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Kainate Receptor Function in Rodent Subcortical Visual Processing

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

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Figures

Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
dLGN	Dorsal lateral geniculate nucleus
EPSC/P	excitatory postsynaptic current/potential
GABA	γ-aminobutyric acid
GABAR	γ-aminobutyric acid receptor
iGluR	ionotropic glutamate receptor
IPSC/P	inhibitory postsynaptic current/potential
KA	kainate/kainic acid
KAR	Kainate receptor
mGluR	metabotropic glutamate receptor
NMDA	*N*-methyl-D-aspartate
NMDAR	*N*-methyl-D-aspartate receptor
RGC	retinal ganglion cell
SC	superior colliculus
SSC	superficial superior colliculus

1 Summary

Glutamate is found throughout the central nervous system and has been shown to be an important excitatory neurotransmitter in the visual system.

There are two subdivisions of receptor on which this ubiquitous neurotransmitter acts, metabotropic (mGluR) and ionotropic (GluR) glutamate receptors. There are eight sub-types of mGluR falling into three groups, and fifteen GluR subunits also divided into three groups. Kainate receptors (KARs) comprise one group of the ionotropic glutamate receptor subdivision.

Relay cells of the lateral geniculate nucleus (LGN) are driven and modulated by a variety of NMDA, AMPA and metabotropic receptors. In addition, investigation into the involvement of mGluR, AMPA and NMDA receptor function in the synaptic processing of the superior colliculus (SC) has been well documented. It has been difficult, however, to establish specific KAR function in these brain structures due to lack of pharmacological agents acting solely at kainate receptors. In recent years such agents have become available, thus enabling the present study of GluR5 involvement in visual processing within the SC and LGN.

The purpose of this body of work has been to assess the involvement of GluR5-containing Kainate receptors (KARs) in synaptic transmission between retinal ganglion cells (RGCs) and subcortical brain structures involved in the processing of visual information; namely

the superficial superior colliculus (SSC) and the lateral geniculate nucleus (LGN). The majority of the work focused on the function of KARs in the SSC.

To elucidate the involvement of KARs in visual processing, both *in vivo* and *in vitro* methods were utilised. *In vivo* electrophysiology was used for extracellular recording of evoked activity of both SSC and LGN neurons in response to visual stimuli. This was carried out during intravenous injection of GluR5 antagonist. *In vivo* recording twinned with iontophoretic administration of GluR5-specific pharmacological compounds was also employed to investigate KAR participation in direct synaptic transmission between RGCs and the SSC neurons. The same technique was used to study KAR involvement in the phenomenon of response habituation exhibited by these neurons.

To parallel *in vivo* protocols, *in vitro* SSC slice experiments were performed to study the effect of GluR5 agonists and antagonists on evoked postsynaptic currents. This enabled the administration of drugs at concentrations specific for GluR5 subunits whilst investigating GluR5 involvement in direct synaptic transmission between RGC input and SSC neurons.

In addition, a paired pulse protocol was employed to propose a presynaptic location of GluR5-containing KARs at retinal input into the SSC. Furthermore, the use of GluR5-specific and GABAR-specific compounds during evoked current recording indicated the involvement of GluR5-containing receptors in the direct modulation of excitatory but not inhibitory input into the SSC.

In summary, therefore, both *in vivo* and *in vitro* electrophysiology techniques were used to indicate a location and function for GluR5 KARs in the subcortical visual system of the rat.

GluR5-containing receptors were found to modulate visual processing of both the LGN and SSC. It was unclear whether these receptors were located in the LGN itself due to the use of systemic injection protocols, however, iontophoresis of GluR5-selective drugs demonstrated a role in modulating visual responses within the SSC. The mechanism by which GluR5 receptors modulated responses in the SSC was further elucidated by a series of whole cell patch-clamp experiments which revealed that GluR5-containing receptors reduced synaptic transmission at excitatory inputs directly onto recorded cells and those connections with the intrinsic inhibitory circuitry of the SSC. In addition a paired-pulse protocol was used to determine that the decrease in excitatory transmission was caused by the presynaptic reduction of glutamatergic transmission.

2 Glutamate Receptors

The status of glutamate as a neurotransmitter was initially uncertain as this amino acid is also involved in intermediary metabolism within the brain, including protein synthesis, as a precursor of γ-aminobutyric acid (GABA) and in the detoxification of ammonia. However, it is now known glutamate exerts a powerful stimulatory effect on neuronal activity via its actions upon specific receptors.

Glutamate is now considered the most ubiquitous excitatory neurotransmitter in the mammalian central nervous system (CNS), and is stored in synaptic vesicles of nerve terminals until its release into the synaptic cleft. This is mediated by a Ca^{2+}-dependent exocytotic process upon depolarisation of the terminal (Cooper *et al.*, 1996).

The receptors which are concerned with glutamatergic synaptic transmission can be classified into two families, the metabotropic glutamate receptors (mGluRs) and the ionotropic glutamate receptors (GluRs).

2.1 Metabotropic Glutamate Receptors

The mGluRs are G-protein-coupled receptors (GPCRs) which can be further subdivided into eight subtypes which, based on sequence homology, coupling to second messenger systems and pharmacology, fall into three groups (Nakanishi, 1992; Conn & Pin, 1997). Group I, mGluR1 and mGluR5, and their splice variants, mGluR1a, b and c (Pin *et al.*, 1992) and mGluR5a and b (Minakami *et al.*, 1993; Joly *et al.*, 1995; Yamaguchi and Nakanishi, 1998), are coupled to post-synaptic phosphoinositide metabolism (Aramori and

Nakanishi, 1992; Abe *et al.*, 1992). Group II receptors (mGluR2 and mGluR3) and Group III receptors (mGluR4a and 4b, mGluR6, 7a and 7b, and 8a and 8b) both appear to down-regulate cyclic AMP (cAMP) metabolism in expression systems (Conn & Pin, 1997). Within each group mGluRs share 70% homology, but only 45% sequence homology is shared between the groups (Conn & Pin, 1997).

Group I mGluRs are involved in phosphoinositide (PI) hydrolysis via phospholipase C (PLC) which results in a rise in intracellular Ca^{2+} (Aramori & Nakanishi, 1992; Abe *et al.*, 1992). However, Group I receptors have been observed to stimulate an increase in cAMP and arachadonic acid in some preparations (Anwyl, 1999; Fagni *et al.*, 2000), while mGluR1 is able to potentiate NMDA currents via a G-protein-independent mechanism linked to Src kinase activation in hippocampal CA3 neurons (Heuss and Gerber, 2000). Furthermore, mGluR1a and mGluR5 are believed to function via a G-protein-independent mechanism with direct interaction with the protein Homer, resulting in raised intracellular Ca^{2+} (Brakeman *et al.*, 1997). Additional differences between the two Group I subtypes include the observation that mGluR1 action is blocked to a greater extent than mGluR5 when exposed to pertussis toxin (PTX), although the action of mGluR1a is only partially PTX sensitive while mGluR1b is PTX-insensitive (Conn & Pin, 1997).

Group II and III mGluRs are able to inhibit forskolin-induced cAMP production (Conn & Pin, 1997). Thus their action is to inhibit adenylyl cyclase (AC) activity, which in turn inhibits cAMP formation (Conn & Pin, 1997). Group II and III mGluRs are all PTX-sensitive and therefore likely to be coupled to G_i-proteins (Conn & Pin, 1997).

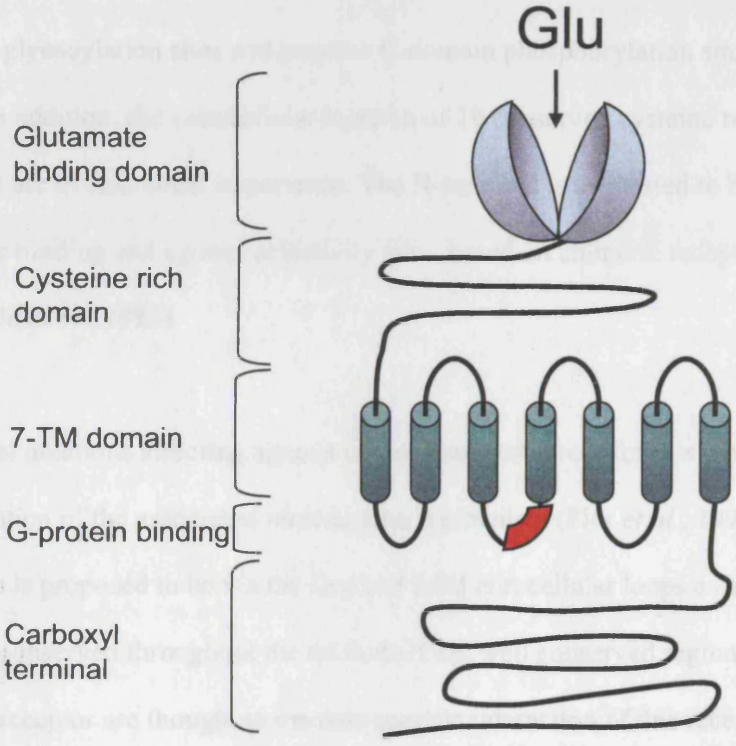


Figure 1. Putative structure of mGluR1 subunit taken from Conn and Pinn, 1997. The structure consists of a 7 transmembrane domain protein with a glutamate binding domain, cysteine rich domain and G-protein coupling segment on the second intracellular loop, and a cytoplasmic carboxyl terminal.

2.1.1 Structure

Metabotropic glutamate receptors consist of a large N-terminal, 7 transmembrane domains and a C-terminal region. From the structure of mGluR1 (figure 1), it has been proposed that the N-terminal is located extracellularly and the C-terminal is situated intracellularly, due to N-glycosylation sites and putative C-domain phosphorylation sites (Masu *et al.*, 1991). In addition, the extracellular location of 19 conserved cysteine residues suggests that these are of functional importance. The N-terminal is suggested to be the location of glutamate binding and agonist selectivity sites, based on chimeric receptor research (Takahashi *et al.*, 1993).

C-terminal deletions affecting agonist potency suggest a role for this terminal is possibly the regulation of the associated transduction mechanism (Flor *et al.*, 1996). G-protein activation is proposed to be via the first and third intracellular loops as amino acid sequence is highly conserved throughout the mGluRs. Less well conserved regions located on the mGluR1 receptor are thought to mediate specific interaction of this receptor with phospholipase C (Pin *et al.*, 1994).

2.1.2 Pharmacology

Glutamate analogues such as ACPD (1-amino-cyclopentane-1,3-dicarboxylate) and L-AP4 (L-2-amino-4-phosphonobutyrate) are specific for mGluRs, but potencies differ between subtypes. Group I mGluRs demonstrate the agonist potency series: Quisqualate > 3,5-DHPG (dihydroxyphenylglycine) > Glutamate ≥ Ibotenate = ACPD (Conn & Pin, 1997).

Along with the potent agonist 3,5-DHPG, the less potent agonist, 3-HPG (3-hydroxyphenylglycine) is specific for Group I, while t-ADA (*trans*-azetidine-2, 4-dicarboxylate) and CHPG (carboxyhydroxyphenylglycine) are more selective mGlu5 agonists (Conn & Pin, 1997).

The phenylglycine derivatives (S)-4-carboxy-phenyl-glycine ((S)-4CPG) and (S)-4C3HPG are relatively potent, specific mGluR1a competitive antagonists (Kingston *et al.*, 1995), however, these also act on Group II receptors at higher concentrations (Hayashi *et al.*, 1994). More specific antagonists include a compound based on (S)-4CPG, LY367385, which is selective for mGlu1, and MPEP (2-methyl-6-(phenylethynyl)pyridine) selective for mGlu5 (Gasparini *et al.*, 1999).

Selective Group II agonists include DCG-IV ((2S, 10R, 20R, 30R)-2-(2, 3-dicarboxycyclopropyl)glycine) and L-CCG-I ((2S, 1'S, 2'S)-2-(carboxycyclopropyl)glycine. However, DCG-IV is also an NMDA agonist (Hayashi *et al.*, 1993), and L-CCG-I is active at mGluR1 and mGluR4 at higher concentrations (Hayashi *et al.*, 1992). Group II antagonists include MCCG (α-methyl-L-CCG-I) and phenylglycine derivatives MPPG (α-methyl-4-phosphonophenylglycine), MSPG (α-methyl-4-sulfonophenylglycine) and MTPG (α-methyl-4-tetrazoylphenylglycine), although MCCG is more potent and specific (Flor *et al.*, 1996). Both Group I and II mGluRs are sensitive to the antagonist LY393053, a compound related to the phenylglycines (Salt *et al.*, 1999; Chen *et al.*, 2000; Kingston *et al.*, 2002).

Among the glutamate receptors, Group III alone shows sensitivity to the agonists L-AP4 and L-SOP (L-serine-O-phosphate). (S)-3,4-DCPG ((S)-3,4-Dicarboxyphenylglycine) is a potent selective mGlu8 agonist (Thomas *et al.*, 2001) (Conn & Pin, 1997; Ozawa *et al.*, 1998; Schoepp *et al.*, 1999). Antagonists for Group III mGluRs include the phenylglycine derivative CPPG ((RS)-a-cyclopropyl-4-phosphonophenylglycine) (Bedingfield *et al.*, 1996; Toms *et al.*, 1996). MAP4 and MSOP, α-methyl derivatives of agonists L-AP4 and L-SOP respectively, are selective competitive antagonists of Group III receptors, but both display some agonist effect at mGluR2 (Gomeza *et al.*, 1996; Thomas *et al*, 1996).

2.1.3 Physiology

Activation of Group I mGluRs is thought to cause the reduction of K^+ leak currents and voltage-dependent K^+ currents, resulting in a slow depolarisation of the membrane potential (Charpak *et al.*, 1990). Along with this depolarisation an increased action potential firing rate has been observed in hippocampal, neocortical and cerebellar neurons. This is possibly due to the inhibition of after-hyperpolarisation via a Ca^{2+} dependent K^+ current (Charpak *et al.*, 1990). Observation of slow repolarisation in hippocampal neurons supports this idea (Hu & Storm, 1991). MGluRs also activate Ca^{2+}-dependent and independent non-specific cation currents, a Na^+/Ca^{2+} ion exchanger and possibly a Ca^{2+}-activated K^+ current (Conn & Pin, 1997;Pin & Duvoisin, 1995). All three groups of metabotropic glutamate receptor are implicated in the inhibition of voltage-dependent Ca^{2+} channels (VDCCs) *in vitro*, but the receptors involved vary between different brain regions (Pin & Duvoisin, 1995).

In the SC the Group I agonist, DHPG, leads to the reduction of evoked EPSP/Cs and action potentials via mGluR1 (White *et al.*, 2003). The inhibition appears to be phospholipase C/protein kinase C independent and independent of Ca^{2+} release from internal stores, and is not caused by an increase in GABAergic transmission. Instead it is suggested that inhibition occurs directly via the activation of an autoreceptor at excitatory terminals (White *et al.*, 2003). It is also revealed that DHPG activity is not via the inhibition of N-type VDCC, however, N-type VDCC inhibition is shown to be mediated by ACPD in CA3 pyramidal neurons (Swartz & Bean, 1992). Alternatively, other groups have shown ACPD administration to result in the inhibition of voltage-dependent K^+-channels in cultured, hippocampal, pyramidal neurons (Wu and Barish, 1999).

PTX-sensitive Group II and III receptors are able to inhibit L-type Ca^{2+} channels which can also be inhibited by a Group I-linked Ca^{2+} dependent, protein kinase-independent pathway. N-type Ca^{2+} channel inhibition is also observed. Again, neuron-specific receptor types mediate this activity and reduction of N- and L-type currents does not occur in all instances, and has even been seen to be elevated in some cell types (Conn & Pin, 1997; Pin & Duvoisin, 1995).

Group I mGluRs are also involved in $GABA_A$ receptor inhibition and AMPA receptor current potentiation in the nucleus of the solitary tract. Group I receptors can also potentiate NMDA and AMPA receptor actions in the spinal cord and potentiate NMDA currents in pyramidal cells (Pin & Duvoisin, 1995).

2.1.3.a Synaptic Plasticity

High frequency stimulation of input can result in a sustained increase in excitability of the postsynaptic cell termed long-term potentiation (LTP). Conversely the cumulative activation of input can also reduce postsynaptic excitability which causes long-term depression (LTD). These are both forms of synaptic plasticity in which glutamate receptors are thought to play an important role.

Metabotropic glutamate autoreceptors are concerned with the reduction of glutamatergic neurotransmission and have some involvement in synaptic plasticity. The Group III agonist, L-AP4 reduces glutamatergic transmission, while Group II receptors reduce both inhibitory and excitatory transmission, probably via the reduction of Ca^{2+} currents (Ozawa *et al.*, 1998). It is unclear whether mGluRs are involved in CA1 LTP, and if so, how they may be involved. Mutation of mGluR1 has also given contradictory results regarding the receptor involvement in NMDA-independent mossy fibre-LTP, however LTD via mGluR2 at this synapse has been observed. MGluR2 is also implicated in olfactory LTP, possibly due to the inhibition of GABAergic transmission at granule cell dendrites (Ozawa *et al.*, 1998).

The potentiation of NMDA receptor activity and excitatory transmission via Group I receptors results in neurotoxic glutamate release and cell death. Conversely, Group II and possibly Group III receptors are involved in presynaptic inhibition of glutamate release which has a neuroprotective action (Pin & Duvoisin, 1995).

2.2 Ionotropic Glutamate Receptors

Ionotropic glutamate receptors are ligand-gated ion channels, and can be subdivided into 3 groups based upon their agonist pharmacology; N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and kainate (KA) receptors (Ozawa *et al.*, 1998;Nakanishi, 1992;Watkins & Evans, 1981). However, kainate receptors (KARs) containing KA2/GluR6 subunit assemblies produce a non-desensitising current to AMPA (Herb *et al.*, 1992;Sakimura *et al.*, 1992) and AMPA receptors (AMPARs) to kainate (Seeburg, 1993), therefore, these two groups have been historically termed non-NMDA receptors.

The AMPAR subunit GluR1 and the NMDAR subunit NR1 were identified by molecular cloning and expression in *Xenopus* oocytes (Hollmann *et al.*, 1989;Moriyoshi *et al.*, 1991). Further cloning and PCR techniques led to the elucidation of additional receptor subunits. To date, four AMPA receptor subunits (GluR1-GluR4), five kainate receptor subunits (GluR5-GluR7, KA1 and KA2) and six NMDA receptor subunits, NR1, NR2A-D and NR3A (also known as NR-like and χ-1 (Ciabarra *et al.*, 1995)) and NR3B (Chatterton *et al.*, 2002) have been demonstrated (Ozawa *et al.*, 1998). Additional variants of these subunits can be formed due to alternative splicing and RNA editing.

All ionotropic glutamate receptors possess an extracellular N-terminus domain and an intracellular C-terminal domain. Between these terminals can be found three transmembrane domains (TMI, TMIII and TM IV) and a region of the protein which either resides in the intracellular leaflet of the plasma membrane or forms a hairpin turn within the

membrane (MII) (figure 2) (Bennett & Dingledine, 1995;Hirai *et al.*, 1996;Z.Galen Wo and Robert E.Oswald, 1995).

2.3 NMDA receptors

2.3.1 Structure

Structurally, 40-50% sequence homology is displayed between NR2 subtypes. 25-29% homology is shown between NR1 and other iGluRs, but only ~18% homology between NR1 and NR2 subtypes (Hollmann & Heinemann, 1994). NR1 can form functional homomeric assemblies but with smaller conductance than native NMDARs. This indicates that native receptors are heteromeric assemblies (Moriyoshi *et al.*, 1991). NR2A-D have also been cloned but must be coexpressed with NR1 subunits to form functional receptor channels (Mori & Mishina, 1995).

NMDAR subunits coassemble into an oligomeric arrangement containing an NR1 subunit and various NR2 subunits which show differential distributions throughout the brain (Monyer *et al.*, 1992). The assemblies produce NMDARs possessing a polyamine modulatory site, Zn^{2+} binding site and modulation sites for reducing agents and protons (Gozlan & Ben Ari, 1995).

2.3.2 Distribution

Ligand binding studies have localised NMDARs throughout the brain with highest levels present in the hippocampal CA1 region (Monaghan *et al.*, 1989). Distribution of different

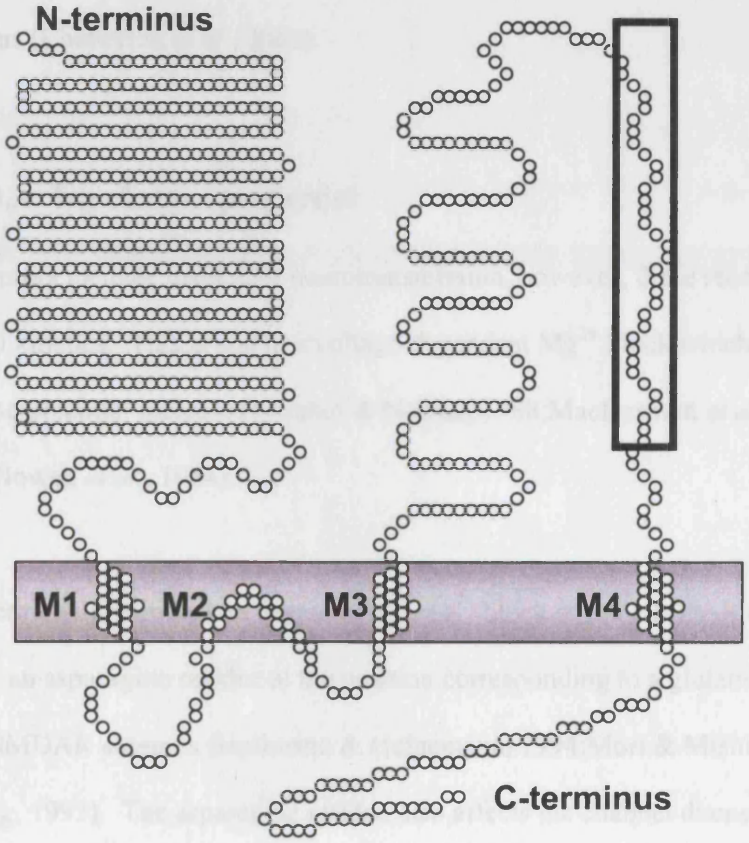


Figure 2. GluR2 structure taken from Ozawa et al., 1998. The protein consists of a large extracellular N-terminal, three transmembrane domains (TMI-III), a domain residing within the membrane, and an intracellular C-terminal. The boxed area shows the region targeted for 'flip' or 'flop' alternative splicing.

subunits is subject to developmental changes in the rodent. These include a postnatal alteration from NR2B and NR2D to NR2A and NR2C (Monyer *et al.*, 1994). Developmental differences may be explained by different functional properties of the subunits (Mg^{2+} block, deactivation kinetics). In addition, the distribution of the NR3A subtype changes with maturation (Ciabarra *et al.*, 1995) while NR3B is found mainly in motor neurons (Chatterton *et al.*, 2002).

2.3.3 Ion channel properties

NMDA receptors mediate excitatory neurotransmission, however, these receptors display slow channel kinetics. This is due to a voltage-dependent Mg^{2+} block which is released as the membrane potential increases (Ascher & Nowak, 1988;MacDermott *et al.*, 1986;Mayer *et al.*, 1984;Nowak *et al.*, 1984).

High Ca^{2+} permeability and some degree of the Mg^{2+} block of the NMDAR channel is conveyed by an asparagine residue at the position corresponding to a glutamine/arginine site in non-NMDAR subunits (Hollmann & Heinemann, 1994;Mori & Mishina, 1995;Seeburg, 1993). The asparagine residue also affects the channel diameter in NR1-NR2A and B assemblies which is the reason suggested for the lower divalent cation conductance of these assemblies (Ozawa *et al.*, 1998). NMDARs are also Na^+, K^+ and Cs^+ permeable (Ozawa *et al.*, 1998).

2.3.4 Pharmacology

The NMDAR agonist binding site appears to involve amino acid residues preceding TMI and between TMIII and IV on NR2 subunits (Laube *et al.*, 1997). The same residues of the NR1 subunit however, appear to affect glycine binding (Hirai *et al.*, 1996). Glycine binding is important as it is necessary as a coagonist for NMDAR channel activation (Ozawa *et al.*, 1998) D-serine, however, has been shown to be active at the glycine binding site in concentrations found normally in rodent frontal cortex (Matsui *et al.*, 1995). Compounds acting at this binding site along with the glutamate binding site, sites within the ion channel and the various modulatory sites can modify NMDAR activity (Sucher *et al.*, 1996).

