocated	Time Used

Identify use of international and national facilities and facilities within HEIs. E.g. CCLRC, SRS etc.

13. PUBLIC AWARENESS OF SCIENCES (PAS):

What activities have been undertaken to promote the public awareness of the scientific results arising from this research, and did you refer to MRC support.

Publication in scientific journals (support acknowledged)

Presentation of data at national and international scientific meetings, symposia and summer schools.

Discussion of data with colleagues in industry

Give details of work/activity undertaken and an indication of the costs. Include details of media coverage, exhibitions, school links, articles in popular journals etc. Please detail any significant publicity.

14. EXPENDITURE:

(i) Has expenditure exceeded or fallen short of the total sum awarded under a particular heading? YES/NO

Expenditure on salaries was lower than predicted because Dr Deuchars drew salary from the grant only for 13 months before he was appointed to a permanent academic position. His replacement took some months to appoint and was then on a lower salary scale. Two short term appointments were made to assist us in minor aspects of the project, but since extension of tenure of the grant was not permitted, there was an excess which was (with agreement from the MRC) used to fund equipment.

If YES, explain the unexpected patterns of expenditure.

(ii) Did you need to vire any funds between heads, if so why?

Yes - see above

15. PUBLICATION AND DISSEMINATION OF RESULTS:	
(i) List titles of papers and internal reports etc. arising from the research (including items in preparation) and did you refer to MRC support. See attached sheets	List publications, (in refereed journals and others) detailing authors, underlining the names of authors funded by this grant, date, title, journal volume, page no's, (where known), conference proceedings, book chapters etc. . Include publications which have arisen through these collaborations. Please detail any significant publicity.
(ii) Has any data been lodged in a public access database. YES/NO	If YES, give details.

16. EXPLOITATION OF RESULTS:	
(i) Who are the likely beneficiaries of the research and have you disseminated any of the results to the User Communities (NHS, Industry etc.) In the long term, our work will help define the most appropriate sites for drug action and appropriate development of therapies. Via a long-term collaboration with Novartis Pharma and through scientific meetings our data are made available to industry.	E.g. other researchers, business and commerce, local or central government and other users.
(ii) Record anything patentable / commercially exploitable arising from the research, in the short, medium or long term? There are no likely short-term exploitable outcomes.	Give details and describe what arrangements have been made or are planned for exploitation of the results.
(iii) What are the implications for improving health and health care or quality of life in the short, medium or long term and what progress is being made towards exploiting these opportunities.	Refer to any actual or potential application or exploitation of research or relevance to Government Department priorities.
(iv) Record any increased collaboration with existing or new industrial commercial partners and any new sponsorship, funding for basic/strategic research.	This part of the report is particularly relevant to holders of ROPAs.

Publications resulting at least in part from recent MRC grant funding.
Support acknowledged.

Full Papers

Deuchars J. and Thomson A.M. (1996) CA1 pyramid-pyramid connections in rat hippocampus *in vitro*: dual intracellular recordings with biocytin filling. *Neuroscience* 74: 1009-1018

Thomson A.M., West D.C., Hahn J. and Deuchars J. (1996) Single axon IPSPs elicited in pyramidal cells by three classes of interneurones in slices of rat neocortex. *J. Physiol.* 496: 81-102

Thomson A.M., West D.C. and Deuchars J. (1996) Neocortical local synaptic circuitry revealed with dual intracellular recordings and biocytin-filling. *J. Physiol.(Paris)* 90: 211-215.

Thomson A.M. (1997) Activity-dependent properties of synaptic transmission at two classes of connections made by rat neocortical pyramidal axons *in vitro*. *J. Physiol.* 502: 131-147.

Ali, A.B., Deuchars J., Pawelzik H. and Thomson A.M. (1998) CA1 pyramidal to basket and bistratified cell EPSPs: dual intracellular recordings in rat hippocampal slices. *J. Physiol.* 507: 201-217.

Ali, A.B. and Thomson, A.M. (1998) Facilitating pyramid to horizontal O/A interneurone inputs : dual intracellular recordings in slices of rat hippocampus. *J. Physiol.* 507: 185-199.

