Modulation of Visual Responses in the Rat Superior Colliculus by Metabotropic Glutamate Receptors

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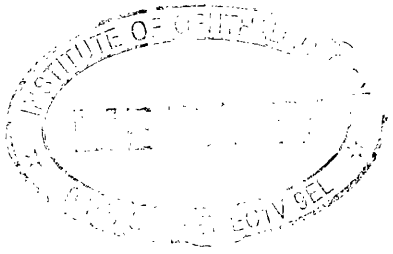
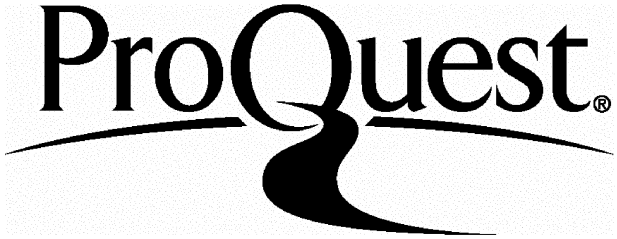
Jennifer Cirone

Department of Visual Science
Institute of Ophthalmology
University College London





ABSTRACT

Neurones in the superficial layers of the superior colliculus (SSC) respond to novel visual events. Cells in the SSC project via neurones in the deep layer of the superior colliculus to motor nuclei which generate appropriate behavioural and avoidance responses to novel sensory stimuli. Glutamate is a neurotransmitter at the retino-collicular and cortico-collicular synapse. Glutamate receptors can be classified as either ionotropic or metabotropic (mGluRs). At present, 8 mGluRs have been cloned (mGluR1 – mGluR8), and these can be divided into 3 groups based on sequence homology, pharmacology and coupling to 2nd messenger pathways. There is evidence that metabotropic glutamate receptors may be present on SSC neurones and SSC afferents. This study examines how mGluRs may modulate the response properties of visually responsive cells in the SSC. Iontophoretic application of pharmacological agents including selective mGluR agonists and antagonists are used to probe the functional effects of mGluR manipulation in an *in-vivo* preparation. All three groups of receptor appear to be activated by endogenous glutamate during visual synaptic transmission. Activation of Group I mGluRs (mGluR1 and mGluR5) cause a depression of the visual response. Activation of both Group II (mGluR2 and mGluR3) and Group III mGluRs (mGluR4, mGluR6, mGluR7 and mGluR8) causes a facilitation or inhibition of the visual response in individual neurones. Neurones in the SSC detect novel visual stimuli by producing a decline in the response to repeated stimuli, this is called habituation. Group III (but not Group I or Group II) mGluRs contribute to response habituation in the SSC and therefore have a functional role in detecting stimulus novelty. Activation of Group II receptors is dependent upon the intensity of the stimulus, probably due to their location away from the central region of the synapse. Immunohistochemical data presented here details the distribution of selected mGluRs in the sub-cortical retinofugal pathway of the rat, ferret and cat. Analysis shows that the distribution in these three species is dissimilar. This suggests that mGluRs may have different functional roles in visual processing in different species.

Declaration

I declare that this thesis submitted for the degree of Doctor of Philosophy is my own composition and the data presented herein is my own original work, unless otherwise stated.

Jennifer Cirone

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Finally, I must thank Simon Jackson for all his endless love and support.

List of Abbreviations

μm	micrometer
1S,3R-ACPD	1S,3R-1-aminocyclopentane-1,3-dicarboxycyclic acid
2R,4R-APDC	2R,4R-4-aminopyrrolidine-2,4-dicarboxylate
3,5-DHPG	(S)-3,5-dihydroxy phenylglycine
4CPG	4-chlorophenylguanidine hydrochloride
5-HT	Serotonin
7TM	7 transmembrane
AA	Arachidonic acid
AC	Adenylate cyclase
AMPA	D,L-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AP	Anterior-posterior
APV	Amino-5-phonopentanoic acid
BK	Big calcium sensitive potassium channel
Ca^{2+}	Calcium ion
cAMP	Cyclic adenosine monophosphate
CAT	Choline acetyltransferase
cd	Candela
Cd^{2+}	Cadmium ion
cGMP	Cyclic guanosine monophosphate
CGP35348	P-(3-aminopropyl)-P-di-ethoxymethyl-phosphonic acid
CHPG	((RS)-2-chloro-5-hydroxy phenylglycine
Cl^-	Chloride ion
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
CPPG	R,S-α-cyclopropyl 1-4-phosphono phenylglycine
CRT	Cathode ray tube
CSF	Cerebrospinal fluid
CTX	Cholera toxin
DAG	Diacylglycerol
D-AP4	D-2-amino-4-phosphonobutanoate
dLGN	Dorsal lateral geniculate nucleus

DSC	Deep layers of the superior colliculus
ECG	Electrocardiogram
EEG	Electroencephalogram
I_{AHP}	Calcium dependent slow after hyperpolarisation current
Ibo	Ibotenate
IGL	Intergeniculate leaflet
iGluR	Ionotropic glutamate receptor
GABA	γ-aminobutyric acid
$GABA_A$	γ-aminobutyric acid receptor A
$GABA_B$	γ-aminobutyric acid receptor B
GCL	Ganglion cell layer of the retina
GDP	Guanosine diphosphate
GIRK	G-protein coupled inwardly rectifying potassium current
Glu	Glutamate
GTP	Guanosine triphosphate
H^+	Hydrogen ion
I_{CAN}	Slow inactivating voltage-dependent current
IgG	Immunoglobulin
$I_{K(slow)}$	Slow potassium current
I_{LEAK}	Potassium leak conductance
I_M	Non inactivating voltage dependent potassium current
Immuno	Immunohistochemistry
INL	Inner nuclear layer of the retina
In-situ	In-situ hybridization
i.p.	Intraperitoneal
IP_2	Inositol biphosphate
IP_3	Inositol triphosphate
IPL	Inner plexiform layer of the retina
IPSC	Inhibitory post synaptic current
K^+	Potassium ion
kg	Kilogram
L-AP4	L-2-amino-4-phosphonobutanoate

L-CCG-II	(2S,1'R,2'R)-2-carboxycyclopropyl glycine
L-CSA	L-cysteine sulfinic acid
LY341495	αS-α-amino-α-(1S,2S)-2-carboxycyclopropyl-9H-xanthine-9-propanoic acid
LY354740	(+)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid
LY367385	S-(+)-α-amino-4-carboxy-2-methylbenzeneacetic acid
MAO	Monoamine oxidase
M-AP4	(S)-2-amino-2-methyl-4-phosphono butanoate
MCCG	(2S,3S,4S)-2-methyl-2-carboxycyclopropyl glycine
MCPG	(2S,3S,4S)-2-methyl-2-carboxycyclopropyl
Mg^{2+}	Magnesium ion
ML	Medio-lateral
mGluR	Metabotropic glutamate receptor
MPEP	2-methyl-6-(phenylethynyl)pyridine
mRNA	Messenger ribonucleic acid
mV	millivolt
nA	Nano amp
Na^{+}	Sodium ion
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NOS	Nitric oxide synthase
NR	NMDA receptor subunit
OH^{-}	Hydroxide ion
ON	Optic nerve
OT	Optic tract
PA	Phosphatidic acid
PBS	Phosphate buffered saline
PI	Phosphoinositide
PIP_2	Phosphatidylinositol-4,5-*bis*-phosphate
PKA	Protein kinase A
PKC	Phosphokinase C
PLA_2	Phospholipase A_2

PLC	Phospholipase C
PLD	Phospholipase D
PPG	R,S-4-phosphonophenylglycine
PSB	Pontamine sky blue
PSTH	Peristimulus time histogram
PTX	Pertussis toxin
Quis	Quisqualate
RF	Receptive field
RGC	Retinal ganglion cell
R,S-MPPG	(RS)-2-methyl-4-phosphonophenyl glycine
SC	Superior colliculus
SGI	Stratum griseum intermediale
SGS	Stratum griseum superficiale
SO	Stratum opticum
SSC	Superficial layers of the superior colliculus
SZ	Stratum zonale
t-ACPD	(±)-1-aminocyclopentane-trans-1,3-dicarboxylic acid
tADA	*trans*-azetidine-2,4,dicarboxylic acid
vLGN	Ventral lateral geniculate nucleus
Zn^{2+}	Zinc ion

Contents in Brief

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1.0 Superior Colliculus

1.1 General overview of the function of the superior colliculus

The superior colliculus (SC) is a phylogenetically ancient midbrain structure where information from different sensory modalities converges to generate an appropriate behavioral response to a novel sensory event (Stein 1981 & 1984). It is a laminated structure and is generally divided into two components, the most superficial layers are entirely concerned with the visual system and are driven by afferents from the retina and visual cortex. In these superficial layers, the SC has a role in primary sensory processing. The deep SC layers contain neurones that are frequently multimodal and are responsive to auditory, somatosensory and nociceptive information. The deep SC layers also receive inputs from the superficial SC although the deep layers receive very little or no direct retinal input. The deep SC integrates these multisensory inputs in order to generate appropriate motor commands in response to changes in the animal's environment (Stein & Meredith 1994).

Studies in SC function began as long ago as 1872, when the role of the SC in the generation of eye movements was investigated (see Stein 1984). Interest in collicular function was revived by a series of experiments starting in the 1960's which described a profound neglect of visual space following ablation of the SC in several species (Sprague & Meikle 1965, Casagrande *et al.* 1972, Goodale & Murison 1975). These lesion experiments led to the proposal of there being "two visual systems" (Diamond and Hall 1969, Schneider 1969) with the retino-tectal pathway concerned with localization, orientation and response to novel visual stimuli – "**Where** is it?", whereas the retino-geniculate cortical pathway is concerned with pattern discrimination of visual stimuli – "**What** is it?".

Based on lesion and stimulation experiments, the SC has been implicated in the generation of many complex behaviours including, the direction of eye movements (McHaffie & Stein 1982), localization of visual events (Goodale *et al.* 1978, Sahibzada *et al.* 1986, Krauthamer *et al.* 1992), avoidance reactions (Olds & Olds 1962, Redgrave *et al.* 1981), defence (Goodale & Murison 1975), turning (DiChiara & Morelli 1982), locomotor exploration (Dean *et al.* 1982, Heywood & Cowey 1987) and approach behaviors (Dean *et al.* 1986, Westby *et al.* 1990). There is evidence that the different behaviors of approach and avoidance are generated by different cell populations in the SC (Dean *et al.* 1986, 1988, 1989, Redgrave *et al.* 1987a, 1987b, Sahibzada *et al.* 1986, Westby *et al.* 1990). Defense behaviors are believed to be generated by the ipsilateral descending projection from the SC to the parabigeminal, cuneiform and pontine nuclei (Kilpatrick *et al.* 1982, Cools *et al.* 1984, Redgrave *et al.* 1987a, Westby *et al.* 1990), whilst approach behaviors are mediated by the contralateral tecto-reticulo-spinal descending projection (Kilpatrick *et al.* 1982, Redgrave *et al.* 1987a, Westby *et al.* 1990). The SC is also able to generate changes in physiological parameters such as heart rate (DaMico *et al.* 1998), blood pressure (Keay *et al.* 1986, DaMico *et al* 1997), and cortical EEG (Dean *et al.* 1984, Redgrave & Dean 1985) in response to novel sensory events. It is therefore apparent that the SC is associated with initiating a full integrated response to novel stimuli and not merely with orienting the head and eyes towards novel visual stimuli for the purpose of analysis by the geniculo-striate system. It is feasible to assume that in small animals such as rats that have many predators, that the responses generated by the SC will be of critical importance to the survival of the animal (Stein 1981).

1.2 Anatomy of the SC

The adult superior colliculus can be divided into seven alternating fibrous (or cell body poor) and cellular (cell body rich) laminae, in the dorsal to ventral direction these are named:-

1. Stratum Zonale (fibrous) }
2. Stratum Griseum Superficiale (cellular) } Superficial Superior Colliculus
3. Stratum Opticum (fibrous) }

4. Stratum Griseum Intermediale (cellular) }
5. Stratum Album Intermediale (fibrous) } Intermediate and Deep Superior Colliculus
6. Stratum Griseum Profundum (cellular) }
7. Stratum Album Profundum (fibrous) }

The superficial and deep layers of the SC have been thought of as being functionally separate entities (Harting *et al.* 1973), however, there are difficulties in describing the SSC and DSC as completely separate structures as visuomotor deficits only appear when lesions of the SC include the deeper layers (Casagrande *et al.* 1972). Also, the topographic organisation of receptive fields is maintained on progression from superficial to deep layers (Siminoff 1966) which might not be expected if the SSC and DSC were separate structures. There is anatomical (Hilbig & Schierwagen 1994) and physiological (Ozen *et al.* 2000) evidence for connections between the superficial and deep SC in the rat.

The data presented in this thesis are concerned with the modulation of visual processing in the SC, therefore this introduction will mainly focus on the anatomy, physiology and pharmacology of the superficial, visually responsive layers.

1.2.1 Retinofugal Pathway

The photoreceptors present in the rat retina consist almost entirely of rods, rare cone photoreceptors contribute <1% of all photoreceptors (Szel & Rohlich 1992). Nearly all of these cones have a photopigment with peak sensitivity in the 500 – 520nm range (a medium wave sensitive pigment), with the other rare class of cone photopigment being maximally sensitive at 370nm (ultraviolet range, Jacobs *et al.* 1991). The distribution of ganglion cells across the rat retina is fairly uniform in contrast with higher order mammals, with the ratio of highest (area centralis) to lowest density being 5:1 (Perry 1979, McCall *et al.* 1987,). The representation of central visual fields in the SSC is not substantially magnified in comparison to the peripheral field (Forrester & Lal 1966, Siminoff *et al.* 1966, Stein 1981).

Output from the retina to the SC is from retinal ganglion cells (RGCs). The number of RGCs in the rat as determined by retrograde labeling of retinorecipient nuclei is approximately 100,000 – 115,000 (Lam *et al* 1982). In mammals, RGCs can be classified into three main groups, in the rat RGCs have been further subdivided into five classes, their properties are described in Table 1.1. Studies examining the conduction velocities of retino-collicular axons identified 3 groups of axons with different velocities (Sumitomo *et al.* 1969, Fukuda 1977). The fastest conducting axons terminate in deep stratum griseum superficiale (SGS) and stratum opticum (SO) and the slower conducting axons terminate in upper SGS. RGC axons enter the SC through the SO. Retino-recipient SSC cells receive multiple retinal inputs (Lo *et al.* 1998).

A very large proportion of RGCs collaterals (approx. 90%) innervate the SC (Linden & Perry 1983, Dreher *et al.* 1985). In the rat, the SC projection is predominantly contralateral, with the ipsilaterally projecting cells confined to a crescent shaped region of the lower temporal retina (Cowey & Perry 1979, Jeffery & Thompson 1986). The small ipsilateral projection is to the centro-medial region of dLGN and the rostral border of the SC and represents central binocular visual space.

Table 1.1 Different types of ganglion cells in the rat retina

Type	Somal size	Axon dia.	Size dendritic field	Notes	Refs
I	16-32μm	0.9μm conduction velocity approx 16.1 m/sec	220-790μm 3-7 primary dendrites	Distributed over whole retina, highest in temporal retina. Estimate of 1-4% of RGCs, form 10–14% of ipsilateral projection. More collaterals project to DLG than SC.	1,2,3,5, 6,7,8,9
IIa	11-22μm	0.6μm	max 250μm 1 – 4 primary dendrites	Project to DLG and SC. Higher density in central retina.	5,6,8
IIb	7-15μm	0.4μm	max 140μm	Largely innervate SC. Higher density in central retina.	5,8
IIIa	6-15μm	0.35μm	max 540μm	Largely innervate SC.. Higher density in central retina.	5,8
IIIb	15-24μm	0.6μm	max 410μm, long primary dendrites with very few branches	Project to both DLG and SC. Higher density in central retina.	5,6,8

References

1. Sefton 1968
2. Sumitomo *et al.* 1969
3. Fukuda 1977
4. Ni & Dreher 1981
5. Dreher *et al.* 1985
6. Martin 1986
7. Reese & Cowey 1986
8. Schall *et al.* 1987
9. Peichl 1989

1.2.2 Cellular Morphology of Cells in the SSC

Cells of the rat SC were first described by Cajal in 1911 (Sefton & Dreher 1995). The cells were named after the orientation of their dendrites (horizontal, vertical and stellate). Several types of interneurones have been identified and these have axons that are confined to the superficial layers. Table 2 describes the location and possible function of cells that have been described in the rat SC.

Table 1.2 – Different cell types of the rat SSC

Cell Name	Location of cell body	Notes	Ref
Horizontal	SZ*, upper SGS,	Interneuron, cell body 10μm in dia, depth of dendritic spread confined locally, but very wide horizontal spread (100μm) , low spine density	2,3,8
Stellate	SZ, SGS, SO	Possible interneuron, dendrites have no overall orientation. Axon is in same layer as cell body.	2,8
Marginal	SZ, upper SGS	Interneuron, 2 main dendrites, many dendritic spines on some cells, smallest cells in SSC 5-8μm dia cell body. Axons have an extensive local distribution.	2,8
Piriform	SZ/SGS junction	Relay cell, 2 ascending & 2 descending primary dendrites, axonal projection to deeper layers, many dendritic spines on some cells. 10-15μm cell body dia.	2,8
Wide Field	SGS/SO junction	Relay cell, receive convergent retinal and cortical inputs. 3 to 7 primary dendrites, axonal projection to deeper layers. cell body 15-25μm dia. Dia. of dendritic field over 400μm. Project to DLG, VLG, LP.	1,2,3,4,6,7 10
Narrow Field Vertical	Upper SGS	Relay cell, receive retinal input. 2 ascending & 2 descending primary dendrites, axonal projection to deeper layers. Project to DLG, VLG, LP.	2,3,6,7,8,9
Vertical Fusiform	SZ, SGS, SO	Narrow vertically oriented dendritic fields (100-200μm wide), elongated cell body, 12-18μm dia. Deep Fusiform cells (SO) have larger cell bodies (20μm). Axon is within same layer as cell body.	2
Pyramidal cells	SZ, SGS	Similar to vertical fusiforms, elongated cell body 15μm, Narrow vertically oriented dendritic fields (100-250μm wide), 1 or 2 thick apical dendrites which branch many times, particularly towards the surface of the SC. Axon projects to deep SC	2

References

1 Humphrey 1968
2 Langer & Lund 1974
3 Tokunaga & Otani 1976
4 Labriola & Laemle 1977
5 Fukuda & Iwama 1978
6 Mason & Groos 1981
7 Mackay-Sim *et al.* 1983
8 Sefton & Dreher 1995
9 Lo *et al.* 1998
10 Hilbig *et al.* 2000

* stratum zonale layer

1.2.3 Cortico-Tectal Projection

The projection to the SSC from ipsilateral areas of visual cortex arises from large pyramidal cells located in the upper two-thirds of layer V (Sefton *et al.* 1981, Olavaria & Van Sluyters 1982, Schofield *et al.* 1987, Hallman *et al.* 1988, Hubener & Bolz 1988, Dori *et al.* 1992). These cortical neurones have a thick apical dendrite, which terminates in cortical layer I (Schofield *et al.* 1987, Larkman & Mason 1990, Mason & Larkman 1990, Rumberger *et al.* 1998). Cortico-tectal neurones send collaterals to other areas including the pons (Hallman *et al.* 1988). Projections from different areas of cortex terminate in different SC laminae (Lund 1966). The principal projection from area 17 terminates in deep SGS and upper SO (although a small projection extends up to the collicular surface). 18a (lateromedial, laterointermediate and laterolateral regions) terminates deep in SO and SGI, with a light input to lower SGS. Area 18 projection does not have a superficial projection, with all terminals being present in deep SC regions (Harvey & Worthington 1990). Retinal and cortical afferents to SSC cells represent the same point in visual space.

1.2.4 Other SSC Afferents

The most important and largest afferent innervation of the SSC is from the retina and areas of visual cortex. However, many other structures in the rat send afferent fibres to the SC although the functional and physiological nature of many of these connections is unknown. Some of these connections to the SSC are shown in Table 3.

Table 1.3 – Afferent projections to the SSC

Projection From:	Reference:
Contralateral SC	Bickford & Hall 1989, Hilbig *et al.* 2000
Intergeniculate leaflet	Taylor *et al.* 1986, Moore *et al.* 2000
Parabigeminal nucleus	Pasquier & Tramezani 1979, Watanabe & Kawana 1979, Stevenson & Lund 1982a, 1982b, Linden & Perry 1983, Sefton & Martin 1984, Taylor *et al.* 1986
Anterior Pre-tectal nucleus	Foster *et al.* 1989
Posterior pretectal nucleus, nucleus of the optic tract	Taylor *et al.*1986
Dorsal Raphe	Waterhouse *et al.* 1993, Villar *et al.* 1988
Locus coeruleus	Waterhouse *et al.* 1993
Posterior commisure	Taylor *et al.* 1986
Prepositus Hypoglossi nucleus	Ohtsuki *et al.* 1992
External ventral lateral geniculate nucleus	Brauer & Schober 1982
Dorsal & Ventral nuclei of the lateral lemniscus	Tanaka *et al.* 1985, Taylor *et al.*1986
Retrosplenial cortex	Garcia Del Cano *et al.* 2000

1.2.5 SSC Efferent Connections

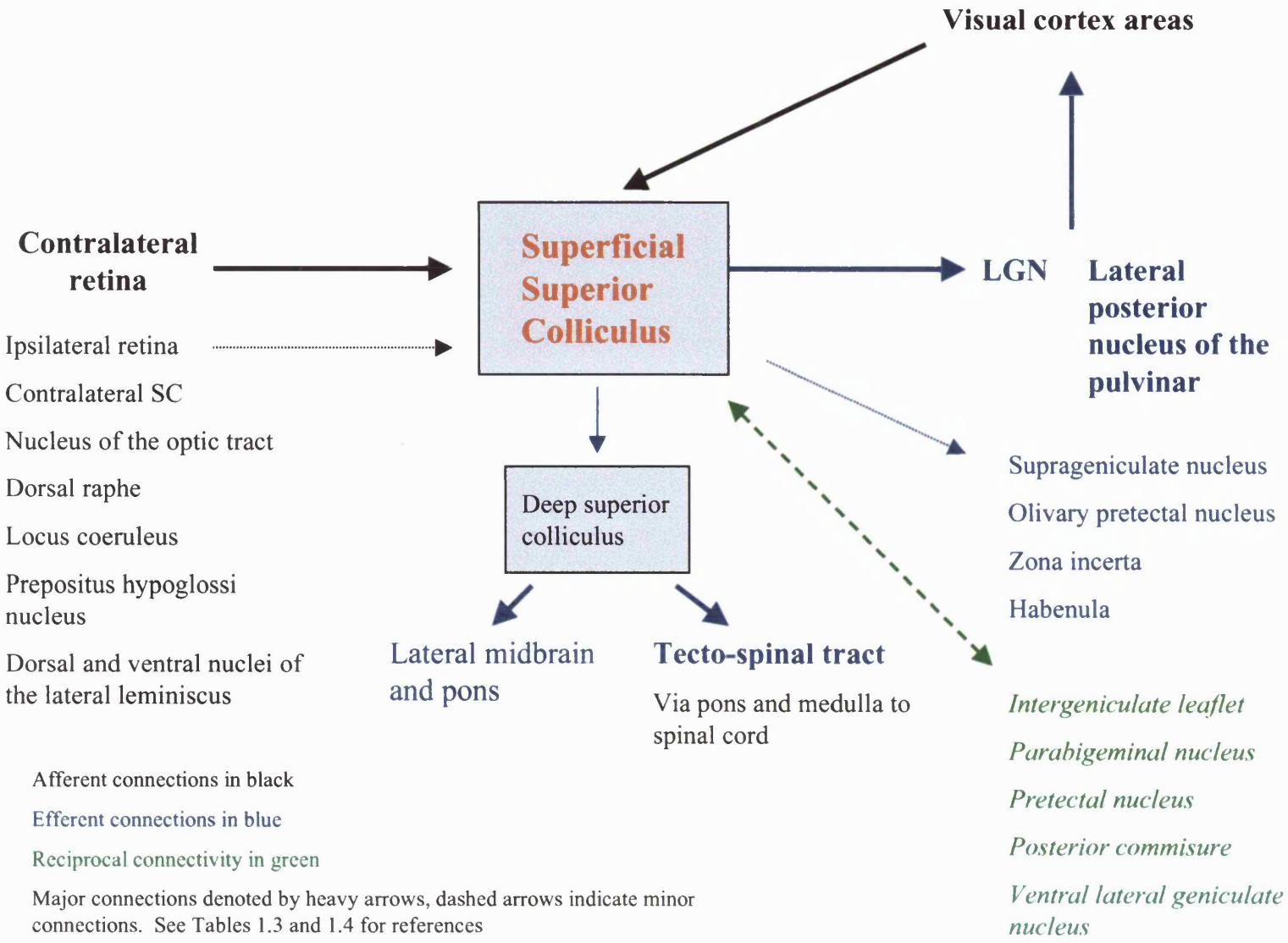
The most important connections from the SSC are to two thalamic nuclei, the lateral geniculate nucleus (Brauer *et al.*1979, Perry 1980, Pasquier & Villar 1982, Mackay-Sim *et al.* 1983, Reese 1984, Taylor et al 1986, Reese 1988, Turlejski *et al.* 1993, Hilbig *et al.* 2000) and the lateral posterior nucleus of the pulvinar (Perry 1980, Mason & Groos 1981, Donnelly *et al.* 1983, Taylor *et al.*1986, Takahashi 1995, Hilbig *et al.* 2000). These thalamic nuclei project to areas of visual cortex. The tecto-geniculate projection originates exclusively from the caudal two thirds of the SC, (Mason & Groos 1981, Harting *et al.* 1991) and terminates in the postero-dorsal part of the dorsal lateral geniculate (dLGN, Reese 1984).

The SSC also projects to other thalamic regions, including the ventral lateral geniculate (vLGN, Pasquier & Villar 1982, Linden & Perry 1983, Mackay-Sim *et al* 1983, Sefton & Martin 1984, Takahashi 1985, Taylor *et al.* 1986,), the intergeniculate leaflet (Taylor *et al* 1986) and the suprageniculate nucleus which originates in the SO layer of the SC (Linke 1999). There are also a number of projections that are functionally poorly characterized, these are summarized in Table 4.

Table 1.4 – Efferent connections from the SC

Projection to:	**Reference:**
Parabigeminal nucleus	Taylor *et al.* 1986, Hilbig *et al.* 2000
Olivary pretectal nucleus	Taylor *et al.* 1986
Anterior pretectal nucleus	Takahashi 1985, Taylor *et al.* 1986
Pretectal nucleus	Martin & Sefton 1981
Posterior commisure	Taylor *et al.* 1986
Zona incerta	Taylor *et al.* 1986
Habenula	Taylor *et al.* 1986

Figure 1.1 Schematic of afferent and efferent connections of the rat superficial superior colliculus



1.3 Receptive Field Properties of Visually Responsive Neurones in the Superficial Layers

Although the response properties of visually responsive cells in the SSC have been examined most extensively in the cat (see McIlwain & Buser 1968, Sterling & Wickelgren 1969, Berman & Cyander 1972, Dreher & Hoffmann 1973), a number of different studies have characterised the response properties of cells in the rat SSC. A summary of findings is shown in Table 5. Although the work has been carried out in a number of different laboratories, there is a high degree of consistency in the observed results.

Table 1.5 – Response properties of rat SSC neurones

Response Properties	Ref
Receptive fields are organised in a topographical fashion with regard to visual space, upper visual space is represented in medial SC, lower visual space in lateral SC, rostral visual space in anterior SC and temporal visual space in posterior SC.	1
The receptive fields can be classified as either ON, OFF, ON-OFF, ON-OFFinhibited and movement sensitive. The majority of receptive fields are organised as a simple circular or elliptical centre surround shape. Spatial location of receptive fields is preserved on moving from dorsal to ventral SC.	1,4
Cells can be divided into 4 classes:- I – Innervated by slow conducting RGC's, mainly ON or ON-OFF receptive fields, respond best to slowly moving stimuli (5-10^0/sec) II – Innervated by intermediate RGC's, mainly ON and ON-OFF type receptive fields, respond best to moving stimuli III – Innervated by fastest conducting RGC's, mainly OFF or ON-OFF type receptive fields, respond to stationary and moving stimuli IV - Innervated by slowly conducting RGC's These different cell classes have a dorso-ventral distribution, with class I found in most superficial layers and Class IV in the deepest SSC layers. Physiological classes may have morphological correlates:- Glass I=Vertical fusiforms, II-pyriforms, III=narrow field, IV=wide field vertical. I-III are more commonly found in anterior SC, with IV being more common in posterior SC.	2,4
Receptive field size range from 2^0-90^0. Receptive field size can be used to classify cells into three groups:- 5.4^0±2.3^0 8.1^0±2.1^0 18.1±15.9^0 Receptive field size generally increases from superficial to deeper SSC layers. Receptive field sizes of RGC's are around 3^0-10^0, SSC cells with large receptive fields are thought to be wide field cells which have extensive dendritic fields and receive convergent retinal input	2,4
Some SSC cells are orientation selective, these are mainly found in the deep SGS/SO with most of these cells responding to a stimulus with moves upwards, the preferred orientation is not sharply tuned.	4 (14%) 5 (16%)
In response to a "maintained" stimulus, the response of SSC cells is reduced. This is more pronounced in the most superficial layers.	2,3,4

References
1. Siminoff *et al.* 1966 2. Humphrey 1968 3. Fukuda *et al.*1978 4 Gonzalez et al. 1992 5. Sefton 1969

1.4 Pharmacology of the Rat Superior Colliculus

There is evidence that a number of different neurotransmitter receptors are present in the SSC and that some of these neurotransmitters are known to affect the responses of neurones in the SSC. Only known effects relating to neurotransmitters and receptors in superficial layers of the rat are discussed at length in this section. For a more thorough discussion of transmitters involved in deep SC and in other species see Binns 1999.

1.4.1 Glutamate

Retinal or cortical deafferentation results in a decrease of glutamate in the SC (Lund-Karlsen & Fonnum 1978, Fosse & Fonnum 1986, Sakurai *et al.* 1990, Sakurai & Okada 1992, Ortega *et al.* 1995, Li *et al.* 1996) and immunohistochemistry in the cat has shown that glutamate is located in retinal and cortical terminals to the SC (Mize & Butler 1996, Jeon *et al.* 1997). Retrograde labelling of the cortico-collicular projection labels cells in the visual cortex which are glutamate and aspartate immunopositive (Dori *et al.* 1992). Immunohistochemical studies show that ionotropic glutamate receptors (NMDA and AMPA) are located in the rat SSC (Petralia & Wenthold 1992, Petralia *et al.* 1994, Kondo *et al.* 2000). Cultured cortico-collicular neurones express both AMPA and NMDA receptors (Grantyn *et al.* 1987, Huettner & Baughman 1988). Metabotropic glutamate receptors are also found in the SSC and this is discussed in detail in Chapter 2.

Synaptic transmission in the SSC is mediated by ionotropic glutamate receptors (Lo *et al.* 1998). Transmission is mainly mediated via the AMPA sub-type although a smaller component is mediated by NMDA receptors (Roberts *et al.* 1991). Experiments in the rat (Roberts *et al.* 1991, Binns & Salt 1998) and cat SSC (Binns & Salt 1996) demonstrate that visual responses are not sensitive to the NMDA antagonist AP5 in all cells. In these same experiments, when the cortex was temporarily inactivated by local anaesthetic, SSC neurones that were previously "AP5 sensitive" became "AP5 resistant". One interpretation of these data could be that NMDA receptors are only involved in cortico-collicular and not retino-collicular transmission, although NMDA receptors are also present at the retino-collicular synapse.

An alternative interpretation is that NMDA receptors are only activated when several inputs co-incide, resulting in membrane depolarisation, thereby relieving magnesium blockade of the NMDA receptor ion channel (Egebjerg 1996). When the cortex is inactivated, temporal overlap of inputs to SSC neurones is less likely to occur, which produces conditions that do not favour NMDA receptor activation. It is proposed that such a model of NMDA receptor activation in the SSC could integrate retinal and cortical inputs which may facilitate the generation of appropriate motor responses in response to an important environmental stimulus (Binns & Salt 1996).

A number of studies have proposed the existence of a synaptic triad whereby retinal terminals form synapses with both the relay cells and inhibitory interneurones, a third synapse is formed by the interneurone and relay cell (Lund 1969, Mize 1992, Lo *et al.* 1998). Feed-forward inhibition is produced by the retinal afferent-interneurone synapse. In the cat (Binns & Salt 1995), it was found that response habituation could be reduced during application of ionotropic glutamate receptor antagonists or i.v. injection of ketamine (an NMDA channel blocker), this could be due to a reduction in glutamatergic drive of inhibitory interneurones which are involved in the generation of habituation.

1.4.2 GABA

Injection of both GABA agonists and antagonists into the SC produces a complex range of behaviours including both approach and avoidance responses (see Dean & Redgrave 1992 and Okada 1992 for reviews). These effects are dependent upon a number of experimental considerations, most importantly the site of injection in the SC. As behavioural responses can be modified by inhibition of GABAergic transmission, this suggests that neurones in the SC that mediate behavioural outcomes are subject to tonic inhibitory control.

The SGS has a large number of intrinsic GABAergic neurons (Lund-Karlsen & Fonnum 1978, Pinard *et al.*1990, Warton *et al.* 1990, Mize 1992) and receives a small number of GABAergic retinal inputs (Caruso *et al.* 1989). Binding studies also indicate that there is a high density of GABA receptors in the SSC (**$GABA_A$** – McCabe & Wamsley 1986, Pinard *et al.* 1990, **$GABA_B$** - Bowery *et al.* 1987, Lu *et al.* 1999, **$GABA_C$** - Wegelius *et al.* 1998). $GABA_A$ receptor sub-unit immunoreactivity has also been found in the SSC (Pirker *et al.* 2000).

Iontophoretically applied GABA has been found to have inhibitory effects on SSC neurones (Kayama *et al.* 1980) although not all cells in this study were sensitive to GABA application. The authors of this study used the anatomical data of Langer and Lund (1974) to suggest which cells were GABA insensitive. Cells defined "GABA insensitive" in the Kayama *et al.* study included pyriform, narrow field and wide field vertical cells - all putative relay cells. These results are surprising given that in a more recent study (Binns & Salt 1997a), visually responsive SSC neurones were shown to be sensitive to GABA agonists. In this study, $GABA_A$ antagonists reduce surround inhibition whilst $GABA_B$ antagonists reduce the extent of response habituation. This study proposes that horizontal cells with their wide horizontal dendritic spread are a possible candidate for mediating surround inhibition, mediating their effect via the $GABA_A$ receptor, with Stellate cells mediating response habituation. In this way, GABA is able to have functional effects that enable the animal to accurately locate a new visual event. An alternative location for $GABA_B$ receptors could include the terminal of the retinal afferent where they may also participate in the generation of response habituation by downregulating glutamate release during high frequency transmission. Pre-synaptic $GABA_B$ receptors are present on retinal afferents to the rat LGN (Emri *et al.* 1996) and this may also be the case for the SSC.

In a slice preparation (Pasternack *et al.* 1999), $GABA_A$ activation inhibits postsynaptic potentials, whilst $GABA_C$ activation appears to potentiate the postsynaptic response. Immunocytochemistry reveals that $GABA_C$ receptors have terminal locations on interneurones in the SGS (Pasternack *et al.* 1999) suggesting that $GABA_C$ receptors could produce an excitatory effect in the SC by disinhibition.

Long term potentiation (LTP) *in-vivo* in the SSC can be elicited when the ipsilateral visual cortex is removed and GABA antagonists are applied suggesting that corticotectal neurones inhibit LTP formation in the SSC via GABAergic inhibitory neurones (Shibata, *et al.* 1990, Hirai & Okada 1993).

1.4.3 Acetylcholine

Fibre and terminal staining for the acetylcholine synthesising enzyme, choline acetyltransferase (CAT, Tan & Harvey 1989) and acetylcholinesterase activity (enzyme which terminates Acetylcholine and peptide activity, Beninato & Spencer (1986) has been shown in the SSC. Retrograde labelling techniques indicate that the source of the CAT labelling arises from cells in the ipsilateral parabigeminal nucleus (Tan & Harvey 1989). Both nicotinic (Hunt & Schmidt 1978, Segal *et al.* 1978) and muscarinic receptors (Rotter *et al.* 1979, Nonaka & Moroji 1984) have been localized in the SSC.

In an *in-vivo* preparation (Binns & Salt 2000) application of the nicotinic agonist lobeline causes a depression of the visual response and reduces the effect of AMPA evoked responses. The effects of lobeline can be blocked by co-application of a $GABA_B$ antagonist. It is likely that parabigeminal afferents release acetylcholine which activates nicotinic receptors on retinal terminals, resulting in an increase in glutamate release. This increase in glutamatergic transmission activates GABAergic interneurones which release GABA onto relay cells in the SSC. The effect of GABA on these relay cells is mediated via the $GABA_B$ receptor and results in a decrease of relay cell activity.

1.4.4 Adenosine

There are high levels of adenosine (A_1) receptors in the SGS (Fastbom *et al.* 1987). A_2 receptors are also present in the SSC although autoradiographic labelling is not lost following enucleation suggesting that these receptors are not located on retinal terminals (Ishikawa *et al.* 1997).

Evoked potentials in the SSC can be enhanced following application of adenosine uptake inhibitors or microdialysis application of adenosine *in-vivo* (Hirai & Okada 1995) or following an increase in the concentration of endogenous adenosine in the CSF (Ishhikawa *et al.* 1997). Application of adenosine or inhibitors of adenosine deaminase increases glutamate release (Hirai & Okada 1994), with the effects mediated via both A_1 and A_2 receptors.

1.4.5 Angiotensin

Two different receptors for Angiotensin II called AT_1 and AT_2 have been localized postsynaptically in the SSC (Gehlert *et al.* 1991, Hunt & Walmsley 1993, Michels *et al.* 1994). Injection of angiotensin II into the SC suppresses visually evoked potentials (Marois *et al.* 1996, Merabet *et al.* 1997) an effect mediated primarily via AT_1 receptors. It is possible that when angiotensin is released into the bloodstream in response to a change in the physiological state of the animal it is able to modify the response properties of cells in the visual centres. Injection of Angiotensin II into the SC results in an increase in blood pressure and bradycardia (DaMico *et al.* 1997).

1.4.6 Catecholamines

Noradrenaline and dopamine are degraded by an intracellular enzyme called monoamine oxidase (MAO). Following enucleation, MAO staining disappears from the SGS (Nakajima *et al.* 1996, 1998) although levels of noradrenaline are increased following visual cortex ablation (Fosse & Opstad 1986). The increase in noradrenaline following cortical ablation is thought to result from sprouting of noradrenergic fibres from the locus coeruleus. Dopamine uptake into membrane preparations from the rat SC has been examined (Weller *et al.* 1987) with the uptake rate about 20% of a striatal preparation. In this same study, dopamine and it's metabolites were also found in low concentrations. Both dopamine and noradrenaline have inhibitory effects on visual responses in the cat SC (Straschill & Perwien 1971). Immunoreactivity for a subtype of dopamine receptor, D5 has been found in the SC (Ciliax *et al.* 2000).

1.4.7 Opioid Peptides

Opioid ligands can be divided into 3 groups, these include the enkephalins and dynorphins. Moderate enkephalin binding in cell bodies and fibres in the SSC has been described in immunohistochemical studies (Watson *et al.* 1977, Kachaturian *et al.* 1983). The SC has one of the areas of highest autoradiographic labelling following administration of radiolabelled diprenorphine (Atweh & Kuhar 1977). Interestingly, in this same study, several other regions associated with visual function also had high levels of diprenorphine binding. The possible role of opioid receptors in visual processing has yet to be investigated.

1.4.8 NAAG

The di-peptide NAAG is released in-vivo following electrical stimulation of the optic nerve (Tsai *et al.* 1990) although in this study, the extracellular concentration of glutamate was not increased following optic nerve stimulation. Immunoreactivity for NAAG is reduced following enucleation in the cat, indicating that NAAG may have a role in retino-collicular transmission (Tieman *et al.* 1991). A physiological role for NAAG has not yet been described in the rat SSC although NAAG may be an agonist at mGluR3 receptors (Ghose *et al.* 1997) which are present in the rat SSC (see Chapter 2).