Competitive antagonists for NMDARs include D-AP5 (D-2-amino-5-phosphonopentanoate), a more potent derivative of the early NMDAR blocker, D-α-aminoadipate binds at NMDARs with the following potencies: NR1/NR2A>B>C>D (Mori & Mishina, 1995). Another competitive antagonist for the glycine binding site, 7-CL-Kynurenate (7-CK), also shows differential binding; NR1/NR2C>B>A=D (Bleakman & Lodge, 1998). The non-competitive antagonists PCP and ketamine, however, block NMDA channels and have similar effects on all NR2 subunits (Mori & Mishina, 1995). Other intrachannel blockers include Zn^{2+}, Mg^{2+} and MK-801, a structurally related compound to PCP, but displaying greater potency and selectivity (Mori & Mishina, 1995).

NMDAR activity can be modulated by alteration in proton, zinc and Ca^{2+} concentrations. A voltage-dependent reduction of NMDA-evoked current can be achieved by the addition of

protons. IC50 values for proton inhibition occur at around physiological pH (6.9-7.3) suggesting native NMDARs are tonically inhibited and may serve as a physiological pH sensor (Kumamoto, 1997). NMDAR activity is also inhibited by low concentrations of zinc, depending on NR1 splice variants, but its inhibition is voltage dependent at higher concentrations (Yamakura & Shimoji, 1999). In addition, increases in intracellular Ca^{2+} ($[Ca^{2+}]_i$) cause the dissociation of an actin filament-associated regulatory protein leading to inactivation of the NMDAR, which may be a possible protective mechanism during ischaemia. Furthermore, calmodulin binds to NR1 when $[Ca^{2+}]_i$ is increased, leading to inactivation of NMDAR (Yamakura & Shimoji, 1999).

2.3.5 Physiology

During synaptic transmission, NMDARs generate a slowly activating current further characterised by a decay at least 100-fold slower than that of AMPARs. The receptor affinity for glutamate and response decay time are, however, influenced by the subunit arrangement (Cull-Candy *et al.*, 2001).

2.3.5.a Synaptic Plasticity

NMDARs are implicated in the cellular basis of memory and learning due to their ability to induce long term potentiation (LTP). Tetanic stimulation of hippocampal CA1 neurons causes a larger, long-lasting depolarisation which removes Mg^{2+} blockade. Ca^{2+} influx follows, which may further increase $[Ca^{2+}]_i$ by calcium-induced calcium release from internal stores and lead to the induction of intracellular signalling cascades (Mori & Mishina, 1995). Blockade of this effect by MK-801, 7-CK or AP5 indicates that NMDAR

action as well as membrane depolarisation are crucial for the occurrence of LTP (Bliss & Collingridge, 1993). Further evidence for NMDA involvement in learning and memory is that AP5 antagonism and NR1 mutation reduces spatial learning in rats (Davis *et al.*, 1992;Sakimura *et al.*, 1995).

2.3.5.b Developmental Plasticity

Involvement of NMDAR channels in developmental plasticity has been demonstrated by chronic AP5 block of kitten visual cortex (Kleinschmidt *et al.*, 1987) and studies of the visual development of dark-reared rats (Binns & Salt, 1998). The NR1 subunit is implicated in such plasticity following mutation studies of the NR-1 subunit (Li *et al.*, 1994;Mori & Mishina, 1995).

2.3.5.c Neuronal Cell Death

Application of NMDAR antagonists provide protection against glutamate neurotoxicity (Olney, 1990). *In vitro* evidence also demonstrates the prevention of NR1 subunit formation reduces NMDA-evoked neurotoxicity *in vitro* and ischaemic damage *in vivo* (Wahlestedt *et al.*, 1993).

2.4 AMPA receptors

2.4.1 Structure

The oligomeric assemblies formed from GluR1-GluR4 can consist of a homomeric or heteromeric arrangement of subunits (Ozawa *et al.*, 1998). It is the subunit configuration of

the receptor assembly which leads to pharmacological and functional differences (Ozawa *et al.*, 1998).

Alternative splicing in all AMPA subunits imparts two versions of a 38 amino acid sequence preceding the fourth transmembrane domain of the receptor, giving AMPA receptors either a 'flip' or 'flop' configuration (Sommer *et al.*, 1990). This confers different pharmacological and kinetic properties to the receptor such as sensitivity to L-glutamate.

2.4.2 Distribution

On a gross level, [^{3}H]AMPA-binding studies have revealed large amounts of high affinity AMPA receptors in the hippocampus, especially CA1, CA3 and pyramidal cell layers, dentate gyrus and superficial cerebral cortex (Monaghan *et al.*, 1984;Olsen *et al.*, 1987). Lower levels are found deeper in the cortex and caudate-putamen. Even smaller levels have been localised in the diencephalon, midbrain and brainstem (Ozawa *et al.*, 1998). All receptor subunit mRNA levels are low in the thalamus, although GluR4 mRNA is the most abundant in the reticular thalamic nucleus (RTN) (Keinanen *et al.*, 1990).

Specific antibody binding studies have revealed GluR1, 2/3, and 4 subunits on the plasma membrane of the cell body and dendrites (mainly postsynaptic locations), the outer mitochondrial and nuclear membranes and on microtubules (Petralia & Wenthold, 1992). GluR1 and 2/3 are located at postsynaptic positions in the dendrites and the dendritic spines after initial embryonic distribution throughout the neurons (Craig *et al.*, 1993). The

alternatively spliced 'flip' subunit configuration can be mainly found in embryonic neurons, while the 'flop' sequence receptor expression increases globally from postnatal day 8 to day 14 when most AMPA receptor assemblies consist of 'flop' subunits (Monyer *et al.*, 1991;Sommer *et al.*, 1990).

2.4.3 Ion channel properties

Homomeric assemblies of the GluR1, 3 and 4 subunits are inwardly rectifying and permeable to Na^+, K^+ and Ca^{2+}, whereas GluR2 assemblies rectify outwardly and show little permeability to Ca^{2+} (Ozawa *et al.*, 1998). Furthermore, heteromeric assemblies containing GluR2 subunits display properties associated with this subunit, ie. outward rectification and Ca^{2+} impermeability (Hollmann & Heinemann, 1994;Seeburg, 1993). Differences in rectification and permeability are due to a single positively charged arginine residue in GluR2 subunits which during RNA editing replaces a neutral glutamine residue present in other subunits (Seeburg, 1993). The edited version of GluR2 is prevalent postnatally, however, embryonic AMPAR subunits display a small amount of unedited GluR2 subunits (Seeburg, 1993). Thus the presence of GluR2 determines Ca^{2+} permeability of AMPA receptors.

2.4.4 Kinetics

Segments SI and SII preceding TMI and TMIV appear to form an extracellular agonist binding site (Stern-Bach *et al.*, 1994). Once bound, AMPA (Trussell *et al.*, 1988), quisqualate (Tang *et al.*, 1989), glutamate (Trussell *et al.*, 1988) and to some extent, kainate

(Kiskin *et al.*, 1986), produce rapidly desensitising currents. These desensitisation rates vary with subunit configuration (Mosbacher *et al.*, 1994); desensitisation after activation is slower in edited receptor subunit assemblies than unedited arrangements (Lomeli *et al.*, 1994). AMPA receptors also display relatively rapid deactivation rates which increase as desensitisation rate increases (Geiger *et al.*, 1995).

2.4.5 Physiology

As the kinetics suggest AMPA receptors mediate fast synaptic transmission at most central synapses. Receptor activation and therefore EPSC kinetics are regulated by glutamate concentration in the synaptic cleft and receptor subunit configuration. The subunit arrangement is crucial in determining receptor affinity, desensitisation, and maybe more importantly, deactivation (Ozawa *et al.*, 1998).

Low Ca^{2+} permeability is conferred by the GluR2 subunit, however, central synapses have been identified which have Ca^{2+} permeable AMPA receptors. This seems to depend on the number of GluR2 subunits within the assembly. The permeability may indicate an AMPA receptor-mediated, Ca^{2+}-dependent long-term modulation in these neurons (Gu *et al.*, 1996; Jia *et al.*, 1996).

2.5 Kainate Receptors

2.5.1 Structure

KARs are thought to be tetrameric structures (Lerma *et al.*, 2001) comprised of five different subunits, low kainate affinitiy subunits GluR5, 6 and 7, and high affinity subunits KA1 and KA2 (Hollmann & Heinemann, 1994). Each subunit consists of approximately 900 amino acids which are thought to share membrane topology similar to that of other ionotropic glutamate receptors (Wo & Oswald, 1995).

These subunits were first identified using cloning techniques based on sequence homology for AMPARs (GluR1-4) (Bettler *et al.*, 1990; Egebjerg *et al.*, 1991; Bettler *et al.*, 1992 ; Morita *et al.*, 1992; Sommer *et al.*, 1992). KARs share only 40% homology with AMPARs and about 20% homology with NMDARs (Hollmann & Heinemann, 1994, Lerma *et al.*, 2001). Within the KAR group itself low affinity subunits share approximately 75% homology, whilst KA1 and KA2 appear to display 68% sequence homology, although the two groups are believed to share only 45% homology with each other (Chittajallu *et al.*, 1999).

Of the KAR subunits GluR5, 6 and 7 can form functional homomeric ion channels in expression systems (Bettler *et al.*, 1990; Egebjerg *et al.*, 1991; Sommer *et al.*, 1992; Schiffer *et al.*, 1997) while KA1 and KA2 are unable to form functional channels on their own (Werner *et al.*, 1991; Herb *et al.*, 1992) but KA2 may assemble with the low affinity GluR5 or 6 subunits to form functional heteromeric channels (Herb *et al.*, 1992; Sakimura

et al., 1992). Additionally, GluR5, 6 and 7 are able to assemble to form functional heteromeric channels (Bortolotto *et al.*, 1999; Cui and Mayer, 1999; Paternain *et al.*, 2000).

GluR5 has two splice variants, GluR5-1 and GluR5-2, with GluR5-1 having a 15 amino acid addition to the extracellular N terminal (Bettler *et al.*, 1990). GluR5-2 possesses additional variants (a, b and c) with differing C terminal domains (Sommer *et al.*, 1992). Alternative C terminal splice variants have also been reported for GluR7 (GluR7a and GluR7b) (Schiffer *et al.*, 1997). In addition, a human splice variant of GluR5 (GluR5-1d) has been identified, along with a splice variant of the mouse GluR6 subunit (GluR6-2). Both produce differences in the C-terminal domain (Gregor *et al.*, 1993).

Thus it is feasible that KARs can consist of a number of receptor subunit combinations.

2.5.2 Distribution

The first proposal for a new receptor group, KARs, was suggested after studies showed DRG fibres depolarised when exposed to KA (Davies *et al.*, 1979;Agrawal and Evans, 1986). It was observed that KA blocked C-fibre action potential conduction (Agrawal and Evans, 1986), which was later validated by patch clamp experiments (Huettner, 1990; Wong and Mayer, 1993; Lee *et al.*, 2001). Molecular biology has since revealed genes encoding KAR subunits and the existence of splice variants and post-transcriptional modification (Chittajallu *et al.*, 1999). In particular the expression of GluR5 and GluR6 subunits has been demonstrated in dorsal root ganglion (DRG) and spinal neurons (Kerchner *et al.*, 2002).

Both high and low affinity kainate binding sites exist throughout the CNS, especially in the CA3 region of the hippocampus and cerebellar granule cells (Roche & Huganir, 1995; Siegel *et al.*, 1995;Ozawa *et al.*, 1998). GluR5 mRNA can be identified in the neocortex, especially the cingulate and piriform cortex, and to some extent in CA1 pyramidal neurons, in the striatum and in some septal nuclei (Wisden & Seeburg, 1993). Within the thalamus GluR5 mRNA is expressed in the anteroventral nucleus and in subdivisions of unidentifiable thalamic nuclei (Wisden & Seeburg, 1993) while GluR6 and KA1 are seen at low levels throughout the thalamus. The reticular nucleus appears to contain high levels of GluR7 mRNA (Bettler *et al.*, 1992; Monyer *et al.*, 1992; Wisden & Seeburg, 1993) with weak expression of GluR6 and KA2 (Wisden & Seeburg, 1993). All kainate subunit mRNA is expressed diffusely throughout the SC, however, a laminar expression of GluR5 mRNA can be seen in the intermediate grey layer (Wisden & Seeburg, 1993).

In situ hybridisation for GluR5, 6, 7 and KA2 has shown these subunits to be expressed throughout the CNS, for example, in the cortex, striatum, hippocampus and cerebellum. In contrast, KA1 is expressed in hippocampal CA3 and dentate granule cells (Bettler *et al.*, 1990; Egebjerg *et al.*, 1991; Werner *et al.*, 1991; Lomeli *et al.*, 1992; Wisden and Seeburg, 1993; Bahn *et al.*, 1994; Tolle *et al.*, 1993; Partenain *et al.*, 2000; Bureau *et al.*, 1999).

GluR5-7 subunits have been identified in hippocampal dendrites (Siegel *et al.*, 1995), whereas GluR6/7 and KA2 receptor subunits have been found post-synaptically in small spine synapses of the stratum oriens, CA1/CA2 and CA3 molecular layers (Petralia *et al.*, 1994). Both GluR6/7 and KA2 immunostaining was also observed post-synaptically in

mossy fibre synapses of the CA3 layer (Petralia *et al.*, 1994). Petralia *et al.*, (1994) also suggest that staining can be observed presynaptically in unmyelinated axons.

2.5.3 Ion Channel Properties

Due to the larger EPSP formation of AMPAR activity, a large proportion of kainate receptor channel research has been performed in recombinant expression systems. These have revealed that homomeric GluR5 or 6 receptors can produce rapidly desensitising kainate-evoked currents, whereas homomeric GluR7, KA1 and KA2 receptor assemblies appear functionless.

Higher kainate binding affinities of KA1 and KA2 (Herb *et al.*, 1992; Sakimura *et al.*, 1992), have led to the proposed idea that heteromeric assemblies in native tissue containing these subunits may form higher affinity receptors than those lacking them (Contractor *et al.*, 2003). This does not in fact occur; GluR5/KA2 heteromers appear to have lower affinity for kainate binding than either KA2 or GluR5 homomers (Herb *et al.*, 1992).

Rapid desensitisation to agonist application is characteristic of KARs (Lerma *et al.*, 2001) the rate of which is both subunit and cell type dependent (Lerma *et al.*, 2001;Paternain *et al.*, 1998). Recovery is also dependent on agonist (Lerma *et al.*, 2001) and subunit composition (Swanson *et al.*, 1998); the presence of the KA2 subunit in the arrangement reduces receptor affinity for the ligand (Swanson *et al.*, 1996) and therefore may confer a faster recovery.

As with the AMPAR subunit GluR2, the KAR subunits GluR5 and 6 may also undergo post-transcriptional editing (Sommer *et al.*, 1991). Once again editing results in the alteration of a glutamine (Q) residue to a positively charged arginine (R) residue in the MII pore-forming region of the subunit (Chittajallu *et al.*, 1999). Unedited forms of these subunits display strong inward rectification propeties (Herb *et al.*, 1992;Sommer *et al.*, 1992), however, edited homomeric GluR5 and GluR6 receptors (Egebjerg *et al.*, 1991) and GluR5/KA2 or GluR6/KA2 assemblies (Herb *et al.*, 1992) show a linear or slight outward rectification. In addition, editing confers a reduction of Ca^{2+}-permeability to homomeric GluR5 and 6 (Burnashev *et al.*, 1995; Egebjerg & Heinemann, 1993). Homomeric assemblies comprised of both edited and unedited forms of the GluR5 and 6 subunit also demonstrate either a linear or outward rectification along with reduced Ca^{2+} permeability (Sommer *et al.*, 1992;Egebjerg & Heinemann, 1993;Kohler *et al.*, 1993; Burnashev *et al.*, 1996). Furthermore, the presence of any unedited versions in the assembly will confer impermeability to Cl^- ions (Bernashev *et al.*, 1996). The edited ion channel properties appear to remain applicable for embryonic hippocampal cultures in addition to the recombinant expression systems in which they were characterized (Ruano *et al.*, 1995).

2.5.4 Metabotropic signalling pathways

In addition to the ion channel properties, KARs may also exert effects via G-protein signalling pathways. To date this has been shown to occur in the hippocampus (Roriguez-Moreno and Lerma, 1998; Frerking *et al.*, 2001; Cunha *et al.*, 2000; Melyan *et al.*, 2002) and cultured dorsal root ganglion neurons (Rozas *et al.*, 2003). In dorsal root ganglia the activation of GluR5-containing KARs have been implicated in the G_i/G_o-dependent release

of Ca^{2+} from intracellular stores resulting in PKC-mediated inhibition of N-type Ca^{2+}-channels (Frerking *et al.*, 2001; Rozas *et al.*, 2003). In addition, a metabotropic effect has been suggested to underlie the KAR-modulation of a postsynaptic K^{+}-current which mediates the slow afterhyperpolarisation in CA1 pyramidal neurons (Melyan *et al.*, 2002). This metabotropic activity, mediated by GluR5 and in some cases possibly GluR6 and KA2 (Melyan *et al.*, 2004; Fisahn *et al.*, 2005), may be due to an intermediate/linker protein between the ion channel subunit and a target G-protein (Coussen *et al.*, 2005).

2.5.5 Physiology

Physiological functions of KARs include the contribution to EPSC/P after glutamate release and the presynaptic modulation of synaptic transmission by the reduction of transmitter release.

2.5.5.a Effect on excitatory transmission

Among the numerous proposed roles of KARs, they have been found to act presynaptically at excitatory synapses to modulate glutamate release.

At mossy fibre-CA3 synapses EPSPs and EPSCs are reduced by the administration of KA (Kamiya and Ozawa, 2000). This effect can also be achieved by endogenous transmitter release upon associational commissural fibre stimulation presumably by the activation of presynaptic KARs (Schmitz *et al.*, 2000).

It is the GluR5 subunit that has been implicated in the modulation of presynaptic mossy fibre and associational/commissural transmission onto CA3 neurons, and also (Vignes *et al.*, 1998; Lauri *et al.*, 2001a; 2001b), although GluR6$^{-/-}$ and GluR5$^{-/-}$/Glur6$^{-/-}$ mice show an absence of KAR-mediated inhibition of input to CA3 neurons, whereas GluR5$^{-/-}$ respond as wildtype (Chittajallu *et al.*, 1996; Kamiya and Ozawa, 1998) suggesting that it is the GluR6 subunit and not the GluR5 subunit that is required to modulate input onto CA3 neurons. Explanations for these anomalies include the proposals that heteromeric KARs are expressed at MF synapses which respond to GluR5 selective drugs, homomeric GluR5 KARs are not expressed in GluR6$^{-/-}$ animals (Huettner, 2001; Lauri *et al.*, 2003), or they do not form functional assemblies (Sommer *et al.*, 1992).

It is suggested that GluR5 reduces mossy fibre transmission indirectly by increasing interneuron firing which activates presynaptic $GABA_BRs$ (Schmitz *et al.*, 2000). However, this block of tert-butyl AMPA (ATPA) effect by $GABA_B$ antagonists has not been observed by other groups and results have been interpreted as the ability of GluR5 to directly modulate mossy fibre transmission or by indirectly affecting GABA transmission or transmission of other neurotransmitters (Lauri *et al.*, 2001b).

At synapses between Schaffer collaterals and CA1 neurons, presynaptic KARs are able to reduce glutamatergic transmission (Chittajallu *et al.*, 1996) which have been linked to reduced presynatpic Ca^{2+} transients (Kamiya and Ozawa, 1998). However, KAR acitivity at this synapse appears to have a bidirectional influence on the modulation of excitatory transmission; the administration of high KA concentration (1-30μM) causes an initial increase in spontaneous EPSCs followed by a reduction in evoked EPSCs at Schaffer

collateral-CA1 synapses, whilst low KA concentration (300nM) produces a small increase in evoked EPSCs (Chittajallu *et al.*, 1996). The GluR5 subunit has again been associated with the modulation of transmission at Schaffer collateral/commissural input at CA1 pyramidal neurons (Vignes *et al.*, 1998).

As at the Schaffer collateral-CA1 synapse, the activation of KARs in lamina II of the dorsal horn results in the increase in sEPSCs (Lee *et al.*, 1999), but the reduction of evoked DRG-dorsal horn transmission (Kerchner *et al.*, 2001a). An increase in spontaneous transmission, coupled to the reduction of evoked transmission could both be a result of the activation of KARs. This activation could result in a rise in intracellular Ca^{2+} causing transmitter release which may be followed by receptor desensitisation, or reduced release by subsequent activation of interneurons and GABA release.

Although previous studies have excluded non-NMDARs from a role in LTP induction (Castillo *et al.*, 1994;Ito & Sugiyama, 1991), additional research has produced results consistent with an involvement of GluR5 activation in mossy fibre-CA3 LTP induction (Bortolotto *et al.*, 1999; Lauri *et al.*, 2001a;Ji and Staubli, 2002). It is possible that plasticity at this synapse may be either via the modulation of presynaptic excitatory input or due to a postsynaptic alteration, although knockout studies appear to suggest that the GluR5 subunit is not an absolute requirement for this at mossy fibre or associational/commissural synapses (Contractor *et al.*, 2000).

In the immature animal (P3-P5) it is the GluR5 subunit which modulates transmission at thalamocortical synapses during high frequency stimulation, suggesting a possible role in mediating frequency-dependent depression of synaptic transmission (Kidd *et al.*, 2002). In addition, this role of GluR5-mediated frequency-dependent depression was not observed in animals aged P7-8 which indicates a possible role in developing neuronal networks (Kidd *et al.*, 2002).

2.5.5.b Effect on inhibitory transmission

The reduction of miniature inhibitory postsynaptic potentials (mIPSPs) by application of kainate has been demonstrated by some (Lerma *et al.*, 2001), while other groups have not observed these results (Ben Ari & Cossart, 2000;Frerking & Nicoll, 2000), therefore their involvement in the reduction of inhibition remains unclear.

A role of KARs in the reduction of evoked inhibitory transmission has been demonstrated in hippocampal CA1 (Clarke *et al.*, 1997;Min *et al.*, 1999;Rodriguez-Moreno *et al.*, 1997) and CA2/CA3 neurons (Fisher and Alger, 1984; Kehl *et al.*, 1984). It is possible that this may indicate the involvlement of KARs in the disinhibition of network activity.

Conversely, however, the activation of KARs has also been implicated in an increase in spontaneous firing of interneurons (Cossart *et al.*, 1998;Frerking *et al.*, 1998;Semyanov & Kullmann, 2001). Furthermore, both facilitation and depression of GABAergic synaptic transmission has been observed depending on the concentration of kainate applied (Braga *et al.*, 2003;Jiang *et al.*, 2001). This bidirectional modulation of GABA release, however, does not occur with differing concentration at interneuron-interneuron synapses. Both high

(Mulle *et al.*, 2000) and low (Cossart *et al.*, 2001) kainate concentrations increase inhibitory transmission at these synapses.

It has been demonstrated that the application of kainic acid and the endogenous release of glutamate are able to increase hippocampal interneuron firing (Ben Ari & Cossart, 2000;Frerking & Nicoll, 2000;Semyanov & Kullmann, 2001). KA administration increases action potential firing in interneurons in the CA1 region (Cossart *et al.*, 1998; Frerking *et al.*, 1998), possibly via GluR5 (Cossart *et al.*, 1998; Mulle *et al.*, 2000). This effect on interneurons was suggested to be mediated by somatodendritic KARs (Frerking *et al.*, 1998; Cossart *et al.*, 1998), although a subsequent study indicated an axonal location was more probable (Semyanov and Kullmann, 2001).

In addition to studies of KARs in the hippocampus, projection neurons in the striatum possess the GluR6 subunit which appears to reduce GABAergic transmission indirectly apparently via adenosine A2A receptor activation (Chergui *et al.*, 2000). KARs may also be located both pre- and postsynaptically in the hypothalamus with the presynaptic KARs regulating inhibitory transmission (Liu *et al.*, 1999).

There are further instances illustrating the role of KARs in the depression of GABAergic transmission onto neurons of the hippocampal region (Rodriguez-Moreno *et al.*, 1997;Rodriguez-Moreno & Lerma, 1998). This was suggested to be caused by an increase of extracellular GABA concentration which can desensitise postsynaptic $GABA_A$ receptors (Benardo, 1993;Overstreet *et al.*, 2000) and activate presynaptic $GABA_B$ receptors

(Frerking *et al.*, 1999;Kerchner *et al.*, 2001). However, the reduction of evoked IPSCs (eIPSCs) in CA1 was not reversed by a $GABA_BR$ antagonist (Clarke *et al.*, 1997), and furthermore, there is evidence that the endogenous release of glutamate by excitatory input appears to reduce eIPSCs by activating presynaptic KARs on the GABAergic terminals themselves (Min *et al.*, 1999).

Functional presynaptic KARs have also been observed at GABAergic interneuron synapses with pyramidal cells in layer V of the motor cortex of the rat. Here the activation of GluR5 leads to a presynaptic reduction of GABAergic transmission (Ali *et al.*, 2001). Additional observations in dentate granule neurons have shown that KA increases sIPSC frequency and decreases eIPSC amplitude via the activation of presynaptic KARs (Behr *et al.*, 2002).