Thomson, A.M. and Bannister, A.P. (1998) Postsynaptic pyramidal target selection by descending layer III pyramidal axons: dual intracellular recordings and biocytin filling in slices of rat neocortex. *Neuroscience* 84: 669-683.

Ali A.B. and Thomson A.M. (1999) IPSPs elicited in CA1 pyramidal cells by putative basket cells in slices of adult rat hippocampus. *Europ. J. Neurosci.* (in the press)

Thomson A.M. and Destexhe A. (1999) Dual intracellular recordings and computational models of slow IPSPs in rat neocortical and hippocampal slices. *Neuroscience* (in the press).

Reviews and Commentaries

Thomson, A.M. (1997) More than just frequency detectors. *Science* 275: 179-180.

Thomson A.M. and Deuchars J. (1997) Synaptic interactions in neocortical local circuits: dual intracellular recordings *in vitro*. Special Edition of *Cerebral Cortex*, 7: 510-522.

Thomson A.M. (1997) Quantal Analysis of Synaptic Processes in the Neocortex. *C.R. Academie des Science - Série III* vol 321, 131-133.

Abstracts

Deuchars, J., West, D.C. and Thomson, A.M. (1995). Functionally related morphology of local circuit connections of sparsely spiny interneurones in slices of rat neocortex. *J. Physiol.* 483: 59P

Ali, A.B. and Thomson, A.M. (1996) Pharmacology of CA1 pyramid to oriens alveus interneurone connections in hippocampus. *Soc. Neurosci. Abs* 1996.

Pawelzik, H.W., Deuchars J., Hahn J. and Thomson A.M. (1996).Barbiturate enhancement of single axon inhibitory postsynaptic potentials in hippocampus. *Soc. Neurosci. Abs* 1996.

Ali, A.B. and Thomson A.M. (1996) Physiology, pharmacology and morphology of CA1 pyramid to oriens alveus interneurone connections in the hippocampus. *Soc. Neurosci. Abs* 1996.

Ali, A.B. and Thomson A.M. (1997) Inhibitory connections made and received by a radial trilaminar interneurone in adult rat hippocampal slices. *J. Physiol.* 504:17P.

Ali, A.B. and Thomson A.M. (1997) Brief train depression and facilitation at pyramid to interneurone connections in slices of rat hippocampus; paired recordings with biocytin filling. *J. Physiol.* 501: 9P

Abstracts cont....

Thomson, A.M. and West, D.C. (1997) $GABA_B$ receptor mediated IPSPS in neocortex and hippocampus: paired intracellular recordings *in vitro*. *Soc. Neurosci. Abs* 1997.

Ali, A.B. and Thomson, A.M. (1997) Characterization of single axon EPSPs elicited in hippocampal interneurones by pyramidal cells: paired recording and biocytin-filling. *Soc. Neurosci. Abs* 1997.

Hughes D.I., Bannister A.P., Ilia M., West D.C. and Thomson A.M. (1998) Physiological immunocytochemical and morphological classification of identified hippocampal and cortical neurones: a dual immunofluorescence and immunoperoxidase approach. *Proc. Physiological Society Sept. 1998, J. Physiol. in the press.*

Pawelzik H. and Thomson A.M. (1998) Fast IPSPs generated by horizontal oriens-alveaus interneurones in the distal dendrites of simultaneously recorded CA1 pyramidal cells in hippocampal slices. *Proc. Physiological Society Sept. 1998, J. Physiol. in the press.*

Ali A.B. and Thomson A.M. (1998) IPSPs elicited by two morphological subclasses of basket cells in pyramidal cells in adult rat hippocampus: paired intracellular recordings and biocytin labelling in slices. *Proc. Physiological Society Sept. 1998, J. Physiol. in the press.*

Thomson A.M., Bannister A.P., Ilia M. and Pawelzik H. (1998) Zolpidem, Diazepam and Zn^{2+} sensitivity of IPSPS: dual intracellular recordings in adult rat hippocampal slices. *Soc. Neurosci. Abs* 1998.

Pawelzik H. Hughes D.I. and Thomson A.M. (1998) Single-axon IPSPs as generated by identified horizontal oriens/alveus interneurones in simultaneously recorded postsynaptic pyramidal cells of adult rat hippocampus *in vitro*. *Soc. Neurosci. Abs* 1998.