1.4.9 Nitric Oxide

Nitric Oxide Synthase (NOS, the enzyme that synthesises NO) has NADPH-diaphorase activity that can be used to detect NOS positive neurones. NADPH-d positive neurones are present in the SSC (Gonzalez-Hernandez *et al.* 1992). NOS immunoreactivity is found in the SSC (Bredt *et al* 1990) and *in-situ* hyrbridization reveals NOS mRNA expression in the SC (Bredt *et al.* 1991). As yet, a functional role for nitric oxide (NO) in visual transmission has not been determined in the SSC although there is evidence for NO being present in the SC. However, injection of nitric oxide synthase (NOS) inhibitor L-NAME causes an increase in blood pressure (DaMico *et al.* 1998).

1.4.10 Serotonin

There is evidence for serotonin (5-HT) being a transmitter in the SSC of the rat. High levels of serotonin have been found in the SC using a biochemical assay (Weller *et al.* 1987) as well as high affinity re-uptake sites (Biegon & Rainbow 1983, De Souza & Kuyatt 1987, Choi *et al.* 2000). Although there are no serotonergic neurones in the SSC of the rat (Bennett-Clarket *et al.* 1991) many immunopositive fibres are found suggesting that there are serotonergic SSC afferents (Dori *et al.* 1998). The SC receives serotonergic projections from the nuclei raphe dorsalis, raphe medianis raphe pontis and periaqueductal gray (Beitz *et al.* 1986). These nuclei also send collaterals to the LGN (Villar *et al.* 1988).

Evidence indicates that some 5-HT receptors are located on retinal terminals as ablation of one eye results in a large reduction in serotonin binding in the SC (Segu *et al.* 1986, Boulenguez *et al.* 1993). It has been suggested that the receptor type present on retinal terminals is the 5-HT1B type (Boulenguez *et al.* 1993 & 1996), this has recently been confirmed by ultrastructural analysis of immunohistochemical data (Sari *et al.* 1999). Ablation of visual cortex does not affect the level of serotonin binding in the SSC (Boulenguez *et al.* 1993). Although enucleation results in a loss of 5-HT1B receptors on retinal terminals, serotonin turnover is increased in the SSC of enucleated animals (Vizuette *et al.* 1993) and 5-HT7 receptor expression also increases (Venero *et al.* 1997). Moderate 5-HT6 receptor immunoreactivity has also been found in the intact SSC (Gerard *et al.* 1997).

1.4.11 Somatostatin

Immunoreactivity for the peptide somatostatin has been identified in a number of visual structures in the rat (Laemle & Feldman 1985). Immunoreactivity in the SC was particularly pronounced in the SSC where labelling of both cell bodies and fibres was observed. The most frequently labelled cell type in the SSC was the fusiform, whereas narrow field vertical cells (one type of relay cell) were never immunopositive.

Although a functional role for somatostatin has yet to be ascribed, due to the intensity of fibre and neuronal staining found in visual centres which have reciprocal connectivity, it has been suggested (Laemle & Feldman 1985) that somatostatin may have a role in modulating the degree of responsiveness of areas involved in visuomotor reflexes.

1.4.12 Neurotransmitters and Development of the SC

Several neurotransmitters and neurotransmitter receptors may be involved in development of the SC as there are changes in the levels of different receptors during key developmental timepoints, **NMDA receptors** (Cline *et al.* 1987, Cline & Constantine-Paton 1989, Constantine-Paton *et al.* 1990, Hestrin 1992, Simon & O'Leary 1992, Shi *et al.* 1997, Binns & Salt 1998, Ernst *et al.* 1998, Binns *et al.* 1999), **Nitric Oxide** (Tenorio *et al.* 1995, Tenorio *et al.* 1996, Mize *et al.* 1997, Ernst *et al.* 1998, Mize *et al.* 1998), **GABA** (Warton *et al.* 1990, Sakatini *et al.* 1992, Shi *et al.* 1997, Lim & Ho 1998), **Serotonin** (Dori *et al.* 1998), **Substance P** (Behan *et al* 1993) and **Angiotensin receptors** (Michels *et al.* 1994) have all been implicated in development of the SC.

1.5 Summary

It is apparent that the SC is able to participate in processing of sensory information at both the primary and secondary level. Studies of collicular function in different species indicate that the basic superficial/deep, multimodal and motor output organization are broadly similar, even when comparing the optic tectum (the non-mammalian SC homologue), with the SC of higher mammals. It is therefore likely that the optic tectum/SC is an evolutionarily ancient centre with functional capabilities that are well conserved in higher order animals. In higher mammals, with their well developed retino-geniculate system, the contribution of the SSC to visual processing is controversial. However, it is likely that the SC still has important visual capabilities, even in man as suggested in patients with substantial lesions of visual cortex (Blythe 1986, 1987) where motion detection remains.

The SC, in particular the deeper layers receive afferents from a wide range of both sub-cortical and cortical structures enabling it to integrate different sensory modalities. Different studies have shown that the SC is capable of employing a large range of neurotransmitter systems which may ultimately result in modulating the behavioural response of the animal. Descending pathways from the SC that are responsible for the generation of motor and autonomic responses make terminal connections with a large number of brain areas suggesting that the SC is able to generate a wide range of appropriate responses to the environment.

2.0 Metabotropic Glutamate Receptors

2.1 Glutamate and Glutamate Receptors

Glutamate is present in high levels in both the central and peripheral nervous system and is an important intermediary in metabolic pathways, and a constituent of proteins. Experiments conducted in the late 1950's and early 1960's demonstrated that application of glutamate had powerful excitatory effects on central neurones in both invertebrate and vertebrates. It is now widely accepted that glutamate is likely to be the most ubiquitous mediator of fast excitatory transmission in mammalian brain (for reviews see Monaghan *et al.* 1989, Nakanishi 1992).

Glutamate is synthesised in the nerve terminal from two sources, one from glucose via the Krebs cycle and subsequent transamination of α-oxoglutatrate. Alternatively, glutamine can be synthesised in glia which is taken up by nerve terminals then converted by glutaminase into glutamate. Glutamate is stored in vesicles in the terminal until released by a calcium dependent exocytotic process. Glutamate activity is terminated by high affinity glutamate transporters in the presynaptic terminals. Glutamate release is also inhibited by autoreceptors as discussed in section 2.7.4.

Glutamate mediates fast excitatory transmission by activating receptors which are directly coupled to ion channels, these are called ligand gated ion channels or ionotropic glutamate receptors (iGluRs). iGluRs are named after their selective agonists, AMPA, Kainate and NMDA (Watkins & Evans 1981, Monaghan *et al.* 1989). In the presence of high concentrations of AMPA and NMDA, binding of radiolabelled glutamate still occurs (Catania *et al.* 1994) indicating that there are other sites for glutamate binding in the brain. Glutamate can also activate receptors that are coupled to G-proteins and activate intracellular second messenger systems, these receptors are called metabotropic glutamate receptors (mGluRs). Due to the existence of ionotropic and metabotropic receptors, glutamate is able to produce fast excitatory effects (over a few msecs) and modulate the response at the same synapse over a longer time course (secs to mins and longer timescale).

In the relatively short period of mGluR research, mGluRs have been shown to be involved in many physiological processes, just a few examples include long term potentiation (O'Connor *et al.* 1994), long term depression (Kamishita *et al.* 1995), glutamate induced neurotoxicity (McDonald *et al.* 1993), neuroprotection (Maise *et al.* 1996) and hyperalgesia (Boxall *et al.* 1996, Fisher & Coderre 1996). The data presented in this thesis demonstrates that mGluRs are involved in synaptic transmission and that the responses of cells in the SSC may be modulated by mGluRs.

2.2 Ionotropic Glutamate Receptors

The "Non-NMDA" receptors (AMPA and Kainate subtypes) are directly coupled to an ion channel which is permeable mainly to Na^+. The ion channel conductance is generally lower than NMDA receptors (200fs-30ps), with a decay time of around 10ms (Colquhoun *et al.* 1992). Conductance varies with sub-unit composition (Sommer & Seeburg 1992, Sommer *et al.* 1990). AMPA receptors are formed of homomeric and heteromeric compositions of GluR1-4 sub units. Kainate receptors are formed of homomeric and heteromeric compositions of GluR5-7 and KA-1 and KA-2 sub units (Seeburg 1993, Edmonds *et al.* 1995). GluR1-4 sub-units have been identified in the SC by immunohistochemistry (Petralia & Wenthold 1992) with moderate staining throughout the SC and slightly denser staining in the superficial grey. AMPA (and to a lesser extent NMDA) iGluRs participate in visual synaptic transmission in the SSC (Roberts *et al.* 1991, see Chapter 1.4.1). Kainate receptors have also been located in the SSC (Wisden & Seeburg 1993, Petralia *et al.* 1994).

NMDA receptors are coupled to ion channels which are permeable to Na^+ and Ca^{2+}. The ion channel has a conductance of around 150ps, with a decay time of approx 200ms (Verdoorn *et al.* 1991). At hyperpolarized potential, the ion channel is blocked by Mg^{2+}. Sustained depolarisation (presumably resulting from persistent non-NMDA receptor activation) will cause the Mg^{2+} to be expelled from the ion channel, thereby allowing Na^+ and Ca^{2+} to enter the cell. The NMDA receptor also has a binding site for its co-agonist glycine, and co-agonist binding must occur before the receptor is activated.

NMDA receptors are formed of NR1 and NR2 sub-units, of which there are 4 NR2 sub-units called NR2A, B, C and D (Egebjerg 1996). In the SC, the NR-1 sub-unit has been localised by immunohistochemistry (Petralia *et al.* 1994) with moderate staining in the superficial grey and denser staining in the deeper layers.

2.3 Structure of mGluRs

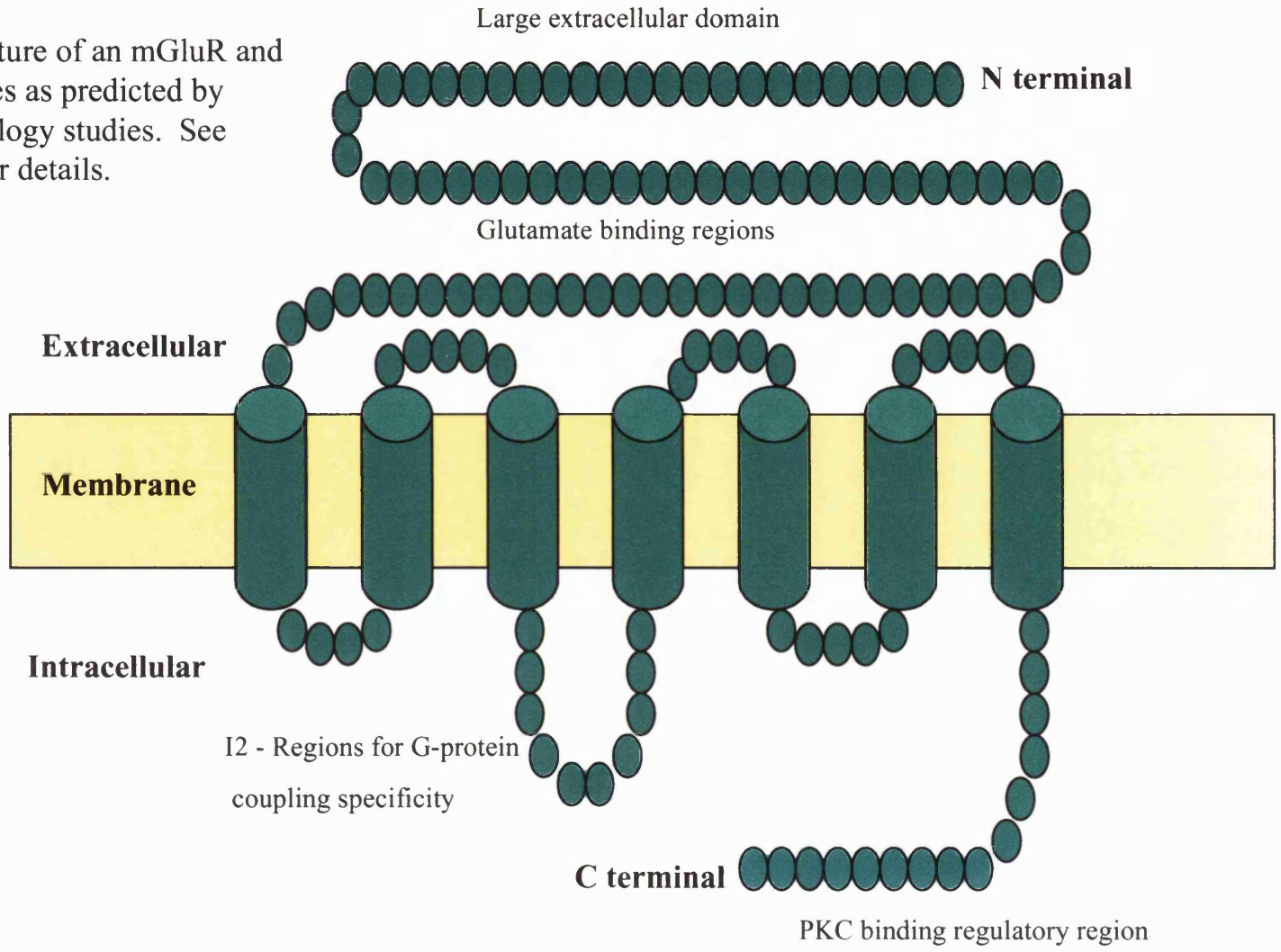
The existence of glutamate receptors directly coupled to second messengers, was first described in the mid 1980's (Sladeczek *et al.* 1985, Sugiyama *et al.* 1987). In experiments with cultured neurons, glutamate and quisqualate were found to hydrolyse inositol phospholipids, which was reversed by inhibitors of G_i and G_o proteins (Sugiyama *et al.* 1987, 1989). In 1991, the first mGluR (now called mGluR1a) was cloned (Houamed, *et al.* 1991, Masu *et al.* 1991), subsequently cloned receptors are named according to their order of discovery.

At present, 8 mGluRs in the rat have been cloned, (called mGluR1 - mGluR8) and these can be divided into 3 groups by sequence homology, pharmacology and 2nd messenger pathway (Conn & Pin 1997). There is approximately 70% sequence identity between receptors of the same group, and approx. 45% sequence identity between the different groups of mGluRs (Pin & Bockaert 1995). Some human mGluRs have also been cloned and these share a surprisingly similar sequence with corresponding rat mGluRs (~94%, Wu *et al.* 1998). Group I includes mGluR1 and mGluR5, Group II mGluR2 and mGluR3, and Group III mGluR4, 6, 7 and 8. There are also splice variants for several mGluRs and these include mGluR1a, b & c (Pin *et al.* 1992), mGluR4a and b (Iversen *et al.* 1994), mGluR5a and b (Minakami *et al.* 1993, Joly *et al.* 1995, Yamaguchi & Nakanishi 1998), mGluR7a and b (Ohishi *et al.* 1998) and mGluR8a and b (Ohishi *et al.* 1998).

Although mGluRs have a classical "7TM" receptor structure, with transmembrane helices passing across the cell membrane seven times, the amino acid sequence and structure is dissimilar to other G-protein coupled receptors. In common with iGluRs, mGluRs have a very large N terminal extracellular domain (Okamoto *et al.* 1998) containing regions critical for agonist selectivity (O'Hara *et al.* 1993, Takahashi *et al.* 1993, Okamoto *et al.* 1998, Kunishima *et al.* 2000). Correct compartmentalisation of mGluRs in the membrane is dependant upon a protein called Homer (Ango *et al.* 2000). There are three intracellular and extracellular loops, the large and variable 2nd intracellular loop contains regions important for G-protein coupling specificity (Francesconi & Duvoisin 1988, Pin *et al.* 1994, Gomeza *et al.* 1996). The receptor is coupled to different G proteins that in the inactive state bind guanosine diphosphate (GDP) and once activated bind guanosine triphosphate (GTP). The cytoplasmic C terminal contains a region where phosphorylation by protein kinase C (PKC) occurs, causing receptor desensitisation (Gereau & Heinemann 1998). The C terminals are not conserved in different mGluR groups (Pin & Duvoisin 1995).

Figure 2.1 General structure of mGluR

Possible structure of an mGluR and regulatory sites as predicted by molecular biology studies. See Section 2.3 for details.



2.4 Transduction Mechanisms of mGluRs

mGluR1 responses appear to be at least partially sensitive to pertussis toxin (PTX, Aramori & Nakanishi 1992) indicating that mGluR1 may couple to G_i proteins, although mGluR5 effects are PTX resistant (Abe *et al.* 1992). Group II and III mediated effects can be inhibited by pertussis toxin indicating that they may be coupled to G_i proteins. PKC may interfere with G-protein coupling as stimulation of PKC has been shown to abolish agonist effects at both Group II and III (Swartz *et al.* 1993, Tyler & Lovinger 1995, Herrero *et al.* 1996, Macek *et al.* 1998) and Group I (Aramori & Nakanishi 1992, Thomsen *et al.* 1993). PKC stimulation may occur due to activation of other neurotransmitter systems such as adenosine indicating the potential for "cross talk" between different neurotransmitter systems.

The transduction mechanisms by which mGluRs mediate effects have been determined in a number of systems, commonly by expressing the receptor in a non-neuronal cell. It is important to consider that under physiological conditions, it is possible that coupling to other second messengers may occur.

The exact transduction mechanisms in intact native neurones and glial cells will be more complex due to the presence of effector systems which are not present in cell lines used in expression systems (Saugstad *et al.* 1994, Prezau *et al.* 1994, Casabona *et al.* 1997, Conn & Pin 1997, Tempia *et al.* 1998). Also, in expressions systems, receptors may be "overexpressed" which may lead to receptors coupling to inappropriate second messengers.

In expression systems, Group I mGluRs stimulate phospholipase C (PLC) which stimulates membrane phosphoinositide (PI) hydrolysis (Houamed *et al.* 1991, Masu *et al.* 1991, Abe *et al.* 1992, Pin *et al.* 1992, Netzeband *et al.* 1997). Phosphatidylinositol-4,5-*bis*-phosphate (PIP_2) is the primary substrate for PLC and results in the formation of inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG, Scholz & Palfrey 1991).

IP_3 releases calcium from intracellular stores (Murphy & Miller 1988), thereby increasing the levels of cytosolic calcium which may then interact with a variety of intracellular targets including protein kinases, phosphatases and ion channels. The kinetics of Ca^{2+} release are variable in mGluR1 splice variants suggesting varying G-protein affinity. Immunocytochemical localization for mGluR1 reveals that in a number of brain areas, mGluR1 receptor protein is present in the absence of mRNA for IP_3 receptors (Fotuhi *et al.* 1993), PKC and IP3 receptors are also differentially distributed (Worley *et al.* 1986, 1989) indicating that Group I mGluRs may also activate other intercellular messengers. DAG stimulates protein kinase C (PKC) by lowering the calcium requirement required for activation. Increases in DAG also activate DAG-specific phospholipases which result in the formation of arachidonic acid (AA). AA serves as a substrate for cyclooxygenase and lipoxygenase, leading to the production of prostaglandins and leukotrienes which have many cellular and intracellular effects. AA formation, coupled with a rise in cytosolic Ca^{2+} may activate guanylyl cyclase, resulting in elevation in cyclic guanosine monophosphate (cGMP). Application of mGluR agonists can result in cGMP accumulation, an effect which is blocked by nitric oxide synthase (NOS) inhibitors indicating that nitric oxide is involved as a downstream messenger in this effect (Okada 1992).

Group I mediated PI hydrolysis may be facilitated by activation of Group II receptors (Schoepp *et al.* 1996) suggesting that there may be a synergistic interaction between these two receptor groups. Group I mGluRs (Aamori & Nakanishi 1992, Gabellini *et al.* 1993, Joly *et al.* 1995, Paolillo *et al.* 1998) and possibly Group II mGluRs (Sortino *et al.* 1996) have also been shown to increase cyclic adenosine monophosphate (cAMP) in some expression systems and native cell types. It is unclear whether mGluRs are directly positively coupled to adenylyl cyclase (AC) or whether increases in cAMP occur as a result of Ca^{2+} release from internal stores which can activate Ca^{2+}/calmodulin sensitive AC.

Group II (Tanabe *et al.* 1993, Schoepp *et al.* 1995, Bruno *et al.* 1995, Wright & Schoepp 1996) and Group III mGluRs (Kristensen *et al.* 1993, Nakajima *et al.* 1993, Tanabe *et al.* 1993, Okamoto *et al.* 1994, Saugstad *et al.* 1994) are predominantly negatively coupled to adenylate cyclase (AC) and inhibit forskolin induced cAMP formation. This effect is inhibited by pertussis toxin indicating that G_i proteins are likely to be involved in this effect (Gilman 1989, Prezeau *et al.* 1992, Baba *et al.* 1993). The extent of AC inhibition is less for Group III mGluRs in comparison with Group II mGluRs which could indicate that Group III receptors may be coupled to different 2^{nd} messengers in native systems.

In the hippocampus (Holler *et al.* 1993, Boss *et al.* 1994, Klein *et al.* 1997), there is evidence of an mGluR which is coupled to phospholipase D (PLD). L-cysteine-sulfinic acid (L-CSA) an endogenous excitatory amino acid potently activates this receptor, whereas glutamate has a lower potency (Boss *et al.* 1994). Activation of PLD results in the formation of phosphatidic acid (PA) which can be converted by phosphatidate phosphohydrolase to DAG which may then activate PKC.

2.5 Evidence for mGluRs in the SSC

Immunohistochemical and *in-situ* hybridization techniques have been used to determine the distribution of different mGluRs in the brain and some of these studies have shown that the protein or mRNA for mGluRs is present in the SSC.

Immunohistochemical studies use antibodies that will label receptor proteins by identifying unique amino acid sequences in the receptor. Potential problems with the use of antibodies are that non-specific binding can occur, splice variants may not be recognised and binding to an uncharacterized protein (which contains the same recognition sequence) may occur.

It should also be remembered that the presence of immunolabel does not necessarily imply the existence of functional receptors, as "internalised" receptors may also be labelled with the antibody. However, immunohistochemical methods are a good way to identify the location of receptors, and once labelling has occurred, it may be possible (with additional processing of the tissue) to determine the cellular location of the labelled receptor using electron microscopy.

In-situ hybridization techniques label specific mRNA. It is important to remember that mRNA is present in the cell body, therefore *in-situ* studies do not give information as to the cellular location of receptors. When looking for mRNA in any particular brain region, inputs to the area of interest should also be examined as the cell bodies of the afferents may be positive for the receptor of interest. For example, when looking for mGluRs in the SSC it will be necessary to examine the levels of expression in the retina and visual cortex, although it is also important to remember that receptors made in the cell body are not necessarily transported to the neurone terminal, but may become functional receptors at the cell body or dendrites. Table 2.1 summarises data from several immunohistochemical and *in-situ* studies which indicate that several mGluRs are likely to be present in the rat SSC.

Examination of other brain areas reveals that certain mGluRs are located in predominantly pre-synaptic or post-synaptic locations. Group I receptors appear to be predominantly post-synaptic (Thalamus: Martin *et al.* 1992, Striatum: Shigemoto *et al.* 1993, Hypothalamus: van den Pol *et al.* 1995, Shigemoto *et al.* 1997) although they are also found in pre-synaptic locations (Romano *et al.* 1995), whilst Group III receptors are predominantly pre-synaptic (Spinal cord: Ohishi *et al.* 1995, Hippocampus: Shigemoto *et al.* 1996, 1997, Olfactory bulbs: Wada *et al.* 1998) although a postsynaptic location for Group III receptors has been shown (Hippocampus: Bradley *et al.* 1996, Retina: Brandstatter *et al.* 1996, Locus Coeruleus: Bradley *et al.* 1998, Cerebellum: Phillips *et al.* 1998). Group II receptors are located in both pre and post synaptic locations (Hippocampus: Neki *et al.* 1996, Shigemoto *et al.* 1997, Cerebellum: Ohishi *et al.* 1994, Neki *et al.* 1996).

There is also evidence for the "extrasynaptic" or "preterminal" location of Group II receptors in some brain areas (Hippocampus: Lujan *et al.* 1997, Shigemoto *et al.* 1997, Cerebellum: Lujan *et al.* 1997, Olfactory bulbs: Wada *et al.* 1998, Spinal cord: Azkue *et al.* 2000). In the hippocampus cerebellum, and dLGN, Group I mGluRs have been shown to be preferentially located at the periphery of the postsynaptic density (Baude *et al.* 1993, Lujan *et al.* 1996, 1997, Vidnyanszky *et al.* 1996). It has been suggested (Lujan *et al.* 1997) that these "extrasynaptic" mGluRs may be activated when there is a high concentration of glutamate at the synapse with "spillover" or diffusion from the central synaptic cleft to extrasynaptic mGluRs (Scanziani *et al.* 1997, Dube & Marshall 2000, Mitchell & Silver 2000). In experiments where glutamate scavengers are used, the involvement of presynaptic mGluR autoreceptors is reduced (Min *et al.* 1998) whilst mGluRs have a greater effect if the diffusion of glutamate within the synaptic cleft is retarded (Maki *et al.* 1994). It has also been suggested (Baude *et al.* 1993) that mGluRs may be located at the periphery of synapses due to spatial constraints involving free movement of G-proteins and associated 2^{nd} messengers. There is also evidence from the hippocampus, that mGluRs are selectively targeted to specific terminals which is dependent on target cell innervation (Scanziani *et al.* 1998).

Table 2.1 – Evidence for the location of mGluRs in the SSC

Receptor	Method	Evidence for presence in SC	Ref
mGluR1	*In-Situ*	High density of moderately labelled neurones in SC, low /moderate levels of label in ganglion cell layer of retina, around 10% of cortical neurones in layers IV-VI labelled	3, 13
mGluR1	Immuno	High levels of label in SC, moderate levels of IP_3 receptor expression	5
mGluR1a	Immuno	High levels in the SC, many immunopositive dendrites and cell bodies, immunopositive RGC's.	2 ,4,16
mGluR2	Immuno	No immunolabel in SC or GCL of the retina	19,20, 25
mGluR2	*In-Situ*	No expression in SC. Weak expression in RGC's, and cortical layer V.	7, 13
mGluR3	*In-Situ*	Moderate expression in SSC, significant expression in all layers of cortex. No expression in the retina	8, 10
mGluR3	Immuno	mGluR3 is not found in the rat retina	19
mGluR2/3	Immuno	Moderate immunolabel in SGS, less in other SC laminae	21
mGluR4	Immuno	No expression in SC or cortex, some immunoreactivity in RGC's	10,19
mGluR4	*In-Situ*	Low levels of expression in the SC, very low levels of expression in cortex, high expression in RGC's	11, 14, 13
mGluR5	Immuno	Heavy labelling in SSC, particularly in SZ. Labelling of SSC more pronounced than deep SC.	9, 17
mGluR5	*In-Situ*	Moderate expression in Cortex, nothing in SC	1
mGluR6	*In-Situ*	mGluR6 is only expressed in ON retinal bipolar cells	6, 13
mGluR7	*Immuno*	mGluR7 is only present in inner plexiform layer of adult retina, nothing in retinal ganglion cells	18
mGluR7	*In-Situ*	High levels of expression in the SGS and visual cortex. Weak expression in RGC's	11, 12, 13, 15, 17
mGluR7a	Immuno	Very intense fibre staining in SC.	23
mGluR7a & mGluR7b	*In-Situ*	Weak expression in SC and cortex	23
mGluR8	*In-Situ*	No expression in SC, weak expression in cortex	22
mGluR8a and mGluR8b	*In-Situ*	No expression in SC or cortex	24

References

1. Abe *et al.* 1992
2. Martin *et al.* 1992
3. Shigemoto *et al.* 1992
4. Baude *et al.* 1993
5. Fotuhi *et al.* 1993
6. Nakajima *et al.* 1993
7. Ohishi *et al.* 1993a & 8. 1993b
9. Shigemoto *et al.* 1993
10. Tanabe *et al.* 1993
11. Akazawa *et al.* 1994
12. Okamoto *et al.* 1994
13. Hartveit *et al.* 1995
14. Kinzie *et al.* 1995
15. Ohishi *et al.* 1995
16. Peng *et al.* 1995
17. Romano *et al.* 1995
18. Brandstatter *et al.* 1996
19. Koulen *et al.* 1996
20. Neki *et al.* 1996
21. Petralia *et al.* 1996
22. Saugstad *et al.* 1997
23. Bradley *et al.* 1998
24. Corti *et al.* 1998
25. Ohishi *et al.* 1998

2.6 mGluR Pharmacology

Early investigations of mGluR function were hampered by a lack of specific mGluR agonists and relied on the use of agonists which also have activity at iGluRs such as L-glutamate, ibotenate and quisqualate, sometimes in the presence of iGluR antagonists. Glutamate is a flexible molecule which is thought to exist in a number of conformations. Many mGluR agonists are rigid glutamate analogs where one carboxyl group is substituted. Such compounds include the first two specific mGluR agonists *trans*-ACPD (of which 1S,3R-ACPD is the active isomer, Irving *et al.* 1990) and L-AP4 (Schoepp & Hilman 1990). Due to L-AP4 reducing glutamate or ibotenate mediated effects, some early studies describe L-AP4 as a "glutamate antagonist" (Koerner & Cotman 1981, Nicoletti *et al.* 1986). 1S,3R-ACPD is an agonist at both Group I and Group II receptors and was widely used in early studies to probe physiological function of mGluR activation. The use of 1S,3R-ACPD in intact neurones which are likely to express a variety of glutamate receptors (rather than expression systems which only express mGluRs from one group) has been problematic and it is likely that the findings of some of these early studies may need to be revised in context of recent knowledge concerning expression of different mGluRs in specific preparations. Pharmacological characterization of putative mGluR ligands has relied heavily upon the activity of ligands at cloned receptors expressed in non-neuronal cell types. Pharmacological characterization in expression systems has an advantage in that the effect at a specific receptor can be examined, whereas in native neuronal systems, neurones may express more than one type of mGluR. However, use of cloned expression systems may be problematic in that reported potencies in expression systems may be different for a receptor in the native environment (Conn & Pin 1997).

Investigation of mGluR physiology have also been compounded by a lack of suitable antagonists, although a series of phenylglycines and their derivatives has yielded some suitable compounds with antagonist and agonist activities. One of these early compounds was MCPG (antagonist at both Group I and II) and has been a useful drug in probing mGluR synaptic function (Eaton *et al.* 1993).

Recently, many compounds have been identified which appear to have good selectivity for the three groups of receptor and in some cases selectivity for individual receptors within each group. Molecular biology and receptor modelling techniques have revealed important information on mGluR ligand binding sites offering the possibility for the design of receptor specific antagonists (Litschig *et al.* 1999). Some of the more useful compounds for investigating the functional role of mGluRs are described in table 2.2. 3,5-DHPG (Group I), 2R,4R-APDC (Group II) and L-AP4 (Group III) are widely agreed to be amongst the most useful selective agonists with which to selectively activate each group of mGluRs.

Table 2.2 Pharmacology of mGluRs

Group	Agonists	Antagonists	Ref
I	**3,5-DHPG** Quis>3,5-DHPG>Glu>1S,3R-ACPD=Ibo>L-CCG-I (S)-3HPG tADA (some mGluR5 selectivity) CHPG (mGluR5) 1S,3R-ACPD – Also Group II mGluRs, more selective for mGluR5 than mGluR1	Most phenylglycines, most potent being - MCPG but also weak activity at Group II and III 4CPG but weak agonist at Gp II, (S)-4CPG, (+)-αM4CPG, (S)-3C4HPG (some agonist activity at Gp III), CPCCOEt (mGluR1) LY367385 (mGluR1), SIB1757, SIB1893 and MPEP (mGluR5 although non-competitive NMDA antagonist?)	1,5,11,14, 15,16,19,23, 24,28,31,33, 34,35,37,38, 40,41
II	**2R,4R APDC** L-CCG-I*> Glu> 1S,3R-ACPD>Ibo>Quis LY354740 Quis is a more potent agonist at mGluR3 than mGluR2 Phenylglycines mentioned as antagonists for Group I are agonists at Group II with varying potencies (S)4C3HPG,(S)3C4HPG, (S)-4CPG *Some evidence of L-CCG-I agonist activity at mGluR8 & Group I receptors	α-methyl-L-CCG-I (MCCG-I) LY341495 (although some antagonist activity at mGluR8) EGLU (R,S)-CPPG (+)-αM4CPG (also Group I) MTPG (more selective for Group II than III)	2,5,6,13, 17,18,23,24, 26,29,36,39
III	**L-AP4** L-AP4>L-SOP>Glu (R,S)-4-phosphonophenylglycine (PPG), particularly mGluR8 Agonists have low potency at mGluR7	α– methyl-L-AP4 (MAP4), also antag at mGluR2 (R,S)-CPPG (R,S)-MPPG (also weak antag at Group II)	4,8,9,10, 12,23,29,30, 38

References

1. Aramori & Nakanishi 1992
2. Hayashi *et al.* 199
3. Thomsen *et al.* 1992
4. Kristensen *et al.* 1993
5. Tanabe *et al.* 1993
6. Cavanni *et al.* 1994
7. Hayashi *et al.* 1994
8. Kemp *et al.* 1994
9. Okamoto *et al.*1994
10. Prezau *et al.*1994
11. Salt & Eaton 1994
12. Saugstad *et al.*1994
13. Thomsen *et al.* 1994
14. Brabet *et al.* 1995
15. Joly *et al.* 1995
16. Kingston *et al.* 1995
17. Laurie *et al.* 1995
18. Schoepp *et al.* 1995
19. Thoreson *et al.* 1995
20. Vignes *et al.* 1995
21. Bedingfield *et al.* 1996
22. Brauner-Osborne *et al.* 1996
23. Bushell *et al.* 1996
24. Gomeza *et al.*.1996
25. Jane *et al.* 1996
26. Salt *et al.*.1996
27. Salt & Turner 1996
28. Sekiyama *et al.*.1996
29. Thomsen *et al.* 1996
30. Toms *et al.* 1996
31. Wermuth *et al.* 1996
32. Acher *et al.* 1997
33. Batchelor *et al.* 1997
34. Doherty *et al.*.1997
35. Klein *et al.* 1997
36. Monn *et al.* 1997
37. Bruno *et al.* 1999
38. Gasparini *et al.* 1999
39. Turner & Salt 1999
40. Varney *et al.* 1999
41. O'Leary *et al.* 2000

2.7 Modulation of Synaptic Transmission by mGluRs

As would be expected for G-protein coupled receptors, mGluR activation produces effects on a longer timescale than iGluR activation and generally mediates slow, modulatory effects rather than fast, excitatory transmission. One exception is at the photoreceptor-ON bipolar synapse in the retina, where mGluR6 mediates fast transmission and hyperpolarizes the ON-bipolar cell (Nakajima *et al.* 1993). It is thought that retinal mGluR6 is responsible for the "ON" response to visual stimuli which can be observed in the SC (Masu *et al.* 1996), although a modified, late onset ON response can still be observed in mGluR6 knockout mice (Sugihara *et al.* 1997). Some of the modulatory effects that mGluR may have upon synaptic transmission are described below.

2.7.1 Modulation of Potassium Currents

In a number of studies, activation of mGluRs produces excitatory effects including slow depolarisation and an increase in firing rate (Charpak *et al.* 1990, Pacelli & Kelso 1991, Batchelor *et al.* 1997, Netzeband *et al.* 1997, Schoppa & Westbrook 1997, Schrader & Tasker 1997). These increases in cell excitability are due to a decrease in potassium conductance. Potassium currents are responsible for setting the resting membrane potential of neurones, repolarising the membrane following actions potentials and modulating the rate of repetitive spike discharge. mGluRs may affect a number of potassium currents including a reduction in potassium leak ($\mathbf{I_{leak}}$, Charpak *et al.* 1990, Desai & Conn 1991, Glaum & Miller 1992, McCormick & Krosigk 1992, Guerineau *et al.* 1994, Schrader & Tasker 1997), calcium dependent slow afterhyperpolarization current ($\mathbf{I_{AHP}}$, Baskys *et al.* 1990, Charpak *et al.* 1990, Desai & Conn 1991, Gerber *et al.* 1992, Glaum & Miller 1992, Ceaser *et al.* 1993, Schrader & Tasker 1997, Zahorodna *et al.* 1998), slow non-inactivating voltage-dependent potassium current ($\mathbf{I_M}$, Charpak *et al.* 1990, Ikeda *et al.* 1995) and slow inactivating voltage-dependent current ($\mathbf{I_{K(Slow)}}$, Luthi *et al.* 1996).

By inhibiting potassium currents, in particular I_{leak}, mGluR agonists can increase the input resistance of the cell which facilitates the effect of other agonists whether they are inhibitory or excitatory (Bond & Lodge 1995). In the hippocampus (Halliwell & Adams 1982), I_M is activated when the membrane is slightly depolarised (~ -60mV). A reduction of I_M by mGluRs would therefore increase the extent of depolarisation and could potentiate the effect of concurrent synaptic inputs.

Inhibition of I_{AHP} results in a decrease in the interval between successive action potentials and a reduction in response accommodation. A decrease in response habituation following mGluR agonist application has been observed in a number of brain areas (Charpak *et al.*. 1990, Pacelli & Kelso 1991, Zheng & Gallagher 1992, Salt & Eaton 1991) it is possible that some of these observed effects are due to a decrease in I_{AHP}. I_{AHP} is generated by influx of calcium from voltage gated channels which then activates low-conductance "SK potassium channels" (Lancaster & Adams 1986). A decrease in I_{AHP} by mGluRs is believed to occur due to a reduction in potassium rather than calcium conductance as low doses of mGluR agonists reduce I_{AHP} without significantly affecting levels of intracellular calcium (Charpak *et al.* 1990, Glaum & Miller 1992). Studies have revealed that neither PKC or protein kinase A (PKA, Gerber *et al.* 1992) are involved in the reduction of I_{AHP} by mGluRs, which raises the possibility that G-proteins may directly couple to potassium channels.

Group I mGluRs appear to be the predominant receptors involved in modulation of potassium currents (Desai *et al.* 1992, Davies *et al.* 1995, Gereau & Conn 1995a, Abdul-Ghani *et al.* 1996, Schrader & Tasker 1997). As well as inhibiting potassium currents, mGluRs have also been shown to activate K^+ currents in some preparations. In the cerebellum, Group I mGluRs activate the calcium sensitive big potassium channel (BK, Fagni *et al.* 1991, Chavis *et al.* 1998), activation of potassium conductances has also been demonstrated in other brain regions (Premkumar & Chung 1995).

Activation of calcium sensitive potassium currents is thought to account for the slow hyperpolarization sometimes observed following ACPD mediated depolarisation (Joels *et al.* 1989, Glaum *et al.* 1992, Takagi *et al.* 1992). mGluRs have also been shown to activate G-protein inwardly rectifying potassium channels (GIRKS, Saugstad *et al.* 1996, Dutar *et al.* 2000).

2.7.2 Modulation of Cation Channels

mGluRs can also mediate excitatory effects by modulating cation currents in a number of brain areas, including calcium activated non-specific cation current (I_{CAN}, charge carried by Mg^{2+}, Cd^{2+} and Zn^{2+}, Crepel *et al.* 1994, Congar *et al.* 1997, Lee & Boden 1997, Guerineau *et al.* 1997), activation of Na^{+}/Ca^{2+} exchanger (Glaum *et al.* 1992, Staub *et al.* 1992, Linden *et al.* 1994, McBain *et al.* 1994) and calcium-independent non-specific cation channel (Pozzo *et al.* 1995).

2.7.3 Modulation of calcium channels

mGluR activation has been shown to reduce calcium currents in different brain regions, although the type of channel affected and receptor group involved is variable in different preparations. L-type channels may be inhibited by all three groups (Sayer *et al.* 1992, Chavis *et al.* 1994, Sayer 1998) whilst inhibition of N-type calcium channels appears to involve Groups I and II (Lester & Jahr 1990, Swartz *et al.* 1992, Trombley & Westbrook 1992, Sahara & Westbrook 1993, Stefani *et al.* 1994, Chavis *et al.* 1995a, Ikeda *et al.* 1995, Choi & Lovinger 1996, McCool *et al.* 1996, Sayer 1998). The kinetics of N type channel mGluR mediated inhibition are fast and may involve a direct effect upon the channel by the activated G-protein (Kleuss *et al.* 1991, Trombley & Westbrook 1992), whereas reduction of L-type channels is relatively slower. P/Q type channels have also been shown to be inhibited by Group II and III mGluR activation (Takahashi *et al.* 1996).