Prolonged kainate application directly increases the amplitude of afferent volleys and antidromic action potentials in mossy fibre axons, which can be reversed by co-application of the quinoxalinedione CNQX. These results were also obtained in response to synaptic glutamate release (Schmitz *et al.*, 2000). Schmitz *et al.* (2000) also demonstrated that synaptic transmission can be inhibited by the activation of presynaptic KARs at mossy fibre terminals with low concentrations of kainate (0.1-0.2 μM) or endogenous glutamate, however, KA administration directly increased afferent volley amplitude. The same depression of synaptic transmission was seen using ATPA although without affecting afferent volleys. Additional experiments suggest the effects of ATPA were via GABA release onto presynaptic $GABA_B$ receptors. These results are supported by the observation that low concentration of kainate (250 nM) increased mIPSC frequency in CA1

interneurons, although this was not mediated via a GluR5-containing receptor assembly (Cossart *et al.*, 2001)

A presynaptic mechanism of modulation has also been hypothesized for synaptic transmission in the reticular nucleus (RTN) of the thalamus. However, no evidence for presynaptic KAR activity or location has been observed at synapses from either the ventral posterior nucleus (VP) or layer VI collaterals from the barrel cortex (Bolea *et al.*, 2001). In contrast kainate autoreceptors mediate an afterdepolarisation in mossy fibre synapses, thus allowing subsequent axonal stimulation to cause a presynaptic facilitation of Ca^{2+} influx. Therefore, KA autoreceptors are believed to be involved in short-term synaptic plasticity at mossy fibre-CA3 synapses (Kamiya *et al.*, 2002).

Presynaptic KAR activation may also increase inhibitory transmission from interneurons of the dorsal horn, possibly by the enhancement of Ca^{2+} entry into the cell due to membrane depolarisation (Kerchner *et al.*, 2001b). This may lead to increased GABA release and activation of GABA autoreceptors resulting in the reduction of evoked IPSCs at these synapses (Kerchner *et al.*, 2001b).

Reports of presynaptic KARs at granule cell input onto stellate cells in the cerebellum, which enhance transmission by activation with low concentration of domoate (5nM) or low frequency stimulation (10-20Hz) but which reduce transmission when higher concentrations of agonist (10-500nM) or high frequency stimulation (100Hz) are administered (Delaney & Jahr, 2002). Interestingly, the activation of presynaptic KARs at

parallel fibre/granule cell input onto Purkinje cells produced an enhancement of transmission at stimulation frequencies of up to 100Hz and agonist concentrations up to 50nM (Delaney & Jahr, 2002). Reduction of transmission was only observed at higher concentrations of domoate (500nM) (Delaney & Jahr, 2002).

Presynaptic GluR5 may also be involved in the regulation of inhibitory transmission onto pyramidal neurons of the basolateral amygdale (BLA) (Braga *et al.*, 2003); low doses of GluR5 agonist increase mIPSC frequency, but higher concentrations reduce the frequency of these events. Furthermore, GluR5 antagonist reduces eIPSC amplitude indicating tonic activation of these receptors, which results in the increase of inhibitory transmission in this brain region.

In summary the literature reflects a versatile role of KARs in the modulation at presynaptic terminals of excitatory and inhibitory inputs into a variety of brain structures. KARs have been reported to reduce excitatory transmission in the DRG and at thalamocortical synapses. A reduction is also observed in the hippocampus which is reported to be as a result of GluR5 activation.

Alternatively reduced inhibitory transmission is described in the striatum and motor cortex, however, increased inhibitory transmission is observed in the RTN and dorsal horn interneurons. Increase inhibitory transmission is also seen in the BLA which is possibly caused by GluR5 activation.

KARs are also able to cause the reduction of both excitatory and inhibitory transmission in the same brain region ie. the hippocampus. Finally, KARs are able to both increase and reduce inhibitory transmission at the same neurons in the hippocampus, cerebellum and the basolateral amygdala via a bidirectional modulation.

2.5.5.c Postsynaptic Kainate Receptors

KARs produce slow EPSCs of small amplitude compared to that of AMPA (Swanson *et al.*, 1996) and are easily ignored during recording (Kidd & Isaac, 1999). However, KARs are able to drive interneuron spikes due to large EPSC formation (Cossart *et al.*, 1998). The slow decay found in CA3 neurons is most probably frequency facilitation caused by mossy fibres or a possible presynaptic affect of KARs (Vignes & Collingridge, 1997).

Repetitive stimulation of hippocampal mossy fibres causes the activation of a slow excitatory current in CA3 neurons (Castillo *et al.*, 1997;Vignes & Collingridge, 1997). The use of pharmacological agents shows that the activity is consistent with the postsynaptic localisation of KARs on CA3 dendrites (Castillo *et al.*, 1997). Subsequent work demonstrated that the GluR5 subunit did not mediate these postsynaptic events, but a presynaptic GluR5 receptor assembly was involved in the modulation of transmission (Lauri *et al.*, 2001a). These conclusions were drawn because the GluR5-selective antagonist, LY382884, did not affect the amplitude of the first mossy fibre EPSC but significantly reduced subsequent EPSCs evoked at 100 Hz.

Although postsynaptic KAR-mediated currents are detectable in CA1 pyramidal neurons, postsynaptic KARs do not appear to mediate excitatory transmission in this region (Chittajallu *et al.*, 1996). Instead, these postsynaptic KARs appear to modulate a slow afterhyperpolarisation (sAHP) by the inhibition of a K^+ current via a GluR5 independent, metabotropic KAR component (Melyan *et al.*, 2002).

In addition, synaptic transmission in the basolateral amygdala produced by stimulation of the external capsule is mediated in part by GluR5-containing KARs (Li & Rogawski, 1998). Here it is thought that GluR5 receptors may have a role in high frequency (50Hz) facilitation and low frequency (1Hz) EPSP enhancement (Li and Rogawski, 1998; Li *et al.*, 2001). There is also possible postsynaptic involvement in nociceptive and thermoreceptive afferent transmission in spinal cord (Li *et al.*, 1999).

Both KARs and AMPARs have been implicated in postsynaptic transmission between cone photoreceptors and subtypes of bipolar cell in the retina of the ground squirrel (DeVries, 2000). GluR5-containing KARs are found to mediate transmission between cone photoreceptors and b3 and b7 off-bipolar cells (DeVries & Schwartz, 1999; DeVries 2000). The differential activation of these non-NMDA receptor types may lead to temporal processing due to the differences in desensitisation recovery rates of the receptor assemblies.

Slower rise time and decay components of KAR-mediated postsynaptic events may indicate a role in temporal integration of excitatory input (Frerking & Ohliger-Frerking, 2002).

Anatomical studies have lead to the idea that differences in KAR-mediated action are due to subunit localisation. GluR6 expression has been localised in pyramidal cells mediating excitatory transmission, while GluR5 has been located mainly in inhibitory interneurons (Bureau *et al.*, 1999).

Thalamocortical synapses exhibit EPSCs which are mediated by AMPARs or KARs only, however, some of these connections are mediated by both AMPAR and KAR components (Kidd & Isaac, 1999). It appears a developmental progression results in the replacement of KARs with AMPARs as mediators of postsynaptic events (Kidd and Isaac, 1999).

Although KARs are not found in abundance in the spinal cord (Tölle *et al.*, 1993; Petralia *et al.*, 1994), electrophysiological studies have revealed postsynaptic KAR function in layer II of the dorsal horn, possibly at Aδ and C-fibre input (Li *et al.*, 1999). Here, trains of stimuli decreased KAR-mediated EPSCs, however, a reduction due to KAR desensitisation cannot be ruled out (Li *et al.*, 1999).

A role for GluR6 has been suggested at cerebellar Golgi neurons (interneurons) postsynaptic to parallel fibre input (Bureau *et al.*, 2000). GluR5 and GluR6 have been located on these neurons while GluR7, KA1 and KA2 are not found here (Bureau *et al.*, 2000).

These observations indicate that KARs indeed have a postsynaptic role in synaptic transmission in the hippocampus, BLA and spinal cord. Additional reports indicate that

postsynaptic KARs have a developmental role in thalamocortical synapses. GluR5 and GluR6 in particular are thought to be involved in postsynaptic events at cone synapses on bipolar neurons and at cerebellar Golgi neurons respectively.

2.5.6 AMPAR and KAR Pharmacology

2.5.6.a Agonists

KAR subunits make up functional homomeric and heteromeric ion channels which are found throughout the CNS, and can be co-expressed with AMPARs (Frerking & Nicoll, 2000). This caused difficulty in distinguishing between the receptor types for some time until the development of specific pharmacological agents i.e. the AMPAR-selective antagonists, 2,3-benzodiazepines (Wilding & Huettner, 1995).

Kainate and domoate are both KAR agonists although these compounds activate non-desensitising responses in AMPAR assemblies. In addition, tert-butyl AMPA (ATPA), although originally developed as an AMPA receptor agonist, is highly selective for GluR5 receptor assemblies (Clarke *et al.*, 1997).

SYM2081 and LY339434 demonstrate weak activity at AMPA receptors and also to some extent at NMDA and mGluRs (Brauner-Osborne *et al.*, 1997;Small *et al.*, 1998). However, SYM2081 displays 100-1000 fold selectivity for KARs over AMPARs. It is also used as a functional antagonist due to rapid desensitisation kinetics at KARs (Clarke *et al.*, 1997).

Halogenated derivatives of willardiine have recently been synthesized which display specificity for AMPA or kainate receptors. (S)-5-fluorowillardiine is AMPA selective,

while (S)-5-iodowillardine (Wong *et al.*, 1994) and 5-iodo-6-willardiine (Jane *et al.*, 1997) show greater KAR selectivity (Jane *et al.*, 1997). Homomeric GluR7, KA1 and KA2 receptors seem unresponsive to agonists, although this may be because of fast desensitisation of the receptor complex (Lerma *et al.*, 2001).

2.5.6.b Competitive Antagonists

The quinoxalinediones CNQX and DNQX are potent non-NMDA competitive antagonists. More selective is NBQX which antagonises AMPARs with a 30 times smaller concentration than is needed to affect KARs (Sheardown *et al.*, 1990).

Decahydroisoquinolines LY382884, LY294486 and LY293558 have displayed higher antagonist activity at GluR5 kainate receptors than AMPA receptors (Bleakman *et al.*, 1996) but no activity at GluR6 kainate receptors (Clarke *et al.*, 1997;Vignes *et al.*, 1997).

2.5.6.c Non-competitve Antagonists

2,3-benzodiazepines (GYKI52466, LY300168 (GYKI53655) and GYKI53784 (LY303070)) are non-competitive AMPA receptor antagonists (Ruel *et al.*, 2002). These compounds are more selective for AMPARs than KARs (Ruel *et al.*, 2002). Another non-competitive antagonist, SYM2206 is thought to act at the same site as the 2,3-benzodiazepines (Pelletier *et al.*, 1996).

2.5.6.d Modulators

Modulators can act on AMPA receptors to reduce agonist desensitisation of the receptor.

Pyrrolidones (eg: aniracetam and piracetam), benzothiadiazides (eg: cyclothiazide and diazoxide) and 1-BCP, a compound related to pyrrolidones, fall into this category (Bleakman & Lodge, 1998). PEPA (Sekiguchi *et al.*, 1997) and S189866 (a pyrolo-benzothiadiazide derivative) (Desos *et al.*, 1996) have also been reported to positively affect desensitisation while thiocyanate ions seem to enhance receptor desensitisation (Bowie & Smart, 1993).

Other modulators include Evans Blue which reduces AMPAR activation (GluR2 and 4), and Joro spider toxin (JSTX) which blocks GluR1, 3 and 4 AMPA receptors with high affinity but has no effect at edited GluR2 receptors (Fletcher & Lodge, 1996).

AMPAR and KAR responses to kainate and quisqualate are both potentiated by Zn^{2+}. Cd^{2+} causes a similar increase in kainate and quisqualate responses while La^{3+} enhances kainate responses via AMPARs and KARs by elevating channel affinity for agonists (Kumamoto, 1997). A specific KAR modulator is the lectin, Concanavalin A (Ozawa *et al.*, 1998).

More recent AMPAR modulators include CX516 (BDP-12) which increases the amplitude of the response (Arai *et al.*, 2002). In addition, CX546 appears to lower the rate of receptor deactivation thus increasing response duration (Arai *et al.*, 2002). Both of these compounds are benzamide-type agents.

3 GABA Receptors

γ-aminobutyric acid (GABA) is the most widely distributed inhibitory neurotransmitter in the vertebrate CNS (Sivilotti & Mistri, 1991). GABA is found at high concentrations throughout the mammalian CNS (μM/g) (Cooper *et al*, 1996). Currently three classes of GABAR have been identified, the ionotropic channels $GABA_A$ and $GABA_C$, and the metabotropic $GABA_B$ receptors (Bormann, 2000).

3.1 Structure and Receptor Properties

$GABA_A$ and $GABA_C$ recptors form channels permeable to Cl^-/HCO_3^- (Johnston, 1996; Enz and Cutting, 1998; Chebib and Johnston, 1999; Bormann 2000). $GABA_A$Rs are pentameric structures composed of subunits belonging to six families (α1-6, β1-4, γ1-4, δ, ε, θ) (Bonnert *et al.*, 1999; Hevers & Luddens, 1998; Mohler *et al.*, 1996; Bormann, 2000). Each receptor is believed to be comprised of at least 1α and 1β subunit (Hevers & Luddens, 1998). $GABA_C$Rs are also believed to be pentameric structures, comprised of rho (ρ) subunits, of which there are three types ρ1 - ρ3 (Cutting *et al.*, 1991; Enz & Cutting, 1998; Shingai *et al.*, 1996). In comparison to $GABA_A$Rs, $GABA_C$Rs produce currents of smaller amplitude and slower activation (Bomann 2000; Boller & Schmidt, 2003).

Cloning studies have revealed two $GABA_B$Rs subunits, R1 and R2, of which two $GABA_B$R1 splice variants have also been identified (R1a and R1b) (Bettler *et al.*, 1998; Kaupmann *et al.*, 1997). These receptor subunits appear to share structural similarities with mGluRs (Kaupmann *et al.*, 1997). Functional $GABA_B$Rs are heterodimeric, comprising

$GABA_BR1$ and $GABA_BR2$ subunits (Jones *et al.*, 1998; White *et al.*, 1998; Kaupmann *et al.*, 1998; Marshall *et al.*, 1999).

$GABA_BRs$ are coupled presynaptically to Ca^{2+} channels and postsynaptically to inwardly rectifying K^+ channels (Couve *et al.*, 2000; Mott & Lewis, 1994) to mediate slow synaptic inhibition in the CNS (Kerr & Ong, 1995).

3.2 Distribution

Autoradiography and Immunohistochemistry have shown $GABA_BRs$ to be expressed in abundance throughout the CNS, especially in the cerebellum (Kerr & Ong, 1995).

$GABA_CRs$ are expressed in the vertebrate retina (Lukasiewiscz, 1996; Matthews *et al.*, 1994; Enz *et al.*, 1995; Matsui *et al.*, 2001; McCall *et al.*, 2002; Qian & Ripps, 2001). In this location, postsynaptic $GABA_CRs$ mediate inhibitory input at the amacrine-giant bipolar cell synapse (Lukasiewiscz & Shields, 1998; Matsui *et al.*, 2001).

These receptors have also been located in other discrete areas of the brain (Boue-Grabot *et al.*, 1998; Enz and Cutting, 1999; Wegelius *et al.*, 1998). Bicuculline- and baclofen-resistant GABA effects have been observed in cerebellum (Drew & Johnston, 1992), amygdala (Delaney & Sah, 1999), dLGN (Zhu & Lo, 1999) and SC (Boller & Schmidt, 2001; Schmidt *et al.*, 2001; Edwards *et al.*, 2002). Furthermore, in situ hybridisation shows that the ρ-subunit is highly expressed in subcortical visual structures, including the SC,

pretectal nuclear complex (PNC) and dLGN (Boue-Grabot *et al.*, 1998; Born & Schmidt, 2004; Wegelius *et al.*, 1998).

3.3 Physiology

GABA is the major transmitter in a wide range of inhibitory interneurons including basket cells of the cerebellum and hippocampus, Purkinje cells of the cerebellum, granule cells of the olfactory bulb and amacrine cells of the retina (Schwartz, 2000). GABAergic neurons are involved in the modulation of network activity throughout the brain therefore an alteration in GABAergic transmission is able to modify network behaviour. Such changes in GABA release can be observed to modify synaptic plasticity in the hippocampus (Wigstrom & Gustafsson, 1983) and contribute to epileptiform activity in models of temporal lobe epilepsy (Hirsch *et al.*, 1999).

The ρ_1-subunit has been colocalised with synaptophysin (Clark *et al.*, 2001) suggesting that $GABA_C$Rs may play a role in synaptic transmission.

3.4 Pharmacology

The $GABA_AR$ has modulatory binding sites for benzodiazepines, barbiturates, neurosteroids and ethanol (Bormann, 1988; Macdonald & Olsen, 1994), although benzodiazepine-insensitive forms of the $GABA_AR$ occur with the subunit arrangement of $\alpha\beta\delta$ or $\alpha\beta\varepsilon$ termed $GABA_{A0}$ (Barnard *et al.*, 1998). Among the most commonly used selective compounds for $GABA_A$Rs is the agonist muscimol (Macdonald & Olsen, 1994), and the competitive antagonist bicuculline (Curtis *et al.*, 1970).

Like $GABA_A$Rs, $GABA_C$Rs can be activated by GABA, but at concentrations 10 times lower than those necessary to activate $GABA_A$Rs (Johnston, 1996; Bormann & Feigenspan, 1995; Feigenspan & Bormann, 1998; Polenzani *et al.*, 1991). $GABA_C$Rs do not respond to either bicuculline or baclofen (Johnston, 1996; Bormann & Feigenspan, 1995), however, the GABA analogue *cis*-4-aminocrotonic acid (CACA) is active as a selective $GABA_C$ agonist (Johnston, 1996). Antagonists to $GABA_C$Rs include the $GABA_A$/$GABA_C$R Cl^- channel blocker, picrotoxin (Feigenspan & Bormann, 1998; Cutting *et al.*, 1991) and the more selective $GABA_C$R antagonist TPMPA ((1,2,5,6-tetrahydropyridine-4-yl) methylphosphinic acid) (Ragozzino *et al.*, 1996; Chebib *et al.*, 1997).

$GABA_B$Rs can be activated by the selective agonist, baclofen (Seabrook *et al.*, 1990) which appears to have no activity at either $GABA_A$Rs or $GABA_C$Rs (Kusama *et al.*, 1993; Kerr & Ong, 1995; Woodward *et al.*, 1993). Antagonists for the $GABA_B$R include CGP35348 (Olpe *et al.*, 1990) and CGP55845A (Davies *et al.*, 1993).

4 The Rat Visual System

The visual system of the rat is typical of nocturnal rodents, and is broadly similar to that of most mammals. Light entering the eye is detected by the photoreceptors of the retina. This visual information is transmitted via the optic nerve to subcortical structures for processing before being relayed to cortical regions.

4.1 Retina

Like that of most rodents, the rat retina predominantly contains rod photoreceptors, with cones accounting for around just 0.85% of photoreceptors. The majority of cones (93%) contain a long-wavelength sensitive photopigment (maximum sensitivity 500-520 nm), the remainder (7%) possess an ultraviolet-sensitive pigment (maximum sensitivity ~370 nm) (Deegan & Jacobs, 1993).

Signals from the photoreceptors are transmitted to retinal ganglion cells (RGCs) via three types of interneuron, the bipolar, horizontal and amacrine cells (Tessier-Lavigne, 2000). These neurons combine signals from a number of neighbouring photoreceptor inputs to initiate visual processing at the level of the retina (Tessier-Lavigne, 2000).

The rat retina contains around 110,000 ganglion cells (Perry *et al.*, 1983). The distribution of these neurons varies across the retina with highest densities (approximately 3000 cells/mm^2) being found in the area centralis, and lower densities (approximately 600 cells/mm^2) in the peripheral retina (Sun *et al.*, 2002). The dendritic trees and thus receptive fields of ganglion cells in the area centralis are no different from those of peripheral cells,

suggesting that objects of interest are not brought into focus on a region of high resolution (Huxlin & Goodchild, 1997).

The ganglion cells projecting exclusively to the SC possess smaller somata than those projecting to the dLGN, although some cells project to both nuclei (Moriya & Yamadori, 1993). Approximately 6% of the ganglion cells projecting to the SC mediate GABAergic neurotransmission (Caruso *et al.*, 1989) suggesting that there is some direct inhibitory transmission from the retina.

4.2 Retinal Output

The optic nerve arises from axons of the retinal ganglion cells, which project through the optic chiasm to relay information via the optic nerve to the visual areas of the brain. Whilst almost all ganglion cells project to the SC (Linden & Perry, 1983a), axon branching occurs in fast conducting and large ganglion cells allowing these neurons to innervate both the contralateral dLGN and SC (Kondo *et al.*, 1993).

Of the ganglion cell projections only approximately 3% form an ipsilateral projection to target nuclei which provides a binocular representation of the visual field (Jeffery, 1984).

4.3 Primary Visual Nuclei

The mammalian visual system operates as a series of parallel pathways, with retinal projections to the dorsal lateral geniculate nucleus (dLGN), superior colliculus (SC),

ventral lateral geniculate nucleus (vLGN), intergeniculate leaflet (IGL), pretectum and accessory optic nuclei (Sefton *et al.*, 2004).

The dorsal lateral geniculate nucleus (dLGN) relays information from the retina to the visual cortex. The major input to the rat dLGN arises from the contralateral eye, although a region of ipsilateral input does exist (Lund *et al.*, 1974). The rat dLGN lacks lamination, although regional variations have been suggested (Reese, 1988). Different regions of the dLGN appear to be innervated by different morphological classes of ganglion cell (Martin, 1986). The retina is mapped topographically in the rat dLGN (Sefton *et al.*, 2004). Projections from the dLGN are to the ipsilateral visual cortex and visual thalamic reticular nucleus (Rt) (Sefton *et al.*, 2004). Rt is involved in focal attention mechanisms, with damage to this nucleus resulting in deficits in orienting tasks (Guillery *et al.*, 1998; Weese *et al.*, 1999). The dLGN is described in more detail in chapter 5.

Virtually all retinal ganglion cells of the rat project to the superior colliculus (SC) (Linden and Perry, 1983). The superficial grey and upper optic strata receive retinal inputs, and in turn project to regions including the intermediate and deep layers of the SC (Harting *et al.*, 1973). Complex circuits link the multimodal intermediate and deep SC layers to areas associated with the selection of appropriate motor responses to novel stimuli. These include the cerebellum, substantia nigra and basal ganglia (Niemi-Junkola & Westby, 1998, 2000). Axons arising from the contralateral eye provide the primary input to the upper layers of the SC (Toga & Collins, 1981), with ipsilateral retinal input to only the rostral SC (Martin *et al.*, 1983). Like most vertebrates, this projection is topographically organised

(Siminoff *et al.*, 1966), although in the rat the central visual field is not magnified compared with the periphery (Siminoff *et al.*, 1966; Toga & Collins, 1981).

The function of the SC is to locate biologically significant stimuli within the visual field, rather than to identify the stimulus (Sefton *et al.*, 2004). The localisation of stimuli by the SC leads to behavioural responses including the orientation of head, eyes or ears towards visual stimuli and the initiation of avoidance, defense or escape behaviours (see Sefton *et al.*, 2004 for more information). The SC is described in more detail in chapter 6.

4.4 Visual Cortex

Seven visuotopically organised areas are found in the rat occipital cortex (for review see Van Essen, 1979), the largest of these being the primary visual cortex (variously termed area Oc1, area 17, striate cortex, area V1).

The projection neurons from the primary visual cortex (pyramidal and some spinous stellate cells) use glutamate and/or aspartate as excitatory transmitters. Glutamatergic neurons are primarily found in layers 2 and 3, and aspartatergic neurons are found in layers 2-6 (Fosse & Fonnum, 1987; Johnson & Burkhalter, 1992). Nonpyramidal cells account for around 15% of the entire neuronal population of the primary visual cortex, and are thought to be GABAergic (Lin *et al.*, 1986; Ribak *et al.*, 1978).

The primary visual cortex of the rat is a granular cortex with 6 layers. The majority of dLGN fibres terminate in layer 4 and lower layer 3 (although some reach layers 1 and 6).

Layer 6 of the primary visual cortex projects to the dLGN (Sefton et la., 2004).The primary neurotransmitter of these fibres may be excitatory amino acids, such as glutamate and aspartate (Johnson and Burkhalter, 1992;Fosse *et al.*, 1984).