Full Papers submitted and in preparation

Ali A.B., Bannister A.P. and Thomson A.M. NMDA receptor components of EPSPs in CA1 interneurones, paired intracellular recordings in slices of rat hippocampus. (submitted).

Thomson A.M. and Bannister A.P. Release independent depression at pyramidal inputs onto specific cell targets: Dual recordings in slices of rat cortex. (submitted)

Pawelzik H., Bannister A.P., Deuchars J., Ilia M. and Thomson A.M. Modulation of bistratified cell and basket cell IPSPs by pentobarbitone sodium, Diazepam and Zn^{2+}. (paper in preparation)

18. DETAILED REPORT ATTACHED: You may also attach:	
(i) a report of not more than six sides* of a A4 typescript (point size 12) with a list of references. It should outline the scientific and / or technological achievements of the research expanding as necessary on the answers provided above.	Do not submit lengthy internal reports or PhD theses. Copies of key publications arising directly from the investigation should be appended, but are not acceptable as a substitute for any part of this report.
(ii) a separate summary (maximum of one A4 page) suitable for publication describing the achievements made on the Research Grant.	Include title, investigators, institutions and the name of a person whom readers should contact.

19. SIGNATURES	SIGNATURES AND DATES
INVESTIGATOR(s): ALEX M. THOMSON	[redacted] 29.01.99.
HEAD(S) OF DEPARTMENT(S):	[redacted] 7.2.99
ADMINISTRATIVE AUTHORITY;	[redacted]
POSITION HELD:	FINANCE DIRECTOR + HEAD OF ADMINISTRATION.

Modulation of Morphologically Defined Inhibitory Synapses by Benzodiazepines, Barbiturates and Steroids. (Lay Summary)

Alex M. Thomson and Jim Deuchars, Dept. Physiology, Royal Free Hospital School of Medicine (now Royal Free and University College Medical School UCL), London NW3 2PF.

Our brains include trillions of nerve cells or neurones. They communicate with each other via synapses, specialized connections between microscopic elements where a chemical (transmitter) released by the pre-synaptic cell influences the electrical behaviour of the post-synaptic cell. This response is mediated by specialized proteins or 'receptors' that recognise that transmitter. Since each neurone receives hundreds to thousands of synapses, the total computing power of the brain is immense. Some neurones excite, others inhibit their targets and the many different classes of both excitatory and inhibitory neurones have subtly different roles. Some important functions of inhibitory cells are apparent when the system malfunctions and inhibition is reduced or lost. A significant generalized loss leads to epileptic seizures, while enhancing the effectiveness of inhibitory synapses can prevent convulsions and induce anaesthesia. Inhibitory neurones are thought to be fundamental to the generation of 'cortical rhythms'. If the brain's electrical activity is recorded from the scalp, oscillations, or 'brain waves' are seen. The frequency and amplitude of these waves depend on the state of consciousness and attention. Different classes of inhibitory neurones may control different cortical rhythms, some generating the rhythms that occur during deep sleep, others those that occur when we explore a novel environment. At a more subtle level, inhibitory cells provide fine tuning of the brain's responses to sensory input, helping us to recognise and attend to novel and behaviourally important events and to respond accordingly.

At many inhibitory synapses, the chemical transmitter is GABA (γ-aminobutyric acid). In addition to the complexities inherent in the wiring of trillions of nerve cells with their own specific inputs and outputs, the receptors that recognise and respond to GABA come in many different varieties and each variety is differentially affected by drugs. A large number of therapeutically important drugs (eg. many anaesthetics, benzodiazepines) act by enhancing the action of GABA. As well as their anti-convulsant effects, these drugs can be sedative, anti-anxiety and anaesthetic and can disrupt motor coordination. Some naturally occurring compounds, including those derived from the sex steroid progesterone, have profound effects on mood and behaviour via GABA receptors and withdrawal of these steroids, which occurs naturally during the menstrual cycle and after giving birth can lead to severe depression. Some of the drugs that potentiate GABA have one major behaviourial effect, others produce different, or multiple effects. In some cases we can explain the behaviourial profile of a drug, eg. some benzodiazepines can be as effectively anti-convulsant and sedative as other drugs but disrupt movement less. This is largely because in a major brain area that controls movement only certain subtypes of GABA receptors occur and these receptors are not affected by benzodiazepines. In other regions many different types of GABA receptors occur and a more subtle explanation of the differential effects of drugs in these regions is required.