Group I mGluRs mobilize Ca^{2+} release from intracellular stores (Courtney *et al.* 1990, Llano *et al.* 1991, Manzoni *et al.* 1991, Bossu *et al.* 1992, Zegarra-Moran & Moran 1993, Chavis *et al.* 1995a & b). In non-neuronal cell lines, mGluR1α activation results in a single-peaked mobilization of Ca^{2+} from intracellular stores, whilst mGluR5a activation resulted in an oscillatory type release (Kawabata *et al.* 1996). This difference in calcium mobilization results from phosphorylation of a single amino-acid in the vicinity of transmembrane 7. Oscillatory type calcium mobilization has also been demonstrated in intact neurones following application of 1S,3R-ACPD (Woodhall *et al.* 1999). Group I mGluRs have also been shown to cause transient increases in calcium ion concentrations which do not result in a change in membrane potential (Takechi *et al.* 1998). Such calcium transients have been suggested to be involved in long term activity dependent changes in synaptic plasticity.

2.7.4 mGluRs Regulate Release of Glutamate and GABA

There is evidence for all three mGluR groups being involved in the downregulation of glutamate release in different brain regions (**all three groups**: Baskys & Malenka 1991, Calabresi *et al.* 1993, Bonci *et al.*1997, Dietrich *et al.* 1997, McCaffery *et al.*1999, **Group I:** Lovinger 1991, Manzoni & Bockaert 1995, Gereau & Conn 1995b, **Group II:** Lovinger 1991, Lovinger & McCool 1995, **Group III:** Koerner & Cotman 1981, Forsythe & Clements 1990, Johansen & Robinson 1995, Gereau & Conn 1995b, Pisani *et al.* 1997a) by functioning as presynaptic autoreceptors. mGluR mediated presynaptic inhibition of glutamate release could reduce the efficacy of high frequency transmission and contribute to response habituation.

mGluR agonists reduce both excitatory post synaptic currents (EPSCs) and inhibitory post synaptic currents (IPSCs) (Calabresi *et al.* 1993, Burke & Hablitz 1994, Bonci *et al.* 1997, Schrader & Tasker 1997). All mGluR groups have been demonstrated to reduce GABAergic transmission in a number of brain areas (**Group I:** Desai & Conn 1991, Pacelli & Kelso 1991, Calabresi *et al.* 1992, Glaum & Miller 1992, Gereau & Conn 1995b, Fitzsimonds & Dichter 1996, Bonci *et al.* 1997, **Group II:** Desai & Conn 1991, Pacelli & Kelso 1991, Desai *et al.* 1992, Glaum & Miller 1992, Llano & Marty 1992, Stefani *et al.* 1994, Poncer *et al.* 1995, Fitzsimonds & Dichter 1996, Salt & Eaton 1995, Bonci *et al.* 1997, Mitchell & Silver 2000, **Group III:** Salt & Eaton 1995, Bonci *et al.* 1997). By reducing GABA mediated inhibition, mGluRs can cause "disinhibition" which may have considerable functional implications.

As previously described, mGluRs may reduce N-type calcium channels (Swartz & Bean 1992, Sahara & Westbrook 1992) and P/Q type channels (Randall et al. 1993) which are involved in neurotransmitter release (Trombley & Westbrook 1992, Turner *et al.* 1993, Herrero *et al.* 1996, Stefani *et al.* 1996) although some studies have shown that L-AP4 inhibition of synaptic transmission is not sensitive to blockade of voltage sensitive calcium channels (Schoppa & Westbrook 1997). In the striatum, t-ACPD reduces the amplitude of high voltage activated calcium currents and GABA-mediated synaptic transmission (Stefani *et al.* 1994) suggesting that inhibition of calcium channels inhibits the release of GABA. 1S,3R-ACPD reduction in excitatory postsynaptic currents in visual cortex can be blocked by 4-AP (potassium channel blocker) suggesting that mGluRs may also modulate potassium channels to reduce glutamate release (Sladeczek *et al.* 1993).

Group I mGluRs may also increase glutamate release from synaptosomes by increasing levels of arachidonic acid (AA, Herrero *et al.* 1992) although a second successive application of Group I agonist (at 5 min interval) does not result in a facilitation of glutamate release (Herrero *et al.* 1998, Rodriguez-Moreno *et al.* 1998). Release facilitation is also inhibited when bath concentration of glutamate is high indicating that mechanisms exist to prevent accumulation of toxic concentrations of glutamate at the synapse.

AA formation is also increased following iGluR stimulation (Dumuis *et al.* 1988, 1990) which may act as a retrograde signal to presynaptic terminals whereby glutamate release is facilitated. In freely moving rats, 1S,3R-ACPD administration results in an increase in glutamate in dialysate from the parietal cortex (Moroni *et al.* 1998) an effect which also appears to be mediated by Group I mGluRs as the Group I agonist 3,5-DHPG also increased glutamate concentration in the dialysate. In the hypothalamus, Group I mGluRs potentiate glutamate and GABA release (Schrader & Tasker 1997), whilst Group III mGluRs produce the opposite effect. Recently, Group III activation has been shown to facilitate glutamate release in the entorhinal cortex (Evans *et al.* 2000).

Group I receptors have also been shown to depolarise inhibitory cortical interneurones resulting in an increase in GABA release (Zhou & Hablitz 1997) which causes an increase in spontaneous inhibitory postsynaptic currents in their pyramidal cell targets (Chu & Hablitz 1998). Other studies have also demonstrated that GABAergic transmission may be enhanced (Llano & Marty 1995, Poncer *et al.* 1995, Sciancalepore *et al.* 1995). The thalamic reticular nucleus contains many GABAergic neurones, Group II mGluRs cause a hyperpolarization of these cells by increasing K^+ conductance (Cox & Sherman 1999) thus causing a disinhibition of thalamocortical relay cells. Group II mGluRs produce a postsynaptic inhibitory effect in other brain regions Holmes *et al.* 1996).

2.7.5 Modulation of Ligand-Gated Ion Channels

Although the use of selective mGluR agonists is essential to investigate the functional role of mGluRs, it is unlikely that under physiological conditions that mGluRs are selectively activated. This raises the possibility that there may be functional interactions of iGluRs and mGluRs by synaptically released glutamate. Application of mGluR agonists can potentiate iGluR mediated currents (Aniksztejn *et al.* 1991, Bleakman *et al.* 1992, Cerne & Randic 1992, Harvey & Collingridge 1993, Glaum & Miller 1993, Fitzjohn *et al.* 1996, Rahman & Neuman 1996, Wang & Daw 1996, Doherty *et al.* 1997, Pisani *et al.* 1997b), reduce iGluR mediated currents (Pacelli & Kelso 1991, Colwell & Levine 1994, Molitor & Manis 1997, Ugolini *et al.* 1997, Yu *et al.* 1997, Martin *et al.* 1998) and reduce ionotropic GABA mediated currents (Pacelli & Kelso 1991, Glaum & Miller 1993).

Group I mGluRs appear to be mainly involved in the modulation of ionotropic responses. Group I (and possibly Group II receptors) by inhibiting K^+ conductances, can increase input resistance and therefore the efficacy of other excitatory and inhibitory inputs (Bond & Lodge 1995) which may explain some of the observed effects where a potentiation of iGluR agonist effect is observed in the presence of mGluR agonists. Such a mechanism could amplify the signal to "noise" ratio resulting in an "amplification" of synaptic transmission. Activation of Group I mGluRs results in di-acyl glycerol (DAG) production which activates PKC and it could be through this mechanism that NMDA responses are potentiated, as PKC has been shown to reduce Mg^{2+} blockade of the NMDA receptor ion channel (Chen & Huang 1992, Kelso *et al.* 1992). In some studies, ACPD mediated enhancement of NMDA responses can be blocked by PKC inhibitors (Aniksztejn *et al.* 1992, Urushihara *et al.* 1992, Pisani *et al.* 1997b, Ugolini *et al.* 1997) although this does not occur in all preparations (Markram & Segal 1992, Harvey & Collingridge 1993).

2.8 Summary

It is apparent that mGluRs are involved in the modulation of a number of important physiological properties including modulation of potassium currents which are responsible for the generation of the resting membrane potential of the cell. Activation of different groups of mGluRs has been shown to have both inhibitory and excitatory effects in different preparations. mGluRs have been shown to modulate the release of both glutamate and GABA and it is feasible that mGluRs, by their effects on calcium currents may also regulate the release of other neurotransmitters. mGluRs are coupled to intracellular 2nd messenger cascades which are able to produce both short and long term effects upon the cell. By activating intracellular molecules there is potential for "cross talk" between different neurotransmitter systems following mGluR activation. Anatomical and *in-vivo* evidence suggests that mGluRs may be located in extra-synaptic locations giving rise to the possibility that mGluRs may be involved in activity dependent processes, being optimally activated when the concentration of glutamate at the synapse is high. The presence of mGluRs at any synapse will add further complexity to the effects of endogenous glutamate as it is necessary to consider the effect at both iGluRs and mGluRs. It is likely that the balance of effect of glutamate will depend upon the extent of glutamate diffusion and re-uptake at the synapse, the location and affinity of different mGluRs and iGluRs and the frequency of neuronal stimulation. There is substantial evidence that mGluRs are located in the SSC and it is possible that mGluRs activation may modify the output of relay cells which may ultimately have behavioural effects

3.0 Project Hypothesis

There is biochemical evidence for glutamate being a neurotransmitter at the rat retino-collicular and cortico-collicular synapse (see Section 1.4.1). Electrophysiological evidence (Roberts *et al.* 1991) has demonstrated that AMPA and NMDA glutamate receptors participate in synaptic transmission in the SSC.

In situ hybridization and immunohistochemical studies provide evidence for the presence of different mGluRs on SSC cells and SSC afferents (see Table 2.1 for a summary of these studies). mGluR1, 3, 4, 5, 7 and possibly 2 and 8 are present in the SSC or afferent connections (the most important being the contralateral retina and ipsilateral visual cortical areas). Studies from other brain regions (see Section 2.5) have also revealed that different mGluRs are predominantly located in post synaptic (Group I mGluRs), pre-synaptic (Group III) or both pre and post-synaptic locations (Group II). Electron microscopy has revealed that some mGluRs can be located away from the main synaptic cleft (see Section 2.5) whilst other receptors have a more central location at the synapse. It is feasible that these extrasynaptic receptors may be activated during periods of intense afferent stimulation which results in glutamate diffusing from the centre of the synapse to these receptors. In this way, mGluRs may modulate synaptic function in an activity dependant manner.

Physiological evidence has revealed that mGluRs may produce multiple aspects of neuronal function. mGluRs have been demonstrated to modulate transmitter release at the pre-synaptic terminal resulting in a reduction of excitatory and inhibitory transmission (see Section 2.7.4). Some of these effects are likely to result from an inhibition of calcium channels which are involved in neurotransmitter release (see Section 2.7.3). Activation of Group I mGluRs may also increase release of glutamate (see Section 2.7.4). If mGluRs participate in regulation of neurotransmitter release in the SSC, then it is likely that mGluRs could produce both excitatory (inhibition of GABAergic transmission or increase in glutamate release) and inhibitory effects (reduction of glutamate release or facilitation of GABAergic transmission). It is particularly important to consider the possible effect that mGluRs may have on GABAergic transmission as the SSC has a very high proportion of GABAergic neurones in comparison with other brain regions (see Section 1.4.2).

mGluRs (Group I in particular) may modify a number of different potassium currents (see Section 2.7.1). These modifications result in slow increases in cell excitability and changes in the firing patterns of neurones. In particular, modulation of one potassium current (I_{AHP}, see section 2.7.1) could have a profound effect on visual processing by reducing response habituation which is a key feature of SSC neurones (Oyster & Takahashi 1974). Inhibition of I_{leak} current could increase the input resistance of the cell thereby facilitating the effect of both inhibitory and facilitatory transmitters (Bond & Lodge 1995, section 2.7.1). In the SSC, modification of this current could again produce both facilitatory and inhibitory effects which would be dependant upon the nature of afferent stimulation at any time point.

mGluR activation (mainly Group I) may modify iGluR mediated currents (both facilitatory and inhibitory effects see Section 2.7.5). Given that iGluRs are involved in synaptic transmission in the SSC it is likely that visual responses may be modified by mGluR/iGluR effects.

As sub-types of each mGluR receptor group are likely to be present in the SSC, it is apparent that mGluRs have the potential to significantly modify the responses of cells in the SSC via a number of different pathways.

The data presented in this thesis explores the possibility that mGluRs may modulate visual responses in the SSC and attempts to answer the following questions:-

- What is the effect of mGluR activation by exogenous agonists?
- Do mGluRs participate in visual synaptic transmission in the SSC ?
- If mGluRs are involved in modulating visual responses, does the effect differ when different types of visual stimuli are used?
- Can mGluRs modulate iGluR responses?
- Drawing upon other anatomical and physiological studies, what are the likely mechanisms by which mGluRs can modulate visual responses?
- Is it likely that mGluR function in visual synaptic transmission is conserved across species?

Previous studies (see Table 1.5 for summary) have investigated the response characteristics of visually responsive neurones in the SSC. In the work described in this study, the effect of mGluR activation on visual processing in the rat SSC will be explored in an *in-vivo* preparation. The use of an intact, *in-vivo* preparation allows different types of physiologically relevant visual stimuli to be used. Iontophoretic application of selective agonists and antagonists will be used as tools to probe the possible role of different mGluR sub-types in aspects of visual processing.

4.0 Materials and Methods

4.1 Overview

Visual stimuli were presented to anaesthetised rats and extracellular activity from single units of the SSC were recorded. Agonist, antagonist and control ions were applied to visually responsive neurones by iontophoresis. The type of visual stimuli used varied according to the experimental protocol in order to investigate different aspects of visual processing within the SSC and how these may be modulated by mGluRs.

4.2 Methodological considerations

The most important advantage of using an *in-vivo* preparation, is that it allows the use of physiologically relevant stimuli. For the preparation described here, it is possible to vary parameters of the stimulus to probe different aspects of visual processing. For example, the size and composition of the receptive field can be investigated by varying the stimulus size and position, mechanisms involved in response habituation can be probed by varying the interstimulus interval. To stimulate *in-vitro* preparations, it is necessary to use electrical stimulation which probably does not exactly mimic physiological inputs in terms of amplitude, temporal events or recruitment of afferents. Another clear advantage of the *in-vivo* preparation is that inputs to the area of interest are relatively intact, it is unlikely that this is the case for an *in-vitro* preparation. Due to the existence of relatively intact connectivity, it is possible that the anaesthetised *in-vivo* preparation allows a good approximation of understanding the processes involved in alert, behaving animals. Although *in-vivo* studies may allow us to form hypothesis of how afferent connections may contribute to an observed effect, an *in-vitro* preparation may permit selective activation of inputs which may give a clearer indication of the involvement of different afferent pathways to observed responses.

It is necessary to use general anaesthetics for *in-vivo* preparations and it is most likely that the use of such agents may modify electrophysiological responses. It is therefore important to consider, that effects observed under anaesthetic may not necessarily occur in an unanaesthetised animal. For example, in an anaesthetized preparation, large cortical lesions resulted in cells of the cat SC loosing directional selectivity (Wickelgren & Sterling 1969), although this did not occur in a different study which did not use general anaesthetic (Hoffman & Straschill 1971). In the experiments carried out for this thesis, urethane anaesthesia is used. Urethane is a good choice for electrophysiological experiments in the rat, Holmes and Houchin (1966) demonstrated that urethane had little effect on sensitivity of neurones to amines and that rate and pattern of cell firing was very similar to recordings obtained in unanaesthetised preparations. Other groups have also confirmed the relative lack of effect of urethane on neuronal responses (Cross & Dryer 1971, Bradley & Dray 1973) although urethane has been shown to antagonise the effect of excitatory amino acid responses in the spinal cord (Evans & Smith 1982).

Another consideration for both *in-vivo* and *in-vitro* studies is that responses may change over time due to a change in the physiological condition of the preparation. Although this is a concern in *in-vitro* studies, the problem is likely to be more complicated for an *in-vivo* preparation due to the effect of anaesthetics and trauma induced by surgery. A particular problem inherent to the preparation described here, is that eye movements can occur which may result in a decline in the visual response. It is possible to overcome this with a variety of methods including paralysing the animal, mechanical immobilisation of the eye or local paralysis of muscles around the eye, although using any of these methods is not without problems. In this study, mechanical immobilisation was performed for a small proportion of studies and was found to adversely affect visual responses. For this reason, in order to control for eye movements, regular mapping of the position of the receptive field in visual space was undertaken and visual stimuli which generate stable responses over a long period of time were used in preference to further mechanical interference with the preparation.

This study uses iontophoresis to apply small quantities of drugs in an *in-vivo* preparation. Although iontophoresis allows the application of small quantities of drugs it is not possible to determine the concentration of drugs in the extracellular environment of the cell and another advantage of *in-vitro* preparations is that known concentrations of drugs can be applied. This is useful where the drug may have selectivity for certain receptors within a defined concentration range or where it may be possible to determine the receptors involved due to the potency of a drug in eliciting a response. In an *in-vitro* preparation it is also possible to determine whether observed effects are mediated by pre or postsynaptic mechanisms, this is commonly investigated by blocking synaptic transmission by TTX.

4.3 Surgery

All procedures were in accordance with the UK Animals (Scientific Procedures) Act 1986 and associated guidelines. Adult male and female Lister Hooded rats (180g - 550g) were used. Animals were housed in conventional animal facility with 12 hour light/dark cycles with free access to food and water. Subjects were initially anaesthetised with gaseous halothane in a sealed chamber until the righting reflex was lost. Urethane (1.25g/kg) was administered via the intraperitoneal route. Additional urethane was administered (0.02-0.04g/kg) as necessary during surgery or during the experiment if the pedal withdrawal reflex was present or heart rate increased. 1% lignocaine with adrenaline was administered subcutaneously at all wound margins: thorax (tracheal cannulation), dorsal skull (surgical entry site), and the right forepaw and right hindpaw if an invasive method was used to monitor the electrocardiogram (ECG).

A tracheal cannulation was made and an appropriately sized glass cannula was inserted. The cannula was securely tied with nylon sutures and the overlying skin was loosely sutured. A piece of tape was attached to the fur behind the cannula to prevent the cannula from getting trapped. The cannula allows the subject to breathe spontaneously and the airway can be maintained as necessary throughout the experiment.

Following transfer to a stereotaxic frame, the ECG was monitored either invasively by subcutaneous implantation of stainless steel needles to the fore and hind-paw or non-invasively by application of gold electrodes to the skin (electrode jelly was applied to ensure good contact between the skin and electrodes) secured with adhesive tape. The subject was warmed with a thermostatically controlled electric blanket to 37^0C. It is important to ensure that anaesthetised animals are kept warm as anaesthetics can interfere with thermoregulatory mechanisms.

During surgery, animals were held in stereotaxic position with blunt earbars (tips coated with lignocaine gel) and a nosebar. A single long incision was made in the skin overlying the dorsal surface of the skull and the overlying muscle was scraped away. The electroencephalogram (EEG) was routinely monitored and was recorded by drilling two small holes and placing small screws in the left hand side of the skull. The two screws make contact with the cerebrospinal fluid (CSF) on the cortical surface and need to be kept electrically isolated. This is achieved by covering each screw in dental cement during fixation of the head holder.

Next, a small craniotomy (4 x 4mm) was made over right superior colliculus (SC) exposing the overlying cerebral cortex. The site for the craniotomy and subsequent electrode entry (with a correction for angled approach) was made according to the stereotaxic co-ordinates of Paxinos and Watson (Paxinos & Watson 1986) (Lambda – 2.0mm anterior-posterior axis and -1.2mm medio-lateral axis). During all drilling, the bone was cooled by application of saline in order to prevent heating and damage to the underlying brain. The overlying dura was carefully removed with a fine gauge needle and pair of forceps. A head holder was mounted onto the skull using screws and dental cement. The headholder allows the head to be held in a stereotaxic position once the ear bars and nose bars have been removed in order to allow greater access to the facial area. Skin around the left hand side eye was sutured as necessary in order to expose the eye. A drop of light mineral oil was applied to the eye in order to prevent corneal drying. In some experiments, a custom made loop device was placed over the eye in order to reduce eye movements. The rat was earthed by a silver wire inserted through muscles in the neck.

At the end of the experiment, the subject received an overdose of urethane administered to the thorax.

4.4 Recording and Iontophoresis

4.4.1 Glass Electrodes

For some of the preliminary characterisation experiments, sharp glass electrodes, tip size approximately 1μm were used. These electrodes were filled with Pontamine Sky Blue (PSB, see table 4.1).

Multi barrelled electrodes (7 barrels) were assembled from four lengths of borosilicate glass capillaries (Clark Electromedical GC150TF, internal diameter 1.17mm). Three lengths were broken in half using a diamond cutter and were arranged around the remaining full length piece of glass. The glass pieces were held in drill chucks and heated, twisted and pulled apart over a Bunsen flame. The un-fused end of each barrel was pulled away slightly from the central barrel over a small Bunsen flame to facilitate filling and minimize possible barrel cross-contamination. The tips of these electrode blanks were then pulled in a vertical gravity-puller set-up to produce fine tips, typically 1μm diameter. Tip pulling consists of three stages, fusing of glass attached to a weight, tip elongation and separation of the fused glass from the tip to produce the final tip shape.

During all stages of electrode manufacture and handling, it is essential that exposure to dust is minimised in order to prevent contamination which may lead to blocking of the electrode barrels. For this reason, prepared electrodes were stored in glass jars for a maximum of three weeks, electrodes unused after this time were discarded. Prior to filling, the electrode tip was broken back to 5-6μm using a microscope and manipulator. This tip size is a reasonable compromise for satisfactory recording of extracellular activity and passage of iontophoretic current. If necessary, the tip can be broken back again in the same way if the resistances of the electrode barrels are too high. Electrodes were filled around three hours before they were inserted into the brain to allow time for solutions to fill the electrode tip and for any small air bubbles to subside.

The resistance to the passage of current of each barrel can be measured by the iontophoretic apparatus, by passing a test current of 50nA through the barrel. If the resistance of the barrel is too low then it is likely that there will be a substantial problem with drug ions leaking out of the electrode. Conversely, barrels with a very high resistance to the passage of current will impede the ejection of drug ions. Barrel resistance may vary throughout the course of an experiment, this may occur due to a patch of membrane or tissue partially occluding a barrel orifice or dust, precipitate or glass particles settling into the electrode tip. If a barrel becomes blocked during the course of an experiment, the LED display on the iontophoretic apparatus will flash as the required current level cannot be applied to the barrel.

4.4.2 Tungsten Electrodes

Tungsten electrodes were used in experiments to characterise the response properties of SSC neurones. Electrodes were made by etching tungsten wires in Levicks Solution ($NaNO_2$/KOH, Levick 1972) and then insulating the wire in glass. Glass was etched off the tip of the tungsten wire with heated borosillic acid to reveal a bare tungsten tip. The tungsten tip was etched as necessary with Levicks solution in order to achieve a satisfactory shaped tip of around 20-50μm.

4.4.3 Recording

Multibarelled electrodes were lowered through the cortex using a stepping micro drive at -15^0 to the vertical in order to reduce damage to the overlying visual cortex which is an important source of afferents to the SC. Multi-barrelled electrodes are relatively large and therefore need to be stepped slowly through the tissue in order to minimise damage and to improve recording stability. Warmed agar solution was applied to the site of the craniotomy during recording in order to protect the cortex from dehydration and to minimize the effect of brain movements which may occur during recording. Visually responsive cells were found approximately 2.7mm from the surface of the brain.

Once the electrode had reached the superficial SC, small steps down were made to identify single cells suitable for recording. Neuronal activity was amplified x1000 (x100 then x10 Axoprobe 1A, Axon Instruments). Inspection of the extracellular wave form gives an indication of the proximity of the recording electrode to the cell (Sefton 1969). As spike size is dependant upon the extent of current flow in the extracellular space, the spikes will become larger as the recording tip approaches the cells membrane. The shape of the wave form is also an indication of distance between the electrode and cell as the waveform may appear as a wholly negative potential at distances of around 50μm from the cell, which usually changes to a wave form with a small positive and larger negative component near the unit (Bishop *et al.* 1962a and 1962b). Recording sites were made through a single electrode track, and were between 50 and 100μm apart. In a typical experiment, data would be obtained from a portion of SC spanning 150-300μm depth. PSB was occasionally used to place dye spots to enable visual inspection of the recording position after removal of the brain.

Extracellular single neurone activity was recorded through the central barrel of the electrodes which contained 4M NaCl. Each drug barrel is connected to the iontophoretic and recording apparatus via a silver wire, before sealing the ends of each barrel with petroleum jelly to prevent evaporation of drug solutions. Action potential spikes were gated with a waveform discriminator and timed and recorded using a CED 1401 computer interface and VS Software (Cambridge Electronic Design, Cambridge, UK) which generated peri-stimulus time histograms (PSTHs).

4.4.4 Iontophoresis

Iontophoresis allows the release of very small quantities of drugs onto neurones (see Stone 1985 and Roberts & Gould 1993, for comprehensive overviews of practical aspects of this technique). Iontophoresis is the only method which allows the direct application of small quantities of compounds of interest onto central neurones. The technique has a distinct advantage over other *in-vivo* methods of drug administration, in that systemic effects are minimized due to local application.

The movement of drug ions within a solvent results from the passage of current, with movement of drug ions out of the electrode tip occurring as they carry the current passed. The technique was first used in the 1950's to apply very small quantities of acetylcholine for very short periods of time at the neuromuscular junction, thus attempting to mimic synaptic transmission (del Castillo & Katz 1955). In 1958, Curtis used multibarelled micropipettes to demonstrate that acetylcholine was the transmitter between motor axon collaterals and the inhibitory Renshaw cells in the spinal cord (Curtis & Eccles 1958).

In order for a drug to be suitable for iontophoretic application, it must be ionised in solution and should ideally be freely soluble in water (although small adjustments in pH may be necessary). It is possible to dissolve drugs in other solvents such as alcohol or DMSO, but the solvents may be biologically active, thus necessitating a control for the solvent. The pH of the drug solution is an important consideration, as the extent of ionisation, solubility and chemical stability is dependant on pH. pK is the pH at which half of the ions will be ionised and a good rule for preparing drug solutions for iontophoresis is to adjust the pH until it is at least pK. However, as pH values move further away from neutrality, an increasing proportion of OH^- or H^+ will be ejected with the drug ions and it is possible that these ions may also alter cellular excitability. It is therefore advisable to try to control for pH effects by ejection of a saline solution which is adjusted to the same pH of a test drug if the test drug solution is highly acidic or basic.

In order to prevent spontaneous efflux of drug ions from the electrode tip, it is necessary to apply a small "retaining" current, which will be the opposite polarity of the drug ion. Thus, over time, the drug ions will move away from the tip of the electrode. Without using a very high retaining current, it is probably not possible to abolish all leakage that occurs due to diffusion and hydrostatic efflux. However, the application of very large retaining currents will result in a significant reduction of drug available for ejection at the electrode tip (Bradshaw & Szabadi 1974), therefore small retaining currents are usually applied (typically 5 to 15nA) in order that a reasonable amount of drug ion is available for ejection. It is important to ensure that a constant response is attained when either retaining or ejection currents or intervals between ejections are changed.

Re-equilibrium and the re-distribution of ions along the length of the barrel may take some time after any of these parameters are changed. A technical problem inherent in iontophoretic experiments, is the inability to quantify the amount of drug ions ejected from the electrode. Therefore, it is not possible to construct conventional dose-response curves or to accurately compare the absolute potency of two compounds. It is also dangerous to assume that there is any linear relationship between the amount of drug ejected and the ejecting current used.

In order to minimize the effect of current passage on the cell, a continuous current is passed through a barrel containing 1M sodium chloride. This current is equal in magnitude but of opposite polarity to the algebraic sum of ejecting and retaining currents that are being applied through the rest of the barrels, therefore the current at the tip of the whole barrel is always zero. This practice is known as current balancing and is automatically carried out by the iontophoretic pump unit (NeuroPhore BH-2 system, Medical Systems Corp.). In addition, to investigate the effect of passage of iontophoretic current on a cell, one barrel can be filled with 100mM NaCl and Na^+ or Cl^- can be ejected at the same currents as those used to eject drugs.

Table 4.1 lists details of all drugs and solutions which were applied by iontophoresis in the experiments described in this thesis.

Table 4.1 - Drugs and solutions applied by iontophoresis

Solution Used	Concentration	Retaining Current	Ejecting Current	Comments
NMDA	200mM in water, pH8	+6 to 10nA	-60 to 200nA	iGluR agonist
AMPA	10mM in 75mM NaCl	+6 to 10nA	-80 to 200nA	iGluR agonist
1S,3R-ACPD	50mM in water pH 8.5	+10nA	-25nA to 100nA	Group I and II agonist
L-AP4	25mM in water pH 7.5	+10nA	-25 to 100nA	Group III agonist
D-AP4	25mM in water pH 7.5	+10nA	-25 to 100nA	Stereo-isomer of L-AP4, control for L-AP4
NaCl	100mM	+ or – 10nA	+ or - 25 to 200nA	For current control, + or – ejecting depending on experimental protocol
Acidified Saline	100mM NaCl + HCl pH3.5	-10nA	+25 to 100nA	pH control for acidic solutions
PSB	2.5% in 0.5M NaCl-0.5M sodium acetate	+20nA	-100μA	Dye for marking recording site
R,S-MPPG	25mM in water, pH 8.5	+10nA	-25 to 100nA	Group III antagonist
CPPG	25mM in water, pH8.5	+8 to 15nA	-25 to 125nA	Group III antagonist
LY367385	25mM in water, pH8.5	+10 to 15nA	-6 to 25nA	mGluR1 antagonist fresh aliquot used each experiment, pH adjusted on thawing
LY354740	25mM in water, pH8.5	+8 to 15nA	-25 to 100nA	Group II agonist
LY341495	25mM in water, pH8.5	+8 to 15nA	-25 to 100nA	Group II antagonist
3,5-DHPG	50mM in water, pH3.5	-8 to 15nA	+25 to 150nA	Group I agonist fresh aliquot used each experiment, pH adjusted on thawing
4CPG	50mM in water, pH8.5	+10 to 15nA	-25nA to 150nA	mGluR5 antagonist
CGP35348	10mM water, pH3	-8 to 15nA	+25 to 100nA	$GABA_B$ antagonist

4.5 Visual Stimuli

A cathode ray tube (CRT, Tektronix 608 monitor) was used to display visual stimuli created by a Picasso visual stimulus generator under computer control (VS Software, Cambridge Electronic Design). The CRT was located in the RF of the neurone, 15cm away from the left eye of the subject. To centre the RF on the CRT screen, spots of light were displayed at different elevations and azimuthal positions. The responses were analysed on line and the position of the CRT was adjusted to centre the RF in the middle of the screen. It was necessary to repeat this procedure a number of times during the experiment for each neurone in order to compensate for eye movements made by the subject.

4.6 Experimental Protocols

4.6.1 Characterisation Experiments

Assessment of size of excitatory component of receptive field – Flashed spots of $2\text{-}32^0$ presented randomly (5 times each).

Orientation and direction tuning – Moving bar (22.5^0/sec), blank screen 3 secs, 2 sec excursion across CRT, blank 4 secs travelling in 8 directions (0^0, 45^0, 90^0, 135^0, 180^0, 225^0, 270^0, 315^0). 5 randomised presentations of each direction.

Response to moving bars of different velocity – Moving bar of preferred orientation and size moving across CRT at 360^0/sec, 180^0/sec, 90^0/sec, 45^0/sec, 22.5^0/sec and 11.25^0/sec. 5 randomised presentations.

Contrast sensitivity – Moving bar of preferred orientation and size (blank screen 4 secs, 2 sec excursion across CRT, blank screen 4 secs). Luminance of foreground object either 20cd m^{-2} or 12.2 cd m^{-2} against background of 7.7 cd m^{-2}. 5 randomised trials.

Response habituation – 5 presentations of moving bars of optimum orientation and size presented for 2secs at interstimulus intervals of 0.5, 1, 2, 4 and 8 secs. Each trial was presented 5 times in random order, with a 10sec inter-trial pause.

4.6.2 Effects of agonists, antagonists and pharmacological controls on visual responses

To investigate the effect of pharmacological agents on visual responses, it is necessary to use a visual stimulus that will produce a stable response over a long period of time. 10 (occasionally 5) presentations of light bars on a dark background were presented (5 x10-15^0, luminance of foreground object 20cd m^{-2}, background 7.7cd m^{-2}) moving at 22.5$^0/s^{-1}$ for 4s every 10s. The moving bars were of preferred orientation and direction as determined by presenting moving bars moving in 8 directions (0^0, 45^0, 90^0, 135^0, 180^0,225^0, 270^0, 315^0). Reproducible control cycles of visual response (120s) were established before two of three cycles of continuous ejection of the substance under investigation. Recovery data were then obtained before collection of new control data and further drug application. To determine if antagonists could block the effect of agonists, the antagonists were continuously ejected for three cycles before the agonist was co-ejected with the antagonist for three cycles. Agonist ejection ceased whilst the antagonist continued to be ejected for two cycles. Antagonist ejection ceased and recovery data was obtained. A timer was used in all protocols to ensure regular ejection cycles.

4.6.3 Effect of antagonists during presentation of low contrast visual stimuli

In order to investigate the possibility that the magnitude of antagonist effect differs during different levels of synaptic activity, moving bars as described above were presented either at "full contrast" (stimulus foreground 20cd m^{-2}, background 3.1cd m^{-2}) or "low contrast" (stimulus foreground 12.2cd m^{-2}, background 3.1cd m^{-2}).

4.6.4 Effects of agonists and antagonists on response habituation

To reliably induce response habituation, the interstimulus interval between presentations was reduced to 0.5 sec. The moving bar was presented 5 times for 5 trials with an inter-trial interval of 10secs. Details of size etc. are as described above. As before, reproducible control cycles of visual responses were established before two or three cycles of continuous ejection of agonist or antagonist, recovery data was then obtained before the collection of new control data and further drug application. To determine the effect of agonists and antagonists in the presence of $GABA_B$ receptor blockade, CGP35348 was continuously ejected for 2 or 3 cycles in order to reduce response habituation before agonists or antagonists were co-ejected with CGP35348 for 2 cycles. Agonist or antagonist ejection then ceased whilst CGP35348 was ejected for a further 2 cycles. Recovery data were then obtained.

4.6.5 Effect of agonists on ionotropic responses

To assess the effects of mGluR agonists on postsynaptic activation of SC neurones, cycles (70s duration) were constructed in which the agonists AMPA and NMDA were ejected for 12s each in order to evoke an excitation. After control data were obtained, agonists were ejected for two cycles, beginning 70s before the first ejection of ionotropic agonist. Recovery data were then obtained following cessation of agonist ejection.

4.7 Data Analysis

Responses were quantified as counts of action potentials evoked by visual stimuli over 5 or 10 repeats of the visual stimulus. Data were observed on-line during data collection and analysed off-line using Spike 2 software (Cambridge Electronic Design). The effect of a test drug is expressed as percentage mean of control cycles of visual responses. For experiments where antagonists and agonists were co-ejected to examine blockade of effects by antagonists, the agonist effect is expressed as percentage mean of continuous antagonist ejection. Non-parametric statistics (Wilcoxon Signed Rank Test) were used to test for significant differences between control data and drug-treated groups. A result was deemed significant at $p<0.05$. All results are expressed as percentage of control ± s.e.m.

For **habituation studies**, the degree of response habituation was calculated by expressing the response evoked by the fifth presentation of a set of five repeated stimulus presentations as a percentage of the response evoked by the first using the formula:-

$$100 \times \left\{ 1 - \frac{\text{(no. of spikes evoked by fifth presentation)}}{\text{(no. of spikes evoked by first presentation)}} \right\}$$

For assessing the extent of **directional or orientation bias**, two indices were calculated. The response "R" to a bar travelling at the angle of θ, can be represented on polar co-ordinates by a vector of length R, inclined at the angle θ. The vector can be represented by the complex number $R=R_{exp}(j\theta)$, where $j=\sqrt{-1}$ (Levick & Thibos 1982, Thibos & Levick 1985, Binns & Salt 1996). The bias vector "B" summarises the mean and spread of the tuning curve and can be calculated as:-

$$\mathbf{B} = B_{exp}(j\theta)=\Sigma R$$

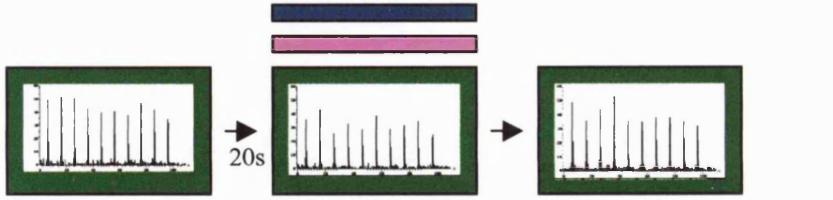
B, will now indicate the magnitude of the directional bias on a scale of 0 – 1.

I, the direction index is calculated according to the following formula:-

$$I = 1- \frac{\text{minimum response – spontaneous activity}}{\text{maximum response – spontaneous activity}}$$

Figure 4.1 – Pictorial description of visual stimuli and drug ejection protocols

4.6.1 Effects of agonists, antagonists and pharmacological controls on visual responses

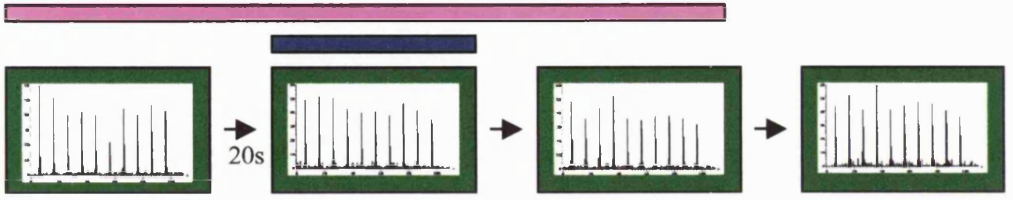


Control 3 cycles 110 secs duration

Agonist, antagonist or control ejection 2 or 3 cycles

Recovery 2 or 3 cycles

4.6.1 Effect of antagonists on blockade of agonist responses



Continuous antagonist ejection 3 cycles 110 secs duration

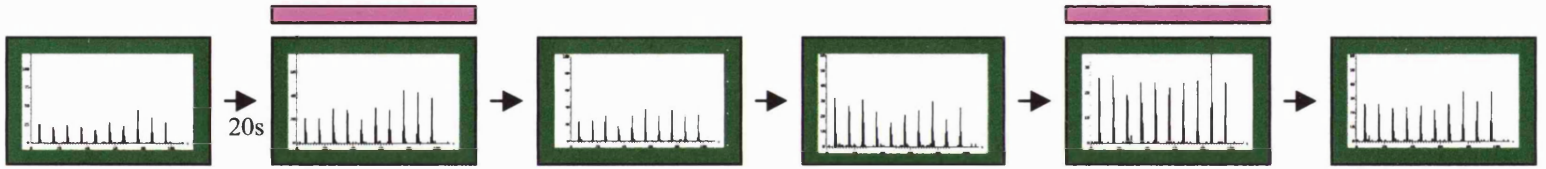
Co-ejection antagonist and agonist 3 cycles

Antagonist ejection only 2 cycles

Recovery 2 or 3 cycles

Coloured bars above panels indicate periods of drug ejection

4.6.2 Effects of antagonists during presentation of low contrast visual stimuli



Control – presentation of low contrast stimuli 3 cycles 110 secs duration

Antagonist application 3 cycles

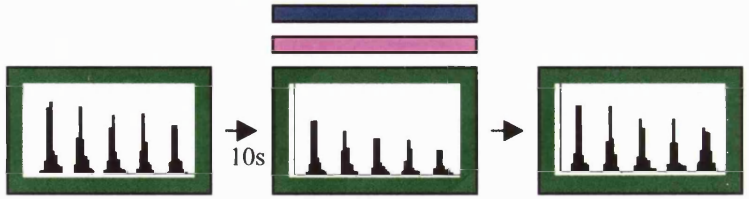
Recovery 3 cycles

New control – full contrast stimuli 3 cycles

Antagonist application 3 cycles

Recovery 3 cycles

4.6.3 Effects of agonists and antagonists on response habituation

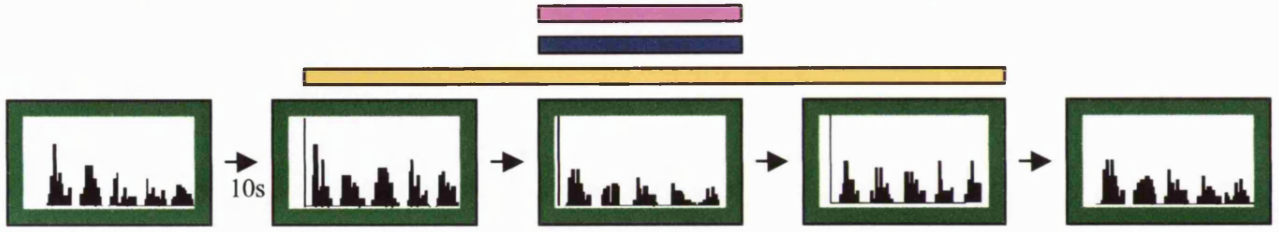


Control – habituation protocol 5x22.5 secs duration

Antagonist or agonist application

Recovery

4.6.3. Agonist/antagonist effect on habituation when $GABA_B$ receptors blocked



Control habituation protocol 112.5 secs duration

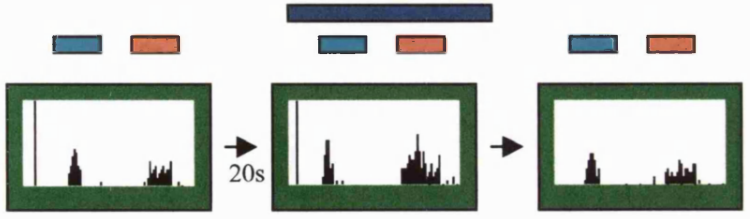
GCP35348 ejection 2 or 3 cycles

CGP35348 and agonist/antag co-ejected 2 cycles

CGP35348 ejection 2 cycles

Recovery 2 or 3 cycles

4.6.4 Effect of agonists on ionotropic responses



Control 3 cycles AMPA ejection and NMDA ejection 12 secs 70sec cycle duration

Agonist application 2 cycles

Recovery 2 cycles

4.8 Immunohistochemical Methods

I acknowledge the assistance of Ms Claudia Sharp who prepared and processed the tissues described here.