5 The Lateral Geniculate Nucleus

The dorsal lateral geniculate nucleus (dLGN) is part of the dorsal thalamus in the diencephalon. In the cat the LGN is a laminated structure receiving input from the retina via three functionally distinct cell types, W, X and Y, which correspond to koniocellular, parvocellular and magnocellular pathways in primate (Sherman & Guillery, 2002). This type of lamination is not seen in the rat although the nucleus appears to be segregated into an inner core of cells with a Y cell-like function, and an outer shell of cells, receiving collicular and retinal input, which may serve a W cell-like function (Reese, 1988).

Ascending input includes that from RGCs, lower brain centres such as the cerebellum and mammillary bodies or from higher order layer V cortical neurons (Sherman & Guillery, 1996).

The thalamus passes on information via relay cells to primary sensory areas of the cortex and other cortical areas such as the motor and cingulate cortex (Sherman & Guillery, 2002), and receives reciprocal projections from each area of the cortex it projects to (Crick, 1984). As well as being modulated by these reciprocal projections, relay cell activity is modulated by inhibitory input from the visual area of the reticular nucleus (part of the ventral thalamus) and neurons of the perigeniculate nucleus (Sherman & Guillery, 1996).

Thalamic neurons exhibit two different patterns of response to excitatory input, burst or tonic firing. This is influenced by T-type Ca^{2+} channels located on both soma and

dendrites, known as I_T, which are voltage- and time-dependent (Destexhe *et al.*, 1998;Zhou *et al.*, 1997).

Burst firing occurs when I_T is activated. The current causes an influx of Ca^{2+} and subsequent membrane depolarisation in the form of a low threshold calcium spike (LTS) which leads to a burst of action potentials. However, when the membrane depolarises but is still below firing threshold for approximately 100 ms or more I_T becomes inactived, which leads to tonic firing. If the cell remains hyperpolarised for 100 ms or more I_T is once again activated and burst firing resumes (Zhan *et al.*, 2000).

The two firing modes mean that relay cells can respond differently to synaptic inputs. It was originally believed that tonic firing occurred only during waking and burst firing only during slow-wave sleep or pathological conditions (Steriade *et al.*, 1993). It is now considered that bursting is part of an attentional mechanism (Crick, 1984) which in the LGN may enhance stimulus detection (Guido *et al.*, 1995;Mukherjee & Kaplan, 1995).

The receptive fields of LGN cell are similar to that of retinal ganglion cells (RGCs), ie. on-centre, off-surround or vice-versa (Hubel & Wiesel, 1961), although rat LGN cells can display an on-off response to visual stimuli (Lennie & Perry, 1981). Such receptive field responses can increase as visual stimulus size increases until the stimulus is large enough to stimulate inhibitory circuitry, thereby reducing the excitatory response (Hubel & Wiesel, 1961). Therefore, LGN output cannot be considered as merely a copy of its input. In addition, centre-surround antagonism is more pronounced in the LGN than in the retina,

which leads to the conclusion that there is more than one input to a geniculate cell (Hubel & Wiesel, 1961).

5.1 Glutamatergic Input

The majority of glutamate present in the LGN is located in the retinal and cortical terminals with less seen in GABAergic terminals, glia, dendrites and geniculate cell cytoplasm (Montero, 1994).

McCormick and Von Krosigk (1992) reported the presence of a metabotropic glutamate receptor on relay cells, the activation of which led to a long lasting EPSP. This EPSP, caused by the reduction of a K^+-leak conductance, was able to alter the firing of the relay cell from burst to tonic mode. Furthermore, patch clamp experiments demonstrate the presence of functional NMDA and non-NMDA ionotropic glutamate receptors on LGN relay neurons (Harata *et al.*, 1999).

Anatomically mGlu4 and mGlu7 have been located in the LGN by in situ hybridisation while mGlu3 can be seen in reticular neurons and possibly also in reticular glial cells (Neto *et al.*, 2000). Functionally, the activation of Group II and III mGluRs leads to a reduction in GABAergic inhibition of ventrobasal (VB) relay cells by reticular cells (Salt & Eaton, 1995) while Group III mGluRs appear to be involved in the modulation of corticothalamic transmission (Turner & Salt, 1999).

Neurons from layer VI of the cortex form a glutamatergic input onto the distal region of the dendrites of relay cells mediated by both ionotropic and metabotropic glutamate receptors (Sherman & Guillery, 2002). The existence of a number of different inputs may indicate the presence of different ionotropic and metabotropic receptors (Sherman & Guillery, 2001).

5.2 Retinal Input

Retinal Input is topographically mapped onto the LGN (Sanderson, 1971) and is located proximally on the relay cell dendritic arbor (Sherman & Guillery, 2002).

Ascending pathways providing input into the LGN include first order input from retinal ganglion cells which activate NMDA, non-NMDA receptors (Crunelli *et al.*, 1987;Scharfman *et al.*, 1990) and metabotropic glutamate receptors (McCormick & von Krosigk, 1992).

The location of mGluR1 has been demonstrated on relay cells, postsynaptic to cortical input (Turner & Salt, 2000), but little is found postsynaptically to retinal afferents (Godwin *et al.*, 1996). Furthermore, mGluR5 has been localised to F2 terminals associated with retinal input to the relay cells. Thus mGluR1 may be involved in corticogeniculate transmission from layer VI while mGluR5 may affect modulation of retinal input via the interneurons of the triadic circuitry (Sherman & Guillery, 2001).

Furthermore Sillito *et al.*, (1990a and 1990b) have demonstrated that retinogeniculate transmission is dependent on NMDA receptors but also involves non-NMDA receptors; the blockade of AMPA receptors prevents EPSP generation by optic tract input (Kielland & Heggelund, 2001), suggesting their importance in transmission of visual information.

Thus it is possible that retinal synaptic transmission is conveyed by ionotropic receptors while it is modulated by both metabotropic and ionotropic receptors (figures 3 and 4) (Sherman & Guillery, 1998).

5.3 GABAergic Input

Interneurons provide two types of inhibitory connection, axonal and dendritic. Axonal connections (F1 terminals) form synapses with dendrites or soma of the relay cells and modulate presynaptic activity (Holdefer *et al.*, 1988). Dendro-dendritic synapses (F2 terminals) are also formed between dendrites of the interneurons and those of the relay cells which have both pre- and postsynaptic effects (Montero, 1986). It is these F2 terminals that appear in the triadic synapses found in the glomeruli of the thalamus (Sherman & Guillery, 1996). The triads consist of a retinal terminal contacting an F2 terminal and a relay cell dendrite, and the same F2 terminal contacting the same relay cell dendrite (figure 3).

TRN cell input to relay cells in the cat is GABAergic, and is mostly found on dendrites outside glomeruli, but TRN cells do not provide a significant GABAergic input to

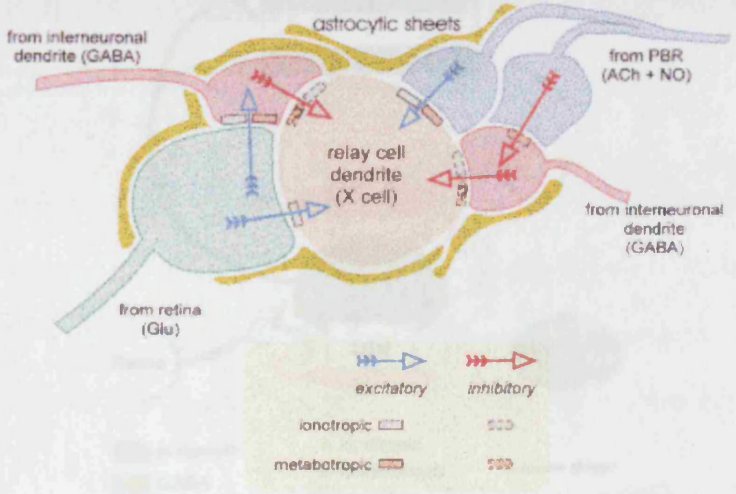


Figure 3. The triadic circuit from the cat LGN. The arrows represent the direction of synaptic transmission. Reproduced from Guillery and Sherman, 2002.

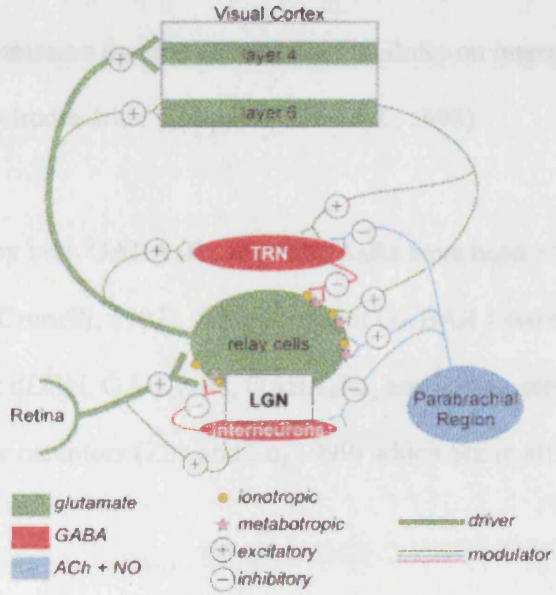


Figure 4. Synaptic circuitry in the cat LGN. Reproduced from Guillery and Sherman, 2002

thalamic interneurons (Wang *et al.*, 2001). TRN cells can also form F terminals on relay cell bodies and dendrites mainly outside thalamic glomeruli (Sherman & Guillery, 1996).

Reticular neurons receive input from collateral branches of thalamocortical and corticothalamic afferents (Bourassa & Deschenes, 1995). The arrangement of projections through the reticular cell layer is topographical as is the reticular projection to thalamus (Crick, 1984). While reticular cells activate both $GABA_A$ and $GABA_B$ receptors on relay cells, interneurons are known only to activate $GABA_A$ receptors (Cox *et al.*, 1998). It is via this GABAergic transmission that the activation of mGluRs on interneurons inhibits relay cell activation via dendrodendritic synapses (Cox *et al.*, 1998).

Responses mediated by both $GABA_A$Rs and $GABA_B$Rs have been recorded in rat dLGN neurons (Soltesz and Crunelli, 1992). Three types of GABAR have been identified on local interneurons in the rat dLGN, $GABA_A$Rs, $GABA_B$Rs, and a class referred to as $GABA_A$-bicuculline-insensitive receptors (Zhu and Lo, 1999) which are in all probability $GABA_C$Rs.

5.4 Physiological Function

Local interneurons in the LGN and thalamic reticular cells are involved in feedback inhibition (Lo & Sherman, 1994). It is thought that the temporal summation of $GABA_A$ receptor activation may be sufficient to de-inactivate I_T and produce burst firing but this has been observed only in slice preparations and anaesthetised animals. Metabotropic $GABA_B$

receptor activation would produce a larger, more sustained hyperpolarisation, thereby de-activating I_T and producing conditions to bring about burst firing.

Attention to a novel stimulus in the environment increases both GABA and glutamate in the LGN (Montero *et al.*, 2001). Visual attention activates the TRN via corticogeniculate neurons (Montero, 2000), therefore, the suggestion is that the increase in glutamate is due to corticogeniculate activity and the rise in GABA is caused by reticular neuron activation by the same corticogeniculate pathway. The possibility that these changes are caused by an increased activity of retinogeniculate afferents, however, is not entirely ruled out.

5.5 Other Input

5.5.1 Nitrergic Input

The release of nitric oxide (NO) from the parabrachial terminals has been suggested to cause a change in firing mode as described earlier (Pape & Mager, 1992). *In vivo* experiments have also demonstrated a nitric oxide mediated enhancement of NMDA receptor activation by RGCs (Cudeiro & Sillito, 1996).

5.5.2 Adrenergic Input

Noradrenergic input to relay cells also arises from the parabrachial region (PBR). The activation of metabotropic α_1 adrenoreceptors causes inositol trisphosphate (IP3) production which leads to the reduction of a K^+-leak conductance. This gives rise to a long, slow EPSP, thus promoting tonic firing (McCormick & Pape, 1990a). The activation of β

adrenoreceptors present on relay cells meanwhile alters the voltage-dependence of a hyperpolarisation-dependent cation conductance (I_h) (McCormick & Pape, 1990a). I_h is activated during hyperpolarisation to cause a slow depolarising current after the de-inactivation of I_T. In this way the production of a low threshold spike (LTS) and burst firing can occur (McCormick & Pape, 1990b).

5.5.3 Cholinergic Input

Cholinergic input from the parabrachial region (PBR) of the brain stem modulates geniculo-cortical transmission. Stimulation of this input causes a fast, ionotropic nicotinic receptor activation followed by a slower and more sustained, metabotropic muscarinic receptor activation (once again caused by the reduction of a K^+-leak conductance) (Sherman & Guillery, 2001). As with the EPSP produced by glutamatergic input, cholinergic parabrachial input is able to switch the relay cell firing mode from burst to tonic firing (McCormick, 1991)

Marks and Roffwarg (1989) state that previous experiments suggested that acetylcholine may increase relay cell excitability during waking and rapid eye movement (REM) sleep. They demonstrated that acetylcholine (Ach) produced response facilitation during waking and REM sleep, but in slow-wave sleep ACh could produce facilitation, inhibition-facilitation or no effect on visual response.

5.5.4 Serotonergic Input

The inhibition of relay cells is the result of serotonergic transmission from the dorsal raphe nucleus *in vivo* (Kayama *et al.*, 1989) probably via the indirect effect on reticular cells (Funke & Eysel, 1995). The effects *in vitro* are the same as those of β adrenoreceptor activation (McCormick & Pape, 1990a).

5.5.5 Histaminergic Input

The activation of histamine receptors via hypothalamic input is similar to that of noradrenergic receptors; a metabotropic effect possibly via H_1 receptors, and an alteration of I_h voltage-dependency mediated by H_2 receptors (McCormick & Williamson, 1991).

5.6 Modulation of Input

Modulation and transmission are via both metabotropic and ionotropic receptors. The slower (>10 ms) response of metabotropic (mGluR, $GABA_B$) receptors is more appropriate for modulation of synaptic transmission as their slower and more prolonged depolarisations may be involved in switching thalamic relay cells between burst and firing modes (Sherman, 2001). The faster activation of ionotropic (NMDA, AMPA, KA, $GABA_A$) receptors means they are more suited to synaptic transmission (Sherman, 2001).

Most synapses located in the thalamus modulate the relay functions of the LGN (Sherman, 2001). One role of this modulation is to alter information transfer depending on current attentional demands. Therefore one can expect the modulation that occurs to affect ascending and corticocortical pathways (Sherman & Guillery, 2001).

Modulatory afferents arise from the cortex and brain stem to have direct actions on the relay cells of the LGN or to act indirectly via local inhibitory interneurons or cells of the thalamic reticular nucleus (Guillery & Sherman, 2002).

It is seen that retinal ('driving') synaptic transmission is conveyed by ionotropic receptors while modulatory input is mediated via metabotropic and ionotropic receptors (see Fig 4 for a summary of receptors, efferents and afferents) (Sherman & Guillery, 1998).

It is possible that GABAergic input from interneurons and reticular cells could alleveate I_T inactivation via hyperpolarising IPSPs to produce burst firing (Kim *et al.*, 1997). It is also possible that the GABAergic modulatory effects can be mediated by retinal afferents, by feedback from relay cells themselves, and by layer 6 and brainstem afferents (Blasdel & Lund, 1983;Humphrey *et al.*, 1985).

6 The Superior Colliculus

6.1 Organisation of the SC

The superior colliculus forms the rostral part of the tectum in the midbrain. It is thought to play a role in integrating sensory information (visual, auditory, somatosensory, nociception) and converting the integrated signal into motor signals that help orient the head toward various stimuli (Stein & Meredith, 1993). Stimulation of SC neurons also initiates non-visual responses to novel stimuli such as changes in heart rate, blood pressure and respiration (Keay *et al.*, 1988). Thus the SC is also involved in producing appropriate defensive behaviour.

The superior colliculus is a laminar structure comprising seven alternating cell and fibre layers (Kanaseki & Sprague, 1974), functionally organised into two regions. The three dorsal layers are known as the superficial subdivisions (stratum zonale, stratum griseum superficiale and the stratum opticum), and the four deeper layers (stratum griseum intermediale, stratum album intermediale, stratum griseum profundum, and stratum album profundum) known as the deep subdivisions. This organisation is based on projections to and from the layers, physiological properties and behavioural response (Harting *et al.*, 1973; Edwards, 1986; Binns, 1999).

Both superficial and deep subdivisions contain topographically arranged, visually responsive neurons which together form a retino-topic representation of contralateral visual space (Chalupa & Rhoades, 1977;Drager & Hubel, 1976).

6.2 Superficial Layer Input

The superficial layers of the SC are exclusively involved in processing visual information (Casagrande *et al.*, 1972). Each colliculus receives contralateral retinal afferents, and also cortical afferents from layer V of the visual cortex which can form synapses on the same neuron in the SGS (Binns, 1999).

Dendrites can extend up to 1.2-1.4 mm (Moschovakis & Karabelas, 1985) and, therefore, are able to cross between the upper layers and even between deep and superficial subdivisions of the SC (Behan & Appell, 1992;Moschovakis *et al.*, 1988;Moschovakis & Karabelas, 1985).

6.3 Deeper Layer Input

The SC receives input from ascending and descending sensory pathways. Both pathways interact to integrate experience with attention and response (Binns, 1999).

Deeper layers receive auditory, somatosensory and nociceptive inputs and inputs from motor-related structures and multi-modal input from the anterior ectosylvian (AES) sulcus (Clemo & Stein, 1982). A large ascending somatosensory input comes from the trigeminal complex, dorsal column nuclei, lateral cervical nucleus and spinal cord (Blomqvist *et al.*, 1978;Edwards *et al.*, 1979;Huerta & Harting, 1984). Other ascending inputs include mainly contralateral auditory projections from the brachium of the inferior colliculus, external nucleus of the inferior colliculus and an area medial to the trapezoid body

(Edwards *et al.*, 1979). Descending input arises from the auditory field of the AES sulcus (Meredith & Clemo, 1989).

Somatosensory cortical afferents are arranged topographically (Clemo & Stein, 1982) from the anterior ectosylvian sulcus (SIV) (McHaffie *et al.*, 1988) and rostral lateral suprasylvian sulcus (Stein *et al.*, 1983).

Visual input to deeper layers includes that from extrastriate regions eg, lateral suprasylvian visual area (Berson, 1985), anterior ectosylvian visual area (Wallace *et al.*, 1991). There is little retinal input to the deep layers (Berson & McIlwain, 1982;Mize, 1983a;Mize, 1983b), but the little there is mainly connects with the contralateral rostral SC (Beckstead & Frankfurter, 1983).

6.4 Superficial Layer Output

Neurons of the upper superficial grey layer SGS of the SSC project to the dorsal and lateral geniculate nuclei (upper SGS), while neurons of the lower SGS project to the lateral posterior nucleus (lower SGS) and the pretectum (Huerta and Harting, 1984 for review). Neurons of the optic layer also provide both excitatory and inhibitory input to the contralateral superior colliculus (Olivier *et al*,. 2000). This commissural glutamatergic/GABAergic input may provide a balance of control for the production of orientation and avoidance behaviour (Olivier *et al.*, 1998, 2000).

The superficial SC also sends topographically mapped excitatory efferents (Wang *et al.*, 1988; Bennett-Clark *et al.*, 1989) to the ipsilateral parabigeminal nucleus (Sherk, 1979; Harting *et al.*, 1980; Taylor *et al.*, 1986). This connection in turn forms an inhibitory feedback mechanism which modulates retinal input onto collicular neurons via its cholinergic transmission onto interneurons (Binns & Salt, 2000). Neurons from the SGS project to the pretectal complex (Kubota *et al.*, 1989), which also provides ipsilateral feedback to the SC (Edwards *et al.*, 1979; Weber & Harting, 1980).

Projection neurons of the SGS also make connections with cells of the dLGN (Kawamura *et al.*, 1980; Harrell *et al.*, 1982; Sugita *et al.*, 1983; Harting *et al.*, 1991). These respond to static or slow-moving stimuli and have small receptive fields which show central summation and centre-surround suppression (Albano *et al.*, 1978). Once again these projections are topographically arranged in all species but targets are species specific due to variation in dLGN organisation (Harting *et al.*, 1991).

6.5 Input to Both Layers

Motor afferents include mainly ipsilateral input from the frontal eye fields (Kawamura & Konno, 1979;Leichnetz, 1981) and the motor cortices (Leichnetz *et al.*, 1987) involved in oculomotor behaviour. More motor input comes from the basal ganglia (Westby *et al.*, 1994) and substantia nigra pars reticulata which synapse with SC efferents. There are also oculomotor inputs from the zona incerta, thalamic reticular nucleus and posterior commissure nucleus.

Other afferents arise from the deep nuclei of the cerebellum and the perihypoglossal nucleus (May *et al.*, 1990), and the contralateral SC, locus coeruleus, raphe dorsalis, parabrachial nuclei, reticular formation and hypothalamus (Edwards *et al.*, 1979). There are additional inhibitory inputs from the substantia nigra and the zona incerta (Appell & Behan, 1990;Araki *et al.*, 1984;Ficalora & Mize, 1989).

It is this variety of inputs which allows the integration of sensory input to produce an appropriate motor response.

6.6 Deeper Layers Output

Deep layer efferent projections can ascend to the thalamus, connect with the contralateral SC, or descend to the brainstem and spinal cord, in a crossed or uncrossed arrangement (Huerta & Harting, 1984). The possible functions of these connections would be for alerting higher centres to activity in the deep layers, coordinating activity in the two colliculi (Edwards, 1977;Edwards & Henkel, 1978), and the production of behavioural responses to stimuli (ie moving eyes, head, limbs, pinnae, whiskers) (Dean *et al.*, 1986;Huerta & Harting, 1984).

6.7 Interlaminar Connection

There is little interconnection between the superficial and deep layers, however, some have found superficial layer projections to the deeper layers in hamster (Rhoades *et al.*, 1989) cat (Behan & Appell, 1992) and monkey (Moschovakis *et al.*, 1988). Also seen are deep layer

dendrites extending into superficial layers in cat (Moschovakis & Karabelas, 1985), suggesting inter-laminar interaction does occur (Mooney *et al.*, 1988).

6.8 Glutamate Receptors

Deafferentation of retinal and cortical input reduces the concentration of glutamate in the SC (Fosse *et al.*, 1984;Sakurai *et al.*, 1990;Sakurai & Okada, 1992). Supporting this evidence of glutamatergic synaptic transmission, the use of immunohistochemistry has localised glutamate in both retinal and cortical synapses in the SC (Mize & Butler, 1996; Jeon *et al.*, 1997).

6.9 Ionotropic Glutamate Receptors

The iontophoretic ejection of NMDA antagonists, AP5 or CPP, affects most neurons in the SGS, however, there appear to be two different populations of neuron present; an AP5-sensitive and an AP5-resistant population (Binns and Salt, 1994). These appear to be located in three sub-layers of the SGS in the cat SSC. In the cat, the inhibition of cortical input into the SGS confers an AP5-resistant state on previously AP5-sensitive neurons of the SGS (Binns & Salt, 1996b). This information along with the localisation of NMDA receptors at both cortico-collicular and retino-collicular synapses may suggest a co-activation of retinal and cortical afferents, possibly for the enhancement of attention to environmental stimuli (Binns, 1996b).

The selective block of cortico-collicular NMDA receptors and lesioning of discrete areas of the visual cortex demonstrates that tuning properties of SGS neurons are due to sub-cortical and not cortical influence (Binns & Salt, 1996a;Mendola & Payne, 1993).

Response habituation to visual stimuli in the SSC is seen if stimuli are presented with a small inter-stimulus interval (Oyster & Takahashi, 1975;Binns & Salt, 1995;Binns & Salt, 1997). The use of locally applied NMDA or non-NMDA antagonists reduces this phenomenon and supports the suggestion that visual stimuli activate both excitatory retino-collicular pathways and intrinsic inhibitory circuits (Oyster & Takahashi, 1975). Both NMDA and non-NMDA receptors may be involved in the inhibitory mechanisms which lead to response habituation in the SSC (Binns & Salt, 1995).

Both NMDA and non-NMDA receptors have been located in the SC (Chalmers & McCulloch, 1991;Kiyosawa *et al.*, 1996). NMDAR1 subunits have been localised by electronmicroscopy postsynaptically on cell bodies and dendrites apposed to retinal and cortical terminals in the SGS and upper optic layers. These subunits are also found in postsynaptic locations of presynaptic dendrites of GABAergic neurons (Mize & Butler, 2000).

Both functional GluR2-containing AMPA receptors and those lacking GluR2 subunits have been located in the SSC (Endo & Isa, 2001).