Using a combination of techniques that are unique to this lab, we are testing the pharmacological profiles of inhibitory synapses involving identified classes of neurones. We find subtle differences in the effects that benzodiazepines have at the different inhibitory synapses on single target cells and can now begin to see how the sedative and anticonvulsant effects of some drugs may result from actions at different GABA receptors located at different sites. In the longer term, this could lead, for example, to the development of therapies that effectively control epilepsy without making the patient somnolent, or sleeping pills which generate less 'tolerance' and less severe effects on withdrawal of therapy.

Modulation of Morphologically Defined Inhibitory Synapses by Benzodiazepines, Barbiturates and Steroids. G9514971N

Project Grant Report, Alex M. Thomson, January 1999

Methods Experiments were performed to explore the properties of inhibitory postsynaptic potentials (IPSPs) elicited by single, identified hippocampal interneurones in single postsynaptic target cells. Experiments involved dual or, more recently, triple intracellular recordings from pairs/triplets of synaptically connected neurones in slices of adult rat hippocampus (typically CA1). If the recorded IPSP was stable for 15 minutes under control conditions, drugs were applied in the bathing medium and their effects assessed by comparing averaged postsynaptic responses recorded during control and test periods. During analysis sequential averages of postsynaptic responses were obtained for each 2-5 minute epoch of the experiment and compared (for details of methods see recent papers and papers in preparation).

$GABA_A$ Receptor Pharmacology

Pentobarbitone and Diazepam To compare the relative efficacies of benzodiazepine agonists, which are anxiolytic, anti-convulsant and sometimes sedative with barbiturates which are powerful anaesthetics, IPSPs were challenged either with sodium pentobarbitone (250μM) or Diazepam (1-2μM). In some experiments, the effect of Diazepam was tested first and after the effect had reached a plateau, pentobarbitone was added.

Diazepam and Zinc To test whether a given inhibitory postsynaptic potential (IPSP) was sensitive to both the broad spectrum benzodiazepine Diazepam, whose efficacy depends on the inclusion of γ subunits (as well as $\alpha_{1/2/3/5}$ subunits) and to Zn^{2+} whose inhibition of $GABA_A$ currents depends on the absence of the γ (or δ) subunit, either Diazepam (1-2μM) or Zn^{2+} (100μM) was added to the bathing medium after a 15 minute stable control period. Once the response to the first drug had reached a plateau, the other drug was added.

Zolpidem and Diazepam To determine whether recorded IPSPs were mediated only by benzodiazepine-1 (BZ_1), only by BZ_2, or by both BZ_1 and BZ_2 receptors, studies with a selective BZ_1 agonist (Zolpidem) and a non-selective, $BZ_{1/2/3}$ agonist (Diazepam) were performed. Zolpidem (typically 0.1-0.2μM) was added to the bathing medium after control data had been recorded. After the effect of this agonist had reached a plateau, Diazepam (1-2μM) was added. The rational being that any response mediated by BZ_1 receptors would be potentiated by the Zolpidem and that any additional enhancement then produced by Diazepam would indicate involvement of $BZ_{2/3}$ receptors.

Zolpidem and Flumazenil Since the time course of the effects of benzodiazepines on some IPSPs was found to be complex (biphasic) a new study is testing the effects of blocking the BZ site after Zolpidem application. The effects of Zolpidem are recorded for one hour and then the low efficacy partial agonist Flumazenil (up to 4μM) is added.

Etomidate To determine whether IPSPs are mediated by $GABA_A$ receptors containing the $\beta_{2/3}$ subunit, Etomidate, which at low doses is selective for receptors containing $\beta_{2/3}$ rather than β_1 subunits, was added to the bathing medium after control data had been collected. A range of concentrations (0.1-3μM) is being studied.