4.8.1 Tissue processing

The materials used for the immunohistochemical studies was obtained from 2 male hooded rats, 2 cats and 5 ferrets. Animals were deeply anaesthetised with sodium pentobarbitone (50mg/kg) and perfused transcardially with 0.9% saline in phosphate buffer (PBS, pH7.2) followed by a solution of 4% paraformaldehyde in 0.2% picric acid in PBS (pH7.2). The optic pathway from the eye to the optic tract was exposed from below without damaging the brain. Each nerve was sectioned directly behind the eye and the nerves and optic chiasm lifted free from the brain. The retinae, nerves and brains were placed in the above fixative for approximately 2h, before being transferred into 20% sucrose in PBS and stored overnight at 4^0C. The following day the nerves were sectioned horizontally and the brains coronally on a freezing microtome at 40μm, and placed in PBS. Retinae were whole mounted (all species) or transversely sectioned (cat and ferret only) as above following embedding in gel albumin for support. Selected brain sections through the LGN and SC of each animal were processed free-floating for immunohistochemistry. Samples were pre-incubated in 0.4% Triton X-100 in PBS for 1h with 5% normal goat serum/0.1% Triton X-100/PBS and then incubated overnight at 4^0C with a panel of affinity purified mGluR antibodies (1a, 1b, 1c, 2/3, 4 and 5), at a working dilution of 1:1000 in 1% normal goat serum/0.1% Triton X-100/PBS. Adjacent sections were incubated in the working solution that did not contain primary antibody to provide negative control slides. The secondary antibody used was biotinylated goat anti-rabbit IgG (vector BA 1000) at a dilution of 1:5000 in 1% normal goat serum/0.1% Triton-X/PBS, incubated for 1h at room temperature. Avidin-biotin complex (Vectastatin Elite ABC Kit, PK6100) with DAB as the substrate used to visualise the staining. The sections were then mounted onto gelatinised slides, dehydrated and coverslipped.

Following initial analysis, coverslips were removed from selected sections, the tissue re-hydrated and Nissl stained, dehydrated and coverslipped. This followed analysis of labelling patterns in both counterstained and un-counterstained material.

In the majority of cases, specific antibody labelling was undertaken on the same day in all three species employed, using common solutions. Details of the generation, preparation and specificity of the antibodies used has previously been described for mGluR1a, 1b, 1c – Grandes *et al.* 1994, mGluR2/3 & 4 – Koulen *et al.* 1996, mGluR5 – Vidnyanszky *et al.* 1994. Briefly, peptides corresponding to the c-terminal of each mGluR were created with polyclonal antisera raised in rabbits. Protein bands detected by immunoblotting against rat tissue were in good agreement with the predicted molecular weight of the corresponding mGluRs.

4.8.2 Analysis

Analysis focused upon the retinae, optic nerves, optic tract, lateral geniculate nucleus of the thalamus and the superficial layers of the superior colliculus. Where markedly heavy labelling was found in non-visual areas of the brain this was also noted. The intensity of labelling was assessed and scored on a graded scale from 0 to 5 where 0=background label only and 5= very heavy labelling. Somal sizes were measured in cat retinae stained with the mGluR4 antibody to determine whether the spectrum of cell types were consistent with their being ganglion cells. One of these strips of retina ran from just below the area centralis at an estimated eccentricity of approximately 0.8-1mm out towards the far retinal periphery with eccentricity of approximately 1.2cm. There was a clear gradient in the density of labelled cells along this strip ranging from around 3000 cells per mm^2 to approximately 125 cells per mm^2. Analysis of the relative number of small, medium and large labelled cells based on measurements of soma sizes were undertaken on stained retinal strips at an eccentricity of approximately 1.5mm. Somal sizes were measured using a X60 oil immersion lens with a total magnification of X600 in the mid nasal retinae and differences in cell populations were compared using a paired Student's T-test.

5.0 Results - Immunohistochemistry

See Table 5.1 for a summary of distribution and relative intensity of immunoreactivity in the different species used in this study. In general, there are many differences in the pattern of immunolabelling between the different species.

5.1 mGluR1a

5.1.1 Rat

No labelling above control levels was seen in the retina. In the optic tract (OT), there was light glial labelling. In the LGN, labelling was more intense in dorsal lateral geniculate nucleus (dLGN) than in ventral lateral geniculate nucleus (vLGN). In the vLGN there was a restricted area of very heavy cellular label (Figures 5.1a and b). In the superior colliculus (SC), immunoreactivity was present throughout superficial, intermediate and deep layers, with quantitatively more labelling in the superficial layers of the superior colliculus (SSC) compared with the intermediate and deep SC (DSC). Label in the SSC had a very dense appearance consistent with cell processes rather than cell bodies. Light label was also present in the pretectum, an area which receives retina and collicular afferents, although the colliculus is the most likely source of this label as no label was observed in the retina.

5.1.2 Cat

Unlike in the rat, in the cat inner nuclear layer (INL) of the retina, around 25-30% of the cells appeared to be labelled (Figure 5.2). Some labelling of small cells was present in the ganglion cell layer (GCL), but in the optic nerve (ON), neither mGluR1a or 1b were present. Throughout the LGN, light background label was visible although it was not possible to attribute this to any specific feature. In the SC, light labelling, consistent with cell processes was evident in the stratum griseum superficiale (SGS) layer but was absent in the stratum zonale (SZ) and stratum opticum (SO) layers.

5.1.3 Ferret

Like the cat, in the ferret, label was present in the INL, but not in any other region of the retina. In the LGN the extent of label was moderate and diffuse in appearance, suggesting that the cell bodies are not labelled with this antibody. Dense, continuous label was present in the SSC, which had a very similar pattern of labelling to that of the rat. There was no obvious labelling of cell bodies and the labelling patterns appeared to be consistent with those in cell processes. There was very little label in intermediate SC layers. In the DSC, the appearance of the label was consistent with it being present in cell processes.

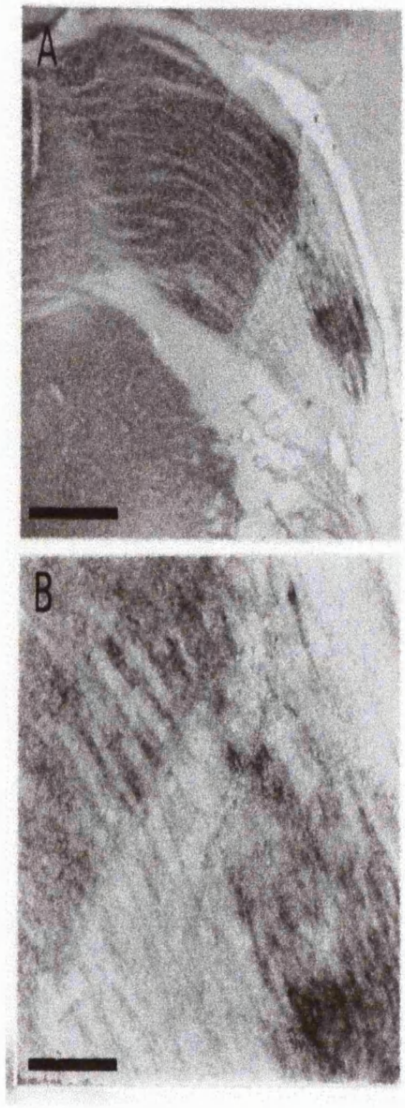


Figure 5.1 – mGluR1a in Rat LGN.

A – Low power micrograph of dLGN and vLGN. Heavy cellular label is present throughout the dLGN. In the vLGN labelling is restricted to a lateral region. Scale bar = 1mm

B – Enlargement of (A) showing the dLGN/vLGN border with label in vLGN on the right side. In each case, label was fibrous rather than cellular. Scale bar = 500μ m.

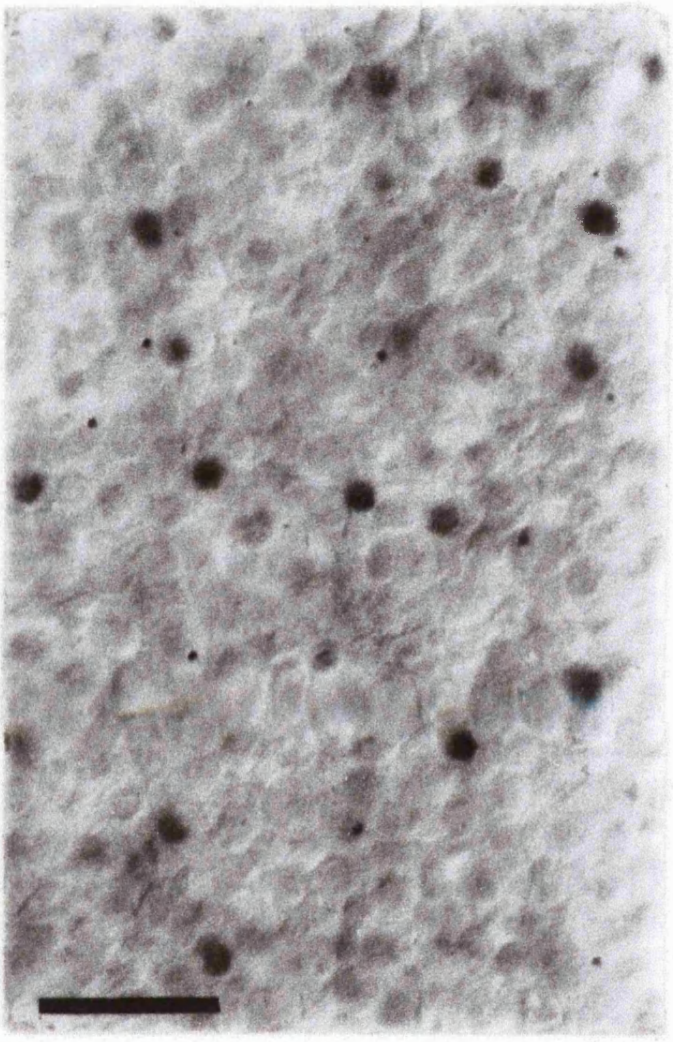


Figure 5.2 – mGluR1a label in cat retina.

Label shown is present in the INL and confined to a subpopulation of cells. Occasionally fine processes were labelled. The label in the ganglion cell layer was lighter. Scale bar = 50μm.

5.2 mGluR1b

5.2.1 Rat

In the retinal GCL layer there was very light labelling of small cells, consistent with their being glia. No label was evident in the OT. In the LGN, there was punctate label in the intergeniculate leaflet. In the SC, cell bodies were lightly labelled in the DSC, but none were present in any other area.

5.2.2 Cat

There was no mGluR1b label in the cat.

5.2.3 Ferret

Labelling patterns for mGluR1b were different in the ferret compared with rat and cat. Label was absent in the retina and OT. Light cellular label was evident in the LGN. In the SC there was moderate, diffuse label particularly in superficial layers, which was not associated with cell bodies and was consistent with labelling of processes.

5.3 mGluR1c

5.3.1 Rat

No label was observed in the retina or OT. In general, labelling throughout the rat brain was more robust in comparison with labelling for 1b. In the LGN there was frequent labelling of large cell bodies which occasionally extended to the primary dendrite (Figure 5.3a). In the SSC there was infrequent cell body label. In the very deep SC, there was clear, though infrequent labelling of cells with large somata. In the pretectum, cell bodies were labelled. Other prominent areas of staining included the substantia nigra, medial geniculate nucleus and the red nucleus where there was clear labelling of somata and primary dendrites.

5.3.2 Cat

As in the rat, no label was observed in the retina with the 1c antibody. There was light labelling of astrocytes in ON sections. In the LGN, stained cell bodes and their primary dendrites were evident at low magnification (Figure 5.3b) although the label intensity was much less than for the rat. Their morphology was consistent with their being LGN relay cells (Godwin *et al.* 1996a). The level of label in the SSC was comparable with other midbrain regions, in that it was fairly light and consistent with cell processes although a few cell bodies in the DSC were clearly labelled.

5.3.3 Ferret

Unlike the cat and rat, in the ferret retina there was occasional labelling in the INL and light cellular labelling in the IPL and OPL although labelling was absent in the ONL. There was also marked label in the GCL. Only very faint label was observed in the OT, suggesting label in the GCL may not be due to ganglion cells. In the LGN there was occasional labelling of cells and their primary dendrites (Figure 5.3c). Stained cell bodies were evident in the SSC, which was more heavily labelled than the deeper SC layers, although the extent of label in the SC was lower than that of the LGN. Other areas of heavy labelling included the ventrobasal nucleus of the thalamus.

Figure 5.3 mGluR1c label in A – rat, B - cat and C - ferret LGN.

In each case label is confined to cell bodies and primary dendrites. Label was more intense in rat and cat LGN than it was in the ferret.

Scale bars = 100μm.

5.4 mGluR2/3

5.4.1 Rat

Immunoreactivity for mGluR2/3 in all brain areas was more pronounced than for any of the mGluR1 antibodies. In the retina, light label spanned the full retinal depth consistent with Muller cell processes. Label in the OT was present only in small cells consistent with their being glia. Throughout the rat brain, there was labelling of glial cells in all areas with morphologies similar to astrocytes. In the LGN, label was present in larger neurones as well as glia. In both the dorsal and ventral LGN there was light labelling of cell bodies and processes. In the SC there was moderate cellular label throughout all layers, especially in the SSC.

A consistent feature of 2/3 immunoreactivity was glial labelling in all species (see Figure 5.4a/b/c).

5.4.2 Cat

In the INL of the retina, there was dense, punctate label. Light axonal and glial label was found in the ON. In each case where the morphology of these glial cells in the ON could be identified, they were consistent with the cells being astrocytes. Labelling with mGluR2/3 was widespread throughout the thalamus and brain stem including the LGN and SC. Labelling was confined to small cells with morphologies similar to astrocytes. These cells were heavily labelled when close to the pial border or in association with blood vessels. The SC was more densely stained than other midbrain areas, such as the medial geniculate nucleus with the SSC label more intense than in intermediate or deep SC.

5.4.3 Ferret

In transverse sections of the ferret retina, mGluR2/3 label was evident in all layers with the exception of the ONL. In the OT, many astrocytes were labelled with a few occasionally heavily labelled axons. Significant levels of label were observed in astrocytes in all regions of the LGN. The level of label in the SC was generally low compared with other midbrain regions. In the SSC, the only observed labelling was of astrocytes. Heavy astrocytic labelling with the 2/3 antibody was a very consistent feature in all of the species examined in this study.

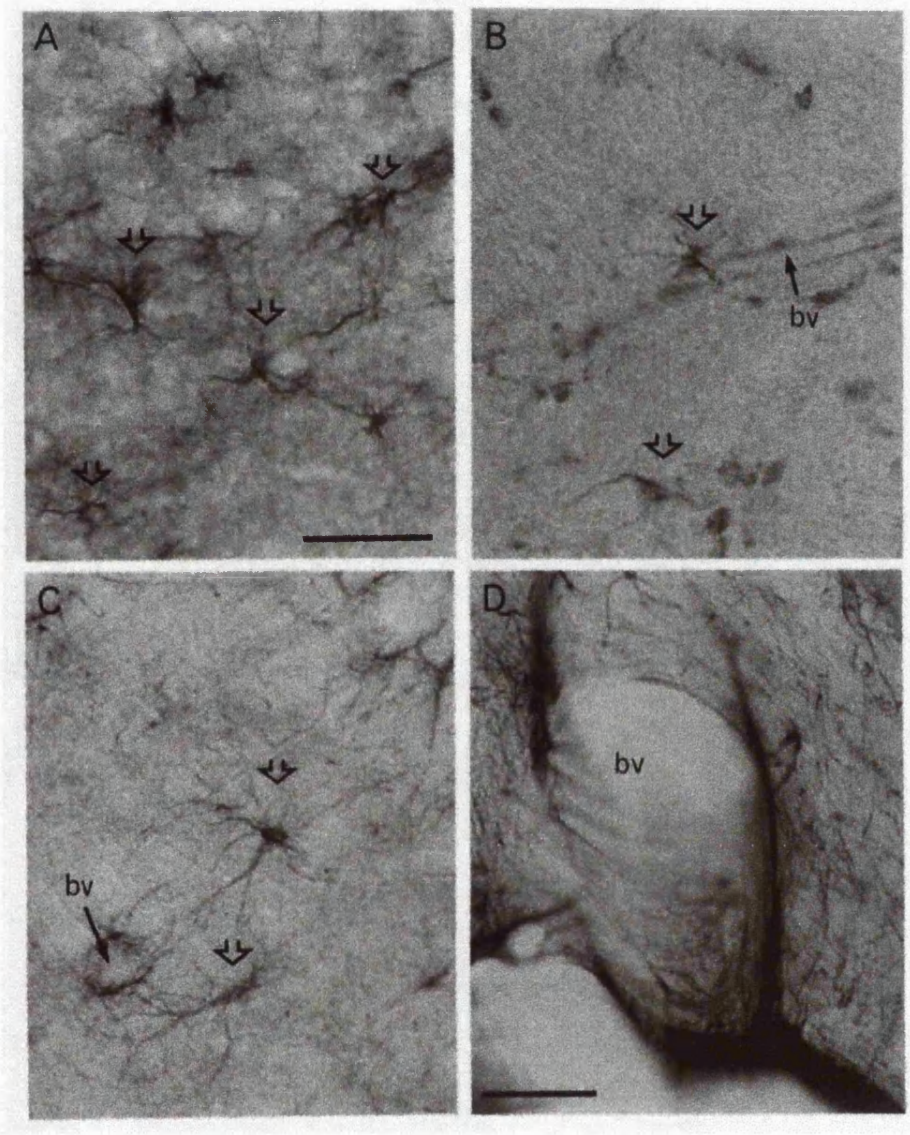


Figure 5.4 Astrocytes (arrows) labelled with mGluR2/3 in Rat (A), ferret (B) and cat (C).

Each micrograph is taken at similar locations in the ventral thalamus. Patterns of labelling with this antibody were almost identical between the different types of animal. Only astrocytes were labelled. These were marked in the location of the blood vessel walls. D shows a higher power micrograph of such processes wrapping around a blood vessel in the ventral thalamus of a rat. Scale bar A, B and C = 50 µm. Scale bar D = 30 µm.

5.5 mGluR4

5.5.1 Rat

Only light label was observed in the retina which may be associated with Muller cells. No label was observed in the OT. In the LGN there was moderate, fibrous label which was more intense in vLGN than dLGN and particularly pronounced in the intergeniculate leaflet (Figures 5.5a and b). There was heavy labelling in all midbrain regions, especially in the SSC, which was fibrous in nature (Figure 5.9a). Small, round unlabelled gaps were present, probably corresponding to the location of unlabelled cell bodies.

The DSC had a moderate level of label. In the pre-tectum there was heavy fibrous labelling. It is possible that given the absence of label in RGC's and the heavy labelling of the SSC that the source of this heavy axonal label in the pretectum is the SC. Alternatively, it may be present on the processes of cells which are intrinsic to this structure.

5.5.2 Cat

Unlike the rat, in the cat many of the cells in the GCL were heavily labelled for mGluR4. The label was mainly confined to the cell body, but in some cells, particularly larger cells, it was also present in the primary dendrites. The distribution of cells labelled was very similar to that seen following retrograde labelling of ganglion cells following HRP injections into the brain (Wassle *et al.* 1983). There were more cells labelled in central regions than in the retinal periphery and labelled cells could be grouped into those with large, medium and small somata. In both cases the large cells formed a distinct population which appeared to be distributed in a rough mosaic pattern. Analysis of the relative number of small, medium and large labelled cells based on measurements of soma sizes were undertaken on stained retinal strips at an eccentricity of approximately 1.5mm. At this location, the distribution of the cell body sizes was bimodal, with the somal size distribution of small and medium cells overlapping, whilst the large sized cells formed a distinctly separate group.

The mean somal size of the small, medium and large cells were 12, 16 and 23 μm respectively (Figure 5.8). Their relative proportions of the total population were approximately 40-45%, 40-45% and 14% respectively. These results are consistent with the cells being RGC's. Further, it is clear that each of the three main ganglion cell types are represented within the labelled population. No other retinal cell types were labelled with mGluR4 antibody in the cat. Label was present in ON and OT, consistent with labelling observed in RGC's. It was identified in all regions of the tract through to the brachium of the SC consistent with mGluR4 being transported down RGC axons to their terminals.

In the SC, the label was fibrous and distributed over the superficial layers providing further evidence of a retinal source for mGluR4 (Figure 5.9c). Minor levels of label were found in the intermediate layers of the SC. In DSC, occasional strong cell labelling was identified in large cells (Figure 5.9D). In these, the label extended throughout the primary dendrites. Label was also present in cell bodies of the LGN, although processes were not labelled (Figure 5.6). Many of the larger cells (mean cell diameter 26±0.11μm) were relatively heavily labelled in contrast to the smaller cells (mean diameter 14±0.11μm) where a relatively small number of cell bodies were labelled. These two cell sizes formed two distinctly different populations as the differences in their sizes were statistically significant (paired T-test $p<0.05$). No obvious differential distribution of label was observed in the different layers of the LGN. Intense cell body and primary dendrite label was identified in the red nucleus and in the adjacent brain stem.

5.5.3 Ferret

There was no clear labelling of cells in the ferret retina. In the whole mounted sections, small punctate labelling was evident although it was not possible to determine its nature. Analysis of the transverse retinal section revealed moderate, occasional labelling of cells in the INL. Label was absent in the OT. In the LGN, there was moderate cellular label throughout the nucleus. Label was particularly pronounced in the C laminae (Figures 5.7a and b) and was mainly of a fibrous appearance although some lightly labelled cell bodies were evident.

All areas of the brainstem had a moderate level of label, with dense labelling in the SSC. Label was low in intermediate SC and moderate in DSC. Label in the SSC was similar to that in the rat and had a punctate fibrous appearance, consistent with terminal processes, with round gaps probably corresponding to the location of cell bodies (Figure 5.9b). Given that labelling was absent in the OT, it is possible that this terminal label is from a cortical source.

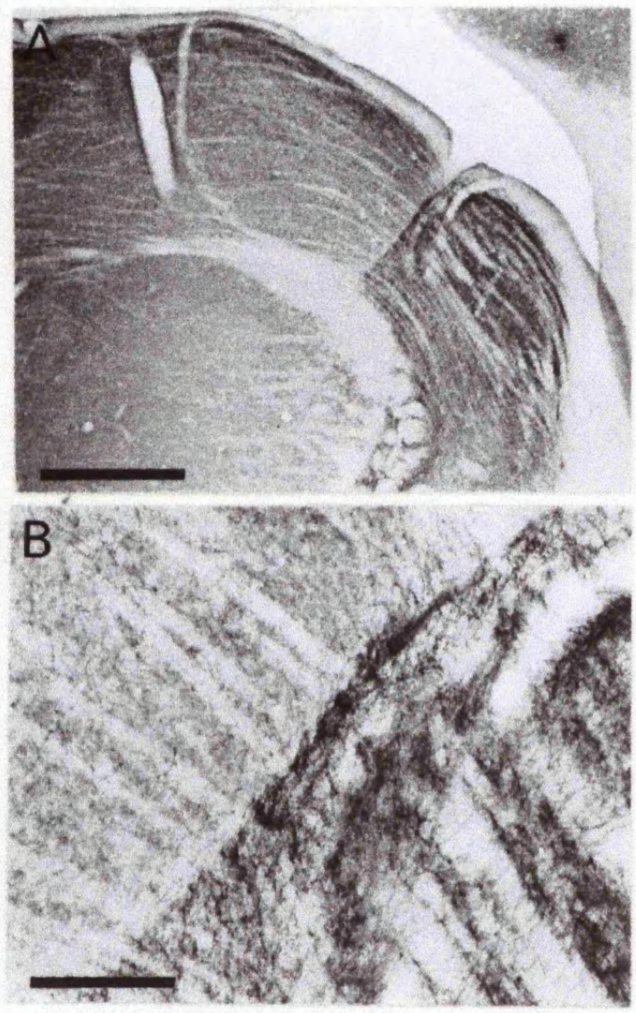


Figure 5.5 – mGluR4 label in rat LGN.

A – Low power micrograph of dLGN and vLGN. In both structures the label is fibrous but is stronger in vLGN than dLGN and pronounced in the intergeniculate leaflet between the two structures. Scale bar = 1mm.

B – Enlargement of A showing border of dLGN/vLGN. Scale bar = 500µm.

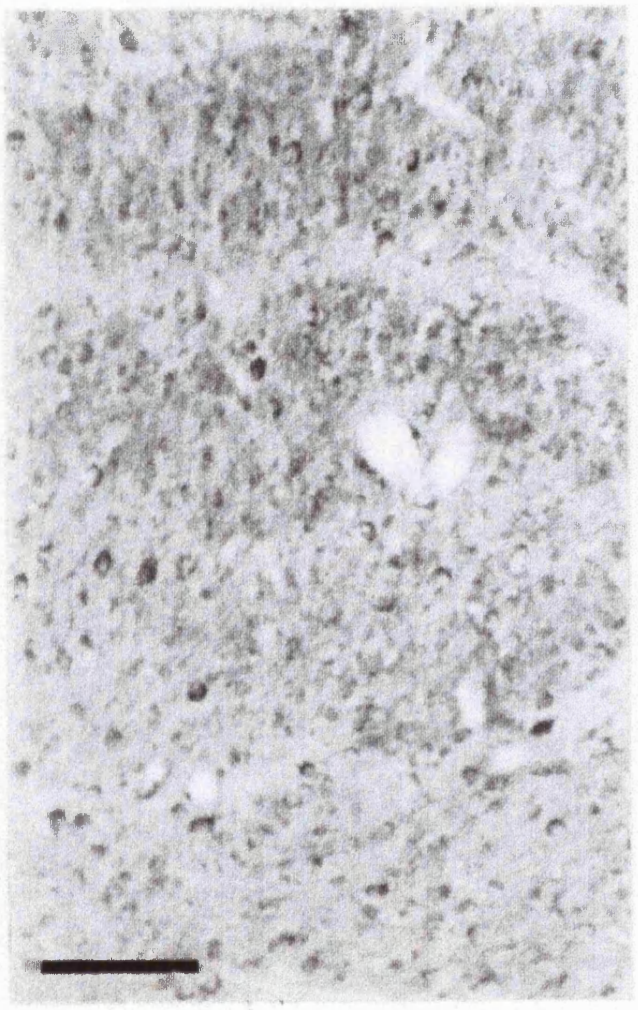


Figure 5.6 – mGluR4 label in cat LGN.

The label was confined to cell bodies and was relatively light. Larger cells appeared to be more heavily labelled. Scale bar = 200μm.

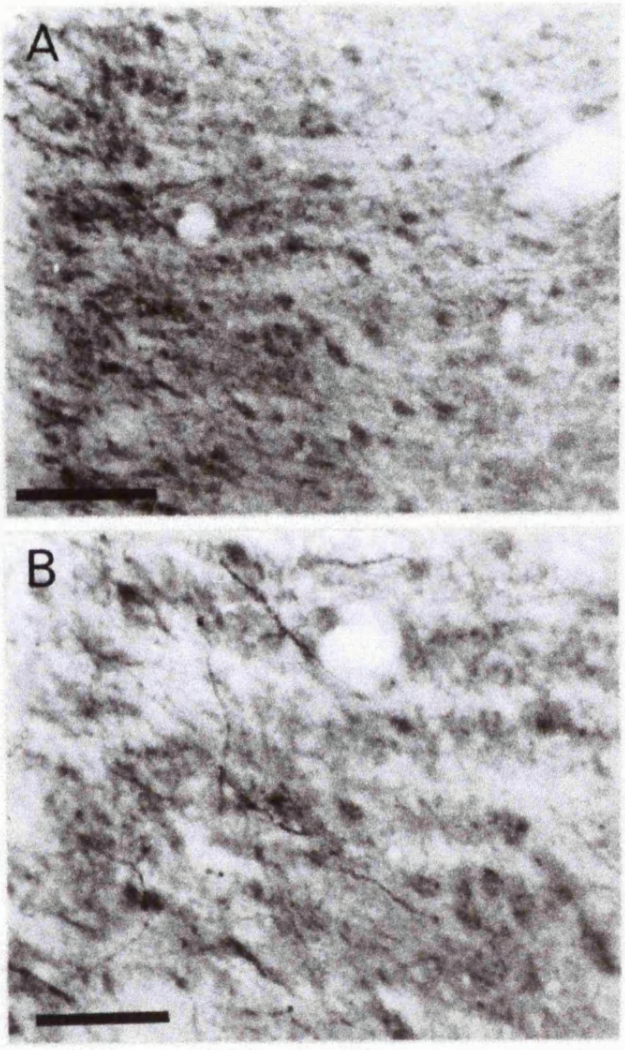


Figure 5.7 – mGluR4 label in ferret LGN.

A – Border of C-laminae (left side) shows heavier label than in adjacent region. Cell bodies are lightly labelled. Scale bar = 200μm.

B – Higher magnification of this region shows that the labelling is due to the presence of labelled fibres. Scale bar = 100μm.

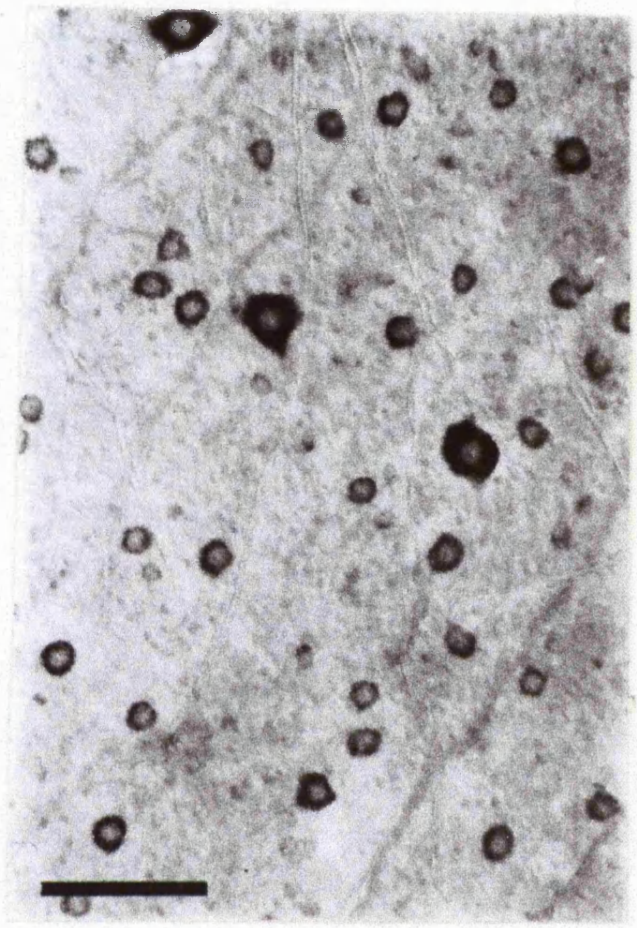


Figure 5.8 – mGluR4 in cat retina.

Strongly labelled retinal ganglion cell bodies were present but no other label was found. All three classes of ganglion cells were labelled in all retinal regions. Scale bar = 80μm.

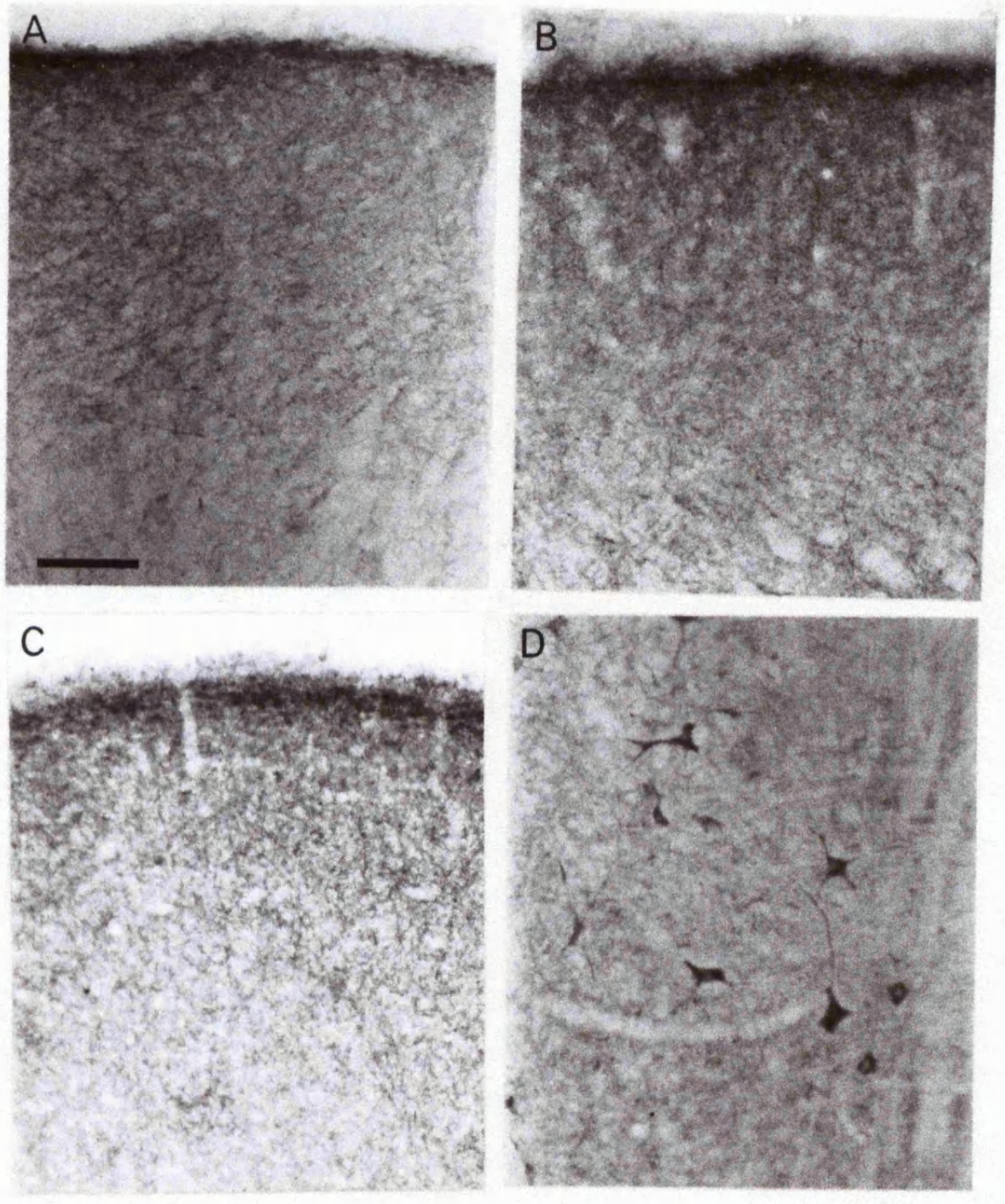


Figure 5.9 – mGluR4 in the SC of A – rat, B – ferret and Cat, C & D.

Similar fibrous label was present in SSC in each of the three species. However, in the cat large cells were labelled in the DSC (D) which were not seen in the rat or ferret. Scale bar = 80μm.

5.6 mGluR5

5.6.1 Rat

A few clearly labelled axons were visible in the GCL, although labelling for mGluR5 in RGC cell bodies was relatively light. There was also light cellular labelling in the ONL of the retina. Around 10% of OT fibres were labelled. In the LGN, heavy fibrous label was observed in dorsal and ventral regions (Figure 5.10a). In the intergeniculate leaflet there was also axonal and terminal like label, with rare somal label. All midbrain areas had moderate labelling, with that in the SSC being a dense, solid band running across the medial lateral extent of the SC. Small, round unlabelled areas, probably corresponding to unlabelled cell bodies were evident. Only poor labelling was found in intermediate SC and moderate in DSC. Label in the SSC was not associated with cell bodies and was consistent with terminal processes (Figure 5.11a) which may have a retinal or possibly thalamic source. Alternatively, this could arise from intrinsic inhibitory interneurones. Fibrous labelling was also present in the pretectum and could be of both retinal and collicular origin.

5.6.2 Cat

As with the rat, mGluR5 label was present in the RGC layer but its density was much lighter compared with mGluR4 and was confined to cell bodies. As with the pattern of labelling observed for mGluR4, the distribution of immunolabelled cells was very similar to that seen following retrograde labelling of ganglion cells following tracer injections into the brain. Similar analysis to that carried out in the mGluR4 stained retina was not undertaken for mGluR5 because the labelling with not sharp enough to guarantee accurate measurements. Rarely, labelled microglia were found throughout the retina (Figure 5.12). Axonal staining for mGluR5 was granular and relatively light. Because of this, it was not possible to estimate the proportion of axons that were labelled, but it was possible to trace the trajectory of occasional heavily labelled larger calibre axons over a considerable distance.

The extent of mGluR5 immunoreactivity in the optic nerve was greater than for mGluR4 with approximately 10% of fibres in the OT being labelled. In the ON, there was also light glial staining.

The label in the cat LGN was relatively strong, but apparently confined to processes rather than cell bodies, consistent with its location within retinal terminals (Figure 5.10c). Counterstaining revealed that immunoreactivity and cell bodies do not localise. Labelling in the OT and brachium of the SC indicates that some mGluR5 label in the SC may result from retinal innervation. In the SC, mGluR5 label was lighter than that found with mGluR4 and confined mainly to the most superficial layers. The nature of the label was mainly fibrous with rare lightly labelled cell bodies. No cellular label was found in the deep layers, but as with mGluR4, there was prominent staining in the red nucleus and the lateral ocular motor nucleus, which was confined only to cell bodies.

5.6.3 Ferret

In the retina, labelled axons were visible, and in the transverse section it was possible to pick out occasionally labelled cell processes and fibres in the INL. Light staining was evident in the OT which was more consistent with axonal labelling than glial label. Label in the C laminae of the LGN was particularly prominent, with labelled cell bodies evident at higher magnification (Figure 5.10b). Labelling in SSC was intense and mainly fibrous with round unlabelled regions likely to correspond with unlabelled cell bodies, although there was occasional light cell body label. Insignificant levels of immunoreactivity were observed in deep SC layers (Figure 5.11b).