Previous slice experiments have shown an interlaminar connection between the superficial and deeper layers of the SC, superficial layer (SGS) stimulation produced a non-linear, NMDA-dependent, burst response in deeper layer (SGI) neurons (Isa & Saito, 2001). The response threshold of this connection can be modulated by the activation of cholinergic nicotinic receptors via input to the SGI layer. It is considered important in the initiation of saccadic eye movements (Aizawa *et al.*, 1999).

Although immunohistochemistry and functional investigation of KARs has yet to be performed, there is a significant expression of GluR5 mRNA in the SGI (Wisden & Seeburg, 1993). In addition all subunits are diffusely but moderately expressed throughout the SC (Wisden & Seeburg, 1993) and autoradiography shows low, uniform KA binding in the midbrain (Monaghan & Cotman, 1982). Enucleation experiments have also suggested that KARs may influence the development of the visual system in the superficial superior colliculus (Kiyosawa *et al.*, 1996; Chalmers an McCulloch, 1991)

6.10 Metabotropic Glutamate Receptors

Both mGluR1 and mGluR5 have been localised in the SC (Cirone *et al.*, 2002b;Shigemoto *et al.*, 1992). A greater amount of mGluR5 reactivity was observed with the possibility of some localisation to retinal afferents in the SSC (Cirone *et al.*, 2002b). Both in vivo and in vitro activation of mGluR1 in the SSC inhibits visual responses, possibly by inhibiting presynaptic glutamate release from retinal or cortical input (Cirone *et al.*, 2002a).

The Group II receptors, mGluR2 and mGluR3, are both located in the optic tract and SSC (Cirone *et al.*, 2002b). The activation of Group II receptors may be involved in contrast adaptation in the SSC (Cirone & Salt, 2001). It has been suggested that Group II mGluRs mediate presynaptic inhibition in retinotectal synapses by the inhibition of N-type calcium channels in retinal ganglion cells (Zhang & Schmidt, 1999).

A high concentration of the Group III receptor, mGluR4, is found in the SSC, possibly in retinal terminals (Cirone *et al.*, 2002b). Immunohistochemistry has also localised mGluR7a in SSC (Kinoshita *et al.*, 1998), possibly in fibre tracts (Bradley *et al.*, 1998). Activation of Group III receptors increases response habituation to visual stimuli, again probably by presynaptic inhibition of glutamate release (Cirone & Salt, 2001). Functional mGlu8 demonstrated in the SGS shown to reduce synaptic transmission on stimulation of the retinal and possibly cortical afferents (Pothecary *et al.*, 2002).

6.11 GABA Receptors

6.11.1 Distribution of GABAergic neurons in the SC

Approximately 50% of SC neurons appear to be GABAergic (Okada, 1992). Immunohistochemistry for GABA and glutamic acid decarboxylase (GAD) indicate a high level of GABA in the SC, especially in the SGS. GABAergic neurons are found throughtout the SC but do not appear to follow the laminar structure of the projection neurons (Mize *et al.*, 1991). However, concentration of GABAergic neurons present appears to be linked to total neuronal density in each layer, therefore, highest distribution is

found in the zonal layer and upper SGS, but they are also located throughout the SC (Mize *et al.*, 1991).

GABA has been localised to the dendrodendritic processes of horizontal cells, which receive around half of their input from the visual cortex, pyriform and stellate cells (Mize, 1992). Autoradiography also shows that $GABA_A$ and $GABA_B$ receptors are present throughout the SC (Bowery *et al.*, 1987) while $GABA_C$ mRNA has been localised in the SGS (Boue-Grabot *et al.*, 1998;Wegelius *et al.*, 1998).

6.11.2 GABAergic projections to the SC

There are at least three extrinsic GABAergic inputs onto neurons of the SC, from the substantia nigra (Araki *et al.*, 1994; Ficalora and Mize, 1989), zona incerta (Araki *et al.*, 1994; Ficalora and Mize, 1989) and contralateral colliculus (Appell and Behan, 1990). In the cat, other GABAergic projections have also been studied, including the cuneiform nucleus, the subcuneiform area, the peri-parabigeminal area, the inferior colliculus, lateral lemniscus nuclei, perihypoglossal nucleus and several pretectal nuclei (Appell and Behan, 1990).

The pathway from the substantia nigra (SN) inhibits neurons of the intermediate and deep grey layers of the SC (Deniau *et al.*, 1978;Chevalier *et al.*, 1981). This pathway is involved in the release of SC neuron inhibition prior to saccadic eye movements (Hikosaka and Wurtz, 1985a; 1985b). Lesioning studies in the SN further support the observation that the

inhibitory input from this nucleus is via GABA transmission; GAD is reduced in the deeper layers of the SC after lesions of the SN (Vincent *et al.*, 1978).

The zona incerta (ZI) similarly provides a GABAergic input to the intermediate and deep grey layers (Ricardo, 1981; Rieck *et al.*, 1986), connections which may play a part in the production of orienting behaviour (Kim *et al.*, 1992). There is also GABAergic commissural input to the intermediate grey layer from the contralateral intermediate grey layer (Edwards, 1977; Edwards *et al.*, 1979) which is thought to provide contralateral suppression of excitation during stimulus presentation (Munoz & Istvan, 1998).

6.11.3 Distribution

$GABA_AR$s appear to be highly expressed in superficial layers, but are also present in the deep layers of the SC (Bowery *et al.*, 1987).

$GABA_BR$ localisation also appears to be denser and more specific to the superficial layers of the SC (Bowery *et al.*, 1987; Gehlert *et al.*, 1985). Baclofen sensitive $GABA_BR$s do not respond to bicuculline application (Hill and Bowery, 1981). Binding studies for $GABA_BR$s using tritiated muscimol demonstrate intermediate binding in the superficial grey layer but lower affinity binding in the deep layers of the rat SC (Palacios *et al.*, 1981).
The $GABA_CR$ subunits, ρ_1 and ρ_2, have been shown by in situ hybridisation and reverse transcription-PCR (RT-PCR) to be present in the rat SC (Albrecht *et al.*, 1997; Wegelius *et al.*, 1998). These inhibitory receptors appear to be selectively expressed by intrinsic SGS interneurons (Schmidt *et al.*, 2001; Boller and Schmidt, 2003).

Thus all three types of GABAR are found in the SC and furthermore, appear to undergo alteration in postnatal distribution indicating a role in SC development (Clark *et al.*, 2001).

6.11.4 Physiology

It has been demonstrated that the SSC contains a high concentration of inhibitory GABAergic interneurons (Mize, 1992), which may be important in centre-surround inhibition and habituation responses of the SC (Binns & Salt, 1997a; Binns & Salt, 1997b).

$GABA_A$ and $GABA_B$ receptors have been shown to have different roles in the modulation of responses in SGS neurons. $GABA_A$ receptors modulate centre-surround responses, while $GABA_B$ receptors are involved in response habituation (Binns & Salt, 1997a). Furthermore, $GABA_B$ receptors may be located pre- or postsynaptically on either inhibitory or excitatory projections in the SSC (Endo & Isa, 2002).

$GABA_C$Rs subunits are activated by concentrations of GABA and muscimol that do not activate $GABA_A$Rs to increase excitatory transmission in cells of the SC thought to be projection neurons (Pasternack *et al.*, 1999; Schmidt *et al.*, 2001).

In the SGS of the SC, $GABA_C$R activation is suggested to exert a disinhibitory effect on projection neurons by reducing GABAergic transmission from interneurons (Boller and Schmidt, 2001; Schmidt *et al.*, 2001).

$GABA_CRs$ appear to be selectively expressed by intrinsic SGS interneurons (Schmidt *et al.*, 2001; Boller and Schmidt, 2003), but they are not activated by optic layer stimulation (Boller and Schmidt, 2003). This suggests, therefore, that input other than that from the retina and visual cortex is able to activate $GABA_CRs$ on GABAergic interneurons located postsynaptically to retinal and visual cortex input in order to disinhibit projection neurons (Boller and Schmidt, 2003; Pasternack *et al.*, 1999; Schmidt *et al.*, 2001).

In addition to postsynaptic $GABA_CR$ activity, a presynaptic location is also suggested by cell culture studies (Kirischuk *et al.*, 2003). Although $GABA_CRs$ do not appear to mediate evoked IPSCs in culture, they do contribute to paired-pulse depression. Therefore, these presynaptic receptors are able to modulate synaptic transmitter release in a frequency-dependent manner (Kirischuk *et al.*, 2003).

7 Aims Of The Investigation

Until relatively recently, pharmaclological tools have not been selective enough to distinguish between AMPARs and KARs. Due to the emergence of new, more selective agents, establishing roles for KARs in synaptic function has now become possible and attracted more interest. In the current study, GluR5 subunits in particular have been studied using pharmacological agents that are specific for these subunits. These compounds include the agonist ATPA (Clarke *et al.*, 1997) and the antagonists LY382884 (Bleakman *et al.*, 1999) and UBP296 (More *et al.*, 2004).

KARs have been located both pre-and postsynaptically and implicated in both the increase and reduction of GABAergic and glutamatergic transmission. Studies have also shown that this receptor group has a possible postynaptic involvement in neurotransmission. In addition there is evidence from both autoradiography (Monaghan & Cotman, 1982) and *in situ* hybridisation (Wisden & Seeburg, 1993) demonstrating that all KAR subunits including GluR5 are found in the SC. With the possibility of diverse roles in synaptic transmission and modulation, KARs may well play an important part in the processing of visual information in the SC and LGN.

To investigate whether GluR5 KARs are directly involved in synaptic transmission from RGCs to relay cells of the LGN and neurons of the SSC.

To elucidate the involvement of GluR5 KARs in visual processing both *in vivo* and *in vitro* methods will be used. *In vivo* electrophysiology will be used for extracellular recording of evoked neuronal activity.

Tungsten electrode recording and i.v. injection of GluR5 antagonist will be used to provide an indication of GluR5 actvity in the LGN and SSC. *In vivo* recording along with iontophoretic administration of GluR5-specific pharmacological compounds will also be employed to investigate the participation of KARs in direct synaptic transmission between RGCs and the LGN and SSC.

To parallel *in vivo* protocols, *in vitro* LGN and SC slice experiments will be undertaken. These will include studying the effect of GluR5 agonists and antagonists on single and paired evoked postsynaptic potentials. This will enable the application of drugs at concentrations specific for GluR5 subunits whilst investigating GluR5 involvement in direct synaptic transmission between RGCs and SSC neurons.

To investigate whether GluR5 KARs are involved in synaptic transmission or modulation in response to different types of visual stimuli *in vivo*.

A variety of visual stimuli can be used to observe phenomena such as response habituation and centre-surround inhibition which occur in both the SSC and LGN. Response habituation can be produced successfully with relatively high stimulus frequency, while increasing the size of visual stimuli can produce centre-surround inhibition. The use of specific compounds may reveal a modulatory role for GluR5-containing receptors in the glutamatergic or GABAergic transmission involved in these responses.

In addition, the evidence for GluR5 involvement in high frequency synaptic activity may be investigated using a contrast sensitivity protocol. As the strength of the stimulus increases, the frequency of RGC-SSC and RGC-LGN transmission will increase. The iontophoresis of GluR5-specific drugs during these stimuli may demonstrate an involvement of these receptors in high frequency synaptic transmission.

To investigate whether GluR5 KARs are involved in (a) postsynaptic visual transmission or (b) presynaptic modulation of visual transmission via an excitatory or inhibitory pathway

Using a paired pulse protocol during the application of GluR5-specific pharmacological agents will give an indication as to the pre- or postsynaptic location of these receptors. Additionally, the use of GluR5-specific compounds during perfusion of glutamate receptor or GABAR-specific antagonists will reveal the involvement of GluR5-containing receptors in either excitatory or inhibitory pathways in the SSC and LGN. Furthermore it may be possible to correlate any KAR activity with either RGC or cortical input into the LGN. This can be done by separately stimulating the retinal or cortical pathways into the LGN in slice preparations.

8 Methods

Pharmacological compounds used in this research are summarised in the appendix.

8.1 *in vivo* investigation

8.1.1 Experimental Preparation

Hooded Lister rats of either sex, weighing 250g - 375g were used for all experiments.

The animal was lightly anaesthetised using halothane until the righting reflex was lost. Longer term anaesthetic was induced using 1 g/kg urethane which was topped up if necessary until the pedal withdrawal reflex disappeared. Subcutaneous injection of lignocaine (2% w/v) with adrenaline (0.011 mg/ml) was used at all wound margins.

To maintain the airway throughout the experiment a tracheostomy tube was inserted. This was achieved by cutting the skin to access muscle around the trachea. The trachea was freed from the surrounding muscle and connective tissue by blunt dissection. Using a scalpel an incision was then made in the trachea between two rings of cartilage through which a glass cannula was inserted. The cannula was secured and the trachea tied off with nylon sutures. The skin was closed over the tracheostomy with three or four sutures, leaving the end of the cannula exposed.

The animal was then transferred to a stereotaxic frame and secured in place with a nose bar and blunt ear bars the tips of which were coated with lignocaine gel.

Throughout the experiment the animal's temperature was maintained at 37°C by means of a temperature probe placed next to the abdomen linked to a heating pad below the animal. In addition the heart rate was recorded throughout via gold electrodes attached to the hind paw and front paw with adhesive tape. A small amount of electrocardiogram (ECG) gel was applied between the electrodes and the skin to aid recording. At this point sterile eye drops were applied to prevent corneal drying.

To prepare for a craniotomy, an incision was made in the skin on the dorsal surface of the skull and held apart with clamps while muscle and connective tissue was gently scraped from the bone. Using stereotaxic coordinates (Paxinos & Watson, 1998) the skull was marked and the bone carefully drilled out to expose the brain. In addition holes were drilled in the bone to which a head holder and electroencephalogram (EEG) leads were attached with screws. The head holder and EEG leads were further secured to the bone using dental cement which also acted as an insulator for the EEG leads.

The *dura mater* was removed from the surface of the brain using a new small gauge syringe needle. Following this the nose bar and one side of the stereotaxic frame and ear bar were also removed and the animal was electrically grounded via a silver wire secured through the skin in the back of the neck.

Warm agar was applied to the surface of the brain once the electrode had been lowered in order to minimise dehydration and recording instability due to brain movement.

Once neurons were located, single unit extracellular recordings were made in response to visual stimuli produced by a Picasso visual stimulus generator controlled by VS software placed in the centre of the cell's receptive field. Stimuli were displayed on a CRT (cathode ray tube; Tektronix) display positioned 10 cm from the contralateral eye.

Additional 0.1 ml injections of urethane were administered when necessary (i.e. when the pedal withdrawal reflex returned or when an increase in heart rate was observed. Animals were sacrificed at the end of the experiment with an overdose of urethane.

8.1.2 Systemic Injection

In order to administer drugs systemically a second incision was made in the skin overlying the femoral vein. Surrounding muscle and connective tissue were removed by blunt dissection and the vein tied off and held taught with a clamp attached to the suture. The outer edge of the vein was held with fine forceps while an incision was made using scissors through which a Krebs-filled cannula was then inserted and tied in place with a suture. The skin was closed with three or four sutures. To investigate the effect of GluR5 antagonist on LGN neurons, 50mg/kg LY382884 was injected via the cannula after control values were recorded. Identical volumes of the vehicle were also injected either before or after drug injections to test for non-specific effects on visual response. The protocol was repeated whilst recording visual responses from RGC axons in the optic chiasm.

Optic chiasm recordings were made in addition to those in the LGN to explore the possibility that the systemically injected GluR5 antagonist could be exerting an effect at

KARs in the retina itself. GluR5 receptors have previously been found postsynaptically on bipolar cells in the retina of the ground squirrel (DeVries and Schwartz, 1999), therefore it seemed logical to examine the possibility of GluR5 involvement in retinal processing in the rat retina using this technique.

8.1.3 LGN and Optic Chiasm Recording

Stereotaxic coordinates were used to position a tungsten electrode over the location of either the LGN or the optic chiasm and lowered using a microstepper control until visually responsive neurons were located.

8.1.4 Iontophoresis

To record from SC neurons a craniotomy made slightly rostral to the position of the SC. This was to ensure the SC was located successfully using an angled penetration which would avoid the overlying visual cortex. To position the tungsten electrode correctly it was lined up at the level of the SC on a spare frame before transferral to the stereotaxic frame and placed according to previously calculated coordinates ready for penetration into the brain.

Silver wire was inserted into the solutions in each barrel and the ends of the barrels were sealed with a small amount of petroleum jelly. The electrode was then slowly advanced through the brain to the SC using a microdrive stepper, at an angle -15° to the vertical in order to avoid the overlying visual cortex (figure 5).

Electrode resistance was measured by passing a 50nA current through the each of the barrels. Low resistances indicated that there is the possibility of drug leakage from the barrels. High barrel resistance would be detrimental to the ejection of drug solutions.

Neuronal responses were recorded via a central barrel containing 4M NaCl, while current balancing was achieved via one of the outer barrels containing 1M NaCl (see figure 6 for experimental setup). Drug solutions were ejected by passing current through the silver wire inserted into the barrels. Drug ejection and current balancing was controlled by a Neurophore iontophoretic pump unit.

8.1.5 Response Detection and Recording

Evoked action potentials were viewed on an oscilloscope with individual cell responses isolated using a waveform discriminator. These gated neuronal responses were amplified x1000 using an Axoprobe amplifier (Axoprobe 1A, Axon Instruments) and connected to a computer via a CED1401 interface to be recorded by VS software (Cambridge Electronic Design, Cambridge, UK). The data were collected as peri-stimulus time histograms (PSTHs) and analysed using Spike 2 software (Cambridge Electronic Design Limited).

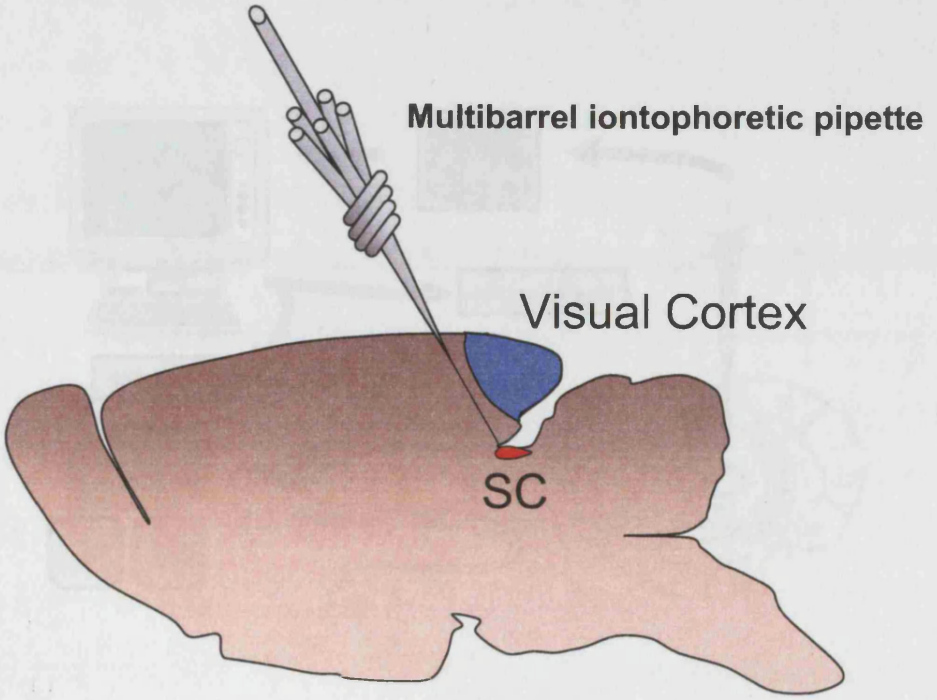


Figure 5. Diagram showing the angled penetration for recording from the SC without damaging the overlying visual cortex.

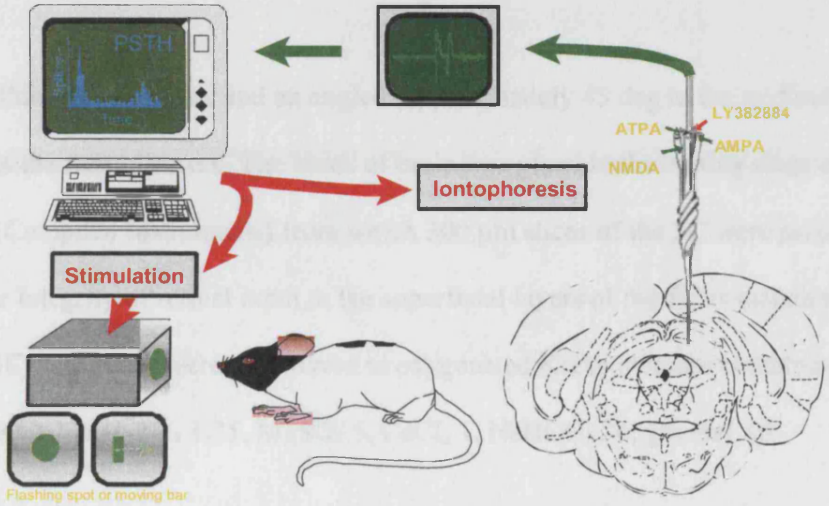


Figure 6. Experimental arrangement for recording from visually stimulated SC neurons

8.2 *In vitro* slice recording

Adult Hooded Lister rats were anaesthetised with halothane and decapitated in accordance with the UK Animals (Scientific Procedures) Act 1986. The brains were rapidly removed and placed in oxygenated (95% O_2/5% CO_2) ice-cold Krebs containing (mM): sucrose 202, KCl 2, KH_2PO_4 1.25, $MgSO_4$ 10, $CaCl_2$ 0.5, $NaHCO_3$ 26, glucose 10. The cerebellum was removed and an angled cut of approximately 45° to the midline was made

The cerebellum was removed and an angled (approximately 45 deg to the midline) cut made across the frontal cortex. The block of brain was glued to the cutting stage of a vibratome (Campden Instruments) from which 300 μm slices of the SC were prepared. In this way the integrity of retinal input to the superficial layers of the SC is maintained as it enters the SC. The slices were transferred to oxygenated Krebs medium containing (mM): NaCl 124, KCl 2, KH_2PO_4 1.25, $MgSO_4$ 5, $CaCl_2$ 1, $NaHCO_3$ 26, glucose 10.

After one hour, slices were transferred to a submerged recording chamber and held in place using a steel washer from which a quarter had been removed and replaced with a strip of nappy liner which had been glued into place using cyanoacrylate adhesive. The liner was used to stabilize the medial part of the SSC during continuous perfusion with oxygenated (95% O_2/5% CO_2) Krebs solution containing (mM): NaCl 124, KCl 2, KH_2PO_4 1.25, $MgSO_4$ 1, $CaCl_2$ 2, $NaHCO_3$ 26, glucose 10. Drugs were applied to the bath in the Krebs perfusate.

For recording IPSCs a monopolar or bipolar tungsten-in-glass stimulating electrode was positioned in the upper half of the SGS. To record EPSCs the stimulating electrode was placed in the optic tract.

Patch recording electrodes were pulled on a Sutter microelectrode puller (Sutter Instrument Co., USA) using filamented capillary tubes (Harvard Apparatus, UK) and filled with an internal solution of (in mM): Potassium gluconate 130mM, NaCl 5mM, EGTA 0.5mM, HEPES 10mM, GTP 0.4mM, ATP 4mM, QX314 10mM, biocytin 0.1%. Electrode resistances varied between 6.5 and 17.5 MΩ.

Responses were recorded with an Axoclamp-2B amplifier (Axon Instruments), digitised (10 kHz) via a CED1401 interface and stored on a computer with Spike2 software (Cambridge Electronic Design). Recordings were filtered at 3 kHz during acquisition.

To investigate GluR5 involvement in the modulation of inhibitory input into SGS neurons, the effect of the GluR5 agonist, ATPA, was observed on IPSCs. In order to isolate KAR affects, all other glutamate receptors were blocked using a cocktail of D-AP5, GYKI52466, CPPG and LY393053, which antagonize NMDA, AMPA, mGlu4, 6, 7 and 8 (Group III), and mGlu1, 2, 3 and 5 (Group I and II) respectively.

An enhancement of the IPSC was achieved by voltage-clamping the membrane potential at –50mV in order to study GluR5 involvement more clearly.

Glutamate antagonists were applied for 20-30 minutes prior to a 10 minute perfusion of ATPA. ATPA was then washed off for a period of 25-30 minutes. During this time, paired IPSCs were evoked with stimulation of between 40-100 volts of duration 0.04ms at 10 second intervals.

To study GluR5-mediated effects on EPSCs, retinal input into the SSC was stimulated with a tungsten-in-glass electrode placed in the optic tract outside the SC. To investigate the affect of GluR5 activation and block on the excitatory transmission alone, GABAergic transmission was blocked with 10 μM bicuculline and 3μM CGP55845A ($GABA_A$ and $GABA_B$ antagonists respectively) before applying ATPA at varying concentrations. The GluR5 antagonist, UBP296 was applied in some experiments. To clearly isolate GluR5-mediated actions some experiments were also carried out in the presence of the AMPAR antagonist GYKI52466.