$GABA_B$ receptor mediated IPSPs A study to determine i) whether 'slow IPSPs' that might be mediated by $GABA_B$ receptors can be activated by the GABA released by a single presynaptic interneurone, ii) whether there are any 'pure slow' IPSPs (ie. IPSPs that might be mediated only by $GABA_B$ receptors), iii) whether some IPSPs are be mediated only by $GABA_A$ receptors, or iv) by both $GABA_A$ and $GABA_B$ receptors. Presynaptic interneurones were driven to fire single spikes and bursts or trains of spikes of different frequencies and durations and postsynaptic responses recorded. Where a fast, short latency ($GABA_A$ receptor mediated) IPSP was elicited by single presynaptic spikes, either bicuculline or picrotoxin was added to the bathing medium to block fast IPSPs. Data were compared with and used to tune

the parameters of kinetic models of the activation of G-protein coupled receptors.

Histological Processing Recorded cells were filled with biocytin, slices fixed, processed histologically (Avidin-HRP-DAB) and presynaptic interneurones drawn in their entirety (100x). Selected axon terminals were cut from the slides, re-embedded and ultrathin sections cut for electron microscopy (for detailed methods see recent publications).

Immuno-fluorescence Most recently, to help classify the interneurones more completely, an additional step in the processing has been added which allows the calcium binding protein and neuropeptide content of biocytin filled cells to be studied (D. Hughes). After re-sectioning at 60μM the fixed slices are permeabilized by freeze-thawing in liquid N_2. Sections are incubated in sodium borohydride to quench free aldehyde groups and reduce background fluorescence, then in normal blocking serum prior to overnight incubation in a cocktail of primary antibodies (eg. mouse anti-PV + rabbit anti-CCK) and then in a cocktail of secondary antibodies tagged with fluorescent labels (eg. goat anti-mouse conjugated to FITC + goat anti-rabbit conjugated to AMCA) and with Avidin conjugated to Texas Red to reveal the biocytin-filled cells. The sections are then exposed sequentially to the relevant wave-lengths to reveal each fluorochrome in the same field and focal plane. Images are 'grabbed' from a CCD camera attached to the microscope and the digital images stored on disc and superimposed as required. Slices are then incubated in anti-Avidin, then in biotinylated goat anti-Avidin-D, and the original biocytin label visualized using the ABC method with DAB and nickel as chromogen. With this method the cells can be identified first as immuno-positive for eg. parvalbumin and then their entire dendritic and axonal arbours revealed and reconstructed.

Results and Discussion

IPSPs elicited in CA1 pyramidal cells by identified interneurones

i) Basket cell IPSPs (A. Ali *et al*, in press)

The IPSPs elicited in CA1 pyramidal cells by 29 morphologically identified basket cells exhibited a wide range of durations. Typically the basket cells that exhibited a fast spiking behaviour elicited the briefest IPSPs (width at half amplitude 30 ± 11 msec, mean ± SD) and burst firing basket cells the slowest (50 ± 12msec), with regular spiking basket cells eliciting IPSPs with a wide range of durations (35 ± 19msec). There was a strong correlation between IPSP rise time and width at half amplitude, but neither parameter correlated with IPSP amplitude. Future studies will test whether PV-immuno-positive Basket cells elicit IPSPs with different properties from those elicited by CCK-immuno-positive Basket cells.

ii) Bistratified cell and Basket cell IPSPs compared (H. Pawelzik, paper in preparation)

In a parallel study, the IPSPs elicited by 33 Basket cells, 16 Bistratified cells (and 2 putative chandelier cells) were compared. The ranges of rise times and widths at half amplitude for both Bistratified and Basket cell IPSPs were wide and overlapped almost totally. However, for a similar width at half amplitude, Bistratified cell IPSPs typically displayed a longer rise time, commensurate with their more distal origin and dendritic filtering. No evidence was found for the suggestion that distally located IPSPs are longer-lasting than proximal IPSPs.

iii) Oriens-Lacunosum-Moleculare (OLM) Interneurones (Pawelzik & Thomson, 1998)

To pursue further questions relating to IPSP time course and origin, the IPSPs elicited by OLM interneurones were recorded. These interneurones target the most distal apical dendrites of CA1 pyramidal cells in *stratum lacunosum moleculare*. Since these distal events are not apparent in somatic recordings, IPSPs were recorded in the distal apical dendrites of postsynaptic pyramidal cells. The IPSPs elicited by these interneurones (n=3) were relatively brief, with 10-90% rise times of 4.4-6.8msec and widths at half amplitude of 14-44msec.