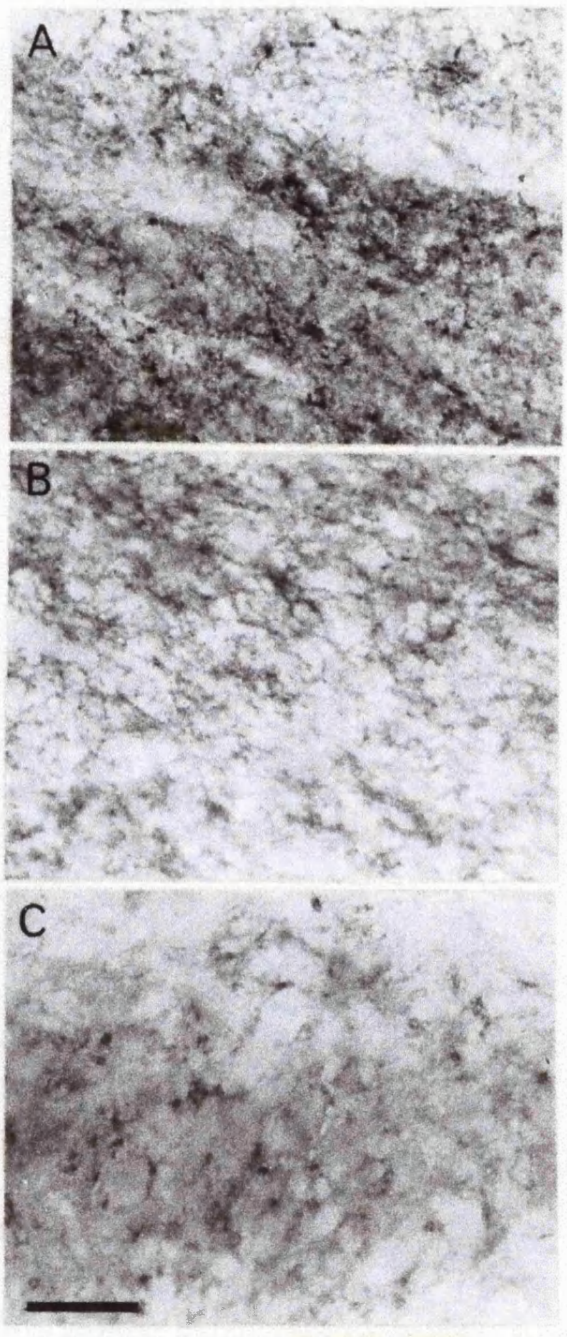


Figure 5.10 – mGluR5 in the LGN of A – rat, B – ferret and C – cat.

In each case label was confined to fibres, not cell bodies. The intensity of the label varied between animals with that in the rat being relatively dense and relatively light in the cat, label in the ferret was intermediate between the two. Scale bar = 70μm.

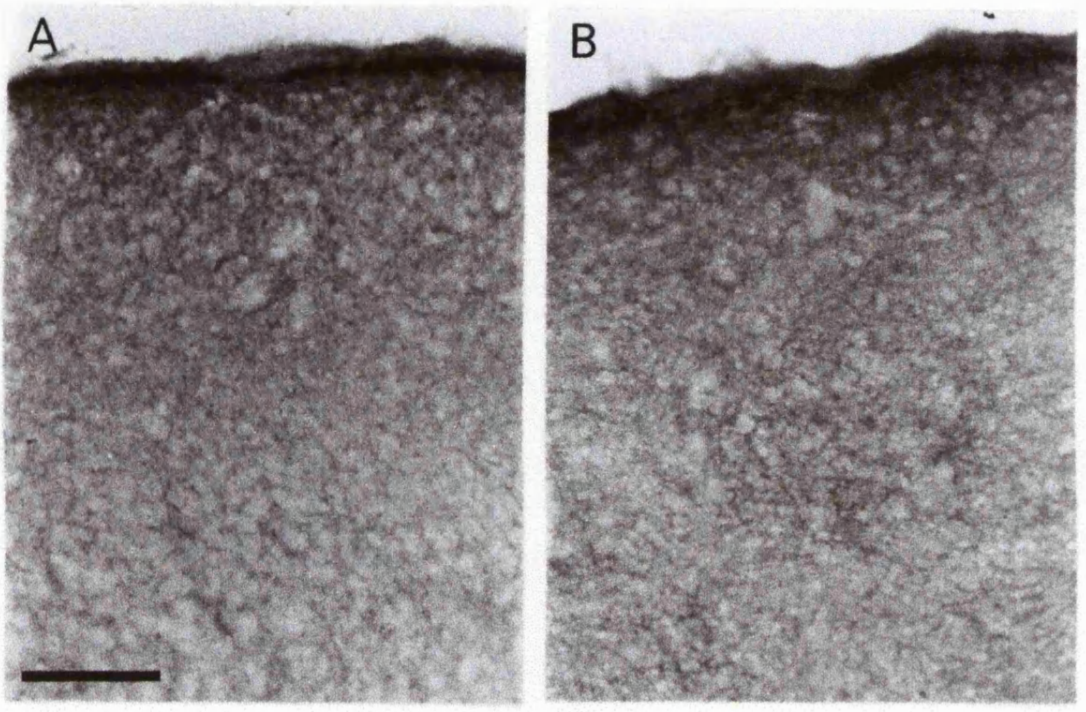


Figure 5.11 mGluR5 in A – rat and B – ferret SC.

Label was predominantly confined to SSC in both animals. It was present in fibres, but not in cell bodies. Scale bar = 100μm.

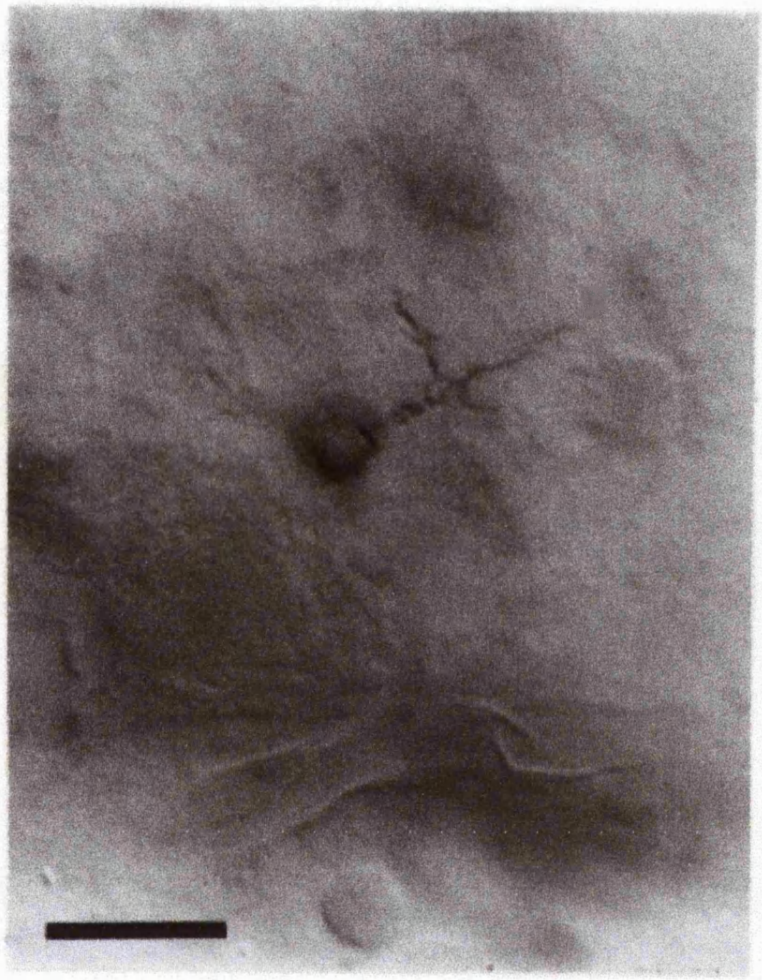


Figure 5.12 – mGluR5 label in cat retina.

In the retina label was present in microglia. These were well labelled but probably only present in a sub-population as labelled cells were relatively rare. Scale bar = 10μm.

Table 5.1 Summary of distribution and relative intensity of mGluR immunolabel in different species

	1a	1b	1c	2/3	4	5
Rat						
Retina	-	••C	-	•P	•P	•C&P
Optic nerve/optic tract	•C	-	-	•P	-	••P
LGN	•••C	•C	••C	•C&P	•••P	•••P •C
SC	••P	-	•C	•••C	••••C	•••P
Ferret						
Retina	•C	-	••C	•C&P	•C	••P
Optic nerve/optic tract	-	-	•P	•C&P	-	•P
LGN	••P	•C	••C •P	••C	•••P	•••••C
SC	••P	••P	••C	••C	••••P	•••P •C
Cat						
Retina	•C	-	-	•C	••C •P	••C
Optic nerve/optic tract	-	-	•C	•C&P	••P	•••P
LGN	•P	-	•C&P	••C	••C	•••••P
SC	•P	-	•P	••C	•••P	•C&P

P=Cell processes
C= Cell bodies

- \- No label or not significantly greater than control
- • Very light label
- •• Light label
- ••• Moderate label
- •••• Heavy
- ••••• Very heavy

6.0 Results – Physiological role of mGluRs in modulation of visual responses

6.1 Overview

The results presented in this Chapter reveal that mGluRs are activated during visual synaptic transmission in the rat SSC and that activation of these receptors can produce both facilitatory and inhibitory effects. To design experiments to probe the role of mGluR mediated modulation of visual responses, some preliminary experiments were carried out to investigate the response characteristics of visually responsive SSC neurones. The data from these experiments are described in Section 6.2. By using different types of physiologically relevant stimuli it has been found that sub-types of mGluRs have distinct roles in modulating different aspects of the visual response (see Sections 6.8 and 6.9).

6.2 Characterisation of response properties of visually responsive SSC neurones

Previous work has investigated the response characteristics of visually responsive neurones in the SSC of different species including the rat (see Section 1.3 and Table 1.5 for details). In order to use appropriate stimuli for pharmacological investigations, some preliminary experiments were carried out to investigate the responses to different types of visual stimuli. Pharmacological modulation of some of the most interesting response types (habituation and contrast sensitivity) have been further investigated. A summary of these preliminary experiments is presented below.

6.2.1 Size of the excitatory component of the receptive field

Presentation of flashed spots of light resulted in a response consisting of "on" and "off" components. Presentation of very small stimuli elicited sub-maximal responses, with the mean maximal response elicited by a spot size of $9.8^0 \pm 1.7$ (n=24).

Stimuli larger than the optimal response size led to a reduction of the response, consistent with the idea of activation of inhibitory surround mechanisms. See Fig 6.1 for data summary.

When receptive fields were arbitrarily divided into "rostral" and "caudal" components of visual space, it was found that there was a small increase in the size of stimulus that elicited the maximal response for caudal receptive fields ($10.5^0 \pm 1.2$) in comparison with rostral receptive fields ($8.8^0 \pm 1.3$) although this difference was not statistically significant. There was no obvious correlation between recording depth and size of stimuli producing the maximal response.

6.2.2 Orientation and directional selectivity

Using two different methods to interpret data (see Section 4.7), it would appear that SSC neurones are not strongly tuned for orientation or direction although a few cells do exhibit a strong preference. Figure 6.2A shows the distribution of I values for all cells. Values of $I>0.5$ represent a ratio of 2:1 of the maximum response compared with the minimum response. Mean I value for all data 0.46 ± 0.3 ($n=24$). From examination of Figure 6.2A, it would appear that the data is categorised into three groups, however, when using another method to quantify the extent of orientation tuning, the cells do not appear to form three distinct populations (see Figure 6.2B).

Another method for calculating the extent of orientation of directional selectivity is by scoring "B". B describes the spread of tuning such that a B value of 0 indicates that there is no bias as all and B value of 1 indicates that cells response only to a stimulus moving in one direction and orientation only. Mean B value for all data is 0.11 ± 0.09. This low B value would suggest that rat SSC cells have little directional or orientation bias. See Figure 6.2B for the distribution of B values.

6.2.3 Response to moving stimuli

In contrast to the response to presentations of flashed objects, presentation of moving objects resulted in a continuous train of action potentials. Small responses occurred when the stimulus moved very fast (180^0/sec) and a decline in the response was observed in some cells when the stimulus moved very slowly across the receptive field (11^0/sec). Optimal responses were observed when stimuli moved in the range of 22.5 – 45^0/sec.

6.2.4 Response habituation

The extent of response habituation was calculated as described in Section 4.7. When the interstimulus interval was very small (0.5secs) the extent of habituation was greatest and decreased as the interstimulus interval increased. See Table 6.1 for data summary, Figure 6.3 for a graphical representation of this data and Figure 6.4 for data from an individual neurone.

Table 6.1 - Summary data of response habituation at different interstimulus intervals

Interstimulus interval (secs)	Extent of response habituation
0.5	60.7%±17.1
1	48.2%±20.1
2	38.5%±20
4	26.8%±18.7
8	13.3%±10.1

Table 6.1 – Summary data for 22 cells showing the extent of response habituation at different interstimulus intervals.

6.2.5 Contrast sensitivity

All cells responded maximally to the highest contrast between stimulus foreground and background (background luminance 7.7cd m^{-2}, foreground luminance 20cd m^{-2}) with much smaller responses (53%±5 of full contrast response) to reduced foreground contrast (12.2cd m^{-2}).

Reduced and full contrast stimuli were used in subsequent experiments in order to vary the level of the synaptic response (see Section 6.9).

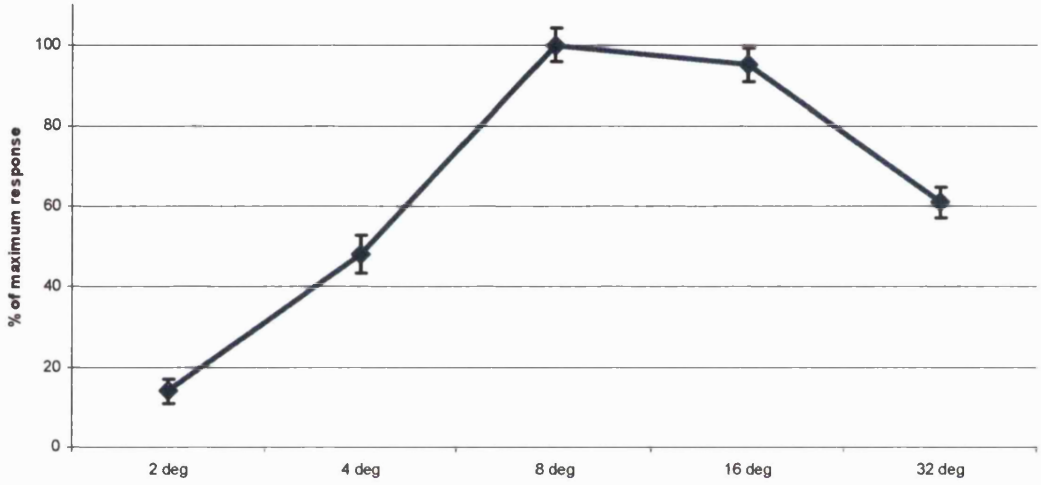


Figure 6.1 – Summary data for 24 cells. The response to flashed spots of light of different sizes is plotted as a percentage of the maximum response. Error bars = s.e.m.

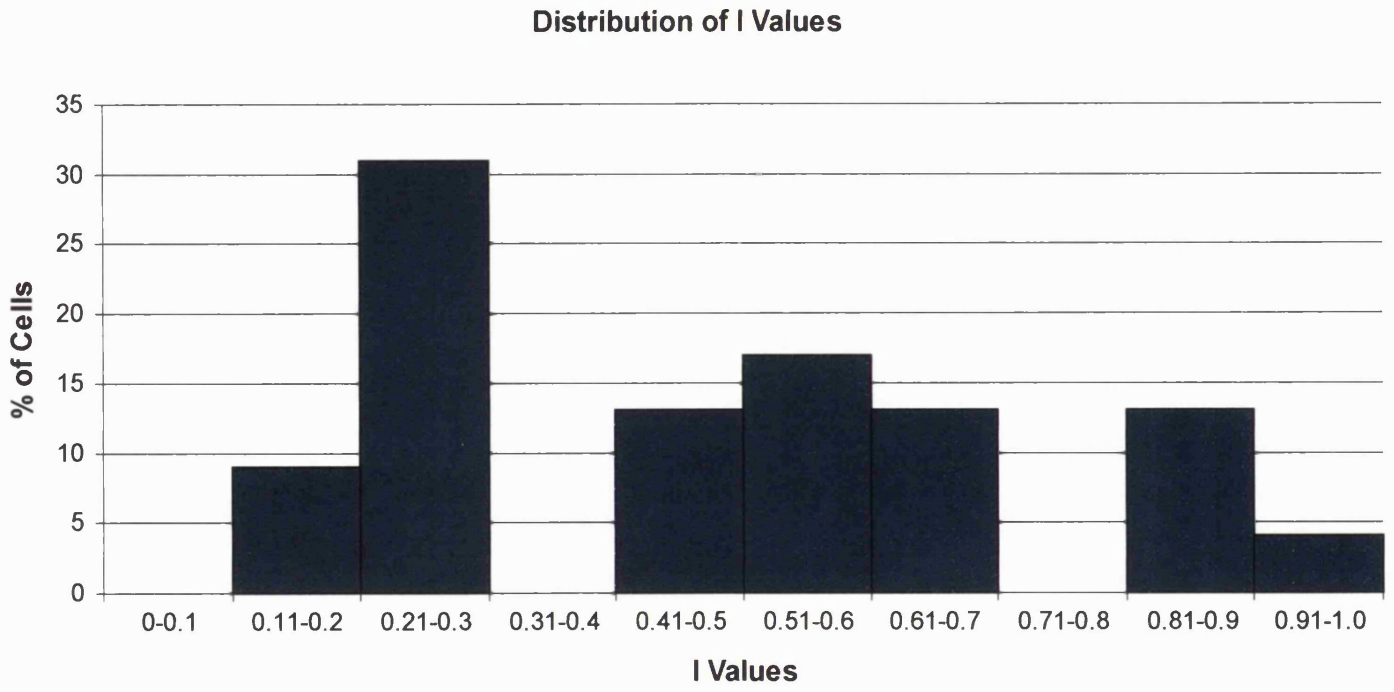


Figure 6.2A - Distribution of "I" values for 23 cells. See Section 4.7 for calculation of I. Values of I >0.5 represent a ratio of 2:1 of the maximum response compared with the minimum response for that cell.

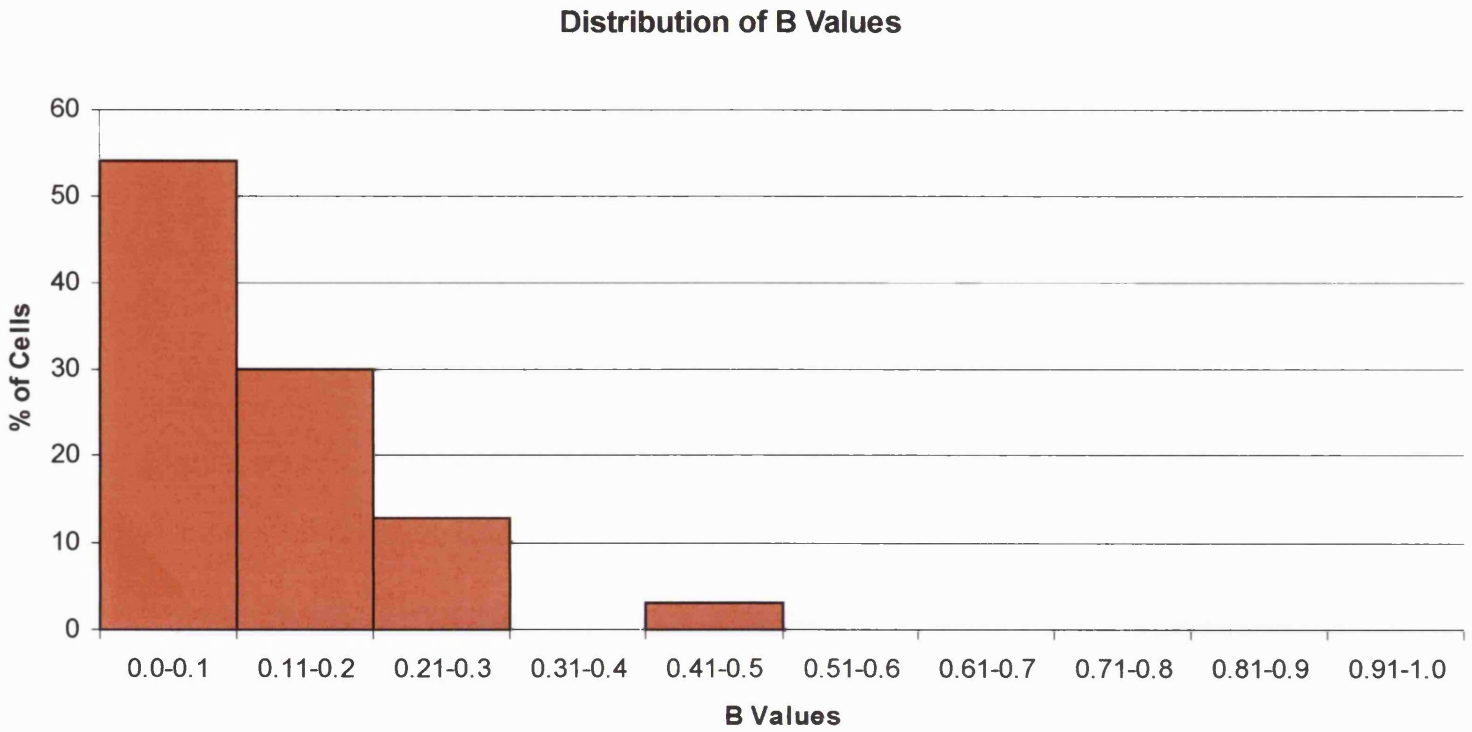


Figure 6.2B - Distribution of "B" values for 23 cells. See Section 4.7 for calculation of B. B value of 0 means no direction bias and B or 1 indicates that a particular cell responds solely to a stimulus presented in only one direction/orientation.

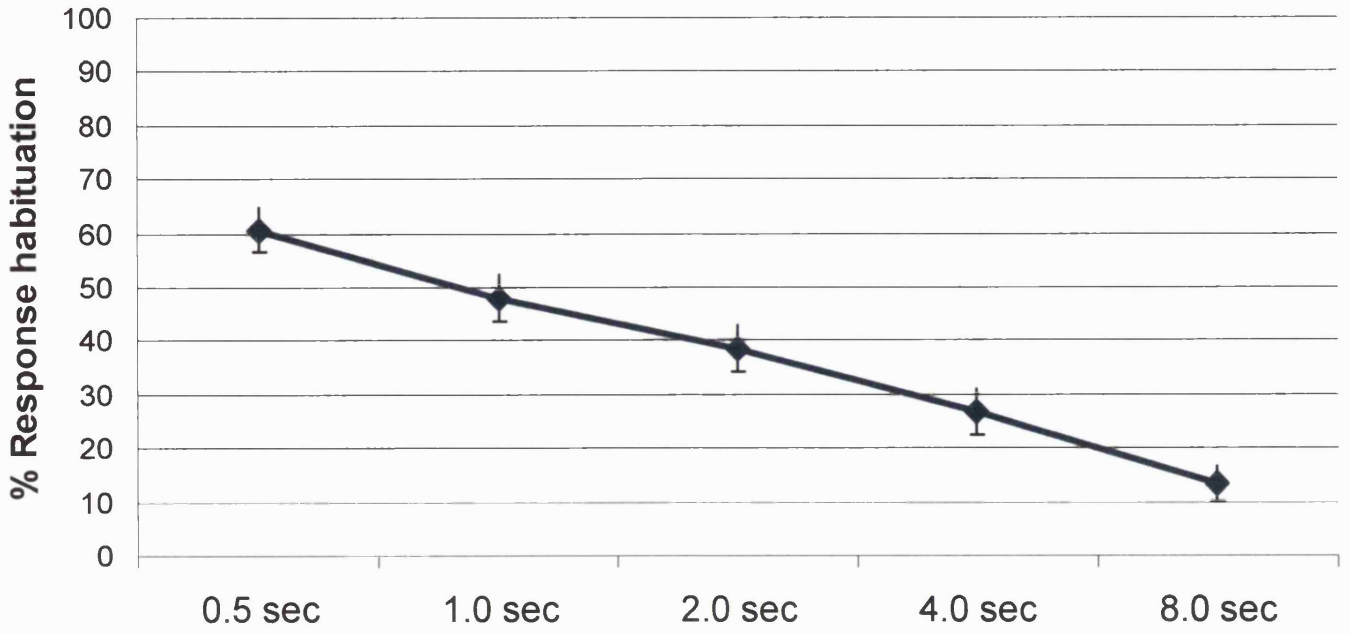


Figure 6.3 - Graphical summary of data from 22 neurones showing the extent of response habituation as calculated in Section 4.7. X axis is interstimulus interval. Error bars = ± s.e.m

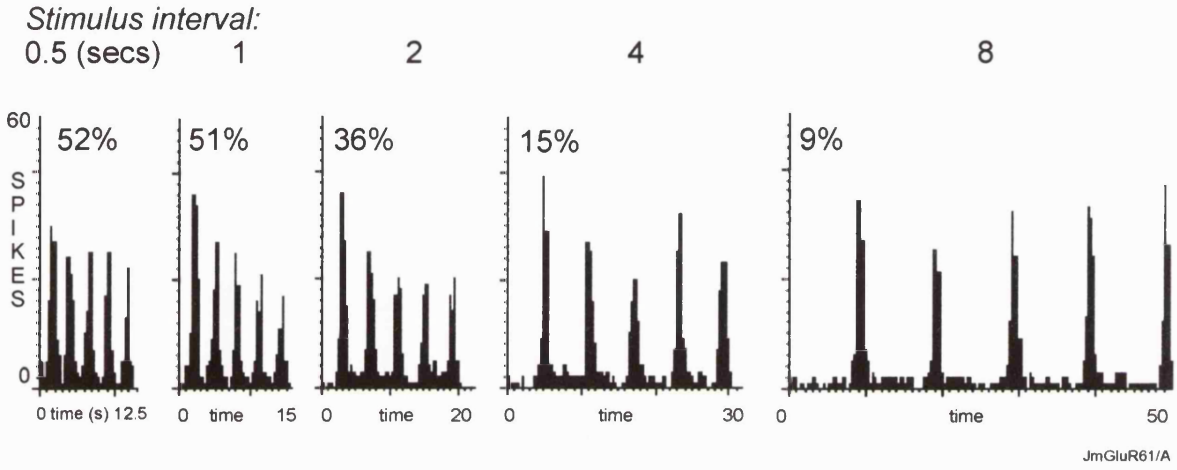


Figure 6.4 -Extent of habituation in an individual neurone when the visual stimulus is presented at different interstimulus intervals as indicated above each plot. Extent of habituation as indicated by percentage values is calculated as in Section 4.7. Histograms show counts of spikes evoked by 5 presentations of identical stimuli (15 x 10deg moving bar), cumulative data from 5 trials is shown for each plot, bin size=200msec.

6.3 Visual responses can be modulated by agonists of all three mGluR groups

To investigate the effect of mGluR activation, group-selective agonists (**Group I:** 3,5-DHPG, ejection currents +25 to 150nA, **Group II:** LY354740, ejection currents –25nA to 100nA, **Group III:** L-AP4,,ejection currents –25 to 100nA) were applied by iontophoresis. As some drugs may have selectivity across a concentration range, the smallest ejection current which would produce an effect was routinely used. Application of agonists of all three mGluR groups resulted in modulation of visual responses, Group I activation resulted in an inhibition of the visual response, with Group II and III agonists producing both excitatory and inhibitory effects. The effect of agonist application in every cell was fully reversible once agonist ejection ceased. See Table 6.2 for data summary. Figures 6.5, 6.6, 6.7A and 6.7B show examples of agonist effect for Group I, II and III respectively. There was no obvious correlation between agonist effect and recording site as revealed by deposit of dye spots. Figure 6.8 shows the distribution of drug effects (as a percentage of control) for both LY354740, L-AP4 and saline controls. From these distributions, it is clear that cells which are "agonist inhibited" form a distinctly separate population from cells that are "agonist facilitated" rather than being a single population distributed around 100% of control. The distribution of effects of saline application do not overlap with that of drug effects and are centered around 100% of control.

Table 6.2 Effect of agonist application on visual responses

Agonist	Effect on visual responses	n=	Effect on spontaneous firing	Saline Controls
Group I				
3,5-DHPG				
Inhibited	71±3.1%**	18	97±5.7%	96±3% (n=8)
Group II				
LY354740				
Inhibited	74±8.6%**	23	77±6.4%**	97±2.7%
Facilitated	140±±4.7%**	20	120±7.5%	(n=11)
Group III				
L-AP4				
Inhibited	70±3.9%**	90	80±5.2%**	96±2.8%
Facilitated	146±3.9%**	41	124±6.6%	(n=18)

Results are expressed as % control ± s.e.m ** $P<0.05$

In the majority of cells, application of the agonist produced either exclusively inhibitory or facilitatory effects, in a small proportion of cells (n=9 for L-AP4 and n=2 for 3,5-DHPG), the agonist could produce both inhibitory or facilitatory effects when different ejection currents were used. See Figure 6.9A for an example where the Group I agonist 3,5-DHPG has mixed inhibitory and facilitatory effects at different ejection currents and 6.9B for an example with the Group III agonist L-AP4.

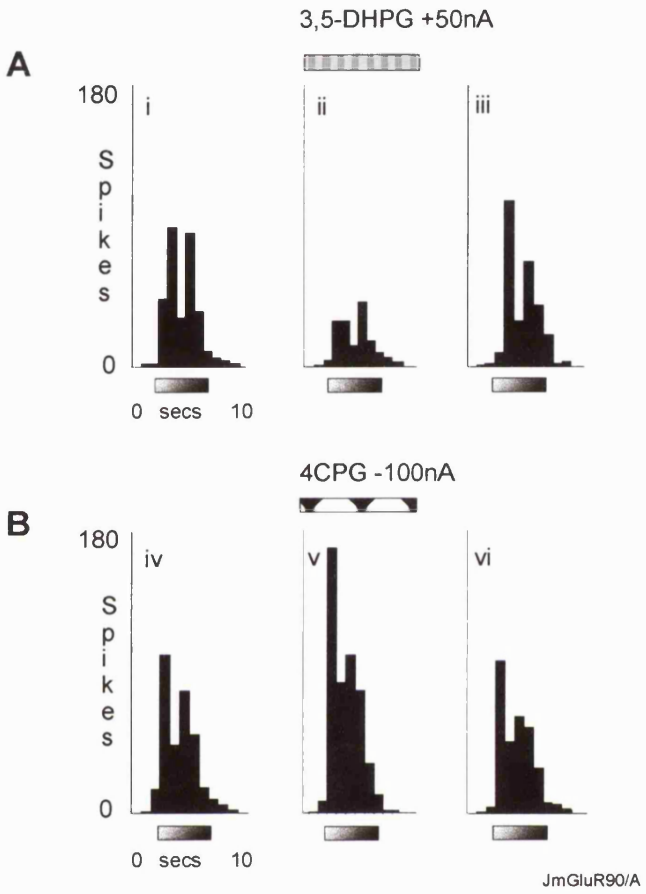


Figure 6.5 - Data from an individual neuron showing that the Group I agonist 3,5-DHPG and Group I antagonist 4CPG have the opposite effect in the same neurone. The histograms show cumulative counts of action potential spikes during (**A)** (i) control, (ii) inhibition of visual responses during DHPG ejection (+50nA), (iii) recovery data obtained 120 secs after 3,5-DHPG ejection ceased. **(B)** (iv) new control, (ii) during 4CPG ejection (-100nA), (iii) recovery data obtained 120s after 4CPG ejection ceased.

Stimulus details:- Presentation of ten visual stimuli (5x10-15 ^{0}) moving at 22.5 ^{0}/sec for 4 secs every 10secs, bin size=200msec, trial duration=110 secs). The moving bars were or preferred orientation and direction. Visual stimuli were presented during the period marked by the shaded bar under each histogram.

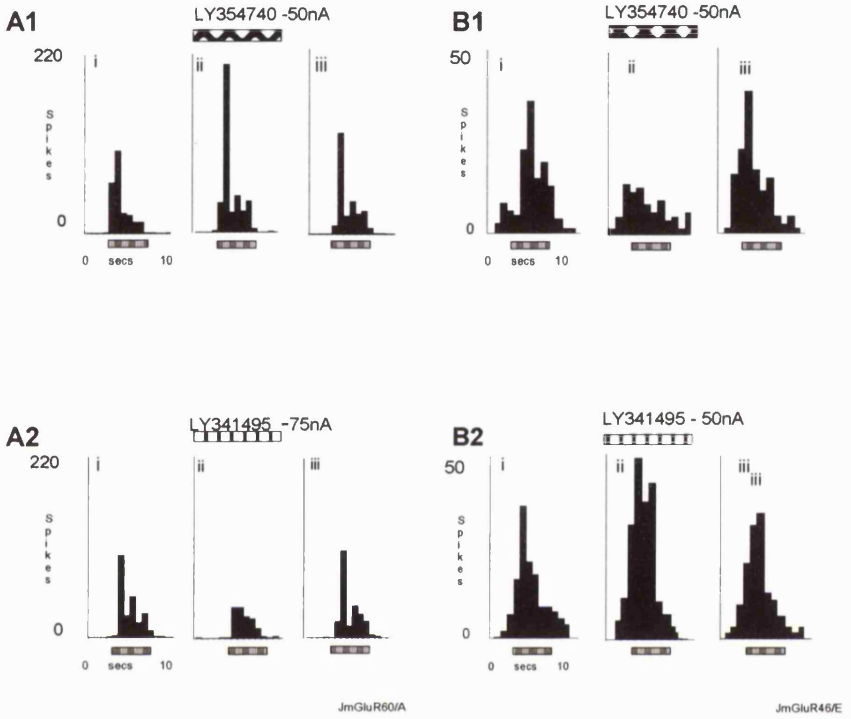


Figure 6.6 - A1 and **A2** -PSTH records from an individual neurone showing that the Group II agonist LY354740 and antagonist LY341495 have the opposite effect in the same neurone. In this neurone, LY354740 has a facilitatory effect, whilst LY341495 causes an inhibition of visual responses. **B1** and **B2** - Data from a different neurone, (compare with A) where LY354740 inhibits the visual response and LY341495 causes a facilitation. The histograms show cumulative counts of action potential spikes under (i) control conditions, (ii) during LY354740 or LY341495 ejection, (iii) recovery data obtained 120 secs after drug ejection ceased. See Figure 6.1 for details of visual stimulus.

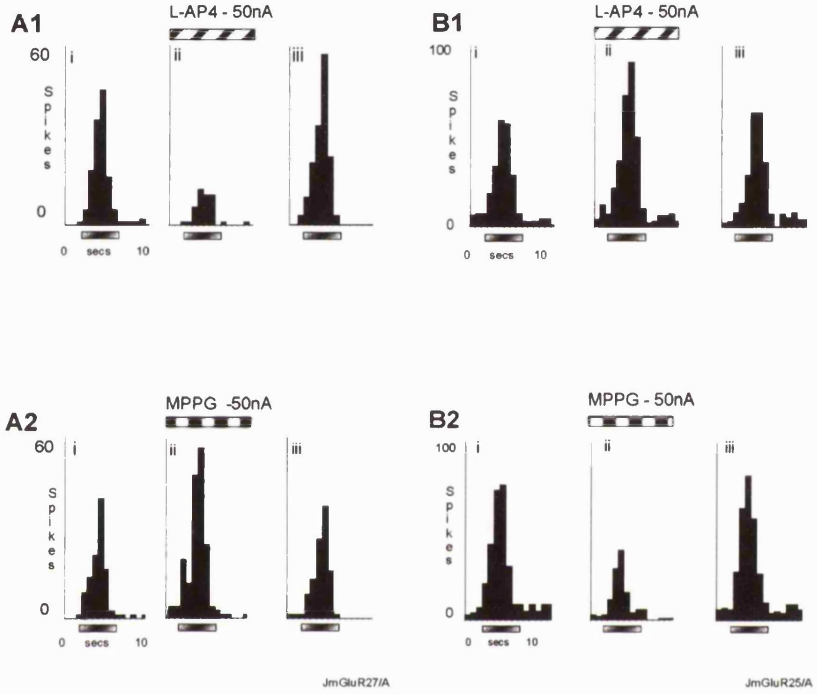


Figure 6.7A - A1 and **A2** -PSTH records from an individual neurone showing that the Group III agonist L-AP4 and antagonist MPPG have the opposite effect in the same neurone. In this neurone, L-AP4 has a inhibitory effect, whilst MPPG causes a facilitation of visual responses. **B1** and **B2** - Data from a different neurone, (compare with A) where L-AP4 potentiates the visual response and MPPG causes an inhibition. The histograms show cumulative counts of action potential spikes under (i) control conditions, (ii) during L-AP4 or MPPG ejection, (iii) recovery data obtained 120 secs after drug ejection ceased. See Figure 6.1 for details of visual stimulus.

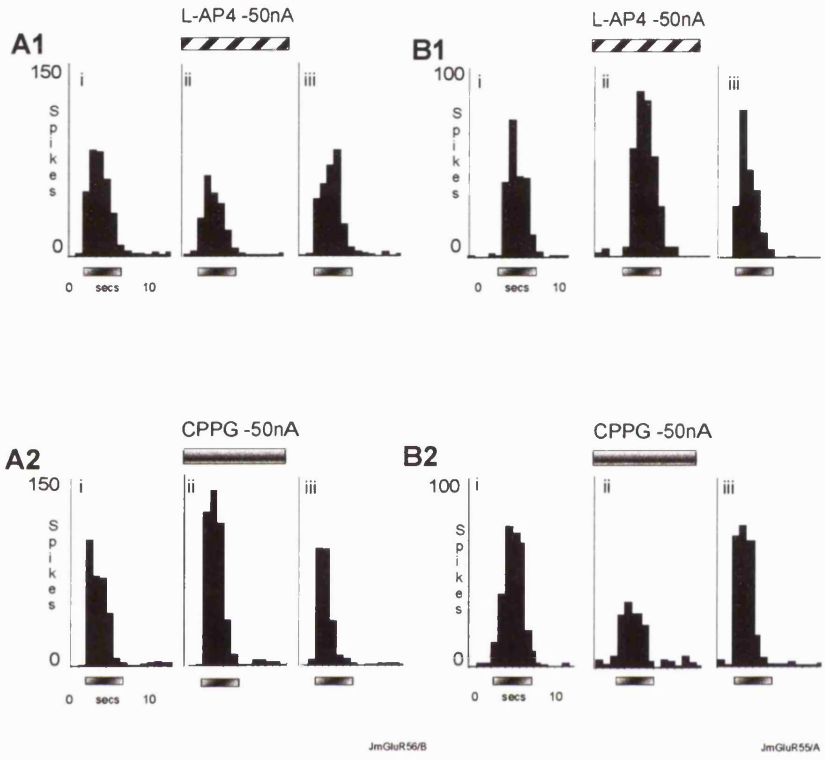
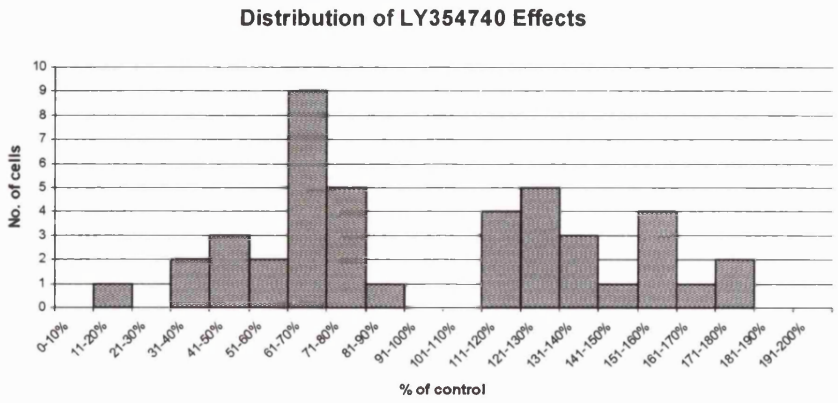
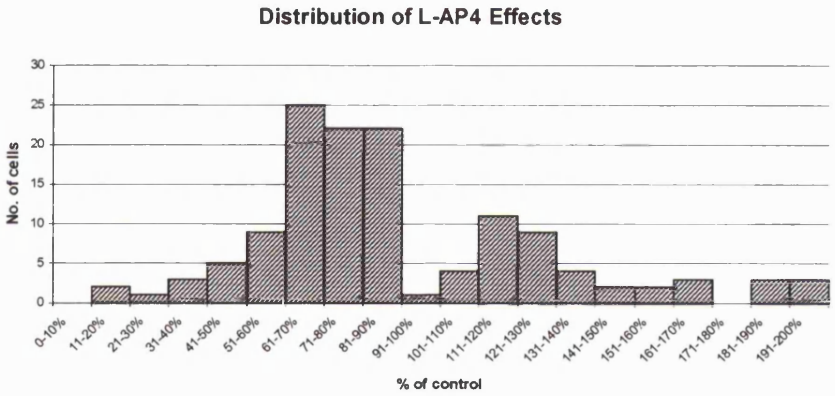


Figure 6.7B - A1 and **A2** -PSTH records from an individual neurone showing that the Group III agonist L-AP4 and antagonist CPPG have the opposite effect in the same neurone. In this neurone, L-AP4 has an inhibitory effect, whilst CPPG causes an facilitation of visual responses. **B1** and **B2** - Data from a different neurone, (compare with A) where L-AP4 facilitates the visual responses and CPPG causes an inhibition. The histograms show cumulative counts of action potential spikes under (i) control conditions, (ii) during L-AP4 or CPPG ejection, (iii) recovery data obtained 120 secs after drug ejection ceased. See Figure 6.1 for details of visual stimulus.