Although $GABA_CR$ subunit expression has been demonstrated in the superior colliculus, they do not appear to be activated by intrinsic GABAergic interneurons (Boller & Schmidt, 2003); evoked IPSCs from optic layer stimulation can be blocked completely by the $GABA_AR$ antagonist bicuculline, indicating that the intrinsic inhibitory input to SGS projection neurons is via $GABA_ARs$ alone. Moreover, afferents activating $GABA_CRs$ in the SC do not appear to be located with the retinal input into the SC and it is not possible to activate them by electrical stimulation of the optic layer (Boller & Schmidt, 2003).

For these reasons it was considered unnecessary to block $GABA_CRs$ as these receptors were unlikely to be activated during the stimulus used in the study of possible GluR5 effects on excitatory transmission in the SGS.

Analysis was carried out using Spike 2 software to produce waveform averages of 6 responses from which EPSC/IPSC amplitude and changes in holding current were measured.

8.3 Histology and Confocal Microscopy

Following whole-cell recording the slices were placed in phostphate buffered saline (PBS) containing 4% paraformaldehyde and stored at 4°C for 24 hours. Slices were then transferred to a solution of 30% sucrose in PBS for 24-48 hours for cryoprotection. Prior to visualisation slices were washed in 30% sucrose + 12% glycerol, twice for 30 minutes, for further cryopreservation.

In order to permeabilise the slices to allow the fluorophore access to the biocytin within the cells, they were subjected to a freeze-thaw protocol. Each slice was placed individually in a foil package and held just above the surface of liquid nitrogen for 30 seconds. They were then brought back to room temperature until the resulting condensation evaporated. This procedure was repeated three times.

The cryoprotectant was removed by washing slices three times for ten minutes with PBS before incubating with a streptavidin, rhodamine red- X conjugated complex (1:200) (Invitrogen, Paisley, UK) for two hours at room temperature.

Finally the slices were washed three times for ten minutes in PBS before transfer to a gelatin-coated microscope slide, and coverslipping with Vectorshield fluorescent mounting medium (Vector Laboratories, Peterborough, UK).

Cells were imaged with a Zeiss laser scanning confocal microscope (LSM510; Carl Zeiss Meditec, Oberkochen, Germany) using a 16x or 40x oil immersion objective at 543nm. Images were analysed using the LSM 5 image browser.

8.4 Electrode Manufacture

8.4.1 Tungsten Electrodes

Tungsten-in-glass electrodes were made by etching pre-straightened tungsten wire in Levick's solution (Levick, 1972), a $NaNo_2$/KOH solution. The wire was then insulated in glass. Excess glass was removed from the tip to expose a small amount of tungsten wire, which was re-etched to create a tip of 20–30 μm.

8.4.2 Multi-Barrel Iontophoresis Electrodes

To create the electrodes for iontophoresis, four thin-walled, filamented, borosilicate glass capillary tubes were taken, three of which were cut in half using a diamond cutter. The six

shorter lengths were then arranged around the longer central tube and the assembly was held together at either end using two pin vices. The central area between the vices was held in a Bunsen burner flame while the tubes were fused together by gently twisting. Once fused, the vices were pulled apart and the resulting blanks were completed by heating the unfused ends and angling them away from the central barrel. This procedure was performed to facilitate barrel filling without drug cross-contamination.

Electrode tips were then pulled to the desired shape and width. This was achieved by fusing a weighted glass tube to the tip. The tip was then elongated and refined by rapidly heating and dropping the unwanted glass from the tip.

Prior to use, the electrode tip was broken back to 8-9 μm under the microscope and filled with recording, current balancing and drug solutions. The electrode was filled before the experiment preparation to ensure the absence of air bubbles and that the tips of the barrels were filled. The solutions used were 4 M NaCl for recording, 1 M NaCl for current balancing, 10 or 50 mM AMPA, 40 mM ATPA and 50 or 100 mM NMDA. In some experiments 40mM D-AP5 was used to validate the technique of iontophoresis. All drug solutions were retained with small positive currents and ejected as anions.

8.5 LGN Stimulus Protocols

8.5.1 Centre-surround

Spot sizes of 5°, 10° and 20° of visual space and luminance of 35 cd m^{-2} were used along with a blank stimulus. Four presentations of each stimulus were presented for a two second

duration in a randomised sequence. These trials were repeated three times in a cycle and the cycle was repeated at 10 minute intervals.

8.5.2 Contrast Sensitivity

A blank screen or a 20° spot of luminances 16, 24 or 32 cd m^{-2} was displayed five times for a duration of two seconds in a randomised sequence. Each trial was repeated three times in a cycle. The cycle was repeated at 10 minute intervals.

One or two control cycles were recorded before the systemic administration of the GluR5 antagonist, LY382884 (50 mg/kg), via the femoral cannula over a period of approximately two minutes.

Data were then collected until a change in response occurred and recovery achieved, or until a change in response had occurred and for 40-60 minutes following this change (4-6 cycles).

8.6 Optic Chiasm Stimulus Protocol

To test for changes in optic tract fibre activation a blank screen or a 20° spot of luminance 32 cd m^{-2} was displayed five times for a duration of two seconds in a randomised sequence. Each trial was repeated three times in a cycle. The cycle was repeated at 5 minute intervals.

Several control cycles were recorded before vehicle or the GluR5 antagonist, LY382884 (50 mg/kg), was systemically administered via the femoral cannula over a period of approximately two minutes. Data were then collected for 30-40 minutes following injection.

8.7 SSC Stimulus Protocols

8.7.1 Iontophoresis visual stimulus and drug cycle protocol

5 repetitions of a 2 second visual stimulus, either a 35 cd m^{-2} spot of 20° visual space or horizontally moving bar (width 4°, length 20° and velocity 40° sec^{-2}), was dispalyed at 10 second intervals. This was followed by 10-13 second ejections of ATPA, AMPA and NMDA at approximately 50 second intervals. Drug ejections were of -35 nA to -120 nA. The cycle was repeated at 5 minute intervals.

Once stable control recordings were achieved, antagonist was continuously ejected using currents of -5 nA or by turning off the retaining current for two cycles. Antagonist ejection was then ceased and recovery data were collected for 3 cycles.

8.7.2 Analysis

Habituation of visual response was calculated with the following calculation:-

$$\left\{1 - \left[\frac{\text{number of spikes evoked during 4}^{th}\text{ presentation}}{\text{number of spikes evoked during 1}^{st}\text{ presentation}}\right]\right\} \times 100$$

Thus the response to the 4th stimulus was expressed as a percentage of the response to the 1st stimulus.

The change in habituation during antagonist ejection was calculated by expressing post-ejection habituation as a percentage of pre-ejection control values.

8.7.3 Statistical Analysis

The most suitable means of analysis would be the Wilcoxon signed rank matched pairs test. The Wilcoxon test has been chosen for three reasons. Firstly the data consist of matched pairs (ie before and after antagonist is administered). Secondly, the data cannot be assumed to follow a normal distribution. Finally the test can be applied to a small sample size. Thus the appropriate non-parametric test is the Wilcoxon signed rank test.

The power of a test is the probability of avoiding a Type II error; if the test is not powerful there is a high chance of Type II error. By their nature non-parametric tests are not as powerful as their parametric counterparts, thus necessitating larger sample sizes. Preliminary testing therefore, will simply return a Type II error; the Null Hypothesis may be accepted when, in fact, it is invalid. Although the Wilcoxon signed rank test can be used to test significance of small groups, six is the minimum number to which a two-tailed test

can be applied. Therefore, for data sets containing less than six the Student t-test was applied.

8.8 Experimental Considerations

8.8.1 *In Vivo*

Although iontophoresis is useful to administer pharmacological agents locally to the cell being recorded from, it was not possible to determine the concentration of the drug in the extracellular environment in the current experimental arrangement. For some agents where concentration determines selective receptor action it is necessary to employ agonists to receptors not directly under scrutiny to evaluate specific receptor interaction. To clarify receptor selectivity future slice experiments confirming receptor involvement in synaptic transmission should be performed. This type of experiment enables the application of drugs at known concentrations, thus the concentration-dependent action of agents can clarify the receptor type involved in responses.

Despite this disadvantage, *in vivo* recording does permit the observation of receptor involvement to physiologically pertinent stimuli in the intact animal.

8.8.2 *In Vitro*

Although slice preparations cannot be considered an intact system, they do have certain advantages over *in vivo* preparations. Primarily, the necessity for anaesthesia for *in vivo* experiments is likely to affect neuronal responses as anaesthetics are centrally acting drugs.

Brain slice recordings in the LGN also allow the possibility of separate retinal and cortical afferent stimulation (Turner & Salt, 1998). This may allow more detailed determination of KAR involvement in visual processing in this structure.

8.8.3 Systemic Drug Administration

Iontophoresis is a valuable method for the observation of neuronal response to locally administered drugs, however as mentioned previously, the concentration of drug ejected cannot be determined. The initial experiments involving the systemic injection of GluR5 antagonist were designed to see if KARs would have any involvement in visual processing in the LGN. The injection of the specified concentration of LY382884 ensured the concentration of drug in the neuronal environment that would selectively affect GluR5 receptors. The disadvantage of this method is that the drug will affect all GluR5 receptors in the brain. Therefore, GluR5 receptors throughout the visual system, from the retina to the cortex, are affected by the injected drug and the specific affect of receptor interaction in the brain structure of interest is not possible to ascertain.

9 Results

9.1 Systemic Injection and LGN Recording

Previous work has observed the involvement of post-synaptic KARs in synaptic transmission (Cossart *et al.*, 1998;Li & Rogawski, 1998). Additional observations suggest that as firing frequency increases, there is activation of post-synaptic KARs to produce a small, slow EPSC (Castillo *et al.*, 1997). Therefore a protocol using visual stimuli of increasing luminance was used to observe neuronal response to an increasing firing frequency of RGCs before and during GluR5 antagonist administration.

Averaged responses to visual stimulus of luminances 16, 24 and 32 cd m^{-2} were produced from peristimulus time histograms (PSTHs). The area of the resulting histograms were calculated and compared before and at maximal effect of systemic injection of GluR5 antagonist.

As mentioned previously, KARs are thought to modulate of GABAergic transmission (Clarke *et al.*, 1997;Min *et al.*, 1999;Rodriguez-Moreno *et al.*, 1997;Rodriguez-Moreno *et al.*, 2000). The centre-surround response observed in the LGN is due to the recruitment of GABAergic circuitry as stimulus size increases (Hubel & Wiesel, 1961;Sillito & Kemp, 1983). A visual stimulus of increasing spot diameter was, therefore, used to attempt to produce the response and observe the effect of systemic GluR5 antagonist injection. Although centre-surround was not observed in the neurons from which the data were produced, the affect of systemic injection on increasing stimulus size was calculated. Wrapping responses to visual stimuli of spot diameters 5, 10 and 20° of visual space, and

calculating histogram area of the responses determined this effect. The PSTH areas were then compared before systemic injection and during the maximal effect of the GluR5 antagonist LY382884.

Preliminary data from 3 cells showed that the time points where maximal drug effect was seen were 20 minutes after injection for spot diameter, and 30 minutes for luminance recordings (figure 7A and B). At the dose of antagonist injected (50 mg/ kg), the brain levels of the drug can only reach concentrations affecting KARs. Therefore, the maximal effect will only be due to LY382884 acting at KARs. The times at which maximal affect was observed were consistent in all experiments and were used to determine changes in visual response in subsequent experiments in the LGN. The time points are at different times for analysis of diameter and luminance because these stimuli were shown alternately in the same experiment.

The preliminary data also demonstrated that the systemic injection of GluR5 antagonist, LY382884, into the femoral vein reduced the visual responses to all stimuli (figure 8A and 8B) with neurons usually recovering to control values within 70-80 minutes (figure 7). The reduction of neuronal responses was similar for all stimuli presentations. Values are summarised in Table 1.

All 5 neurons showed a marked decrease in visual response to 48.7±8.24% of control values and to ensure that this reduction was due to specific drug affects, vehicle injections where carried out in the same way. Systemic administration of vehicle alone did not reduce

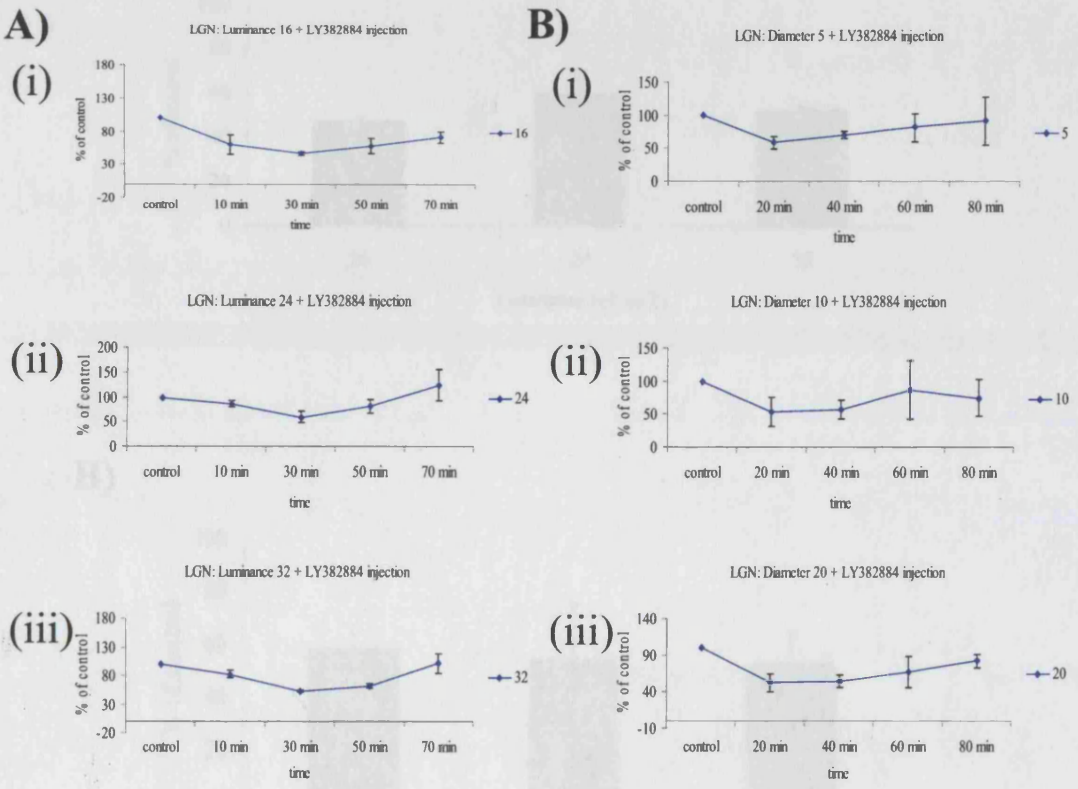
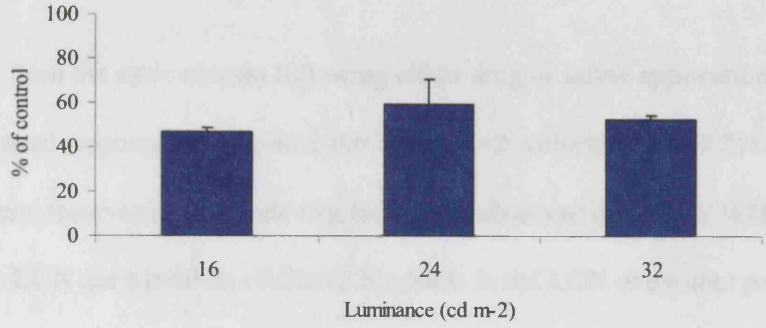


Figure 7. The effect of GluR5 antagonist injected systemically. **A)** The graphs show responses to luminance levels of **(i)** 16 cd m-2 **(ii)** 24 cd m-2 **(iii)** 32 cd m-2. Responses are expressed as the percentage of control responses prior to drug injection. n = 3. Stimulus cycles were repeated at 10 minute intervals with a maximal antagonist effect at 30 minutes post-injection. **B)** The graphs show responses to spot diameters of **(i)** 5° **(ii)** 10° **(iii)** 20 ° of visual space. n = 3. Stimulus cycles were repeated at 10 minute intervals with a maximal antagonist effect at 20 minutes post-injection. Values are given in Table 1.

A)



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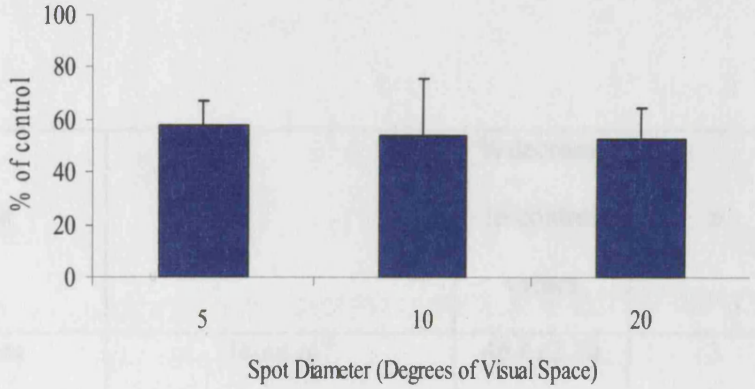


Figure 8. **A)** The histograms represent visual responses to luminance levels 16, 24 and 32 cd m-2 after antagonist injection, expressed as the percentage of control values (n=3). **B)** visual responses to spot diameters 5, 10 and 20° after antagonist injection, expressed as the percentage of control values (n=3). The graphs show data at the point of maximal antagonist effect. (At 20 minutes for spot diameters and 30 minutes for luminance levels) Values are given in Table 1.

visual responses to a stimulus of 20° of visual space (92.4±9.61%, n=2) (figure 9) thus demonstrating that the effects on visual response are produced by the antagonist and not by indirect influences on the cardiovascular system.

Measurements from the optic chiasm following either drug or saline application resulted in no change in visual responses (antagonist 100.4±8%, n=2; vehicle 103.5±9.71%, n=3) (figure 9). These observations indicate that the effects observed during LY382884 injection in the LGN are a product of GluR5 blockade in the LGN or another part of the brain influencing the processing of retinal input in the LGN rather drug influence on retinal processing.

Table 1

Stimulus		% decrease in control values	n
Luminance	16 cd m^{-2}	46.6±2.30	3
	24 cd m^{-2}	59.6±10.97	3
	32 cd m^{-2}	52.2±2.04%	3
Spot Diameter	5° of visual space	58.2±8.94	3
	10° of visual space	54.0±21.78	3
	20° of visual space	52.3±12.31	3

Data are expressed as mean±sem.

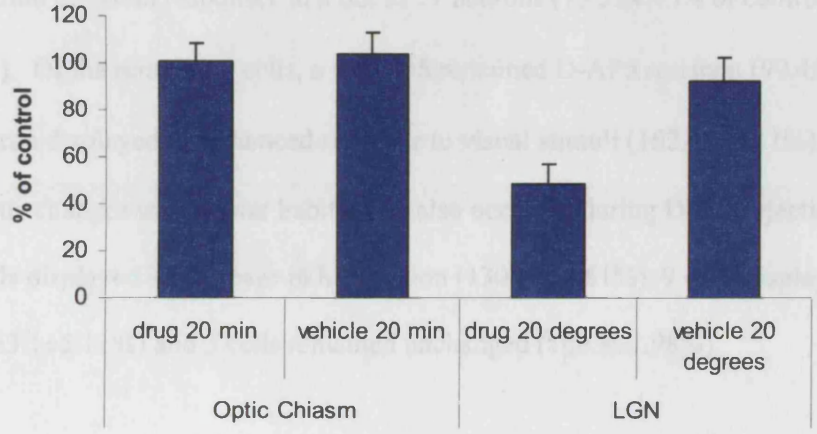


Figure 9. Histogram showing changes in visual responses recorded in the LGN during systemic LY382884 injection (48.7±8.24% of control values, n=5) and vehicle injection (92.4±9.61% of control values, n=2). Thus illustrating the reduction of visual response in the LGN is due to drug affect. Neither drug nor vehicle injection reduced visual responses recorded at the optic chiasm (100.4±8.00%, n=2 and 103.5 ±9.71%, n=3 of control respectively)

9.2 Iontophoresis in the SC

Initial studies on visual responses and response habituation were conducted using the NMDA antagonist D-AP5 to demonstrate reliable iontophoretic recordings from the rat SSC. The ejection of the NMDAR antagonist during presentation of visual stimuli, resulted in the reduction of visual responses in 8 out of 17 neurons (73.5±4.45% of control values) (figure 10A). Of the remaining cells, a further 8 remained D-AP5 resistant (99.4±2.50%), while 1 neuron displayed an enhanced response to visual stimuli (162.41±5.11%). Heterogenous changes in response habituation also occurred during D-AP5 ejection (figure 10B). 5 cells displayed an increase in habituation (130.3±10.61%), 9 cells displayed decreases (63.1±5.11%) and 3 cells remained unchanged (100.8±1.98%).

The results are comparable to previous work which has shown that visual response in a proportion of SGS neurons of the cat is reduced by NMDAR antagonists, while some remain 'AP5-resistant', and some responses may be slightly enhanced (Binns and Salt, 1994). Moreover the same study by Binns and Salt (1994) also revealed that these results were achieved during antagonist ejections at currents that would selectively block NMDA-mediated responses. These findings demonstrate that the technique of iontophoresis was carried out sufficiently well to accept those results collected during ejection of LY382884.

To determine direct KAR contribution to synaptic transmission between RGCs and collicular neurons, the GluR5 antagonist was iontophoretically administered whilst neurons

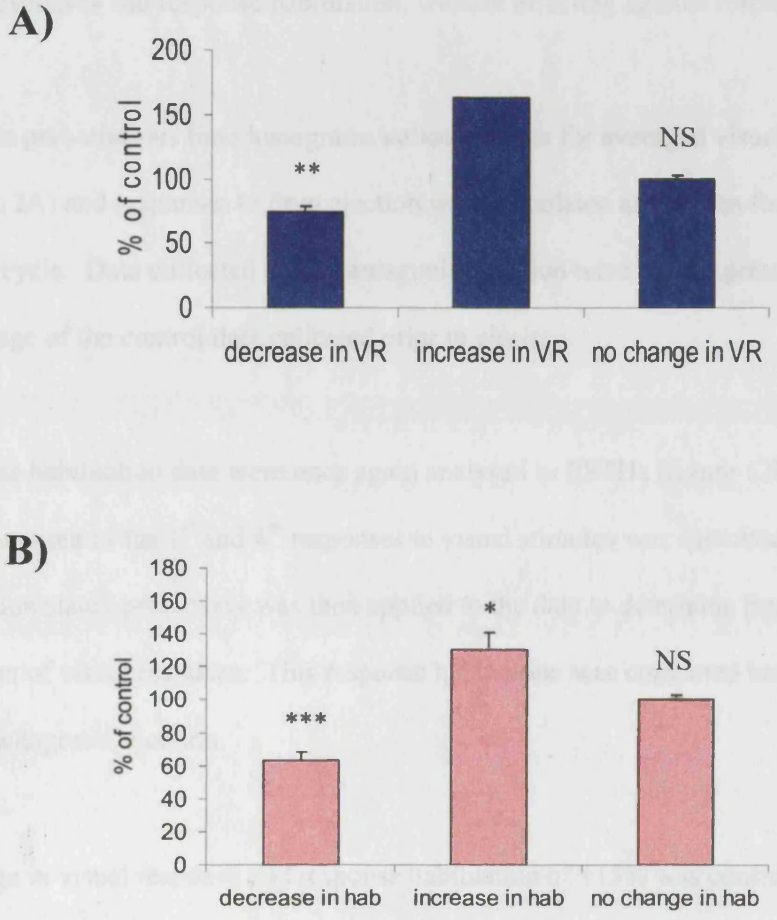


Figure 10. The NMDA antagonist causes heterogeneous effects on visual responses and response habituation. **A)** Visual responses were reduced (73.5%±4.45, n=8), increased (162.41%, n=1) or remained D-AP5-resistant (99.4%±2.50, n=8). **B)** Response habituation was reduced (63.1%±5.11, n=9), increased (130.30%±10.61, n=5) or remained D-AP5-resistant (100.8%±1.98, n=3). Student paired t-test, *<0.05, **<0.01, ***<0.001, NS=not significant.

were subject to visual and glutamate receptor agonist stimuli (figure 11). Application of the GluR5 antagonist LY382884 to SSC neurons produced reversible, specific effects on visual responses and response habituation, without affecting agonist responses (figure 11).

From the peri-stimulus time histograms collected, data for averaged visual responses (figure 12A) and responses to drug ejection were calculated as the area for the histograms in each cycle. Data collected during antagonist ejection were then expressed as a percentage of the control data collected prior to ejection.