Pentobarbitone and Diazepam compared (H. Pawelzik, paper in preparation)

All IPSPs tested were enhanced by Diazepam and by sodium pentobarbitone. Between 15 and 20 minutes after adding pentobarbitone, IPSPs elicited by 1 chandelier, 2 bistratified, and 8 basket cells were increased in amplitude by 82 ± 56%, in rise time by 150 ± 101% and in

width at half amplitude by 71 ± 29%. The effect of pentobarbitone was consistently greater than that of Diazepam and there was no difference apparent in the enhancements produced by pentobarbitone at the different types of inhibitory connections studied. With Diazepam however, Bistratified IPSPs were enhanced more than Basket cells IPSPs (average IPSP amplitude increased by 66 ± 48 cf. 19 ± 11, 10-90% rise time by 14 ± 24, cf. 2 ± 28% and width at half amplitude by 32 ± 35, cf. 3 ± 15%). This would suggest either that Basket cell IPSPs are mediated by a lower proportion of BZ-sensitive $GABA_A$ receptors than Bistratified cell IPSPs, or that the GABA released by Bistratified cell terminals saturates their postsynaptic receptors less effectively than the GABA released from Basket cell terminals. This because in addition to increasing deactivation time course, benzodiazepines increase $GABA_A$ receptor affinity for GABA.

Diazepam and Zinc (H. Pawelzik, paper in preparation)
Although Bistratified cell IPSPs were enhanced more by Diazepam than Basket cell IPSPs (see above), they were also more consistently (4 of 5 Bistratified, versus 4 of 10 Basket cell IPSPs) and more powerfully depressed by Zn^{2+} (to 71 ± 9% of control amplitudes n=4) than were Basket cell IPSPs (to 90 ± 12% n=4). This was the case whether Zn^{2+} was added alone or after Diazepam. Neither the postsynaptic cell's response to current injection, nor the proportion of apparent failures of transmission was affected by the added Zn^{2+}, suggesting that the IPSP depression was not mediated by a change in postsynaptic membrane properties or by a change in the probability of GABA release. Bistratified cell IPSPs therefore appear to be mediated in part by γ- (or δ-) subunit lacking receptors, despite the sensitivity of these IPSPs to Diazepam. The large enhancements of Bistratified cell IPSPs by Diazepam may therefore be best explained by incomplete saturation of the receptors by GABA at these connections. Their concomitant sensitivity to Zn^{2+}, may be explained by their mediation via both γ-containing and γ-lacking receptors. In contrast, Basket cell IPSPs were relatively insensitive to Zn^{2+} and appear to be mediated largely via γ-containing receptors. Their less powerful enhancement by Diazepam may be because the receptors are more fully saturated with released GABA. The larger size of many Basket cell terminals may represent a larger number of release sites per terminal and result in release of more than one vesicle at a time.

Zolpidem and Diazepam (H. Pawelzik, study in progress)
Of 14 stable IPSPs challenged with Zolpidem, 8 were enhanced within the first 5-15 minutes. The amplitude and duration of these IPSPs then remained stable until 25-30 minutes after the addition of Zolpidem. Six IPSPs were not enhanced during this first 30 minutes. This would suggest that some IPSPs are mediated by α_1 subunit containing $GABA_A$ receptors, while others are not. The four largest early responses to Zolpidem (23-50% increase in average amplitude) involved IPSPs elicited by classical Basket cells with their axons confined to *stratum pyramidale* (SP). The other 4 Zolpidem-enhanced IPSPs (10-15%) involved wide arbour Basket cells whose axons also innervated proximal *stratum oriens* (SO) and *stratum radiatum* (SR) and a putative Chandelier cell. The IPSPs that were not enhanced by Zolpidem in the first half hour were elicited by 3 Basket cells, one putative Chandelier cell, 2 Bistratified cells and an atypical SO Basket cell.