A



B



C

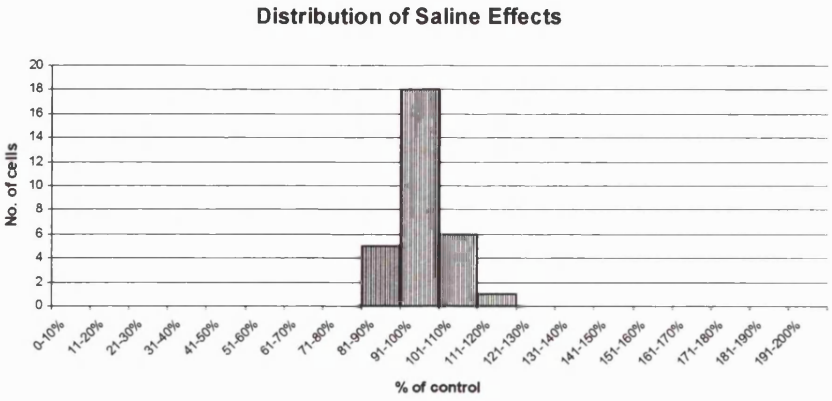


Figure 6.8 – Histograms showing the distribution of effect of (A) LY354740, (B) L-AP4, (C) Saline. Inhibitory and facilitatory effects of both LY354740 and L-AP4 form two different populations which are not centrally distributed around 100% of control. Contrast with saline effects.

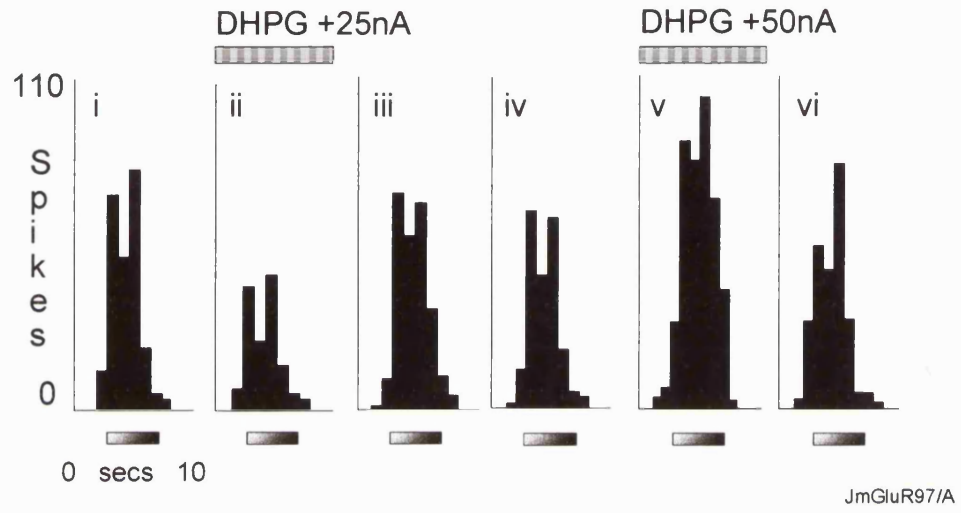


Figure 6.9A - The Group I agonist 3,5-DHPG can cause different effects on the visual response when the ejection current is varied. In this neurone DHPG has an inhibitory effect when ejected at +25nA, whilst a facilitatory effect is observed when the ejecting current is increased to +50nA. The plots show cumulative counts of action potential spikes during (i), control, (ii) during ejection of 3,5-DHPG (+25nA), (iii) recovery 120 secs after DHPG ejection ceased, (iv) new control, (v) during ejection of 3,5-DHPG (+50nA), (vi) recovery data obtained 120 secs after DHPG ejection ceased. See Figure 6.1 for details of visual stimulus.

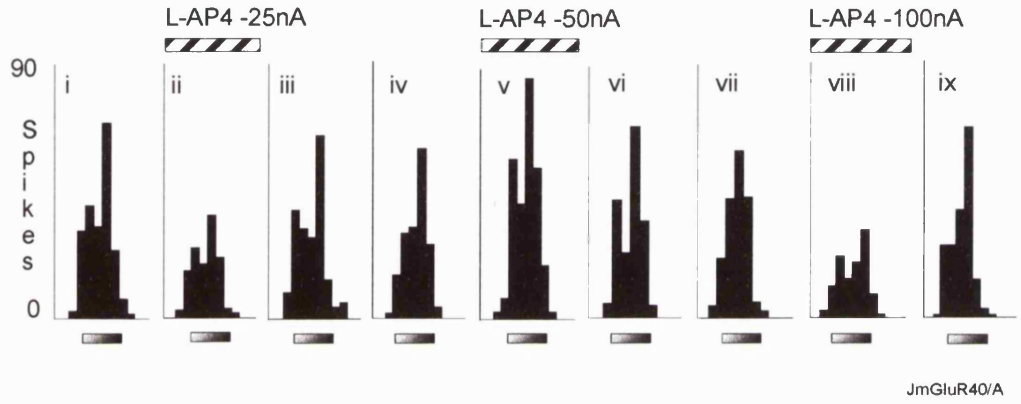


Figure 6.9B - The Group III agonist L-AP4 can cause different effects on the visual response when the ejection current is varied. In this neurone L-AP4 has an inhibitory effect when ejected at -25nA, whilst a facilitatory effect is observed when the ejecting current is increased to -50nA and when the current is increased to -100nA inhibition occurs The plots show (i), control, (ii) during ejection of L-AP4 (-25nA), (iii) recovery 120 secs after L-AP4 ejection ceased, (iv) new control, (v) during ejection of L-AP4 (-50nA), (vi) recovery data obtained 120 secs after L-AP4 ejection ceased., (vii) new control, (viii) during L-AP4 ejection (-100nA), (ix) recovery obtained 120 secs after L-AP4 ejection ceased See Figure 6.1 for details of visual stimulus.

6.4 Visual responses are also modulated by antagonist application

To investigate the effect that release of the endogenous mGluR ligand (glutamate) has on visual synaptic transmission in the SSC, group-selective antagonists (**Group I:** LY367385 ejection currents –6 to –25nA and 4CPG -25 to 150nA, **Group II:** LY341495, ejection currents –25nA to 100nA **Group III:** MPPG ejection currents –25nA to 100nA and CPPG –25nA to 125nA) were applied by iontophoresis. As some drugs may have specificity within a concentration range, the smallest ejection current which elicited an effect was routinely used. Application of antagonists of all three groups affected the visual response indicating that mGluRs are involved in synaptic transmission during visual stimulation. LY367385 (selective mGluR1 antagonist) did not significantly affect visual responses indicating that mGluR5 is the Group I receptor involved in synaptic transmission in the SSC. The effect of antagonist application was also compared with the effects of the corresponding agonist in the same cell. In very nearly every example, the antagonist effect was the opposite of the agonist effect, both in cells where the agonist inhibited visual responses and where visual responses were potentiated following agonist application. See Table 6.3 for data summary. See Figures 6.5, 6.6, 6.7A and 6.7B for examples of the contrasting effect of agonist and antagonist application in the same cell for Group I, II and III respectively.

Table 6.3 Effect of antagonists on visual responses

Antagonist	Agonist Inhibited	Agonist Facilitated	% of cells with opposite agonist/antagonist effect
Group I			
4CPG	131±4.5%**	N/A	86%
LY367385	106±3.0% (n=7)	N/A	N/A
Group II			
LY341495	124±3.5%** (n=20)	74±4.1%** (n=23)	95%
Group III			
MPPG	140±5.4%** (n=26)	65±3.9%** (n=14)	93%
CPPG	128±3.4%** (n=11)	77±3.2%** (n=8)	100%

Results are expressed as % control ± s.e.m. The antagonist effect is compared with the effect of agonist application in the same cell (agonist inhibited or agonist potentiated).

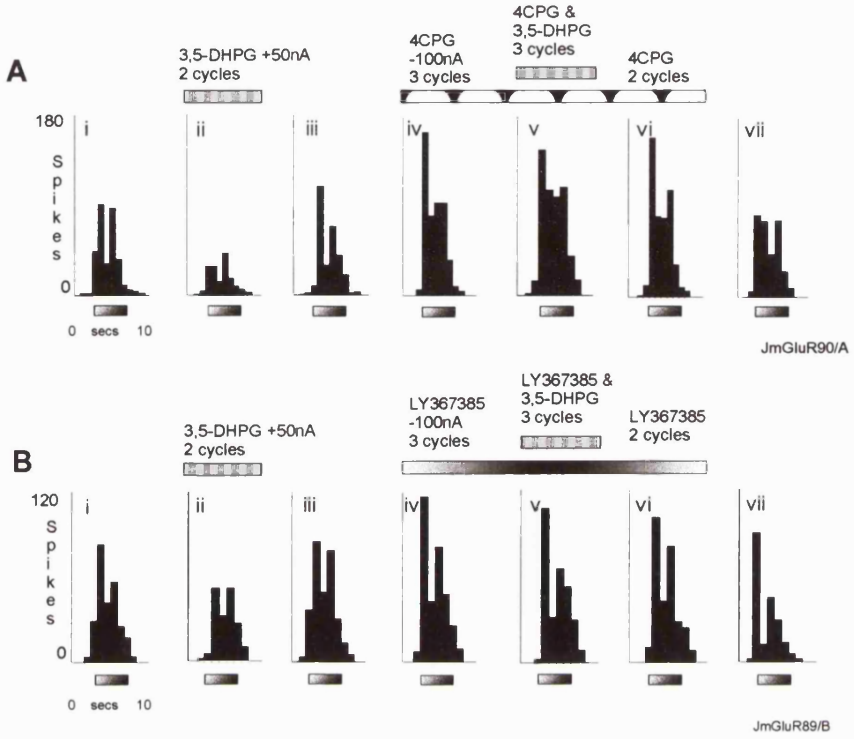


Figure 6.10 - Data from two different neurones showing that the Group I antagonists 4CPG (**A**) and LY367385 **(B)** block the effects of applied 3,5-DHPG. (i) control, (ii) inhibition of the visual response during DHPG ejection (+50nA), (iii) recovery data obtained 120 secs after DHPG ejection ceased, (iv) 4CPG or LY367385 (both -100nA) is ejected for 3 cycles, note the facilitatory effect on the visual response, (v) DHPG is now co-ejected with 4CPG or LY367385 for three cycles and there is little effect, (vi) DHPG ejection is ceased whilst 4CPG or LY367385 ejection continues, (vii) 4CPG or LY367385 ejection ceases and recovery data is obtained. See Figure 6.1 for stimulus details.

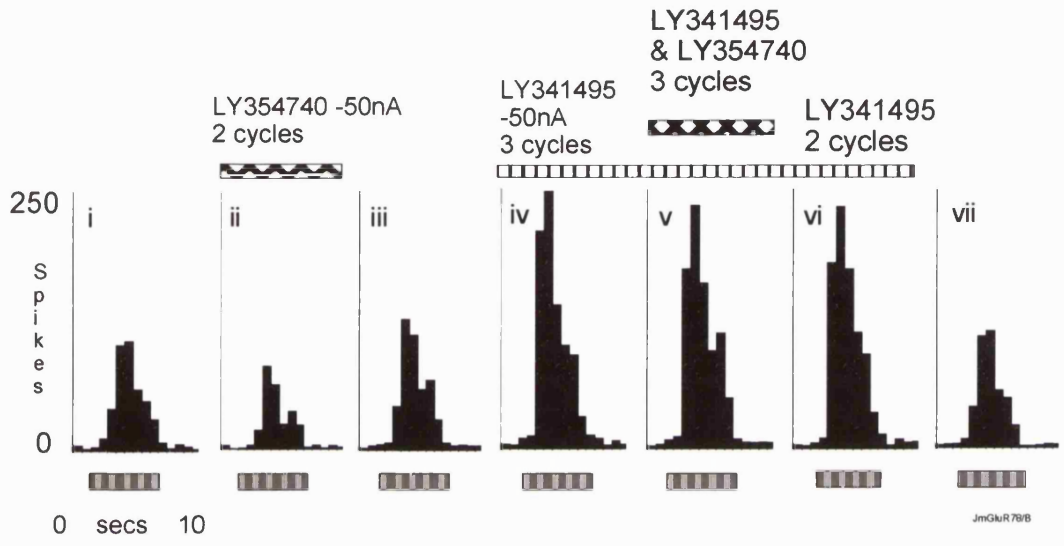


Figure 6.11 - The Group II antagonist LY341495 is able to block the effects of the Group II agonist LY354740. The plots show (i) control, (ii) during LY354740 ejection (-50nA), (iii) recovery data obtained 120 secs after LY354740 ejection ceased, (iv) the antagonist LY341495 (-50nA) is ejected for 3 cycles, note the facilitatory effect on the visual response, (v) LY354740 is now co-ejected with LY341495 for three cycles and there is little effect, (vi) LY354740 ejection is ceased whilst LY341495 ejection continues, (vii) LY341495 ejection ceases and recovery data is obtained. See Figure 6.1 for details of visual stimulus.

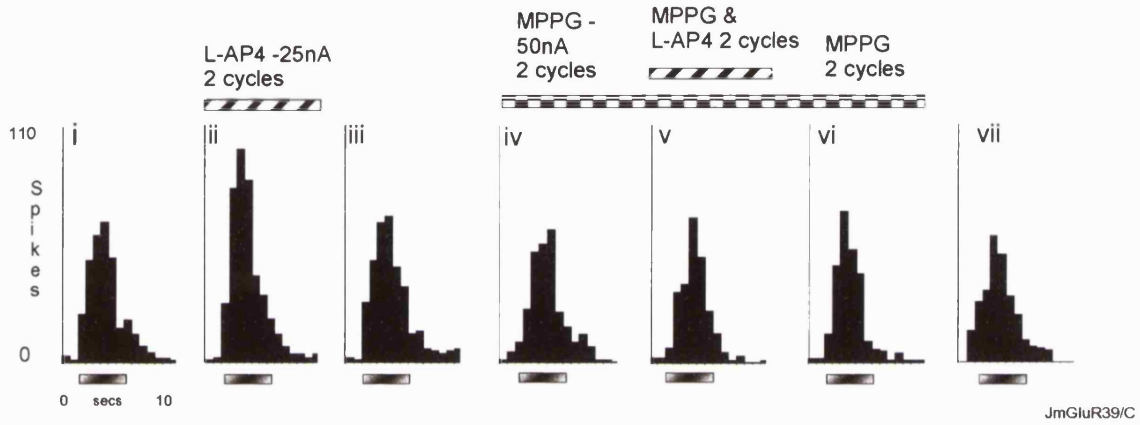


Figure 6.12 - The Group III antagonist MPPG blocks the effects of applied L-AP4 when co-ejected (i) control, (ii) facilitatory effect of L-AP4 (-25nA), (iii) recovery data obtained 120 secs after L-AP4 ejection ceased, (iv) MPPG (-50nA) is ejected for 2 cycles, (v) L-AP4 is now co-ejected with MPPG for two cycles and there is little effect, (vi) L-AP4 ejection is ceased whilst MPPG ejection continues, (vii) MPPG ejection ceases and recovery data is obtained. See Figure 6.1 for stimulus details

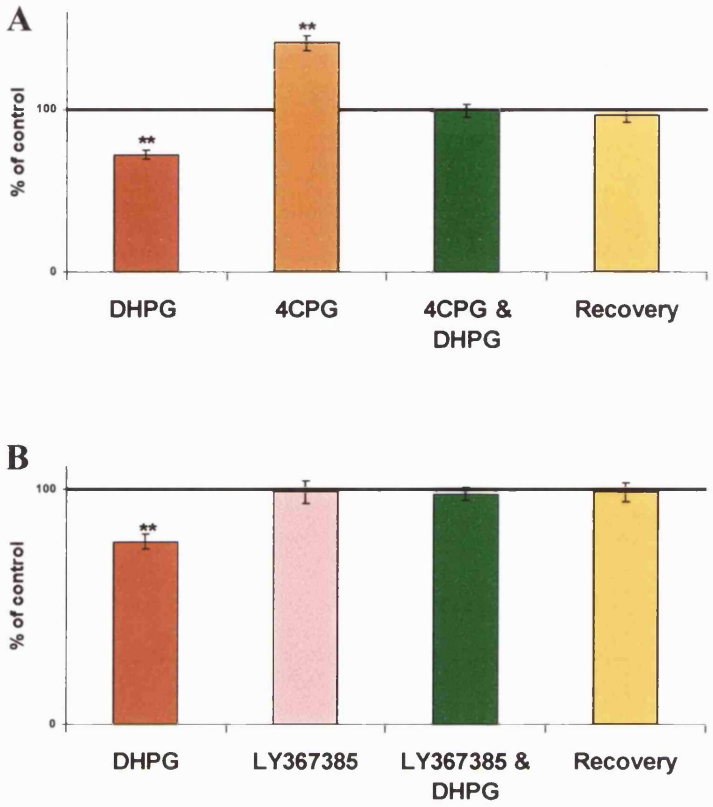


Figure 6.13 – Summary histogram of 3,5-DHPG and antagonist effects. 3,5-DHPG has a significant inhibitory effect on visual responses, whilst the mGluR5 selective antagonist 4CPG has a significant facilitatory effect on visual responses. The mGluR1 selective antagonist LY367385 has little effect on visual responses when applied alone but is able to block the effect of applied 3,5-DHPG. DHPG does not have a significant effect when applied with the antagonists 4CPG or LY367385 and no change is seen once drug ejection ceases (recovery). **(A)** n=9, **(B)** n=8; error bars are s.e.m; **P<0.05.

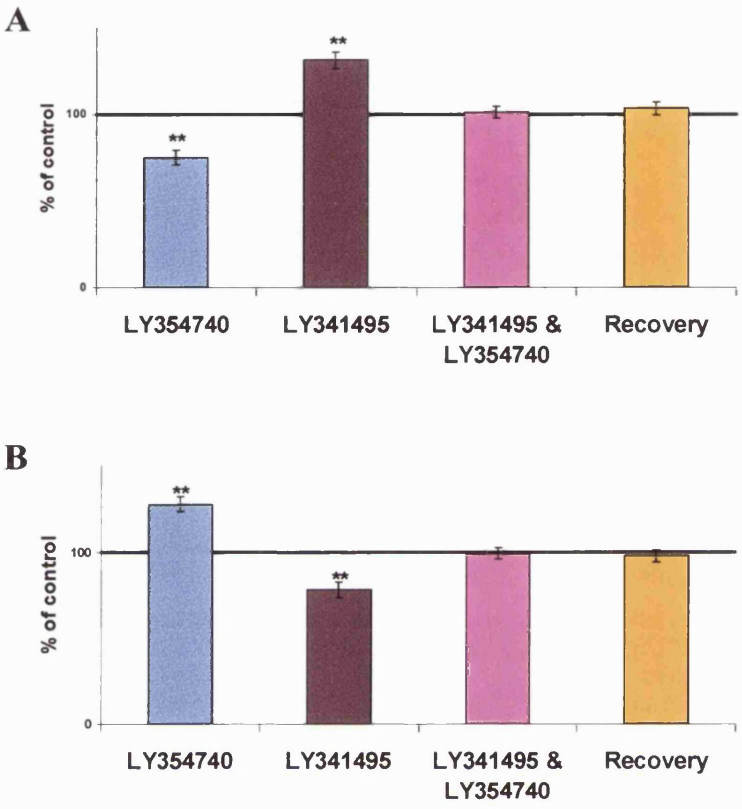


Figure 6.14 – **(A)** Summary histogram of LY354740 and LY341495 effects on cells where LY354740 has an inhibitory effect. The antagonist LY341495 has the opposite effect and causes significant facilitatory effects in the same cells. LY354740 does not cause a significant effect when ejected together with LY341495 and no change is seen once drug ejection ceases (recovery). LY341495 is therefore able to block the inhibitory effect of LY354740. n=6; error bars are s.e.m; **$p<0.05$. **(B)** Summary histogram of LY354740 and LY341495 effects on cells where LY354740 causes facilitatory effects. LY341495 has the opposite effect and causes significant inhibition in the same cells. LY354740 does not cause a significant effect when ejected together with LY341495 and no change is seen once drug ejection ceases (recovery). LY341495 is therefore able to block the facilitatory effect of LY354740. n=9; error bars are s.e.m; **$p<0.05$.

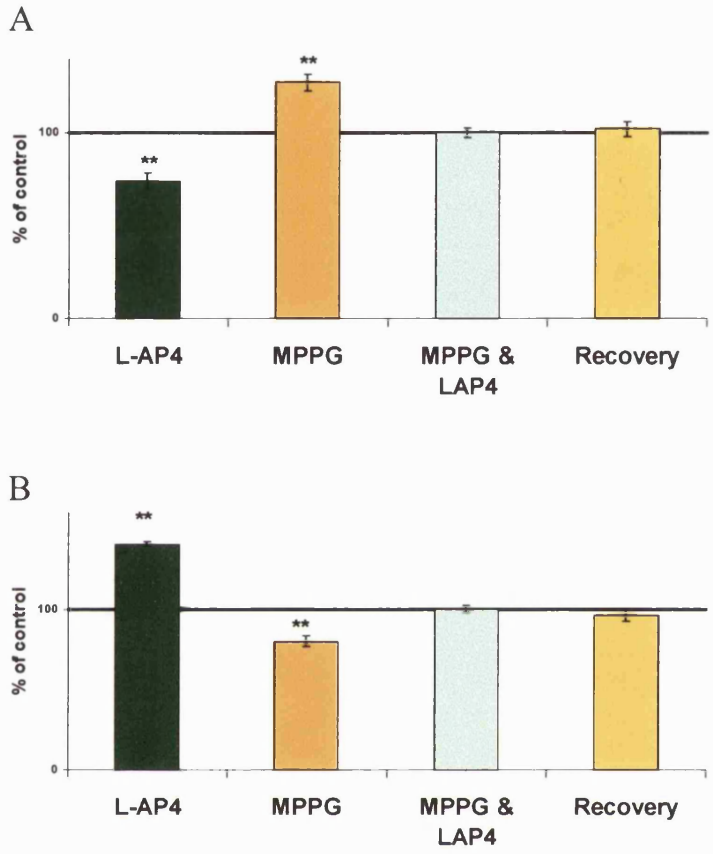


Figure 6.15 – **(A)** Summary histogram of L-AP4 and MPPG effects on cells where L-AP4 has an inhibitory effect. The antagonist MPPG has the opposite effect and causes significant facilitatory effects in the same cells. L-AP4 does not cause a significant effect when ejected together with MPPG and no change is seen once drug ejection ceases (recovery). MPPG is therefore able to block the inhibitory effect of L-AP4. n=16; error bars are s.e.m; **$p<0.05$. **(B)** Summary histogram of L-AP4 and MPPG effects on cells where L-AP4 causes facilitatory effects. MPPG has the opposite effect and causes significant inhibition in the same cells. L-AP4 does not cause a significant effect when ejected together with MPPG and no change is seen once drug ejection ceases (recovery). MPPG is therefore able to block the facilitatory effect of L-AP4. n=8; error bars are s.e.m; **$p<0.05$.

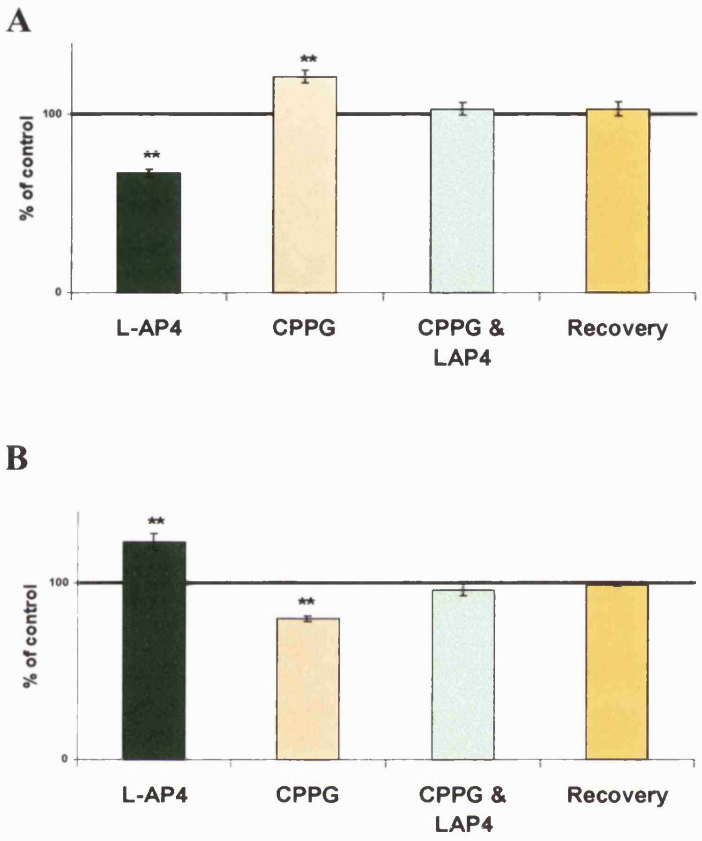


Figure 6.16 – **(A)** Summary histogram of L-AP4 and CPPG effects on cells where L-AP4 has an inhibitory effect. The antagonist CPPG has the opposite effect and causes significant facilitatory effects in the same cells. L-AP4 does not cause a significant effect when ejected together with CPPG and no change is seen once drug ejection ceases (recovery). CPPG is therefore able to block the inhibitory effect of L-AP4. n=11; error bars are s.e.m; **$p<0.05$. **(B)** Summary histogram of L-AP4 and CPPG effects on cells where L-AP4 causes facilitatory effects. CPPG has the opposite effect and causes significant inhibition in the same cells. L-AP4 does not cause a significant effect when ejected together with CPPG and no change is seen once drug ejection ceases (recovery). CPPG is therefore able to block the facilitatory effect of L-AP4. n=8; error bars are s.e.m; **$p<0.05$.

6.5 Selective antagonists are able to block the effect of agonists

To investigate whether group selective antagonists could block the effects of the corresponding agonist, antagonists were ejected for three cycles before the agonist was co-ejected with the antagonist for three cycles. Agonist ejection then ceased whilst antagonist were ejected for a further two cycles, antagonist ejection then ceased whilst recovery data was obtained. For all three mGluR groups, the group selective antagonists were able to block the effect of the specific agonist for that group, irrespective of whether the agonist produced an inhibitory or facilitatory effect. The data is summarised in Table 6.4. See Figures 6.10, 6.11 and 6.12 for examples of data with Group I, Group II and Group III drugs respectively and Figure 6.13, 6.14, 6.15 and 6.16 for summary histograms of the data.

Table 6.4 Group specific antagonists are able to block the effect of group specific agonists

Agonist effect	n	Agonist alone	Agonist + antagonist co-ejected
Group I			
4CPG & 3,5-DHPG			
Inhibition	9	72±2.8%**	99±4%
LY367385 & 3,5-DHPG			
Inhibition	8	78±3.5%**	98±4.8%
Group II			
LY341495 & LY354740			
Inhibition	6	75±3.3%**	101±2.8%
Facilitation	9	128±4.1%**	99±3.4%
Group III			
MPPG & L-AP4			
Inhibition	17	71±4.1%**	99.6±2.5%
Facilitation	9	140±1.5%**	100.3±2.0%
CPPG & L-AP4			
Inhibition	11	67±2.0%**	103±3.7%
Facilitation	8	123±4.6%**	95.6±3.4%

"agonist alone" values are percentages of control values. Co-ejection values are percentages of effect of agonist co-ejected with antagonist compared with three cycles of continuous ejection of the antagonist. **$p<0.05$.

6.6 Control Experiments

6.6.1 Effect of passage of iontophoretic current

In order to mimic the effect of passing current through the electrode, current was passed through a barrel filled with 100mM NaCl solution and the effect compared to controls. The polarity of the ejecting current was the same as ejecting current for the agonist under investigation (either – or + 25 to 200nA). The results are summarised for each group of experiments in Table 6.2 in the column headed "saline controls". Ejecting current applied through this barrel did not significantly affect visual responses in any of the cells tested. See Figure 6.17 for examples from each agonist group.

6.6.2 D-AP4 as a pharmacological control for L-AP4

As a pharmacological control for L-AP4, the stereoisomer D-AP4 was applied to 32 cells (ejection currents –25 to –200nA) to which L-AP4 had also been applied. The results are summarised in Table 6.5. Although D-AP4 is a weak ionotropic (NMDA) glutamate receptor antagonist (Evans *et al.* 1982), no statistically significant inhibition of visual responses was observed. Figure 6.18 show the effect of D-AP4 and L-AP4 on the same cell. D-AP4 had little effect, whilst L-AP4 had an inhibitory effect.

Table 6.5 Effect of D-AP4 versus L-AP4 effect in the same cells

Effect of L-AP4	n	L-AP4 effect	D-AP4 effect
Inhibition	21	71±5%**	97±4%
Facilitation	11	180±8%**	97±4%

Results are expressed as % control ± s.e.m. **$p<0.05$

6.6.3 Specificity of LY341495 as a Group II Antagonist

Although LY341495 has nanomolar antagonist potency Group II mGluRs (Kingston *et al.* 1998), LY341495 at higher concentrations also has antagonist activity at other groups of receptors (particularly Group III mGluRs, Johnson *et al.* 1999, Turner & Salt 1999, Fitzjohn *et al.* 1999). To investigate the possibility that LY341495 may block Group III responses, LY341495 was co-ejected with the Group III agonist L-AP4. L-AP4 (-50nA) was still able to produce an effect during the continuous ejection of LY341495 (-25nA to 100nA), indicating that LY341495 has specificity for Group II mGluRs in these experiments. See Table 6.6 for data summary and Figure 6.19 for example.

Table 6.6 Application of the Group II antagonist LY341495 does not block the effect of the Group III agonist L-AP4

L-AP4 effect	L-AP4 alone	L-AP4 & LY341495
Inhibited (n=10)	70±3.6%**	69±4.1%**
Facilitated (n=2)	134±2.1%	131±4.1%

"L-AP4 alone" values are % control ± s.e.m. "L-AP4 and LY341495" values are percentages of effect of L-AP4 co-ejected with LY341495 compared with three cycles of continuous LY341495 ejection. **$p<0.05$.

6.6.4 pH Control

To assess the effect of ejected H^+ ions on SSC neurones in experiments where 3,5-DHPG was applied, positive current was applied to a barrel containing 100mM saline, which had been adjusted to around the same pH value as the 3,5-DHPG solution (approx pH 3.5) by addition of small amounts of 1M HCl solution. Ejection of H^+ and Na^+ ions from this barrel (ejection currents +25nA to 100nA) did not significantly affect visual responses (102±2.1% of control n=6), See Figure 6.20 for example.

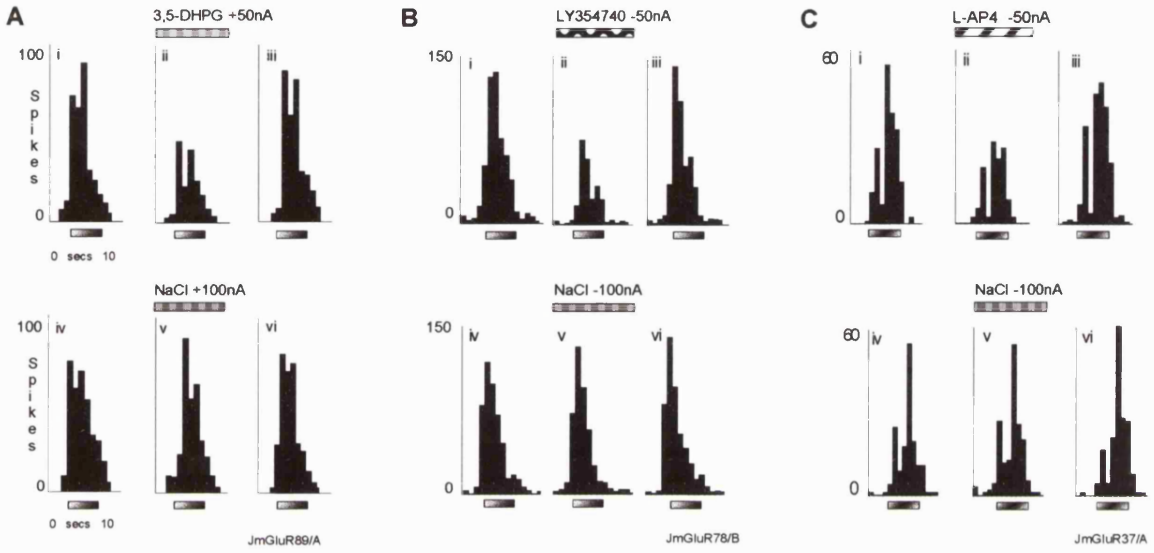


Figure 6.17 - Agonists of all three groups affect visual responses, but ejection of current through 100mM NaCl solution does not affect the visual response in the same neurone. The plots show **(A)** the effect of 3,5-DHPG **(B)** LY354740 and **(C)** L-AP4 on visual responses. (i) control, (ii) during agonist application, (iii) recovery data obtained 120 secs after agonist application, (iv) new control, (v) during NaCl ejection (100nA), (vi) recovery data obtained 120 secs after NaCl ejection ceased. See Figure 6.1 for stimulus details.

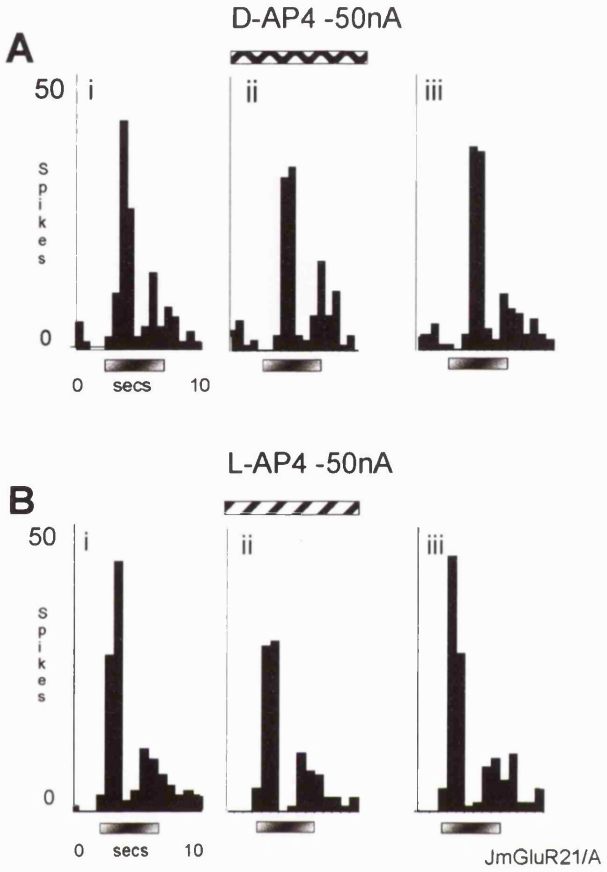


Figure 6.18 - Data from an individual neurone where L-AP4 significantly reduces the visual response but the stereoisomer D-AP4 does not affect the visual response in the same cell. The plots show cumulative counts of action potentials during **A** - (i) control, (ii) little change during D-AP4 ejection (-50nA), (iii) recovery data obtained 120 secs after D-AP4 ejection ceased. **B** -Data from the same neurone as A, (i) new control, (ii) inhibitory effect during L-AP4 ejection (-50nA), (iii) recovery data obtained after 120secs after L-AP4 ejection ceased. See Figure 6.1 for stimulus details.

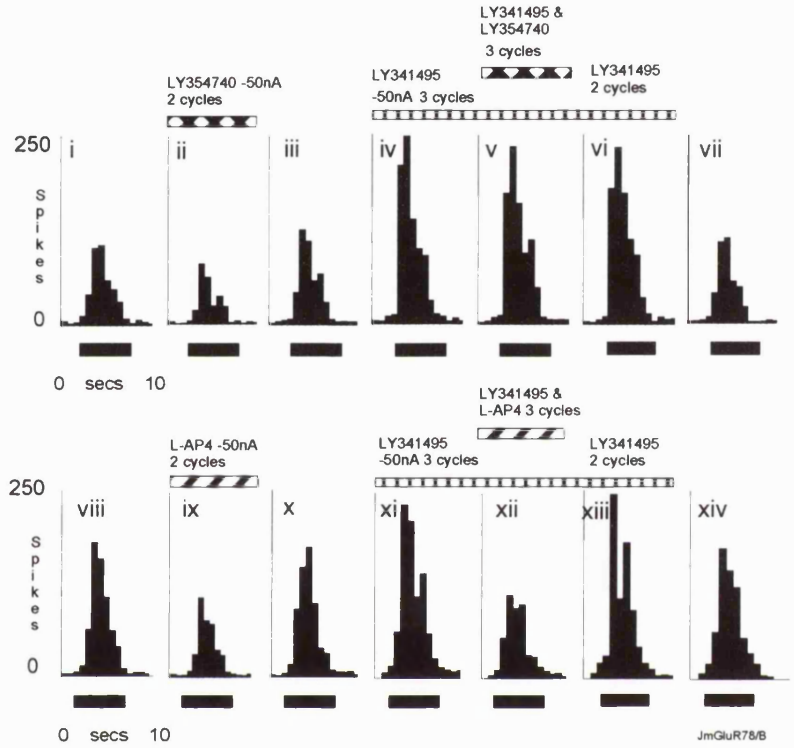


Figure 6.19 - Data from a single neurone showing that LY341495 blocks the effect of LY354740 but not the effect of L-AP4 and therefore has a specific effect upon Group II mGluRs. The plots show cumulative counts of action potential spikes during (i) Control, (ii) inhibitory effect of LY354740 (-50nA), (iii) recovery data obtained 120 secs after LY354740 ejection ceased, (iv) LY341495 is continuously ejected for 3 cycles, note the potentiation of the visual response, (v) LY354740 and LY341495 are now co-ejected for 3 cycles and there is little change, (vi) LY354740 ejection now ceases whilst LY341495 is ejected for a further 2 cycles, (vii) drug ejection ceases and recovery data is obtained, (viii) new control, (ix) inhibitory effect of L-AP4 (-50nA), (x) recovery data obtained 120 secs after L-AP4 ejection ceases, (xi) LY341495 is ejected for 3 cycles before (xii) co-ejection of L-AP4 with LY354740 for three cycles note that L-AP4 is still able to produce an inhibitory effect on visual responses, (xiii) L-AP4 ejection ceases whilst LY341495 ejection continues for a further 2 cycles, (xiv) recovery data is obtained following cesation of all drug application. See Figure 6.1 for details of visual stimulus.