Response habituation data were once again analysed as PSTHs (figure 12B), and the histogram area of the 1st and 4th responses to visual stimulus was calculated. The calculation stated previously was then applied to the data to determine the percentage reduction of visual response. This response habituation was compared before and after GluR5 antagonist ejection.

A change in visual response and response habituation of ±15% was considered to be due to antagonist affect, as the variation in control responses to stimuli prior to antagonist ejection was typically well below this value (±15% represents 2 standard deviations from control response values). Using this criterion data were separated into one of three groups: increase in response, decrease in response, or no change in response.

Organising the results in this way it appeared that the iontophoresis of LY382884 caused a heterogeneous effect on both visual responses and response habituation. Figure 13 A

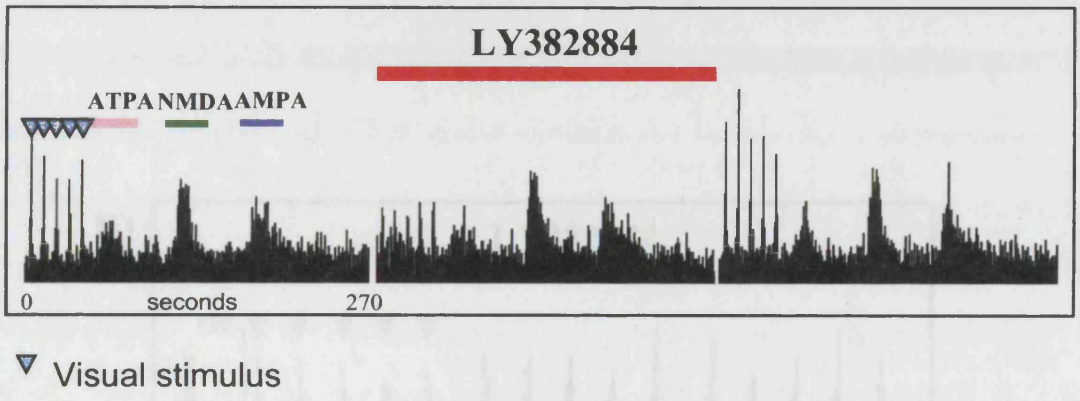


Figure 11. PSTH recorded from a single SSC neuron showing response to 5 presentations of visual stimuli followed by ATPA, NMDA and AMPA responses. The example illustrates a selective reduction in both visual responses and response habituation without affecting agonist responses, thus demonstrating the selective effect of LY382884.

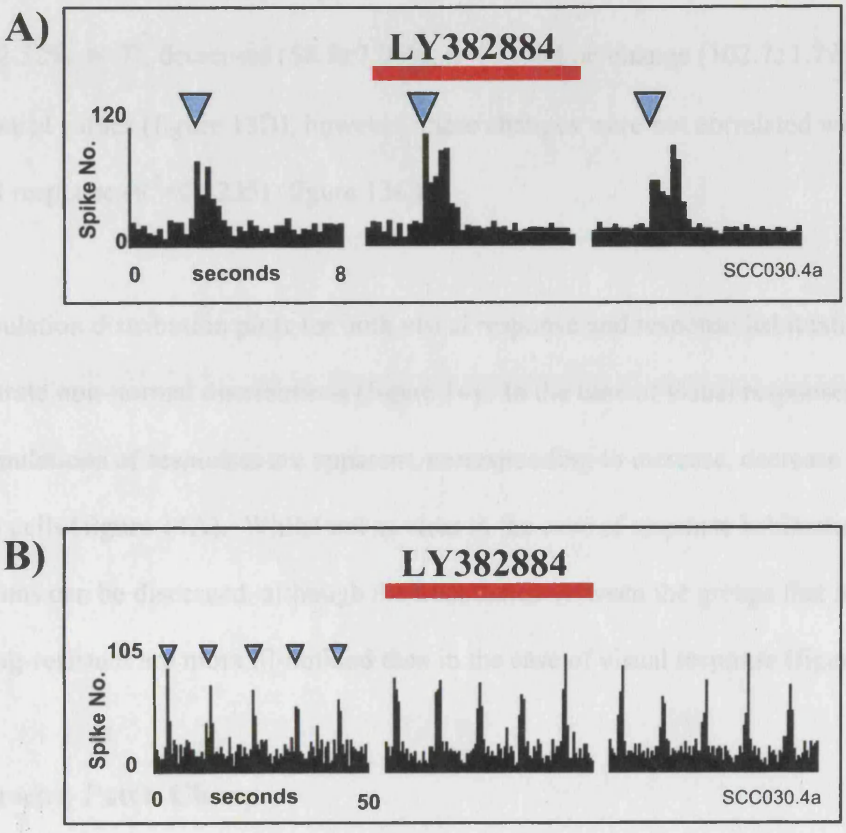


Figure 12. PSTHs showing the effect of LY382884 on **A**) visual response and **B**) response habituation before, during and after antagonist ejection. The example is recorded from a single SSC neuron demonstrating **A**) an increase in visual response and **B**) a reduction in response habituation between the 1st and 4th response.

shows that visual response could increase (127.8±5.2%, n=7), decrease (68.7±2.86%, n=11) or remain unchanged (99.6±2.02%, n=13) with respect to control values during the ejection of GluR5 antagonist. Similarly response habituation also demonstrated increases (118.8±2.52%, n=7), decreases (58.8±7.06%, n=13) and no change (102.7±1.79%, n=10) from control values (figure 13B), however, these changes were not correlated with changes in visual response (R^2=0.0235) (figure 13C).

The population distribution plots for both visual response and response habituation demonstrate non-normal distributions (figure 14). In the case of visual responses three clear populations of responses are apparent, corresponding to increase, decrease and drug-resistant cells (figure 14A). Whilst not as clear in the case of response habituation, multiple populations can be discerned, although the boundaries between the groups that increase and were drug-resistant are more ill-defined than in the case of visual response (figure 14B).

9.3 *In vitro* Patch Clamp

9.3.1 Agonist Experiments

The intrinsic circuitry of the SSC causes some difficulty when trying to show the effects of receptor modulation on separate excitatory or inhibitory input onto neurons within the SSC. To overcome the problem of recording a compound EPSC/IPSC response it was necessary to use pharmacological tools to isolate the initial fast EPSC and slower IPSC responses.

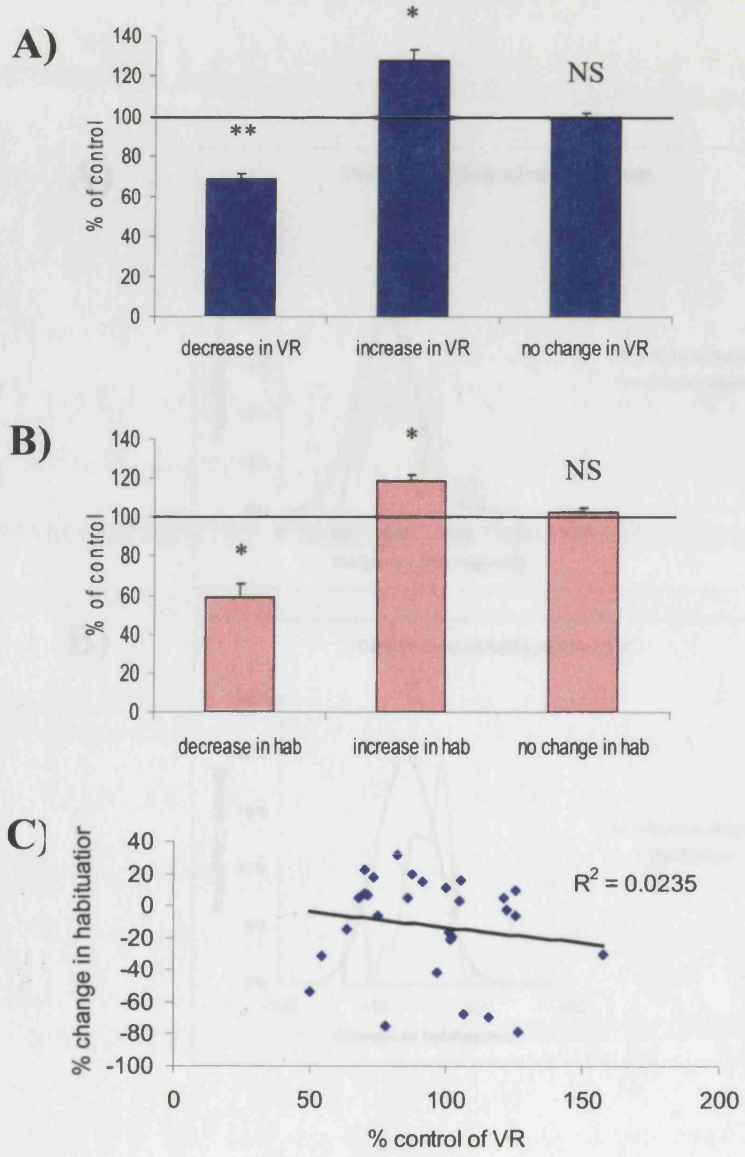
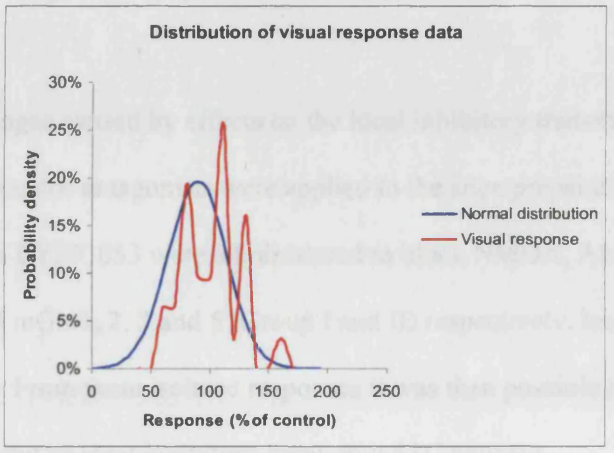


Figure 13. The GluR5 antagonist causes heterogeneous effects on visual responses and response habituation. **A**) Visual responses were reduced (68.7%±2.86, n=11), increased (127.8%±5.20, n=7) or remained LY382884-resistant (99.6%±2.02, n=13). **B**) Response habituation was reduced (58.8%±7.06, n=13), increased (118.8%±2.52, n=7) or remained Ly382884 resistant (102.7%±1.79, n=10). **C**) Correlation showing the affect of LY382884 on visual response did not correlate with observed affects on response habituation (R2=0.0235,n=28). This indicates visual responses and response habituation are mediated via different mechanisms. Student paired t-test, *<0.05, **<0.01, ***<0.001, NS=not significant.

A)



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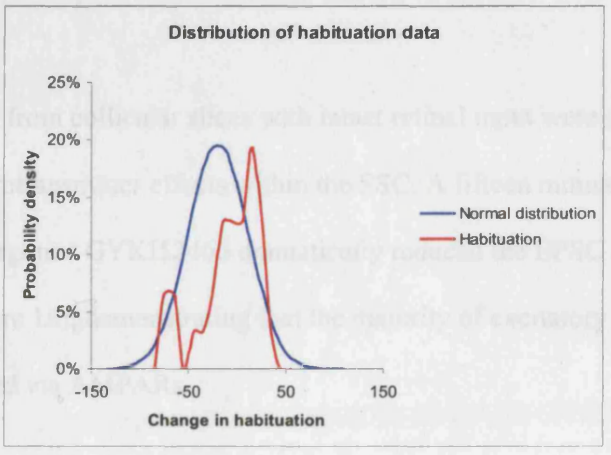

Figure 14 **A)**. Distribution of visual responses following LY382884 iontophoresis (n=31). Normal distribution is shown in blue. Responses in the SSC were non-normal, demonstrating at least three clear categories – decreased response, increased response and LY382884 insensitive groups. Bin size = 10%. **B)**. Distribution of response habituation following LY382884 iontophoresis (n=31). Normal distribution is shown in blue. Cellular responses in the SSC were non-normal. Bin size = 10%.

With the aid of $GABA_A$ and $GABA_B$ receptor antagonists (bicuculline and CGP55845A respectively) it was possible to isolate the EPSC response in order to measure effects of compounds on the excitatory, retinal input to the SSC (figure 15A).

In order to measure changes caused by effects on the local inhibitory transmission, a cocktail of glutamate receptor antagonists were applied to the slice preparation. D-AP5, GYKI52466, CPPG and LY393053 were administered to block NMDA, AMPA, mGlu4, 6, 7 and 8 (Group III), and mGlu1, 2, 3 and 5 (Group I and II) respectively, leaving clear IPSC responses (figure 15B). From these isolated responses it was then possible to observe changes in excitatory input or local inhibitory input onto SSC neurons.

Patch clamp recordings from collicular slices with intact retinal input were subsequently used to investigate neurotransmitter effects within the SSC. A fifteen minute application of the specific AMPA antagonist GYKI52466 dramatically reduced the EPSC amplitude to 14.10% of control (figure 16), demonstrating that the majority of excitatory transmission to SSC neurons is mediated via AMPARs.

To examine the possible modulation of the AMPAR-driven excitatory input by the activation of GluR5 receptors ATPA was then applied to the slice preparation. Application of the GluR5 agonist ATPA resulted in a dose-dependent reduction of EPSC amplitude (figure 17), as well as a dose-dependent increase in inward current (figure 18).

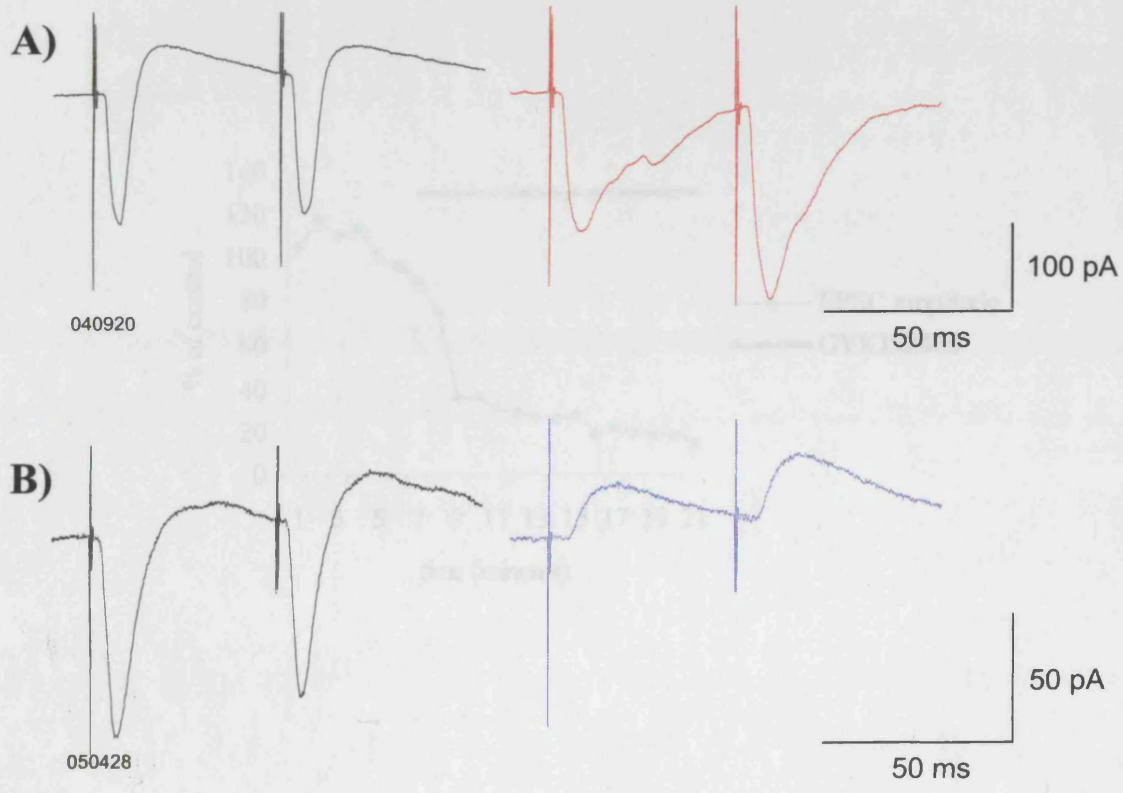

Figure 15. The isolation of direct excitatory input and local inhibitory input into the SSC. Waveform-averaged (n=6) whole-cell EPSCs in response to optic tract stimulation **A)** before (black) and during (red) administration of $GABA_A$ and $GABA_B$ antagonists and **B)** before (black) and after (blue) administration of AMPA, NMDA and mGluR antagonists.

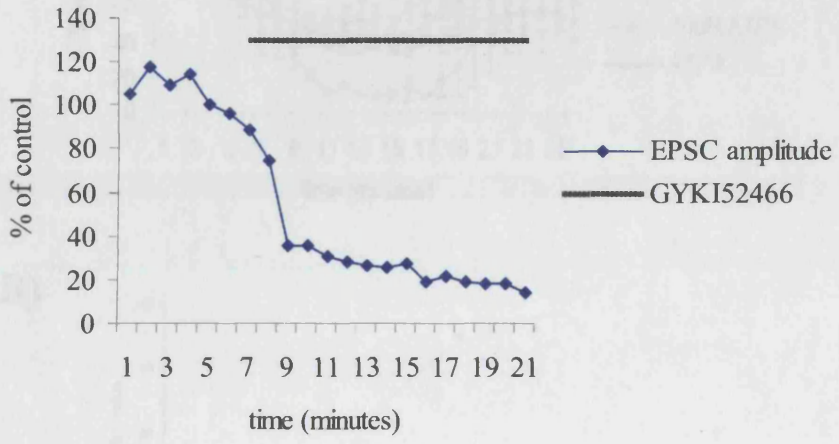

Figure 16. Data recorded from a single neuron showing that the addition of the AMPAR antagonist, GYKI52466, reduces the EPSC amplitude dramatically (in this case by 85.9%). This indicates that the majority of the excitatory transmission to SSC neurons is mediated via AMPARs.

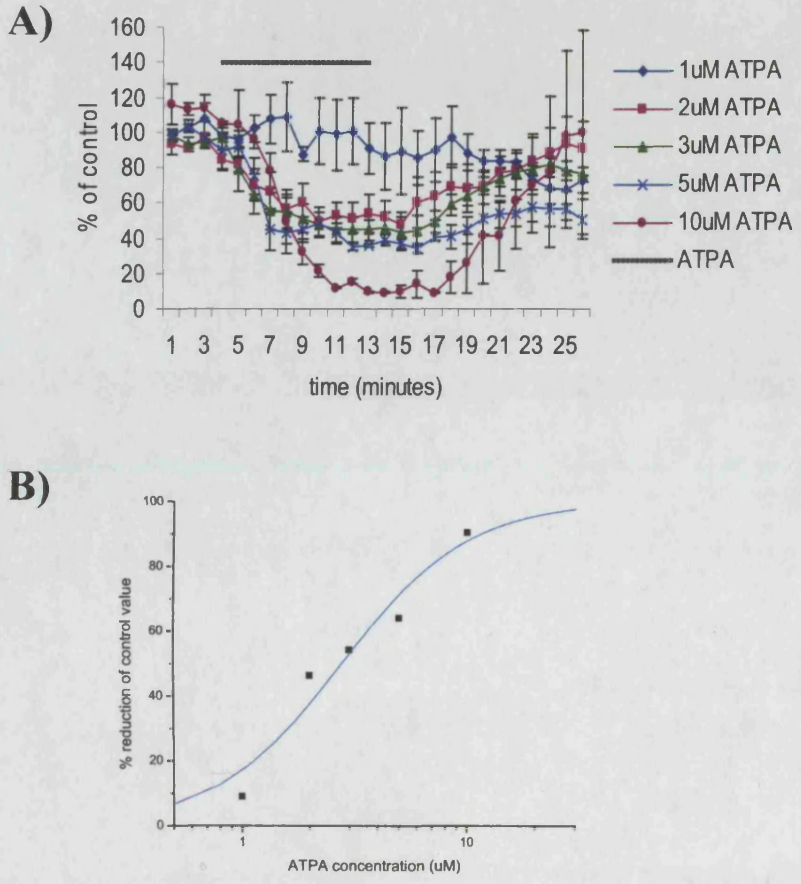

Figure 17. Affect of increasing concentrations of ATPA on EPSC amplitude. **A)**Application of ATPA results in a dose-dependent reduction of EPSC amplitude. Concentrations of ATPA used were 1uM (diamonds, n=2), 2uM (squares, n=5), 3uM (triangles, n=6), 5uM (crosses, n=3) and 10uM (circles, n=2). ATPA application is indicated by shaded bar. **B)** ATPA reduces EPSC amplitude in dose-related manner. Concentration response curve obtained for the action of ATPA (1-10uM) on synaptic response. Points are mean±sem (n=2-5). From the dose-response curve the calculated EC50 value is 2.7±0.34uM.

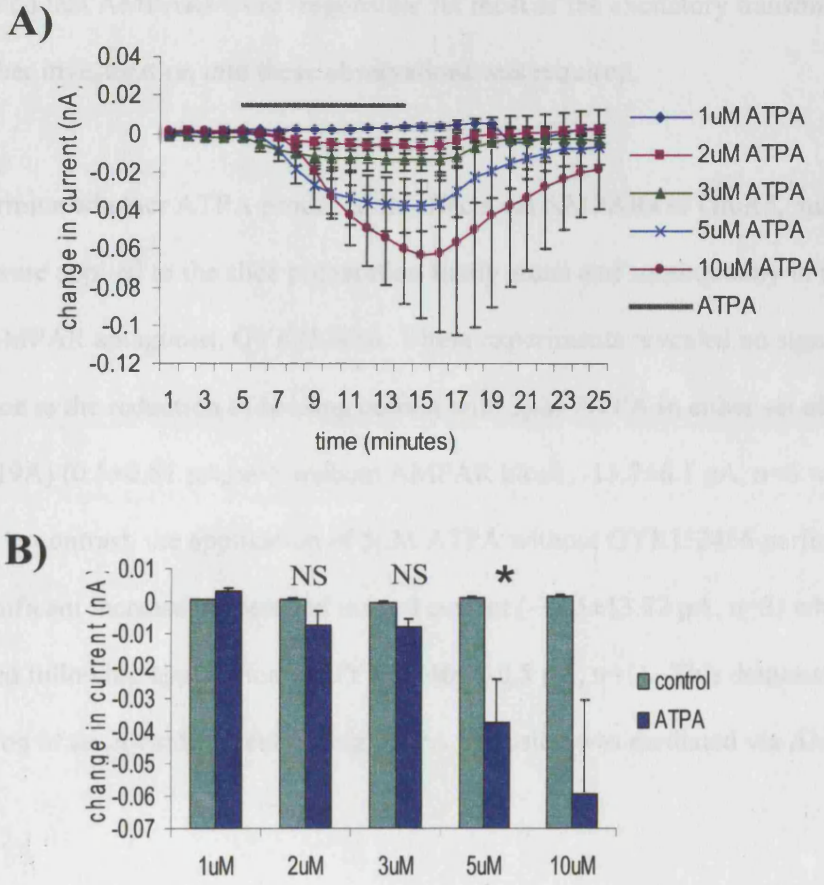

Figure 18. Affect of increasing concentrations of ATPA on holding current (mA). **A)** Application of ATPA results in a dose-dependent increase in inward current, with low drug concentrations having little affect. Concentrations of ATPA used were 1uM (diamonds, n=2), 3uM (squares, n=3), 5uM (triangles, n=3) and 10uM (crosses, n=2). ATPA application is indicated by shaded bar. **B)** Application of the KAR agonist, ATPA (1-10uM, n=2-6), causes a dose-related increase in inward current which is significant with the application of 5uM ATPA but not significant during application of 2uM or 3uM ATPA.

Although the GluR5 agonist is relatively selective, it is possible that ATPA may able to activate AMPARs at the higher concentrations used (Clarke *et al.*, 1997). Having established that AMPARs were responsible for most of the excitatory transmission (figure 16) further investigation into these observations was required.

To determine whether ATPA produced its effects via AMPARs or GluR5, 3μM and 5μM ATPA were applied to the slice preparation firstly alone and subsequently in the presence of the AMPAR antagonist, GYKI52466. These experiments revealed no significant difference in the reduction of holding current with 3μM ATPA in either set of experiments (figure 19A) (0.5±0.81 pA, n=5 without AMPAR block, -13.7±6.1 pA, n=6 with AMPAR block). In contrast, the application of 5μM ATPA without GYKI52466 perfusion resulted in a significant increase in recorded inward current (-37.5±13.72 pA, n=3) which was abolished following application of GYKI52466 (-0.5 pA, n=1). This demonstrates that the generation of an inward current during ATPA perfusion was mediated via AMPARs (figure 19B).