Four of the IPSPs that had exhibited no early enhancement by Zolpidem were exposed to Diazepam (after equilibration and development of the 2nd Zolpidem phase see below). All 4 were further enhanced, suggesting that these IPSPs are mediated at least in part by BZ_2 receptors. One of these IPSPs, which resulted from the only connection involving a proximally targeting presynaptic interneurone (an atypical SO basket cell) that showed neither an early nor a late enhancement by Zolpidem, nevertheless increased in amplitude after the addition of Diazepam. This connection may therefore have been mediated solely by BZ_2 or BZ_3 (α_5 subunit containing) receptors. Only one of the 3 IPSPs that did exhibit an early Zolpidem enhancement and was then tested with Diazepam was further enhanced, suggesting perhaps that some Basket cell connections are mediated only by BZ_1 receptors.

Some bias therefore in the location of α_1-subunit-containing receptors ? Although the present data are preliminary and require further confirmation there is some bias, if no absolute distinction apparent in the present data. Classical Basket cells elicited the IPSPs that were most strongly enhanced by Zolpidem in the first 30 minutes, while the IPSPs elicited by putative Chandelier cells, non-classical Basket cells and Bistratified cells were enhanced less, or not at all during the first half hour. However, 3 classical Basket cells also elicited IPSPs that were not enhanced by Zolpidem in the first 30 minutes. It remains to be determined whether another property of the presynaptic basket cell (eg. immunocytochemical profile) correlates with the sensitivity of the IPSPs it elicits in pyramidal cells to Zolpidem. Further studies are also required to test whether the pharmacological profile of the IPSPs elicited in more than one target pyramidal cell by the same interneurone are identical.

Possible implications in the efficacy of sedative and anti-convulsant drugs

Although preliminary, the bias or pattern that has so far emerged correlates with a recent study of the behaviourial effects of Diazepam in a mouse with a point-mutated α_1 subunit which forms BZ-insensitive receptors (Rudolph *et al*, 1998). In other respects the mouse is normal, but while Diazepam is anti-convulsant in half of the animals, it is not sedative. Basket cells innervate pyramidal cell somata and have a powerful effect on the firing of hundreds to thousands of postsynaptic cells. They can synchronize the firing of their targets by shunting excitation and then by eliciting 'rebound firing' as they decay. If the hypnotic effects of some neuroactive drugs relate to their ability to promote, slow or modify cortical rhythms, Basket cell inputs to pyramidal cells would be a very effective place for them to act. Conversely, for a drug to be an effective anti-convulsant, it does not need to block somatic firing, but would be effective if it reduced excitatory input to the dendritic tree (pyramids do not receive excitatory inputs on their somata or proximal dendrites) by shunting excitatory inputs there. A prediction could therefore be that effective sedatives would need to enhance proximally located BZ_1 receptors, while effective anti-convulsants could work simply through the more distal BZ_2 receptors and need not, if selective drugs were developed, be sedative. Clearly these predictions are very preliminary and require considerably more detailed investigation.

Enhancement of IPSPs by Zolpidem (and Diazepam) can be biphasic

Four of 5 IPSPs that had exhibited an early enhancement with Zolpidem and then remained stable for another 15-20 minutes, increased in amplitude again sharply between 25 and 30 minutes after Zolpidem addition. The mechanism underlying this second increase is unclear, but might be important in the therapeutic use of these drugs as for example 'premeds'. The biphasic effect was not dependent on Zolpidem as agonist and is unlikely to be simply a concentration effect, since 3 of 4 Basket cell IPSPs exhibited a similar biphasic enhancement with Diazepam alone. Nor was the late enhancement confined to IPSPs that showed an early enhancement. Five of the 6 IPSPs that did not change in amplitude for the first 30 minutes after Zolpidem addition nevertheless increased sharply in amplitude during the next 5 minutes.

This biphasic enhancement was not, however, observed at all connections, IPSPs elicited by 2 Bistratified interneurones exhibited a gradual increase in amplitude and duration during exposure to Diazepam. Nor was the enhancement of IPSPs by other $GABA_A$ receptor modulators biphasic. Pentobarbitone (n=13 IPSPs) and Etomidate (n=5) produced monophasic enhancements that reached a plateau. The biphasic effect may therefore be BZ_1 dependent.

Preliminary studies with Flumazenil (H. Pawelzik).

After equilibration with Zolpidem (60 minutes) and stabilization of the second phase of enhancement, Flumazenil decreased IPSPs to control levels (n=3). These very preliminary data suggest that both phases of the Zolpidem enhancement are mediated by BZ-sensitive receptors and also that the IPSPs studied so far do not involve α_5-subunit containing receptors.