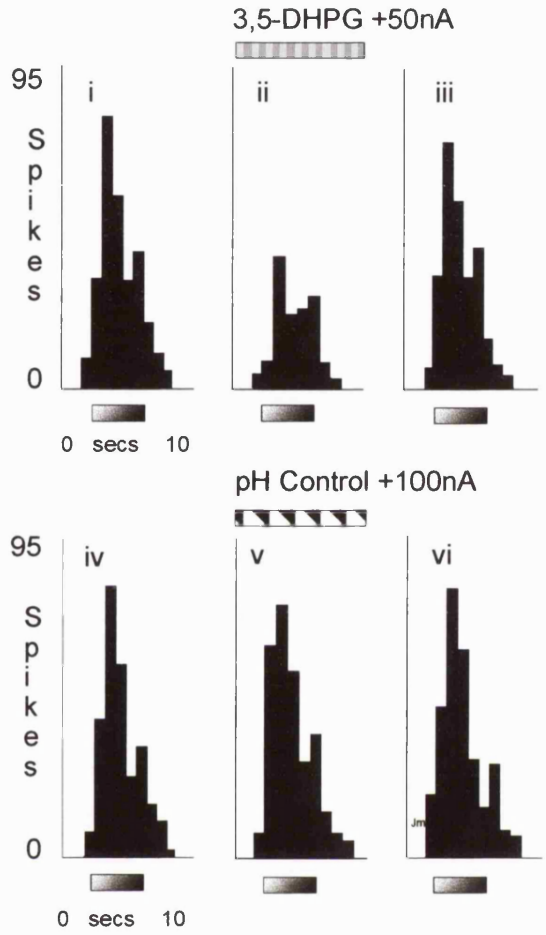


Figure 6.20 - Data from a single neurone showing the inhibitory effect of DHPG application, whilst ejection of H^+/Na^+ from a solution of the same pH as the DHPG solution does not significantly affect the visual response. The plots show (i) control, (ii) inhibition of visual responses during DHPG ejection (+50nA), (iii) recovery data obtained 120secs after DHPG ejection ceases, (iv) new control, (v) little effect during ejection of H^+/Na^+ (+100nA), (vi) recovery data obtained 120secs after drug ejection ceases. See Figure 6.1 for details of stimulus.

6.7 Effect of agonists on ionotropic glutamate evoked responses

The effects of agonists on NMDA (ejection currents –60 to 200nA) and AMPA (ejection currents –80nA to 200nA) evoked responses were examined for each mGluR group. The data is summarised in Table 6.7. This experiment determines if mGluRs are having an effect at the post synaptic site of the SSC relay cell.

Table 6.7 Effect of agonists on AMPA and NMDA evoked responses

Agonist	n	AMPA	NMDA	Effect Vision
3,5-DHPG				
Inhibited	8	116±6.7%	82.5±4.2%	82±1.9%**
LY354740				
Inhibited	8	121±4.4%	88±3.9%	75±3.7%**
Facilitated	1	148%	108%	119%
L-AP4				
Inhibited	10	107±4.6%	110±4.7 %	76±3.3%**
Facilitated	6	151±5.6%**	146±5.8%**	139±4.8%**

Results expressed as % control ± s.e.m.

6.8 Effect of agonists and antagonists on response habituation

To investigate the possible involvement of mGluRs in response habituation, a visual stimulation protocol which resulted in response habituation (five single stimuli presented at 0.5sec intervals, Binns and Salt 1997) was used to assess the extent of habituation in the control situation before selective agonists and antagonists were applied. In controls, the response to the last stimulus was typically 40±3.6% ($n=93$, $p<0.05$) less than the response to the first stimulus.

The average extent of habituation observed using the full contrast stimuli was not significantly different from that observed in the same cells using the lower contrast stimuli.

The Group III agonist L-AP4 produced an increase in the extent of response habituation in cells where L-AP4 inhibited the visual response (ejection currents –25nA to 100nA, Figure 6.21 for example) whereas application of the Group III antagonists MPPG or CPPG decreased the extent of response habituation in these same cells (ejection currents –25nA to 125nA). In contrast, the Group I and Group II agonists 3,5-DHPG (ejection currents +25nA to 150nA) and LY354740 (ejection currents –25nA to 100nA) or Group I and Group II antagonists 4CPG (ejection currents –25 to 150nA) and LY341495 (-25 nA to 100nA) did not have any such effect upon the extent of response habituation (Figure 6.21). The data for effect upon response habituation by mGluR agonists and antagonists is summarised in Table 6.8.

As $GABA_B$ (but not $GABA_A$) receptors are also involved in the generation of response habituation in the SSC (Binns & Salt 1997), the effect of response habituation produced by L-AP4 and inhibited by MPPG was examined in the presence of the $GABA_B$ antagonist CGP35348. CGP35348 ejected alone (+25 to +100nA) caused a reduction in response habituation by 16.3±3.4%, consistent with $GABA_B$ antagonism as previously reported (Binns & Salt 1997). In the presence of CGP35348, L-AP4 (ejection currents –25nA to 100nA) still increased the extent of habituation (Figure 6.22, Table 6.8), whilst MPPG was still able to reduce the extent of habituation (ejection currents –25 to 100nA, Figure 6.22, Table 6.8). The data indicate that Group III mGluRs are involved in generating a component of response habituation in the SSC which is distinct from that mediated by $GABA_B$ receptors.

Table 6.8 Effect of group-specific drugs on response habituation.

Group	Effect upon Habituation	Effect on Habituation with $GABA_B$ antagonist
Group I		
3,5-DHPG	+1.6±3.9% (n=9)	-
4CPG	-0.2±2.7% (n=9)	-
Group II		
LY354740	+2.1±2.1% (n=18)	-
LY341495	-5.5±2.4% (n=18)	-
Group III		
L-AP4	+21.8±3.7%** (n=16)	+21±2.7%** (n=7)
CPPG or MPPG	-19.7±3.2%** (n=16)	-23.7±3.5%** (n=16)

Values are increases or decreases in response habituation from pre-drug values. Neither of the Group I or Group II compounds affected habituation, whereas the Group III agonist L-AP4 significantly increased the extent of habituation and the Group III antagonists CPPG or MPPG reduced habituation. In the presence of the $GABA_B$ antagonist CGP35348, the effect of the Group III drugs were not occluded. **significantly different from pre-drug habituation value ($P<0.05$).

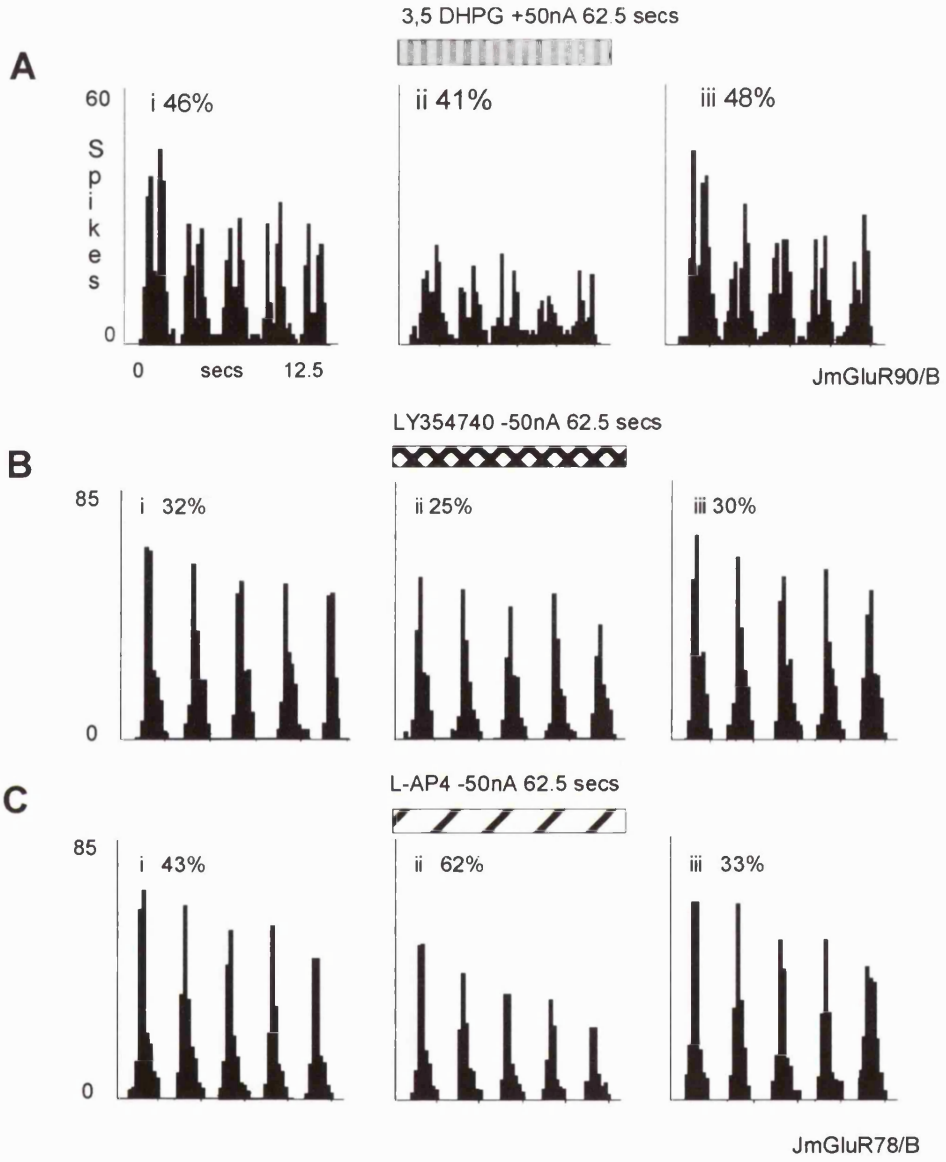


Figure 6.21 - Group I and II agonists do not significantly affect the extent of response habituation although the Group III agonist L-AP4 significantly increases response habituation. Data from B and C are from the same neurone. The histograms show counts of spikes evoked by 5 presentations of identical stimuli (15 x 10^{0} moving bar, moving at 44^{0}/sec for 2 secs with interstimulus interval of 0.5secs, bin size=200msec, cumulative data from 5 trials is shown for each plot. In each panel, the extent of response habituation (calculated as described in Section 4.7) is shown. A - (i) control, (ii) little effect on response habituation although the overall visual response is significantly inhibited during DHPG ejection (+50nA), (ii) recovery data obtained 75secs after DHPG ejection ceased. B - data from a different neurone (i) control, (ii) little effect on response habituation during LY354740 ejection (-50nA), (iii) recovery data obtained 75secs after LY354740 ejection ceased, C - data from the same neurone as B (i) new control, (ii) significant increase in response habituation and inhibition of the visual response during L-AP4 ejection (-50nA), (iii) recovery data obtained 75secs after L-AP4 ejection ceased.

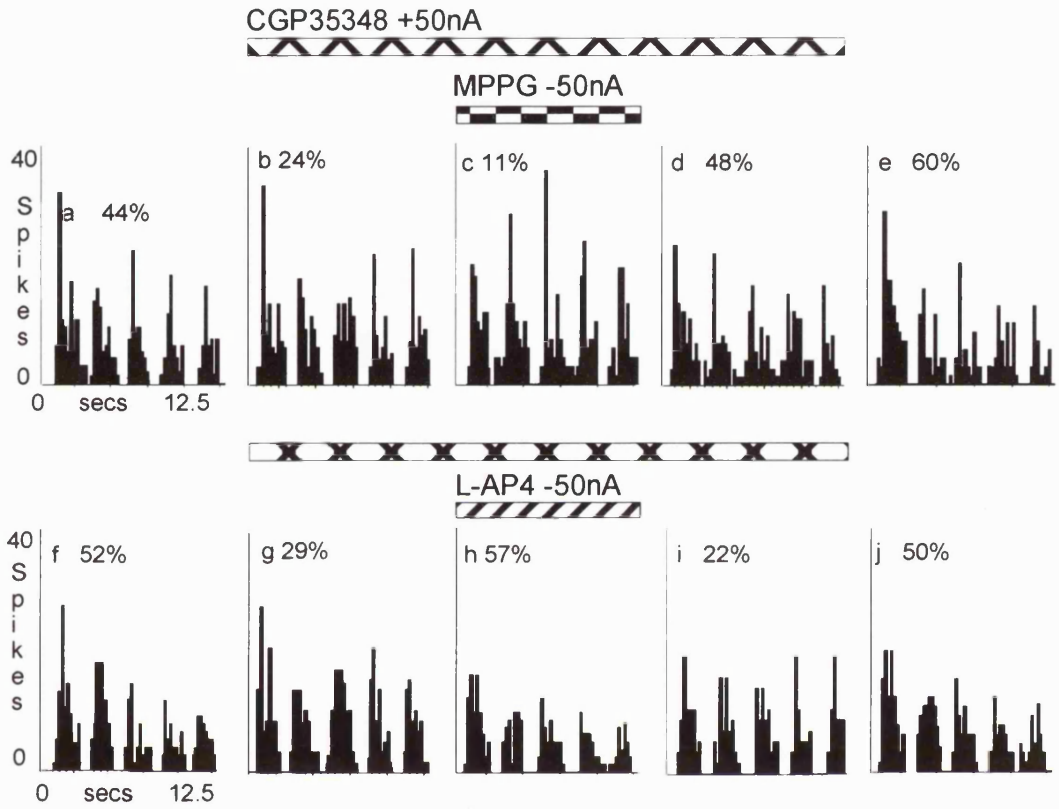


Figure 6.22 - PSTH records from a single neurone showing the reduction in response habituation by MPPG in the presence of CGP35348 and increase in response habituation by L-AP4 in the presence of CGP35348. See Figure 6.12 for stimulus details. The plots show (a) control, (b) reduction in response habituation following CGP35348 ejection (+50nA), (c) a further reduction in response habituation following co-ejection of MPPG (-50nA) with CGP35348, (d) an increase in response habituation in observed following cessation of MPPG ejection, (e) response habituation increases further following cesation of CGP35348 ejection, (f) new control, (g) reduction in response habituation following CGP35348 ejection, (h) increase in response habituation following co-ejection of L-AP4 with CGP35348, (i) response habituation reduces following cesation of L-AP4 ejection, (j) response habituation increases after recovery from CGP35348 application.

6.9 The effect of antagonist application when the magnitude of visual response is varied

In-vitro studies have shown that activation of mGluRs may occur under conditions of intense synaptic activity (Scanziani *et al.*, 1997, Rusakov and Kullmann 1998, Mitchell and Silver 2000). It is possible to modulate the response magnitude of neurones in the SSC very simply by adjusting the contrast between stimulus foreground and background. Reduction of the foreground contrast results in a visual response which is on average 55±3.2% of the response under normal full contrast. At low contrast, the effects of the Group II antagonist LY341495 (ejection currents –25nA to 100nA) were less prominent than under high contrast conditions, irrespective of whether the antagonist inhibited or reduced visual responses (Table 6.9, Figure 6.23). In contrast, when the Group I (4CPG ejection currents –25nA to 150nA) or Group III antagonists (CPPG or MPPG, ejection currents –25nA to 100nA) were applied, little difference in effect was seen between high and low contrast stimulus conditions (Table 6.9, Figures 6.23).

Table 6.9 Effect of antagonists at low and high contrast

Antagonist Effect	Low contrast stimuli	High contrast stimuli
Group I		
4CPG Facilitated (n=10)	121±4.7%	124±3.4%
Group II		
LY341495 Inhibited (n=6)	76±4.4%**	62±4.7%**
LY341495 Facilitated (n=6)	130±5%**	144±4.3%**
Group III		
CPPG/MPPG Inhibited	73±3.6%	67±4.4%
CPPG/MPPG Facilitated	129±4.5%	139±5.4%

Values are percentages of control visual responses ±s.e.m. All antagonists had significant effects on visual responses under both low and high contrast stimulus conditions ($P<0.05$). However, the Group II antagonist effect was significantly different at low contrast from the effect at high contrast (** $P<0.05$), whereas there was no significant difference between low and high contrast groups during application of the Group I or Group III antagonists.

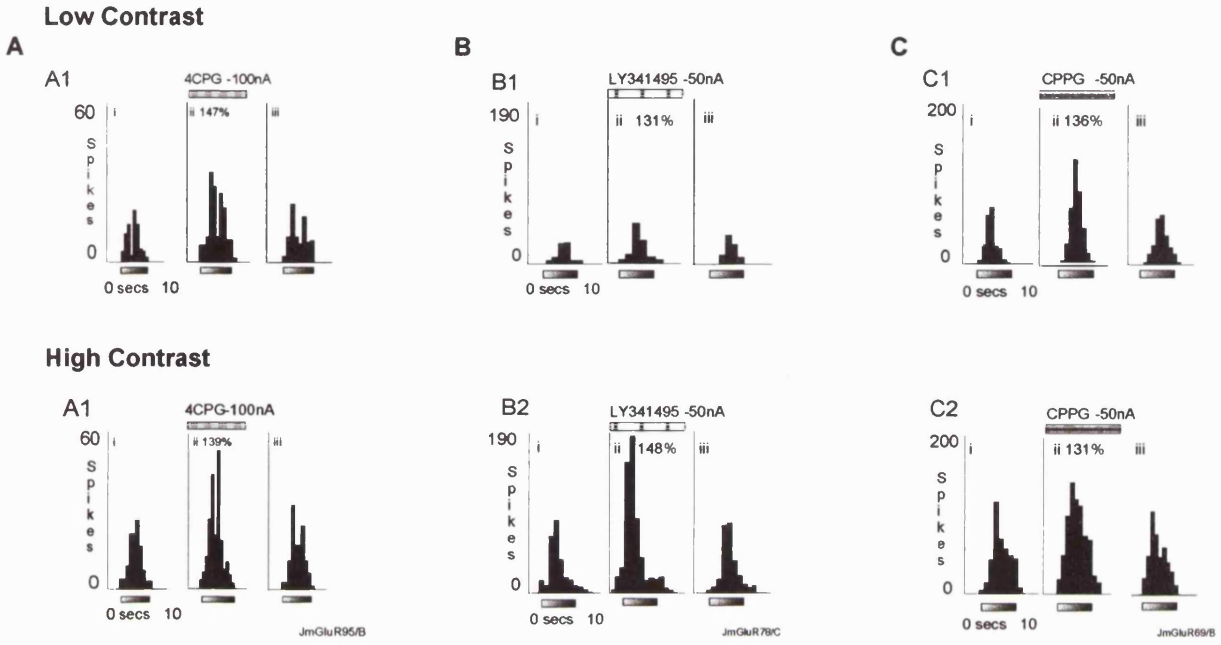


Figure 6.23 - Data from 3 different neurones showing that the magnitude of effect at low and high stimulus contrast is different for the Group II antagonist LY341495, but not the Group I (4CPG) or Group III (CPPG) antagonist. See Figure 6.1 for stimulus details. Luminance of foreground object 20cd/m ^{2} (high contrast) or 12.2cd/m ^{2} (low contrast). **A** - The Group I antagonist 4CPG produces a similar effect on visual responses under conditions of either low contrast (A1) or high contrast (A2). **B** -LY341495 has a greater effect on the visual response under conditions of high stimulus contrast (B2) than under conditions of low contrast (B1). **C** -The Group III antagonist CPPG produces a similar effect on the visual response under conditions of low contrast (C1) and high contrast (C2). The plots show (I) control, (ii) during antagonist application, (iii) recovery data obtained 120secs after anatagonist ejection ceased.

6.10 Conclusions

- All three groups of mGluRs are involved in modulating visual transmission in the rat SSC. mGluR5 rather than mGluR1 appears to be the receptor activated by endogenous glutamate released during visual stimulation.

- Activation of Group II and III mGluRs can produce both inhibitory and facilitatory effects.

- Activation of Group I mGluRs would appear to produce mainly inhibitory effects.

- In the majority of cells, application of agonists produced exclusively inhibitory or facilitatory effects however, in a few cells both inhibitory and excitatory effects could be observed when the ejection current was varied.

- The Group III agonist L-AP4 is able to modulate iGluR evoked responses and therefore have some activity at the post-synaptic site of the SSC relay cell. Application of Group I and II agonists has little effect on iGluR evoked responses.

- Group III mGluRs have a role in response habituation which is distinct from previously reported $GABA_B$ mediated mechanisms.

- Activation of Group II mGluRs appears to be activity-dependent and have a functional role in detecting stimuli contrast.

7.0 Discussion of Results – Immunohistochemistry

7.0 Overview

In general, the results reveal that differences predominate in patterns of mGluR labelling in the three species examined and that there are few similarities in the distribution and intensity of mGluR immunoreactivity. In many cases, the same antibodies labelled completely separate features in different species. Hence, it is highly unlikely that mGluRs subserve similar functions in visual processing in these three species. Consequently, considerable caution should be taken in assuming that physiological or anatomical data obtained for one species will have parity with other species. This is particularly important in view of the common use of these three species in investigations of visual function.

Given that there is a very high degree of sequence homology (around 96%) in cloned mGluRs from species as diverse as drosophila (Parmentier *et al.* 2000), rat and human (Emile *et al.* 1996, Wu *et al.* 1998) there is little question that mGluR antibodies will fail to label ferret and cat mGluRs (i.e. false negatives). Unfortunately, no immunoblot data is available for the antibodies used in this study and therefore some question remains as to the degree of non-specific cross-reactivity that these antibodies may have in the species used (i.e. false positives). With this in mind, it should be noted that there are a number of cases where labelling for a specific antibody in a particular region was found in only one species (such as mGluR1a labelling in the rat retina). It is likely that in these cases that the absence of label in the other species is an important difference. It should be noted that since this study was carried out, new antibodies have become commercially available. It would be interesting to compare labelling produced by these newer antibodies with the results of this study. Another problem inherent with immunohistochemical studies is the presence of "edge effects" therefore caution should be taken when interpreting labelling which occurs close to the edge of a section.

7.1 mGluR1

Antibody staining for mGluR1a showed a pattern, distribution and intensity that was similar in the rat and ferret and different in the cat. Where labelling occurred in the cat it was of a much weaker intensity than in the rat or ferret. Immunoreactivity for mGluR1b was dissimilar in all species and mGluR1c showed a pattern of label which was broadly similar for all species.

In-situ hybridization and immunohistochemical studies have shown that mGluR1 is widely expressed and distributed in the adult rat brain (Shigemoto *et al.* 1992). There are high levels of mGluR1mRNA expression in the GCL of the retina (Hartveit *et al.* 1995) and high levels of mGluR1a immunoreactivity in RGC's (Peng *et al.* 1995). High levels of mGluR1 (Fotuhi *et al.* 1993) and mGluR1a immunoreactivity (Baude *et al.* 1993) are present in the SSC and LGN (Fotuhi *et al.* 1993). In this study, very little mGluR1b immunolabel was found in the rat and it is therefore more likely that in earlier studies which have used pan mGluR1 antibodies (antibodies that recognise all splice variants of mGluR1) show labelling for mGluR1a or 1c receptors. High levels of immunoreactivity were observed in the SC in a study which used a specific mGluR1a antibody (Martin *et al.* 1992) in the rat, similar to that observed in this study. mGluR1a immunoreactivity was reported in all thalamic nuclei, which presumably includes the LGN although it was not specifically mentioned in Martin *et al.* 1992. In this same study, high levels of immunoreactivity were particularly pronounced in brain areas which received primary sensory afferents including the SC. This evidence, together with the high level of labelling of the LGN observed in this study would indicate that mGluR1 and in particular mGluR1a is associated with modulation of synaptic transmission in rat sensory systems. mGluR1 has been shown to be involved in modulating responses *in-vivo* in another sensory thalamic nuclei called the ventrobasal nuclei which receives somatosensory input (Salt & Eaton 1994).

mGluR1a (1199 amino acids) is comparatively larger than mGluR1b (907) or 1c (897) (Pin *et al.*, 1992, Tanabe *et al.*, 1992, Pickering *et al.* 1993) and has a longer intracellular C-terminal.

In the cat (Godwin *et al.* 1996a), mGluR1a immunolabel is located in dendrites of relay cells in the LGN, which receive retinal inputs. Activation of these receptors by iontophoretic application of mGluR agonists changes the firing rate of these cells from burst to tonic mode (Godwin *et al.* 1996b). mGluR1a immunolabel is also present on dendrites of relay cells in the dLGN which receive cortical input from area 17 of visual cortex (Vidnyanszky *et al.* 1997). In this study, light labelling was observed throughout the cat LGN, which is in agreement with the findings of both the Godwin (1996b) and Vidnyanszky (1996) studies. In this study light labelling was observed in the SGS layer of the SSC which was consistent with cell processes. Retinal axons terminate in the SGS where cortical inputs also form synapses. Given that there was no labelling in the optic nerve, it is likely that the labelling in the SSC is of cortical afferent origin or is present on the processes of intrinsic SSC cells.

Although labelling was observed in the GCL of the ferret retina, it is unlikely that these cells are ganglion cells, as little label was observed in the ON (optic nerve) and the labelling observed in the LGN and SC was cellular and not consistent with terminal labelling. An alternative explanation is that the labelled cells observed in the GCL are indeed ganglion cells, but that synthesised protein is not transported to central terminals.

7.2 mGluR2/3

A very consistent theme in all areas and species examined was the heavy labelling of astrocytes with the mGluR2/3 antibody.

In general, the distribution of label suggested more similarities between the ferret and cat although there were a few marked differences between these animals.

mGluR3 has a widespread distribution in the brain and is strongly expressed in glia although some neurones also express mGluR3 (Tanabe *et al.* 1993). Immunoreactivity for mGluR2/3 was weak in the rat retina in agreement with studies which reveal that there is no mGluR3 mRNA expression (Hartveit *et al.* 1995, Tanabe *et al.* 1993) or immunoreactivity (Koulen *et al.* 1996) in the rat retina.

However, weak mGluR2 mRNA expression has been demonstrated in the rat GCL (Ohishi *et al.* 1993a, Hartveit *et al.* 1995). In a study examining expression of mGluR mRNA in the rat retina (Hartveit *et al.* 1995) no mGluR expression was observed in Muller cells although the results described in Section 5.4.1 and 5.5.1 indicate that immunoreactivity for mGluR2/3 and mGluR4 was present on Muller cells in the rat retina.

An immunohistochemical study (Neki *et al.* 1996) observed marked immunoreactivity for mGluR2 in some brain areas, but not the SSC or LGN. An *in-situ* hybridization study (Ohishi *et al.* 1993a) showed only background levels of mRNA expression in the SSC and LGN. It is therefore most likely that the immunoreactivity observed in this study is primarily mGluR3 rather than mGluR2.

In-situ hybridization (Ohishi *et al.* 1993b) showed moderate levels of expression of mGluR3 mRNA in both the SSC and visual cortex, although only background levels were s observed in the LGN. In this same study, no significant level of mRNA expression in the retina was shown, indicating that the source of mGluR3 immunoreactivity seen in the present study in the SSC is either from cortical afferents or intrinsic SSC cells. Here, mGluR2/3 SSC immunoreactivity was observed in cell bodies rather than cell processes indicating that the most likely location for mGluR3 immunoreactivity is intrinsic SSC neurones. Interestingly, in the mouse, 2/3 immunopositive cells are only seen in the SO layer of the SC (Kim & Jeon 1999). Although there was marked neuronal labelling in the LGN, it was not possible to determine the morphology of labelled cells. Another study (Petralia *et al.* 1996) which used a combined mGluR2/3 antibody also revealed moderate levels of label in the SSC, LGN and cortex.

Combined mGluR2/3 antibodies have also revealed that levels of mGluR2 and 3 protein are regulated during development in the kitten visual cortex by visual experience (Beaver *et al.* 1999). In animals aged 3-4 weeks, mGluR2/3 immunoreactivity disappears from layer 4 with dark rearing postponing this developmental change. It has been suggested that the reduction in Group II (i.e. mGluR2 and mGluR3) receptors results in the formation of ocular dominance columns in layer 4 of visual cortex.

7.3 mGluR4

The distribution of mGluR4 immunolabel highlighted differences between all three species. The only similar patterns of label were found in the ferret and cat SC, although the intensity was heavier in the ferret. *In-situ* hybridization studies have described a fairly restricted level of mGluR4 mRNA expression in the rat brain (Kristensen *et al.* 1993, Ohishi *et al.* 1995), with only low levels of expression in the SC and visual cortex.

In the rat, expression of mGluR4 mRNA is high in RGC's (Akazawa *et al.* 1994, Hartveit *et al.* 1995) although in the current study, no immunolabel was observed in RGC's, label was observed in the ON and OT (optic tract) and in retinorecipient areas of the brain, suggesting that mGluR4 mRNA in RGC cell bodies may be transported to the central terminals. There was heavy fibre labelling in the pretectum (which receives inputs from the retina and SC) and it is feasible that this could originate from either retina or SC afferents. mGluR4 labelling was also detected in the ON of the cat, with heavy labelling of the LGN and SSC indicating that one source of mGluR4 is from the retina.

In the ferret, heavy C-laminae label was observed. As no label was found in the retina or ON it is possible to assume that C-laminae labelling is present on relay cells or that it is from the relatively small cortico-geniculate projection to this lamina (Claps & Casagrande 1990). Label observed in the SSC was consistent with terminal processes and it is likely that a significant proportion of the origin of this label in the SSC is of cortical origin given the absence of labelling in the OT.

7.4 mGluR5

Immunoreactivity for mGluR5 was broadly similar in all species, with all animals having light label in the ON's and heavily labelled LGN and SC. The most notable difference between the species was that the C-laminae of the ferret LGN were very heavily labelled, with no similar regional variation in the distribution of label in either the rat or cat LGN. The C-laminae of the ferret receives mainly retinal input although the ON was only lightly labelled. It is possible that mGluR5 label is present on relay cells or arises from the cortico-geniculate projection (Claps & Casagrande 1990).

In the rat and cat, labelling was identified in the retina and ON suggesting that at least some of the observed label in the LGN and SSC may be of retinal origin, although an *in-situ* hybridization study indicated that there is no mGluR5 mRNA expression in rat RGC's (Hartveit *et al.* 1995). Heavy axonal label was observed in the IGL and terminal like label was present in the SSC, it is possible that the terminal labelling in the SSC may result from IGL afferents which project to the SGS (Moore *et al.* 2000).

An immunohistochemical study (Shigemoto *et al.* 1993) in the rat brain has demonstrated that mGluR5 has a widespread distribution with intense staining in the SSC, although it is not possible to determine intensity of label in the LGN of this study.

mGluR5 immunolabel in the cat LGN has been reported to be associated with the dendrites of retinorecipient LGN neurones and interneuronal dendrites (Godwin *et al.* 1996a). The labelling in the LGN was not consistent with soma, but was consistent with terminals, it is feasible that this may result from retinal input, although this may also have resulted from terminal processes of interneurones.

7.5 Data Summary

- In the retina, mGluR subtypes 2/3, 4 and 5 were found in different regions of each species. Additionally mGluR1b was found in the rat retina, mGluR1a and mGluR1c in the ferret retina and mGluR1a in the retina of the cat.

- In the LGN all mGluR subtypes were found in the rat and ferret but there were differences in the distribution and intensity of labelling between species. In the cat LGN only mGluR1c, mGluR2/3, mGluR4 and mGluR5 were found.

- In the SSC all sub-types were found in the rat and ferret, but again there were differences in the labelling patterns between these species. In the cat all subtypes were found with the exception of mGluR1b.

- A consistent feature in all species was labelling of astrocytes with the mGluR2/3 antibody.

7.6 Conclusion

Although there were some similarities between different species (for example, heavy astrocytes label with the mGluR2/3 antibody), in general there are broad differences in the distribution, intensity and type of mGluR labelling between species. This is somewhat unexpected given the relatively close phylogenetic relationship between the ferret and cat. It would be expected that the results for the cat and ferret would share more similarity in comparison with the rat data. Both ferrets and cats are carnivorous predatory animals where vision is the primary sensory system. The organisation of the ferret visual system would appear to be share more similarities with the cat than the rat. For example, cells in the LGN form well ordered cellular laminae in both the ferret (Linden *et al.* 1981, Johnson and Casagrande 93, Zahs & Stryker 1985) and cat (Hickey & Guillery 1974), and other members of the order *Carnivora* (Sanderson 1974). The rat LGN is not obviously laminated (Brauer *et al.* 1979). Developmental landmarks such as time of innervation of sub-thalamic visual nuclei and time of eye opening are similar in the ferret and cat (Linden *et al.* 1981). However, there are a few key differences in the ferret LGN including a much larger monocular region in the ferret and large and well differentiated C-laminae. The visual system is the primary sensory system in the ferret and cat with relatively large areas of cortex involved in visual processing, this is in contrast with the rat which is predominantly an olfactosensory animal. However, some aspects of the ferret visual system are similar to that of the rat for example, the extent of the ipsilateral projection in rat (approx. 1%, Potts *et al.* 1982) and ferret (approx. 7%, Henderson *et al.* 1988) is very different to that of the cat (approx. 25% Aebersold *et al.* 1981).

The findings of this immunohistochemical study are consistent with the hypothesis that there are large divergences in receptor types and distributions between relatively closely related animals.

8.0 Discussion – Physiological role of mGluRs in modulation of visual responses

8.1 Comment on characterisation of SSC neurone response properties

Preliminary experiments were carried out to investigate the response properties of visually responsive neurones in the SSC. Although the physiological characterisation of such cells has previously been extensively investigated (see Table 1.5 for a summary of such experiments) such experiments are very useful for designing the parameters of visual stimuli that are used in pharmacological studies.

The results presented in Section 6.2 are generally consistent with the findings of previous studies of SSC visual responses. Receptive fields are found to be topographically organised in visual space. For example, when the recording electrode was in the anterior SC, the receptive fields were located in rostral visual space, and when the electrode was in the medial SC, the receptive fields were located in upper visual space. Previous studies have suggested that the response characteristics change upon progression from dorsal to ventral SSC and that this is likely to occur due to differences in the distribution of cell types within these layers (Humphrey 1968, Gonzalez *et al.* 1992). In general, no significant change in any of the response properties was observed upon transition to deeper recording sites in the SSC. This is most likely to have resulted from a sampling bias of the most superficial cells, with poor penetration into "deeper" SSC regions. In pharmacological experiments, there appears to be no correlation between agonist effect and recording position of the electrode within the SSC (see Section 8.2).

8.1.1 General receptive field properties

The exact composition of the receptive field, i.e. size and shape of the excitatory and inhibitory components is difficult to investigate in an un-paralysed preparation as small movements of the eye may lead to misleading results. However, it is apparent in this preparation that most cells have receptive fields with an excitatory "core" and inhibitory surround, with an increase in spot size beyond the optimal response resulting in a decrease in the response. Surround inhibition in the SSC is mediated by $GABA_A$ receptors (Binns & Salt 1997).

Previous studies (Humphrey 1968, Gonzalez *et al.* 1992) have suggested that the size of the receptive field can be used to classify cells into three groups (see Table 1.5). It would appear that the group with the smallest receptive field size (mean size 5.4^0) were not observed in this study, although cells with intermediate (mean size 8.1^0) and large receptive field sizes (mean size 18.1^0) were apparent. It is possible that cells with the smallest class of receptive field were sampled, but that they do not form a distinctly separate population which is separate from cells with intermediate sized receptive fields. The mean optimum spot size in the characterization experiments ($9.8^0 \pm 1.7$) is very similar to a study from the same laboratory ($9.6^0 \pm 1.2$, Binns & Salt 1997).

In this study it was noted that the mean stimulus size eliciting the largest response increased slightly upon transition from rostral to caudal visual space (although this was not statistically significant). This is consistent with previous reports (Humphrey 1968, Gonzalez *et al.* 1992) and the distribution of wide-field cells (Table 1.5) that are more frequent in posterior SC than anterior SC and have large dendritic fields that receive convergent inputs.

8.1.2 Orientation and directional selectivity

Two methods were used to quantify orientation or direction selectivity. From examination of the "I" score (see Sections 4.7 and 6.2.2) it would appear that some visually responsive SSC neurones have some degree of orientation selectivity with 48% of cells having a score of I >0.5. In contrast, in a previous study in the cat SSC from the same laboratory (Binns & Salt 1996), 93% of cells had an I value greater than 0.5, indicating that rat SSC cells are less selective for orientation or direction than those of the cat. This is consistent with other studies (Gonzales *et al.* 1992, Sefton 1969) reporting that only a small number of cells in the rat SSC show strong orientation preference.

The distribution of "B" values (see Figure 6.3B), has an extreme left skew, with a very high percentage of cells showing no preference to bars moving in any direction or orientation. The mean B value of 0.11 is slightly smaller than that obtained for the cat SSC in the same laboratory (B=0.16, Binns & Salt 1996). In the cat, neurones in the visual cortex have high B values (B value of 0.9, Nelson *et al.* 1977) whilst retinal ganglion cells (B = 0.16, Levick & Thibos 1982) and LGN cells (B= 0.14, Leventhall & Schall 1983) are only weakly selective. It is possible that SSC neurones are not highly selective for orientation or direction as they are concerned with the immediate detection of the presence of a visual object ("where is it?") rather than detailed analysis of the stimulus ("what is it?", see Section 1.1).

8.1.3 Response habituation

A consistent feature of cells in the SSC of a number of species, including the rat is that the response declines to a sustained stimulus resulting in "habituation" (Humphrey 1968, Fukuda *et al.* 1978, Gonzalez *et al.* 1992,). In this study it was very easy to evoke habituation by reducing the interstimulus frequency of a series of moving bars or by moving a bar very slowly ($<11^0$/sec) across the receptive field.

Response habituation is a key feature underlying the role of the SC as a "novelty detector" and it is possible that mGluRs may have a role in modulating this response (see Chapter 3). For these reasons, the role of mGluRs in modulating response habituation was investigated (see Section 8.3.5).

It is possible that the response may decline due to factors other than "habituation" such as a small eye movement. A test that could have been applied during the experiments described in Sections 6.2.4 and 6.8 is the presentation of a "novel" stimulus (for example a moving bar travelling across the RF in a different direction or a flashed spot) shortly after presentation of the "habituating stimuli". Such a test would determine if the system response is declining due to a persistent stimuli but is still able to evoke a robust response to a novel event.

Another interesting observation was that it was very easy to modulate the level of response of cells by changing the contrast of the stimulus foreground, with a small reduction in the foreground contrast resulting in a significant decrease in the response.
This stimulus was employed in experiments where the effect of antagonist application during different levels of synaptic activity was investigated (see Section 8.3.3).

8.2 All three groups of mGluRs are involved in modulating visual synaptic transmission in the rat SSC

The data presented in Section 6.3 demonstrates that agonists of all three mGluR groups are able to modulate the response of visually responsive neurones in the SSC. Activation of Group I mGluRs results in an inhibition of the visual response (although facilitation occurred in a small number of cells when the ejection current was varied) whilst activation of Group II and III mGluRs produces both inhibitory and excitatory effects. Furthermore, the data presented in Section 6.4 shows that antagonists of all three groups are able to produce an effect on visual responses when applied alone, suggesting that all three mGluR groups can be activated by an endogenous ligand during visual synaptic transmission in the SSC. This is the first indication that mGluRs are involved in synaptic transmission in the SSC.

Interestingly, in a small proportion of cells, Group I and Group III agonists were found to have both inhibitory and facilitatory effects in the same cell when the agonist was applied at different ejection currents. For the Group II agonist, mixed inhibitory/facilitatory effects were not observed, although an increase in the magnitude of effect was seen when the ejection current was increased. Although it is not possible to rule out "non-specific effects" when high ejection currents are used, it is most likely that different effects result from activation of receptors on different cells as ejection currents are varied. Although only one cell is being recorded from, it is important to remember that iontophoretic application of any drug will influence many cells in the vicinity of the recording electrode. It is feasible that these "mixed" effects might occur due to the agonist binding to different types of receptors as the drug concentration changes, with the observed effect dependent upon the precise spatial relationship between different cells and the source of the exogenous drug at the electrode tip. In a physiological context, the effect of mGluR activation will also depend upon the spatial relationship of receptors with respect to the site of endogenous transmitter release.

There was no observed correlation between agonist effect and location of the electrode in the SSC (as revealed by dye spot deposit). It is feasible that such a relationship may exist as some SSC cells have been described as having different dorso-ventral and rostro-caudal distributions (see Table 1.2). Also, retinal afferents (see Section 1.2.1) and cortical afferents (see Section 1.2.3) terminate in slightly different locations within the superficial layers. Attempts to identify a possible relationship between agonist effect and recording depth did not reveal any observed correlation. This analysis was only undertaken for cells where the recording depth could be assessed accurately, i.e. where little cortical inflammation had occurred due to multiple electrode entries. Additionally, analysis of the recording position on the rostro-caudal and medio-lateral axis did not reveal any relationship between agonist effect.

8.3 Possible mechanisms for modulatory effects of exogenous agonists

There is substantial evidence that all three mGluR groups can downregulate **glutamate** (Koerner & Cotman 1981, Forsythe & Clements 1990, Baskys & Malenka 1991, Lovinger 1991, Calabresi *et al.* 1993, Gereau & Conn 1995b, Johansen & Robinson 1995, Lovinger & McCool 1995, Manzoni & Bockaert 1995, Macek *et al.* 1996, Pisani *et al.* 1997a, Dietrick *et al.* 1997) and **GABA** (Desai & Conn 1991, Pacelli & Kelso 1991, Calebresi *et al.* 1992, Desai *et al.* 1992, Glaum & Miller 1992, Desai *et al.* 1994, Stefani *et al.* 1994, Gereau & Conn 1995b, Llano & Marty 1992, Poncer *et al.* 1995, Salt & Eaton 1995, Fitsimons & Dichter 1996, Mitchell & Silver 2000) release at different synapses.

Glutamate (Roberts *et al.* 1991) and GABA (Binns & Salt 1997) have previously been demonstrated to be involved in synaptic transmission in the rat SSC. It is very likely that some of the inhibitory effects of mGluR agonist application in the SSC result from an inhibition of glutamate release, either at the retino-collicular synapse or the cortico-collicular synapse. It is also possible that facilitation of visual responses occurs by reducing the extent of GABAergic transmission.

Possible mechanisms could involve a reduction of GABA release by inhibitory interneurones which form synapses with SSC relay cells of by reducing the glutamatergic drive of these GABAergic neurones (Binns & Salt 1995).

8.3.1 Group I

Figure 8.1 provides a pictorial summary of the possible location of Group I receptors in the SSC. Group I mGluRs include mGluR1 and mGluR5.

Application of 3,5-DHPG was able to produce an inhibition of the visual response in all cells. Interestingly, in a small number of these cells, 3,5-DHPG produced both inhibitory and facilitatory effects when applied at different currents, although for the majority of cells, 3,5-DHPG application resulted in purely inhibitory effects.

Following cessation of 3,5-DHPG application, complete recovery to control values was achieved irrespective of effect. This is in contrast to other results which indicate that 3,5-DHPG mediated inhibition is persistent (McCaffery *et al.* 1999). As the solution of 3,5-DHPG used was of a low pH (~3.5), pH controls (see Section 6.6.4) were carried out. Although some studies have demonstrated that ejection of H^+ has an excitatory effect (Krnjevic & Phillis 1963, Hewes & Frederickson 1974), ejection of H^+ in these experiments did not significantly affect the visual response. Group I mGluRs do not appear to be involved in modulating response habituation in the SSC as 3,5-DHPG application did not alter the extent of response habituation.

Application of the Group I antagonist 4CPG produced a facilitatory effect in every cell, indicating that Group I mGluRs may be activated by an endogenous ligand during synaptic transmission in the SSC. The magnitude of 4CPG effect was not different when the low and high contrast stimuli were used indicating that activation of Group I mGluRs is not dependant upon glutamate concentration and also indicating that Group I receptors in the SSC probably occupy a central synaptic location.

Interestingly, in other brain regions there is ultrastructural evidence for Group I mGluRs being located at the periphery of synapses (Baude *et al.* 1993, Lujan *et al.* 1996, 1997, Vidnyanszky *et al.* 1996) although the physiological data presented here would suggest that this is not the case for the SSC. Application of LY367385 (mGluR1 selective antagonist) did not produce an effect when applied alone (but see Fig 6.10 where LY367385 has a small excitatory effect) although LY367385 did block the effect of exogenous agonist.

It is therefore likely that the receptor involved in synaptic transmission in the SSC is mGluR5 rather than mGluR1. The possibility that LY367385 was not getting out of the electrode can be ruled out as LY367385 application block the effect of 3,5-DHPG. This data indicates that the exogenous agonist 3,5-DHPG has an effect at both mGluR1 and mGluR5, but that the receptor activated by glutamate released during synaptic transmission is mGluR5.

Immunohistochemical studies indicate that there is heavy fibrous mGluR5 labelling in the SSC (Shigemoto *et al.* 1993, Romano *et al.* 1995). In Section 5.6.1, heavy fibrous labelling in the SSC was also described. Although the pharmacological evidence suggests that mGluR1 does not participate in visual synaptic transmission, studies have shown that mGluR1 expression (Shigemoto *et al.* 1992) and immunolabel (Martin *et al.* 1992, Baude *et al.*1993, Fotuhi *et al.* 1993, Peng *et al.* 1995, see also Sections 5.1.1 and 5.3.1) is present in the SSC.

Although no ultrastructural studies looking at the synaptic location of Group I mGluRs has been undertaken in the SSC, studies from other brain regions suggest that Group I mGluRs predominantly occupy postsynaptic locations (Martin *et al.* 1992, Shigemoto *et al.* 1993, van den Pol *et al.* 1995, Shigemoto *et al.* 1997) although presynaptic Group I receptors have also been found (Romano *et al.* 1995). Application of 3,5-DHPG did not have a significant effect upon iGluR evoked responses (see Section 6.7 and Table 6.7) suggesting that modulation by Group I receptors may involve receptors which are not located in the postsynaptic membrane of the SSC relay neurone.

Activation of Group I mGluRs in other preparations produces mainly facilitatory effects due to inhibition of potassium currents (see Section 2.7.1) or activating cation currents (see Section 2.7.2). Group I receptors (by reducing I_{AHP}) reduce response accommodation in a number of different brain regions and preparations (see Section 2.7.1, Charpak *et al.* 1990, Pacelli & Kelso 1991, Zheng & Gallagher 1992, Salt & Eaton 1991). It is tempting to predict that Group I may play such a role in modulating response habituation in the SSC although the data demonstrates that activation by 3,5-DHPG does not significantly affect response habituation (Section 6.8).

3,5-DHPG could inhibit visual responses by depolarising inhibitory interneurones resulting in an increase in GABA release (Zhou & Hablitz 1997, Chu & Hablitz 1998). Other studies have also demonstrated that GABAergic transmission may be enhanced (Llano & Marty 1995, Poncer *et al.* 1995, Sciancalepore *et al.* 1995) by Group I mGluRs. If Group I modulation does involve mGluR5 rather than mGluR1 (as indicated by the lack of effect of LY367385 application), it could be possible to discount the hypothesis that inhibition mediated by 3,5-DHPG results from depolarisation of the inhibitory interneurone as there is no evidence for mGluR5 expression in cell bodies of the SC (Abe *et al.* 1992). However, fibrous mGluR5 immunoreactivity is present (Shigemoto *et al.* 1993, Romano *et al.* 1995) in the SSC which probably corresponds to cortical terminals (Shigemoto *et al.* 1993, Romano *et al.*1995) or possibly retinal afferents (see Section 5.6.1).

Group I mGluRs have been demonstrated to reduce glutamate release (Lovinger 1991, Manzoni & Bockaert 1995, Gereau & Conn 1995b) and it is perhaps more likely that the inhibitory effect of Group I activation results from an inhibition of glutamate release at the cortical or possibly retinal terminal rather than facilitating GABAergic transmission at inhibitory interneurones.

In this study, in a small percentage of cells (around 10%) application of 3,5-DHPG was found to have a bi-phasic effect at different ejection currents. Application of 3,5-DHPG in the hippocampus caused facilitatory effects at low concentrations (2.5-5μM), whilst at higher concentrations (20 - 50μM), 3,5-DHPG had an inhibitory effect (Zahorodna *et al.* 1998). The inhibitory effect at the high concentration range was associated with an inhibition of excitatory synaptic transmission. Unfortunately, it is not possible to determine the concentration of drug ions applied by a given ejection current when applying drugs by iontophoresis. It would be interesting to investigate the effect of different concentrations of 3,5-DHPG application to the SSC *in-vitro*.

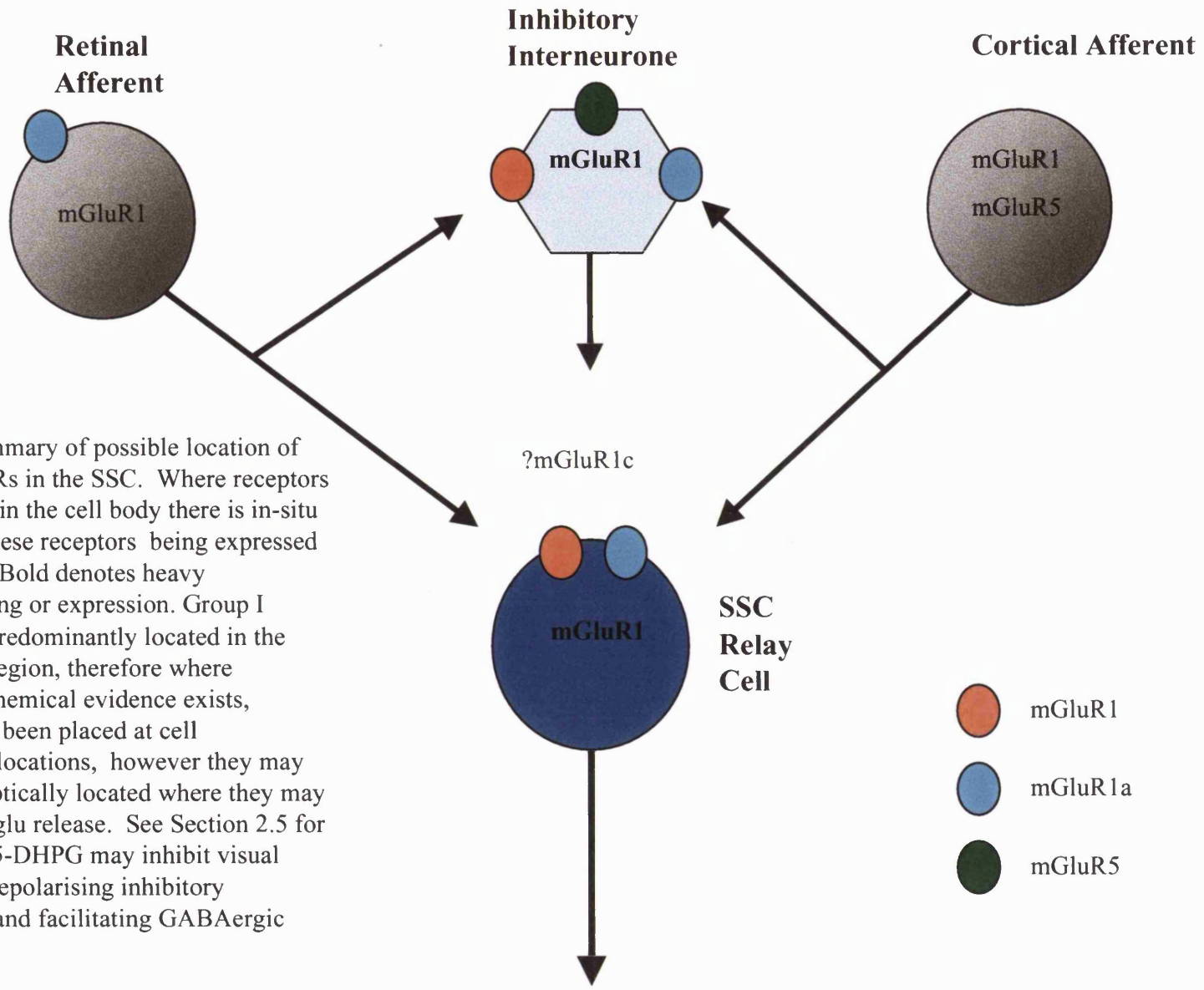


Figure 8.1 Summary of possible location of Group I mGluRs in the SSC. Where receptors are mentioned in the cell body there is in-situ evidence for these receptors being expressed in these cells. Bold denotes heavy immunolabelling or expression. Group I receptors are predominantly located in the post synaptic region, therefore where immunohistochemical evidence exists, receptors have been placed at cell body/dendrite locations, however they may have pre-synaptically located where they may downregulate glu release. See Section 2.5 for references. 3,5-DHPG may inhibit visual responses by depolarising inhibitory interneurones and facilitating GABAergic transmission.

8.3.2 Group II

Figure 8.2 provides a pictorial summary of the possible location of Group II receptors in the SSC. Group II mGluRs include mGluR2 and mGluR3.

Expression data has revealed that there is no mGluR3 expression in the rat retina (Koulen *et al.* 1996) and only weak mGluR2 expression in the ganglion cell layer of the retina (Hartveit *et al.* 1993). It is unlikely that mGluR2 is transported to central terminals of RGC's as no mGluR2 immunoreactivity is observed in the SC (Neki *et al.* 1996, Ohishi *et al.* 1998). Given this evidence, it is reasonable to conclude that Group II receptors are not present on the retinal projection to the SSC and to therefore rule out involvement of the retinal projection in Group II modulation. There is immunohistochemical (Petralia *et al.* 1996) evidence for the presence of Group II mGluRs in the SGS layer of the SC. Expression studies reveal that mGluR2 (Ohishi *et al.* 1993a) and mGluR3 (Ohishi *et al.* 1993b) is present in the cortex whilst cells in the SC also express mGluR3 (Ohishi *et al.* 1993b, Tanabe *et al.* 1993).

Examination of other brain regions reveals that Group II mGluRs are located in both pre and postsynaptic locations (Ohishi *et al.* 1994, Neki *et al.* 1996, Shigemoto *et al.* 1997). However, the Group II agonist LY354740 did not significantly affect iGluR evoked responses in the SSC (see Section 6.7 and Table 6.7 for data) indicating that modulation of visual responses by Group II mGluRs involves predominantly pre-synaptic mechanisms.

It is conceivable that the inhibitory actions of LY354740 are predominantly due to an effect on terminals of cortico-collicular afferents, thus reducing visual responses by inhibiting glutamate release (Lovinger 1991, Lovinger & McCool 1995). Facilitatory effects of LY354740 application may result from disinhibition. This could occur directly by activating Group II mGluRs on interneurones and reducing GABAergic transmission (Calabresi *et al.* 1992, Hayashi *et al.* 1993, Salt & Eaton 1995, Cox and Sherman 1999) or by reducing excitatory input to the interneurone. LY354740 could reduce excitatory input to the interneurone by inhibiting glutamate release from the cortico-collicular afferent.

Interestingly, systemic administration of LY354740 in the conscious rat results in a significant increase in glucose metabolism in the SSC and regions of the limbic system (Lam *et al.* 1999).

mGluR3 is highly expressed in glia (Ohishi *et al.*, 1993b, Testa *et al.*, 1994, Jeffery *et al.*, 1996, Petralia *et al.*, 1996, Mineff & Valtschanoff 1999) therefore, it is also possible that Group II agonists may have a modulatory effect on synaptic transmission in the SSC via receptors located on glial cells (Winder & Conn 1996).

8.3.3 Group II receptors may be activated by glutamate spillover

Section 6.9 describes how the magnitude of Group II antagonist LY341495 effect is smaller during periods of reduced synaptic response. This effect was not observed with antagonists of Group I and Group III. There is evidence for the "extrasynaptic" location of Group II receptors in several brain areas (Nusser *et al.* 1994, Lujan *et al.* 1997, Mineff & Valtschanoff 1999, Azkue *et al.* 2000) and it has been proposed that these receptors are only activated when there is "spillover" of glutamate out of the main synaptic region, possibly during periods of intense synaptic activity (Scanziani *et al.*, 1997, Rusakov & Kullmann 1998, Dube & Marshall, 2000, Mitchell & Silver 2000). However, it has not been clear how relevant this may be to synaptic processing under physiological conditions. Data showing that the effect on visual responses of a Group II antagonist varies with stimulus intensity within the physiological range indicates that the extrasynaptic receptors play a role in synaptic processing during visual responses. It is noteworthy that the contrast-dependent effect of LY341495 was observed both in neurones where the agonist LY354740 enhanced visual responses or inhibited visual responses.

Interestingly, for both the Group I and Group III antagonists, there was no significant difference between effects at low and high contrast. This indicates that the involvement of Group I and Group III receptors in synaptic transmission in the SSC is not so dependent upon synaptic concentration of glutamate and could also suggest that Group I and Group III receptors have a more "central" synaptic location (Shigemoto *et al.*, 1996, Ottersen & Landsend 1997).

Although LY341495 has nanomolar antagonist potency for Group II mGluRs (Kingston et al. 1998) it has antagonist activity at other mGluRs at higher concentrations. When LY341495 was co-ejected with L-AP4, L-AP4 was still able to produce an effect indicating that in these experiments, LY341495 is having specific antagonist activity at Group II receptors only and not Group III receptors (see Section 8.4.3).

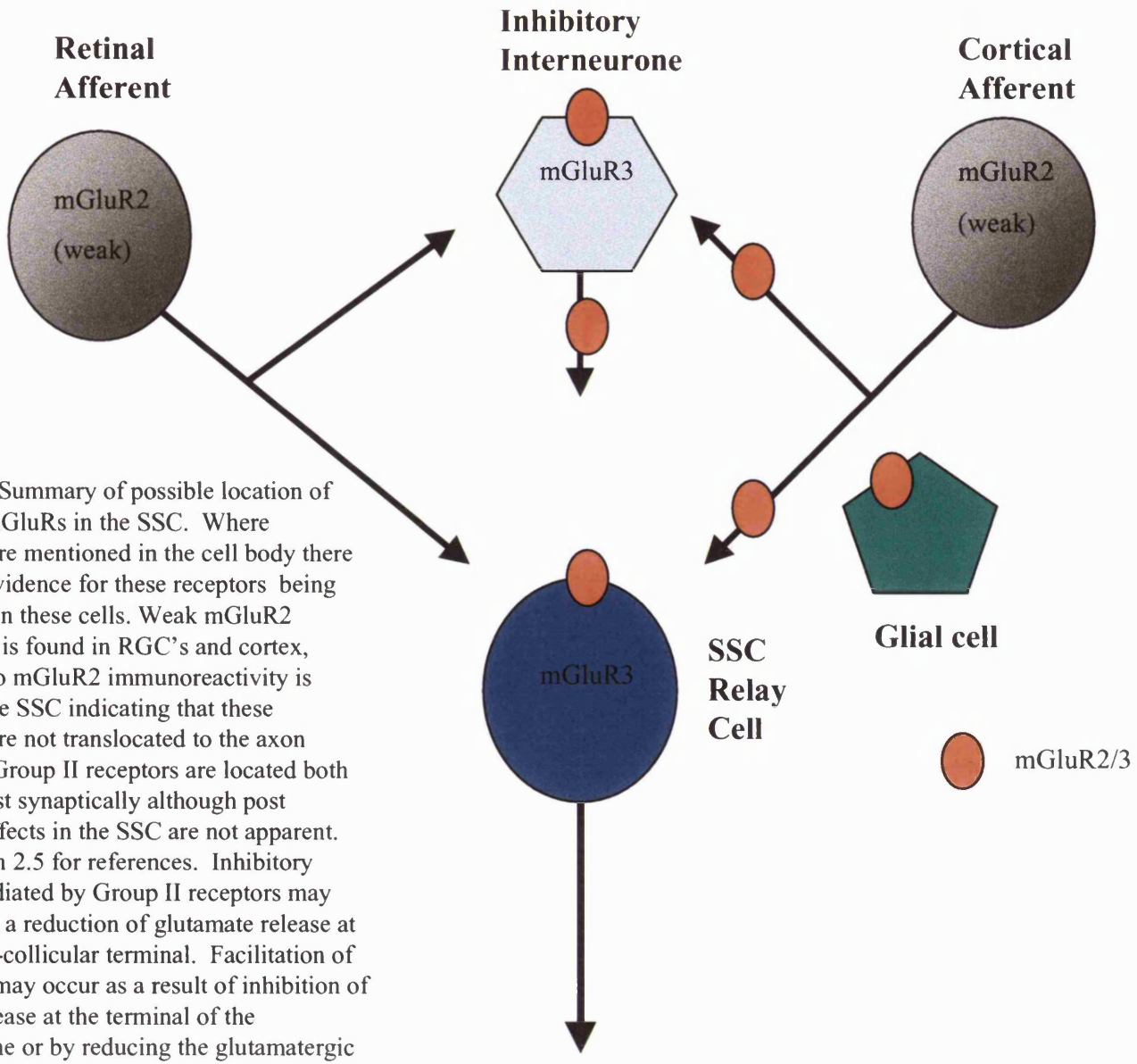


Figure 8.2 Summary of possible location of Group II mGluRs in the SSC. Where receptors are mentioned in the cell body there is in-situ evidence for these receptors being expressed in these cells. Weak mGluR2 expression is found in RGC's and cortex, however no mGluR2 immunoreactivity is found in the SSC indicating that these receptors are not translocated to the axon terminal. Group II receptors are located both pre and post synaptically although post synaptic effects in the SSC are not apparent. See Section 2.5 for references. Inhibitory effects mediated by Group II receptors may result from a reduction of glutamate release at the cortico-collicular terminal. Facilitation of responses may occur as a result of inhibition of GABA release at the terminal of the interneurone or by reducing the glutamatergic drive of the interneurone.

8.3.4 Group III

Figure 8.3 provides a pictorial summary of the possible location of Group III receptors in the SSC.

Group III mGluRs include mGluR4, mGluR6, mGluR7 and mGluR8. Although no analysis of SSC immunolabelling for Group III mGluRs has been undertaken at the electron microscope level, results from other brain regions reveal that Group III mGluRs are predominantly located pre-synaptically (see Section 2.5 for details, Ohishi *et al.* 1995, Shigemoto *et al.* 1996, 1997, Wada *et al.* 1998). However, Group III receptors have also been found in postsynaptic locations (see Section 2.5 for details, Bradley *et al.* 1996, Brandstatter *et al.* 1996, Bradley *et al.* 1998). As L-AP4 was shown to modulate iGluR responses (see Section 6.7) it is likely that some Group III receptors could have a postsynaptic location in the SSC and that these postsynaptic receptors may be involved in modulating the visual response. In the nucleus accumbens (Martin et al. 1997), L-AP4 inhibits the NMDA component of the EPSC in the presence of TTX, suggesting that L-AP4 can have a postsynaptic effect in this preparation.

In an *in-vivo* preparation it is difficult to determine whether a drug has an effect at pre or post synaptic sites. However, it is possible to test for a post synaptic effect by examining the effect upon responses evoked by application of iGluR agonists. This is a particularly suitable investigation for mGluR ligands as mGluRs have been demonstrated to inhibit K^+ channels (see Section 2.7.1) which would result in a decrease in input resistance (Bond & Lodge 1995) which would result in a potentiation of iGluR evoked responses.

Using *in-situ* hybridization methods, low levels of mGluR4 were found in the SC and cortex, whilst mGluR7 was moderately expressed in the SC and visual cortex (Okamoto *et al.* 1994, Ohishi *et al.* 1995). Using an antibody for mGluR7a, intense fibre labelling is found in the SC (Bradley *et al.* 1998), this could correspond to axonal labelling of cortical afferents or intrinsic SSC neurones.

In Section 5.5.1, strong fibrous labelling for mGluR4 was observed in the SSC. mGluR4 (but not mGluR7, Brandstatter *et al.* 1996) is highly expressed in retinal ganglion cells (Hartveit *et al.* 1995, Koulen *et al.* 1996) and it is possible that these receptors may be located at the axon terminal where they may participate in synaptic transmission.

However, the results described in Section 5.5.1 report that no mGluR4 label was observed in the RGC layer or in the optic tract of the rat.

mGluR8 expression is not apparent in the SSC (Corti *et al.* 1998), although moderate levels are found in the cortex (Saugstad *et al.* 1997) and it is therefore possible that mGluR8 may be present on terminals of cortico-collicular afferents. mGluR8 immunoreactivity is also found in RGCs (Koulen et al. 1999) and some receptors may be transported to their central terminal mGluR6 is almost exclusively distributed in retinal bipolar ON cells (Masu *et al.* 1995) and it is therefore most unlikely that mGluR6 contributes to L-AP4 mediated modulation of synaptic transmission in the SSC.

In most of the cells studied, iontophoretic application of L-AP4 resulted in a reduction of visual responses. The most feasible mechanisms by which L-AP4 can reduce visual responses in the SSC is by reducing glutamate release. Presynaptic L-AP4 mediated depression of glutamate release has been well characterised in a number of studies (Koerner & Cotman 1981, Harris & Cotman, 1983, Forsythe & Clements 1990, Baskys & Malenka 1991, Gereau & Conn 1995). The effects of L-AP4 can be inhibited by pertussis toxin (Herrero et al. 1996), suggesting that the receptor is coupled to a G_i protein. Although the exact downstream effects are not known, L-AP4 has been shown to reduce glutamate release by inhibiting a P/Q type calcium conductance in other brain regions (Takahashi et al. 1996).

L-AP4 also produced a facilitatory effect in some cells, it is possible that this may occur as a result of a decrease in GABAergic inhibition. L-AP4 has previously been demonstrated to reduce the extent of GABAergic inhibition in the thalamus (Salt & Eaton 1995).

As for Group II mGluRs, a reduction in GABAergic transmission could result from a direct inhibition of GABA release at the terminal of the inhibitory interneurone which synapse onto visually responsive neurones, or a reduction of the glutamatergic excitation which drives the GABAergic interneurones.

L-AP4 has a high potency for mGluR4 and mGluR8 (Conn & Pin 1997) and a low potency for mGluR7 (Okamoto *et al.* 1994). It is therefore likely that mGluR4 or 8 may be more likely than mGluR7 to be activated during iontophoretic application of L-AP4. Unfortunately, it is not possible to quantify the amount of drug released by iontophoretic application and it is therefore not possible to determine the dose of L-AP4 required to elicit the effects observed in these experiments. Use of an *in-vitro* preparation where known concentrations of drugs may be applied may give a clearer idea of the Group III receptors involved by examining the concentration of L-AP4 required to produce effects. The effect of L-AP4 is stereospecific as application of the stereoisomer D-AP4 did not significantly affect visual responses.

8.3.5 Role of Group III mGluRs in response habituation

$GABA_B$ (but not $GABA_A$) receptors contribute to the process of response habituation (Binns & Salt 1997). Data presented in Section 6.8 show that activation of Group III mGluRs with L-AP4 enhances habituation, whereas habituation is reduced by the Group III antagonist MPPG. As the effects of L-AP4 and MPPG are still evident in the presence of the $GABA_B$ antagonist CGP35348, it would appear unlikely that receptors located on GABAergic interneurones or input to these neurones contribute to the modulation of habituation by Group III receptors. Although such data is not available for the rat, in the rabbit, response habituation does not depend on cortical input (Horn & Hill 1966, Stewart *et al.* 1973). In the experiments described in section 6.8, damage inevitably occurs to the visual cortex as a result of penetration by the multi-barelled electrode with the extent of damage being variable from preparation to preparation.

It is noteworthy that it was possible to generate habituation in every example regardless of the extent of cortical damage and this suggests that in the rat, like the rabbit that response habituation does not involve cortical mechanisms. Based on the evidence that visual cortex does not appear to be involved in generating habituation, it is possible that habituation arises due to activation of mGluRs on the retinal terminal.

During high rates of afferent stimulation (such as during generation of response habituation) the elevated level of glutamate released by the retinal afferent could activate pre-synaptic mGluR autoreceptors resulting in a downregulation of glutamate release which results in a decline in the response and generation of habituation. Binns & Salt (1997) suggested that $GABA_B$ receptors located on interneurones could produce habituation. An alternative mechanism could involve $GABA_B$ receptors sited at the retinal terminal. During high rates of stimulation glutamate released by the retinal afferent could provide extra input to the inhibitory interneurone where GABA may feedback to a $GABA_B$ receptor on the retinal terminal, thereby reducing glutamate release (Emri *et al.* 1996). Additional inhibition of glutamate release from the retinal terminal may occur due to metabotropic autoreceptor activation.

Consistent with the hypothesis that the mGluR mediated component of response habituation is mediated by Group III (and not Group I and II) mGluRs located on the retinal terminal, Group I and II receptors are unlikely to be located at the retinal terminal. As previously discussed, there is no evidence for Group II mGluRs being located at the retinal terminal.

Group I receptors are predominantly located post-synaptically (Martin *et al.* 1992, Shigemoto *et al.* 1993, van den Pol *et al.* 1995, Shigemoto *et al.* 1997) although they are also found in pre-synaptic locations (Romano *et al.* 1995). Sections 5.1.1, 5.2.1 and 5.3.1 indicate that RGC's are not immunoreactive for mGluR1a, mGluR1b and mGluR1c respectively and that labelling for all three mGluR1 antibodies in the SSC was not consistent with retinal terminal label. However, some immunoreactivity for mGluR5 was found in RGC's and their axons (see Section 5.6.1).

As discussed previously, it is tempting to think that Group I mGluRs may have a role in the modulation of response habituation given that Group I receptors have been shown to inhibit a potassium current involved in neuronal repolarisation (I_{AHP}, Baskys *et al.* 1990, Charpak *et al.* 1990, Desai & Conn 1991, Gerber *et al.* 1992, Glaum & Miller 1992, Ceaser *et al.* 1993, Schrader & Tasker 1997, Zahorodna *et al.* 1998). mGluRs have been shown to reduce response habituation in a number of brain areas (Charpak *et al.*. 1990, Pacelli & Kelso 1991, Zheng & Gallagher 1992, Salt & Eaton 1991) and it is thought that some of these effects are due to an inhibition of I_{AHP}. However, application of the Group I agonist 3,5-DHPG did not affect response habituation (see Section 6.8, Table 6.8 for data).

Response habituation during visual recognition tasks occurs in the rat perirhinal cortex (Zhu *et al.* 1996). Application of mGluR agonists of all three mGluR groups (1S,3R-ACPD, 3,5-DHPG, 2R,4R APDC and L-AP4) to an *in-vitro* preparation (McCaffery *et al.* 1999) results in an inhibition of evoked responses. Furthermore, this effect persisted after drug washout from the preparation for all agonists tested apart from L-AP4. The authors of this study suggest that the long term inhibitory effects of Group I and II mGluR activation may underlie response habituation in the perirhinal cortex *in-vivo*.

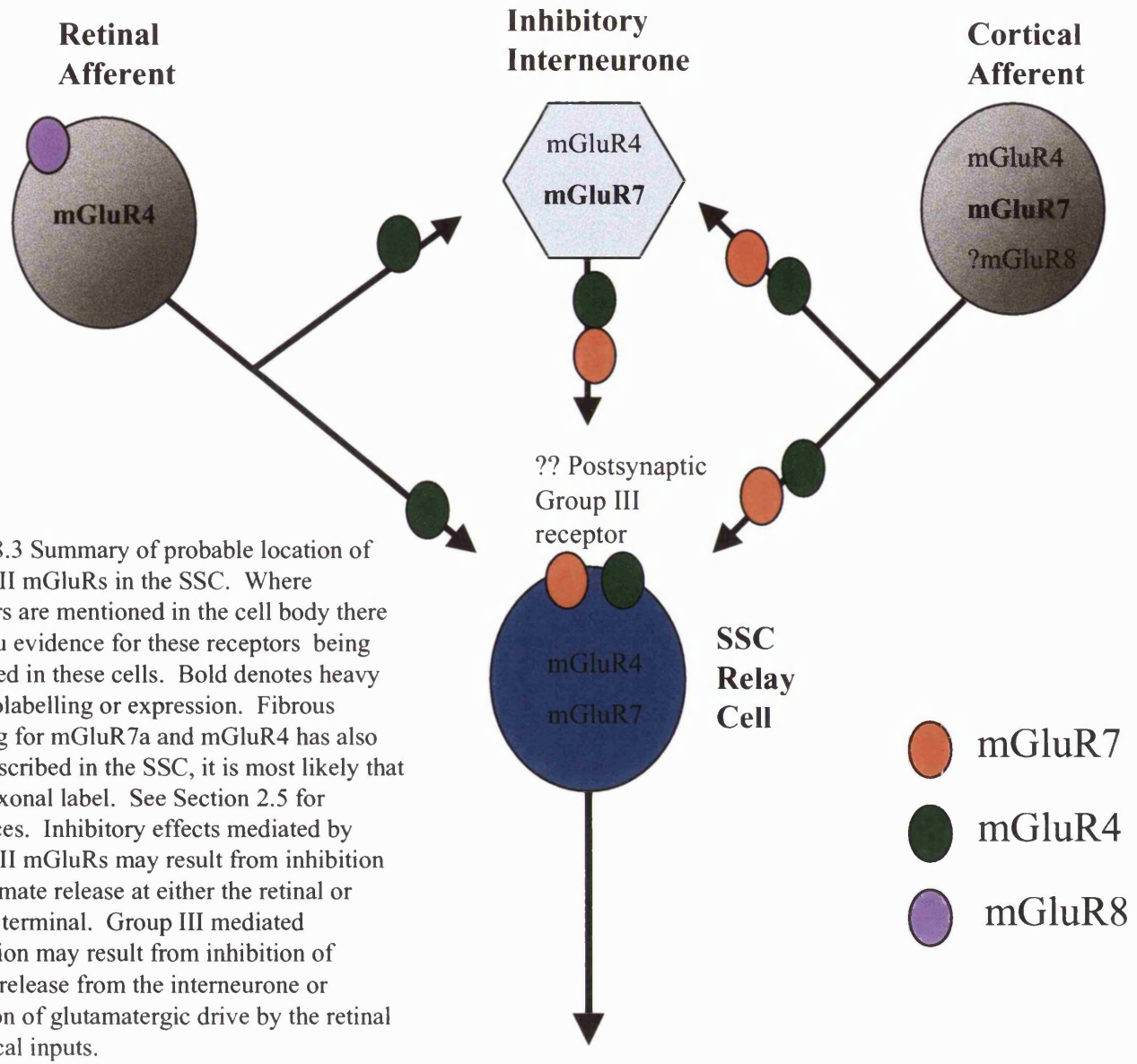


Figure 8.3 Summary of probable location of Group III mGluRs in the SSC. Where receptors are mentioned in the cell body there is in-situ evidence for these receptors being expressed in these cells. Bold denotes heavy immunolabelling or expression. Fibrous labelling for mGluR7a and mGluR4 has also been described in the SSC, it is most likely that this is axonal label. See Section 2.5 for references. Inhibitory effects mediated by Group III mGluRs may result from inhibition of glutamate release at either the retinal or cortical terminal. Group III mediated facilitation may result from inhibition of GABA release from the interneurone or reduction of glutamatergic drive by the retinal or cortical inputs.

8.4 Comment upon control experiments

8.4.1 Passage of iontophoretic current does not affect neuronal responses

It is reasonable to assume that the drug effects observed are specific effects due to drug ion ejection and not passage of iontophoretic current. It is possible to rule out "current artefacts" as ejection of current through a barrel containing 100mM NaCl solution did not affect visual responses. Currents selected for these saline controls were at least the sum total of the highest ejection currents used during agonist/antagonist co-application experiments and in most cases ranged from 100 to 200nA. See Section 6.6.1 and Table 6.2 for data summary.

8.4.2 Effect of L-AP4 is drug specific

For some experiments where L-AP4 was used, an additional pharmacological control was used. The stereoisomer D-AP4 did not have a significant effect in cells where L-AP4 significantly affected the visual response (see Section 6.6.2 and Table 6.5 for details). Although D-AP4 is not active at mGluRs, D-AP4 is a weak NMDA antagonist (Evans et al. 1982) and it is perhaps surprising that D-AP4 did not have a small inhibitory effect in some cells. The lack of D-AP4 effect in the SSC could be explained by the relatively small component of visual synaptic transmission that is mediated by NMDA type receptors (Roberts *et al.* 1991).

8.4.3 LY341495 has antagonist activity at Group II receptors only

Although nanomolar potency for the Group II antagonist LY341495 at Group II mGluRs has been described (Kingston *et al.* 1990) at higher concentrations, LY341495 may also have antagonist activity at other mGluRs (Johnson *et al.* 1999, Turner & Salt 1999, Fitzjohn *et al.* 1999). A serious drawback with applying drugs by iontophoresis is that it is not possible to quantify the concentration of drug applied to the tissue.

In order to minimize problems with drugs having non-specific effects it is sensible to routinely use the lowest ejection current possible to obtain effects. Where it is know that some drugs have specificity over a concentration range it is possible to test their specificity against other drugs. In order to test the specificity for LY341495 as an antagonist at Group II mGluRs, LY341495 was co-applied with the Group II agonist LY354740 and it was found that the Group II antagonist successfully blocked the effect of the Group II agonist (see Section 6.5 and Table 6.4). However, when LY341495 was co-ejected with the Group III agonist L-AP4 it was not possible to block the effect of the Group III agonist (see Section 6.6.3 and Table 6.6). This data indicates that in these experiments, LY341495 is having antagonist activity at Group II receptors only and that antagonist activity at Group III mGluRs can be disregarded.

8.4.5 Ejection of H^+ does not affect the visual response

As previous studies have indicated that ejection of H^+ from drug solutions can affect neuronal responses (Krnjevic & Phillis 1963, Hewes & Frederickson 1974) pH controls were carried out in some experiments. In experiments where 3,5-DHPG was used, positive current was ejected from a barrel containing 100mM NaCl adjusted to ~ pH3.5 which is the same as the DHPG solution. Ejection of Na^+ and H^+ from this barrel did not result in a significant effect upon visual responses (see Section 6.6.4 for data). Although is it satisfying to note that H^+ ejection does not appear to have an effect in these experiments, it is important to consider that ejection of H^+ from saline solution may not result in the same localised change in pH when drug ions and H^+ are ejected.

When a drug ion is ejected into a medium where the pH is significantly different to that of the parent solution (i.e. the drug ion moving out of the barrel into extracellular fluid), then the drug ion can either release or take up H^+ depending on the nature of the drug ion and relative pH values of the two solutions (Curtis 1964). It is therefore unlikely that the change in pH in the extracellular environment is exactly the same when ejecting current is applied to the drug or control solutions.

8.5 Conclusion

- All three groups of mGluRs appear to be involved in visual synaptic transmission in the rat SSC.

- Activation of Group I mGluRs produces mainly inhibitory effects, this inhibition may occur as a result of inhibition of glutamate release from the cortical terminal. An alternative mechanism could involve depolarisation of inhibitory interneurones resulting in an increase in GABAergic transmission.

- Activation of Group II and III receptors can cause both facilitation and inhibition. Inhibition of the visual response may occur due to an inhibition of glutamate release from the cortical afferent (Group II and III) or from the retinal afferent (Group III).

- Facilitation of visual responses by Group II and III mGluRs may result from a reduction of glutamatergic inhibition. This may occur directly by inhibiting GABA release from the terminal of the inhibitory interneurone or more indirectly by inhibiting the glutamatergic drive of the interneurone, either at the retinal input (Group III) or cortical afferent (Group II and III).

- Group II mGluRs are differentially activated during different levels of synaptic response. This may occur as a result of glutamate spillover away from the centre of the synapse resulting in activation of extrasynaptic receptors. This could have a functional role in the SSC in contrast discrimination.

- Group III mGluRs mediate a component of response habituation which is distinct from that mediated by $GABA_B$ receptors. mGluR mediated response habituation could be generated by activation of autoreceptors located on retinal terminals during high frequency stimulation. In this way, mGluR activation may result in a behavioural effect by allowing the rat to distinguish what constitutes a new visual event and thereby allow the generation of an appropriate behavioural response.

9.0 Project Conclusions

The work presented in this thesis has demonstrated for the first time that mGluRs are involved in visual synaptic transmission in the rat SSC. The data indicates that activation of mGluRs from a single group can mediate both facilitatory and inhibitory effects. This probably occurs due to mGluRs being located on different cell types and afferent SSC inputs. In a physiological context, It is most unlikely that selective activation of any mGluR occurs and that the effect of synaptically released glutamate will depend on a number of factors. This may include the extent of glutamate diffusion and re-uptake at the synapse, location and affinity of different mGluRs and iGluRs and the frequency of afferent stimulation.

Immunohistochemical data presented in Chapter 5 and discussed in Chapter 7 suggests that mGluRs are distributed very differently in the visual system of different species. It is therefore unlikely that activation of mGluRs in other species will result in exactly the observed effects described here for the rat. Nevertheless, this study has indicated that mGluRs have interesting functional roles. This includes activity dependent activation (Group II receptors) and the generation of response habituation (Group III receptors).

It is possible that activation of mGluRs under different environmental situations may ultimately result in the generation of different types of behavioural response that are appropriate to the nature of the visual event.

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