Preliminary studies with Etomidate (H. Pawelzik)

Etomidate enhanced all 5 IPSPs tested to date, but was effective at very different concentrations (from 0.1 to 1μM). Studies are in progress to assess the minimally and

maximally effective doses with IPSPs elicited by all major classes of interneurones.

Slow, putative $GABA_B$ receptor mediated IPSPs (Thomson and Destexhe, in press)
In most dual recordings tested in which an interneurone was presynaptic to a pyramidal cell, the IPSP was readily elicited by a single presynaptic interneuronal spike, was of brief latency and was blocked entirely by a $GABA_A$ antagonist (bicuculline or picrotoxin). In 3 of 85 dual recordings in neocortex however, single presynaptic spikes elicited no response in the postsynaptic pyramidal cell, but trains of at least 3 or 4 spikes elicited a longer latency (>20msec) IPSP that was insensitive to bicuculline in the 1 IPSP so tested. These IPSPs increased steeply in amplitude with an increase in the number of spikes in the train, up to 10 spikes. Thereafter increasing numbers of spikes produced no further increase in the IPSP. Computational models (Dr Destexhe) of GABA release, diffusion and uptake suggested that extracellular accumulation of GABA cannot alone account for the non-linear relationship between spike number and slow IPSP amplitude. However, cooperativity in the kinetics of $GABA_B$ transduction mechanisms provided non-linear relations similar to the experimental data. In the CA1 region of the hippocampus, 8 dual recordings in which a presynaptic interneurone elicited a fast, $GABA_A$ IPSP were challenged with a $GABA_A$ antagonist. In 6 the IPSP was totally blocked. In 2 however, blockade of the fast IPSP revealed a slow IPSP that was largely blocked by CGP35348. These IPSPs exhibited the same dependence upon spike number as the slow IPSPs studied in neocortex. These results suggest that i) the firing of a single interneurone can release sufficient GABA to activate $GABA_B$ receptors, though whether $GABA_B$ receptors are more typically activated by synchronous release from several interneurones remains to be determined, ii) that some inhibitory connections in both CA1 and neocortex are mediated solely by $GABA_A$ receptors when the presynaptic interneurone fires alone, iii) that other inhibitory connections in neocortex are mediated solely by slow (probably $GABA_B$) receptors and iv) that other connections are mediated by both types of receptors.

Triple intracellular recordings have recently been implemented. Preliminary studies have demonstrated, for example, that the IPSPs elicited in simultaneously recorded pyramidal cells by the same presynaptic interneurone are extremely similar in time course, despite large differences in average amplitude. It has proved possible to record 2 synaptic connections simultaneously for up to 3 hours during drug applications.

Immuno-fluorescence (D. Hughes, M. Ilia and A.P. Bannister)
Triple fluorescence to reveal biocytin-labelled cells and cells that are immuno-positive for parvalbumin (PV), Calbindin (CB) and/or cholecystokinin (CCK) is now working well, even in slices that have been incubated in the recording chamber for up to 12 hours and in cells recorded for up to 2 hours. We can now distinguish PV-positive and CCK-positive Basket cells and confirm that PV-positive cells are immuno-negative for CB, while Bistratified cells are more typically immuno-negative for PV, but immuno-positive for CB. Somewhat surprisingly, we find a number of wide arbour Basket cells, ie. cells that innervate SO and SR as well as SP that are immuno-positive for PV. It will be of interest to determine whether the axon terminals of these interneurones are immuno-positive for PV, since dendrite-targeting axon terminals are rarely reported to be PV-immuno-positive. To date, as expected, the axonal arbours of CCK-immunoreactive cells have been confined to SP.

Excitatory inputs onto interneurones (Ali & Thomson, 1998, Ali *et al* 1998)
The dependence of synaptic efficacy on presynaptic firing rate and pattern has been studied and compared for excitatory pyramidal inputs onto a range of identified interneurones in hippocampus and neocortex. Certain types of interneurones were found to receive excitatory postsynaptic potentials (EPSPs) that facilitated on repetitive activation, other specific classes received EPSPs that depressed. Studies of a novel form of presynaptic depression apparent at only certain subsets of connections are in progress (paper in preparation).

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