The concomitant reduction in EPSC amplitude remains apparent even during perfusion of the AMPAR antagonist (figure 20). There is indeed no significant difference between EPSC reduction before (44.6±6.12% of control, n=6) and during GYKI52466 administration (47.2±5.90% of control, n=5). These data support the suggestion that the reduction of EPSC amplitude is a product of a GluR5-specific effect of ATPA.

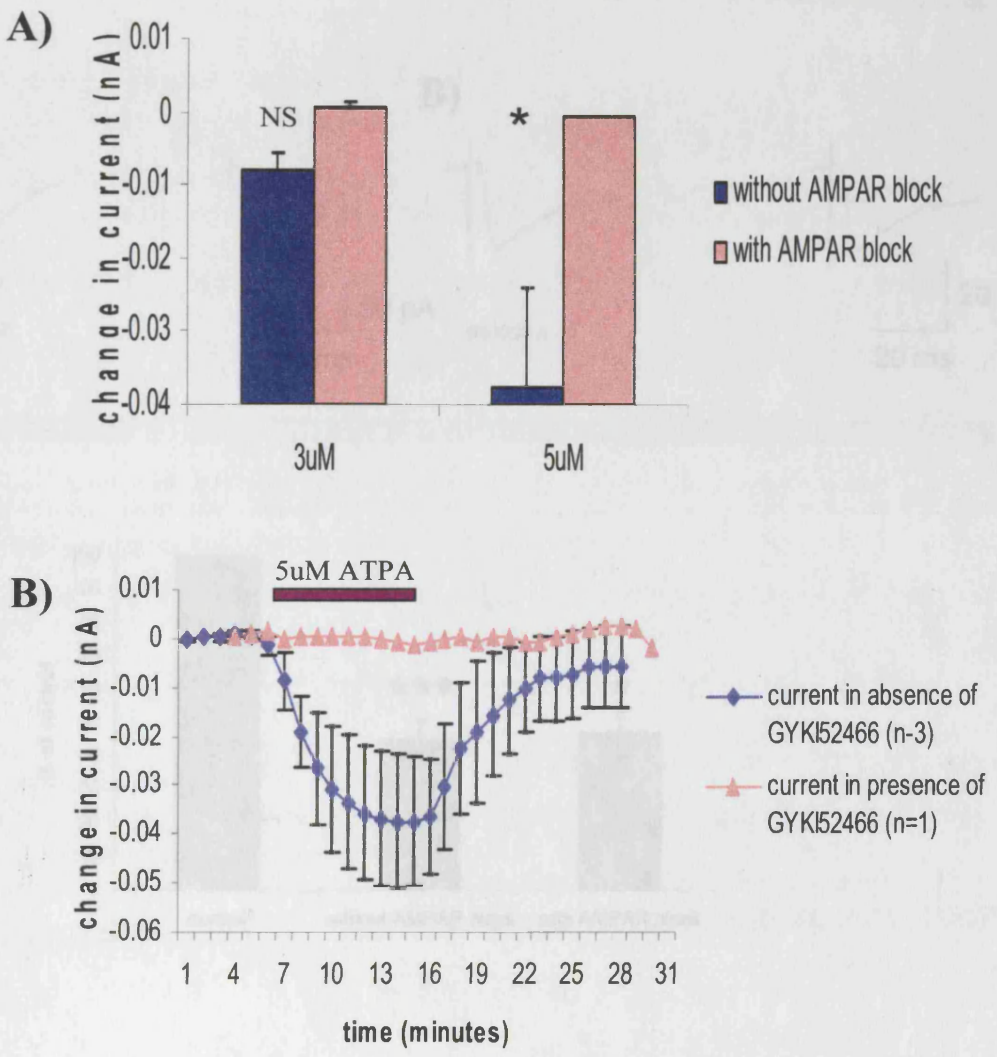

Figure 19. High concentrations of ATPA cause an inward current. **A)** There in no significant change in holding current produced by 3uM ATPA, however, 5uM ATPA results in a significant increase in inward current which is blocked by the AMPAR antagonist. This demonstrates that increases in the inward current with high concentrations of the agonist are due to the affect of ATPA on AMPARs rather than KARs. **B)** Time course showing affect of AMPAR block on holding current following application of 5uM ATPA.

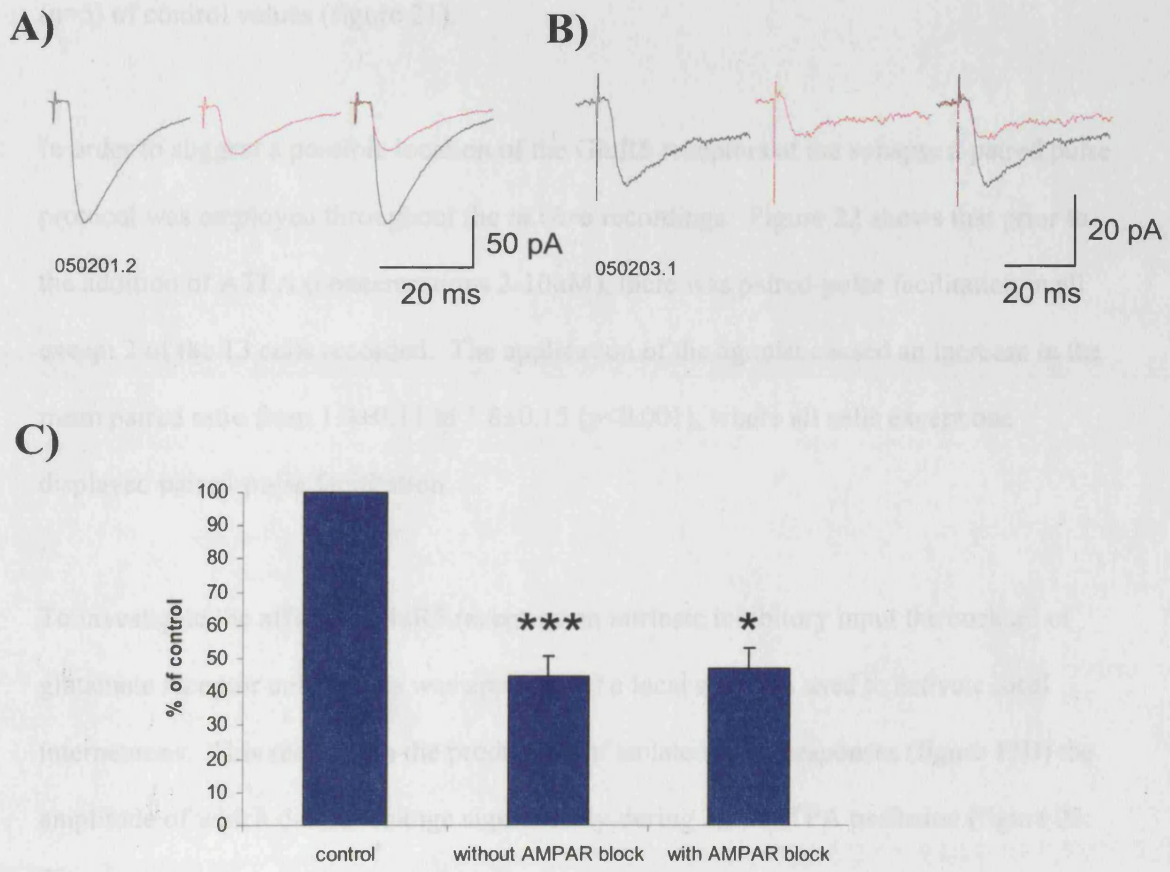

Figure 20. ATPA reduces EPSC amplitude both with and without AMPAR block by GYKI52466. Waveform averages (n=6) representing the reduction in EPSC due to the application of 3uM ATPA. Averages are taken before (black) and during (red) ATPA application, without AMPAR block **(A)** and with AMPAR block **(B)**. **C)** In both the absence and presence of the AMPAR antagonist, 3uM ATPA reduces EPSC amplitude to 44.6±6.12% and 47.2±5.90% of control values respectively. This demonstrates that reduction of EPSC is due to the activation of GluR5-containing KARs. Student paired t-test, *<0.05, ***<0.001

For the majority of the *in vitro* experiments 2μM ATPA was used because there was less effect of this concentration on the holding current than 3μM ATPA (figure 18) while the amplitude of EPSC reponses was also significantly and reversibly reduced to 53.7±10.96% (n=5) of control values (figure 21).

In order to suggest a possible location of the GluR5 receptors at the synapse a paired pulse protocol was employed throughout the *in vitro* recordings. Figure 22 shows that prior to the addition of ATPA (concentrations 2-10μM), there was paired-pulse facilitation in all except 2 of the 13 cells recorded. The application of the agonist caused an increase in the mean paired ratio from 1.4±0.11 to 1.8±0.15 ($p<0.001$), where all cells except one displayed paired-pulse facilitation.

To investigate the affect of GluR5 receptors on intrinsic inhibitory input the cocktail of glutamate receptor antagonists was applied and a local stimulus used to activate local interneurons. This resulted in the production of isolated IPSC responses (figure 15B) the amplitude of which did not change significantly during 2μM ATPA perfusion (figure 23: 96.9±10.87% of control, n=6).

It is observed that stimulating the optic tract input into the SSC results in the generation of a compound EPSC/IPSC response (figure 15). This suggests that the retinal ganglion cell input activates both excitatory and inhibitory transmission onto the same cell simultaneously. The final set of experiments using the GluR5 agonist was designed to test whether the activation of GluR5 receptors affects both the direct excitatory input

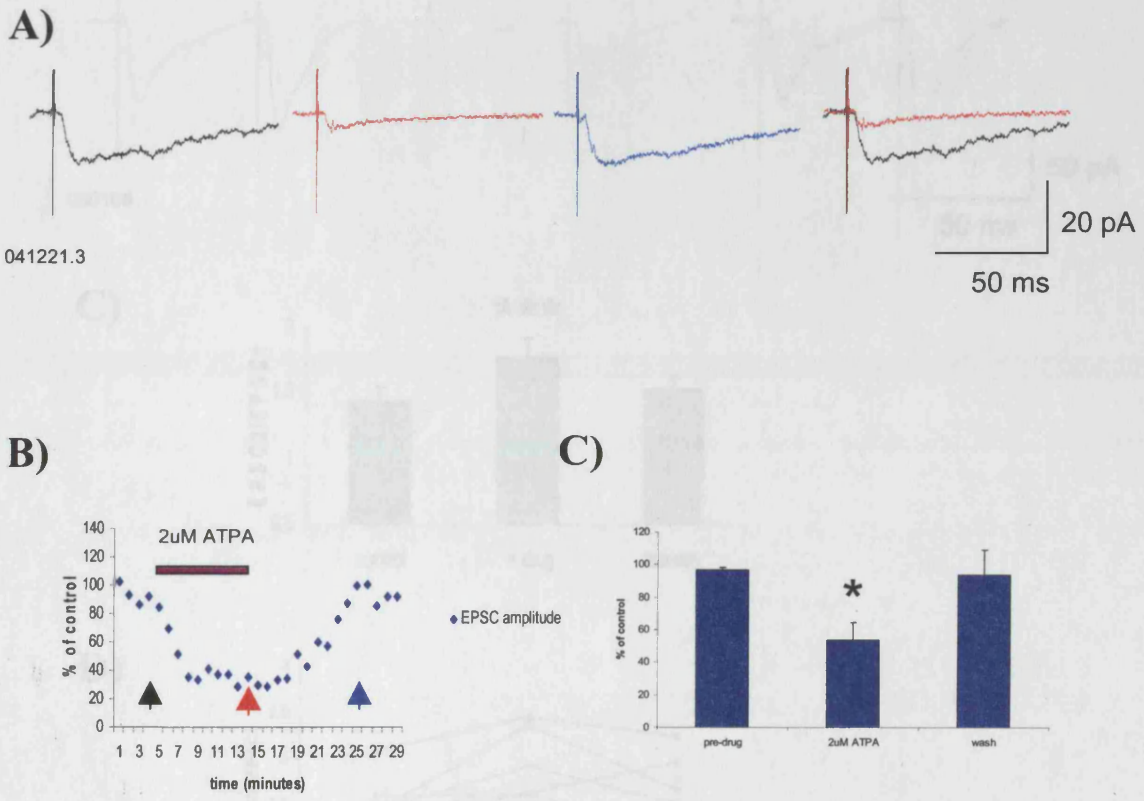

Figure 21. ATPA reversibly reduces EPSC amplitude. **A)** Waveform-averaged (n=6) whole-cell EPSCs in response to optic tract stimulation before (black), during (red), and after (blue) perfusion with 2uM ATPA. **B)** Representative plot of EPSC amplitude from a single cell illustrating the effect of ATPA on excitatory synaptic transmission (the pink bar signifies ATPA administration). Time points where waveform averages were taken are indicated by arrows. **C)** 2uM ATPA significantly reduces EPSC amplitude to 53.7±10.96% of control values (n=5). The data are displayed as mean±sem ($p<0.05$). Student paired t-test, *<0.05

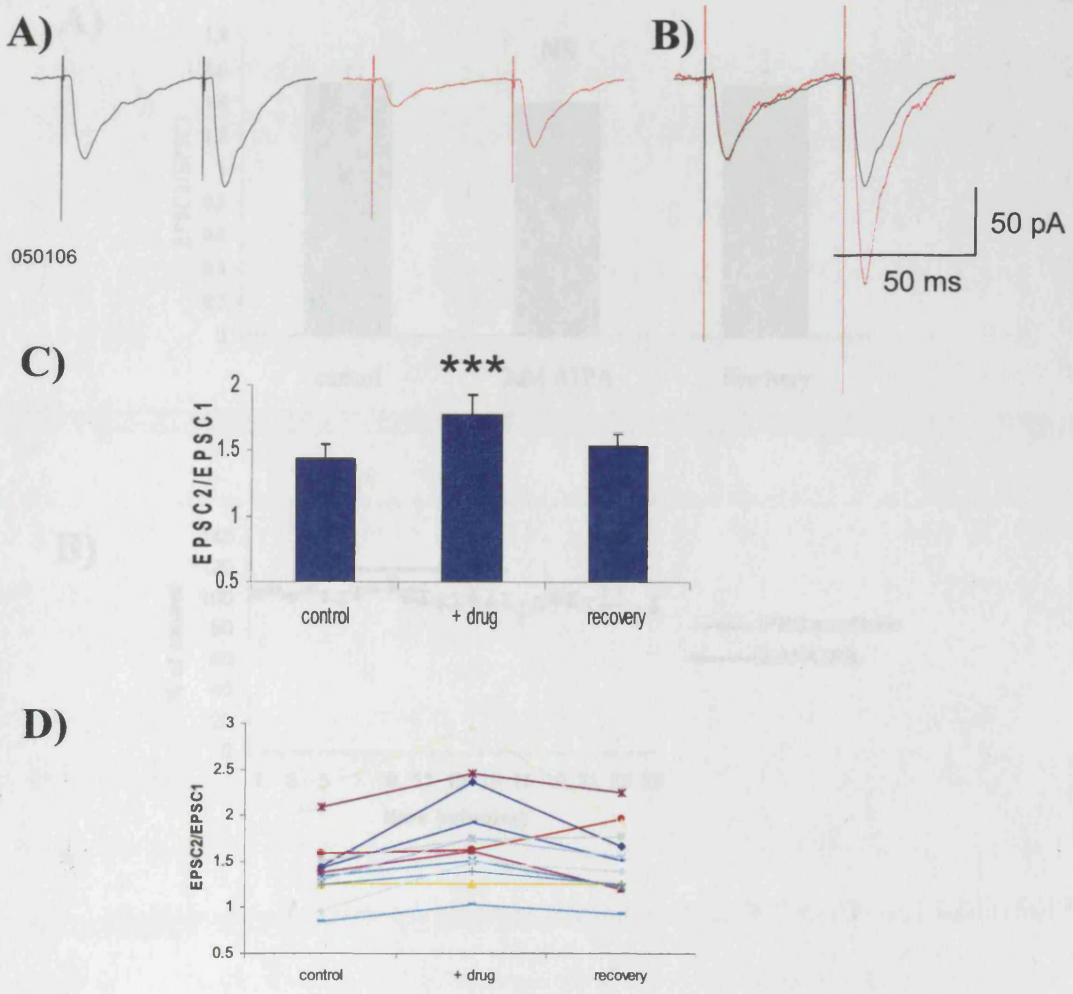

Figure 22. ATPA causes an increase in paired pulse ratio. **A)** Waveform averages illustrating paired-pulse responses before (black) and after (red) 2uM ATPA application. **B)** Scaling the responses to the first EPSC under control conditions shows that ATPA administration increases paired-pulse ratio. **C)** ATPA reversibly increases paired-pulse ratio ($n = 13$; $P < 0.001$). **D)** Under control conditions the paired-pulse ratio differed from cell to cell, with 2 out of 13 cells exhibiting paired-pulse depression and the remaining 11showing paired-pulse facilitation. In all except 1 of the cells tested application of ATPA (2-10uM) increased the paired pulse ratio. Student paired t-test, ***<0.001

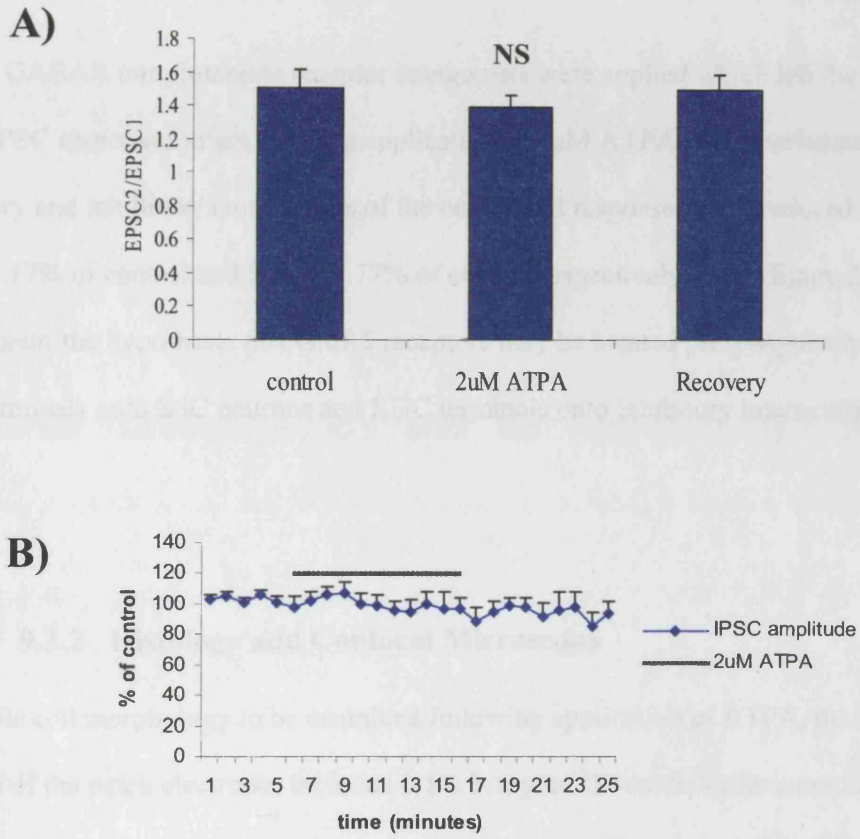

Figure 23. Effect of 2uM ATPA on IPSC amplitude. **A)** 2uM ATPA does not cause a significant change in IPSC amplitude. **B)** Plot of IPSC amplitude over time. The plot shows that IPSC amplitude remains unchanged with the application of 2uM ATPA. Each point represents an average of six evoked responses. Data is displayed as mean±sem. Student paired t-test, NS=not significant.

from the retina and the excitatory RGC input onto the local interneurons, thus also influencing inhibitory transmission via an indirect route.

Neither GABAR nor glutamate receptor antagonists were applied which left the compound EPSC/IPSC responses intact. During application of 2μM ATPA in the perfusate, both excitatory and inhibitory components of the compound responses were reduced (50.8±6.17% of control and 58.3±17.77% of control respectively, n=5) (figure 24). These data support the hypothesis that GluR5 receptors may be located presynaptically at both the RGC terminals onto SSC neurons and RGC terminals onto inhibitory interneurons of the SSC.

9.3.2 Histology and Confocal Microscopy

To enable cell morphology to be examined following application of ATPA, the internal solution of the patch electrodes included 0.1% biocytin. Given the differences in cellular responses recorded in vivo, this approach was to enable cells to be subsequently analysed to determine a correlation between certain response types (increase, decrease or drug resistant) and morphological characteristics of SSC neuron subtypes. A selection of neurons from which recordings were made are shown in figure 25, although ultimately this method was not required as all cells behaved in a similar way after agonist application and therefore analysis of cell morphology not undertaken.

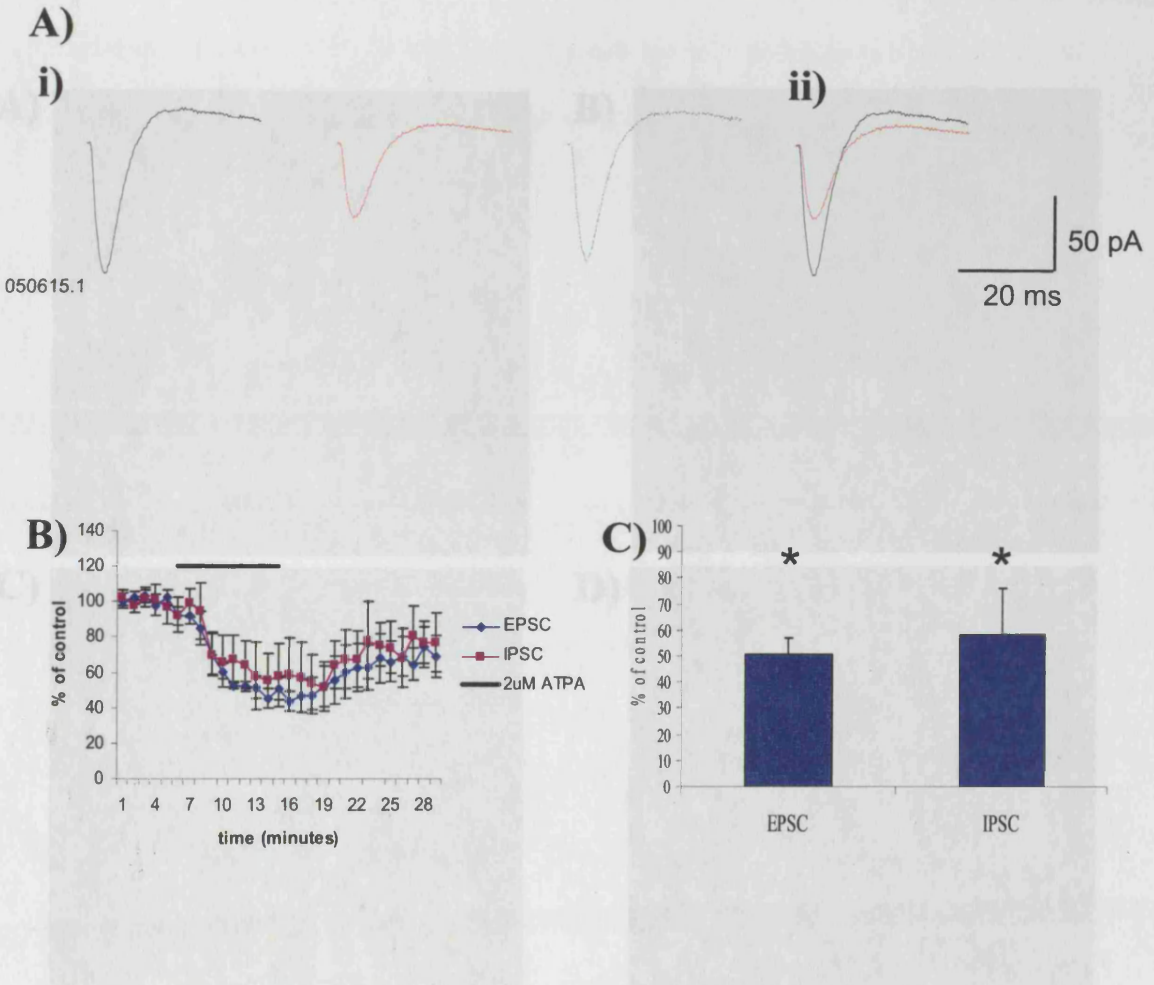

Figure 24. Affect of 2uM ATPA on evoked compound EPSC/IPSCs. **A)** i) Waveform averages illustrating compound EPSC/IPSC responses before (black) during (red) and after (blue) 2uM ATPA application. ii) Overlay of responses before and during drug application. **B)** Representative plot of EPSC amplitude from a single cell illustrating the effect of ATPA on excitatory synaptic transmission (the bar signifies ATPA administration). Time points where waveform averages were taken are indicated by arrows. C) 2uM ATPA significantly reduces EPSC amplitude to 53.7±10.96% of control values (n=5). The data are displayed as mean±semStudent paired t-test, *<0.05

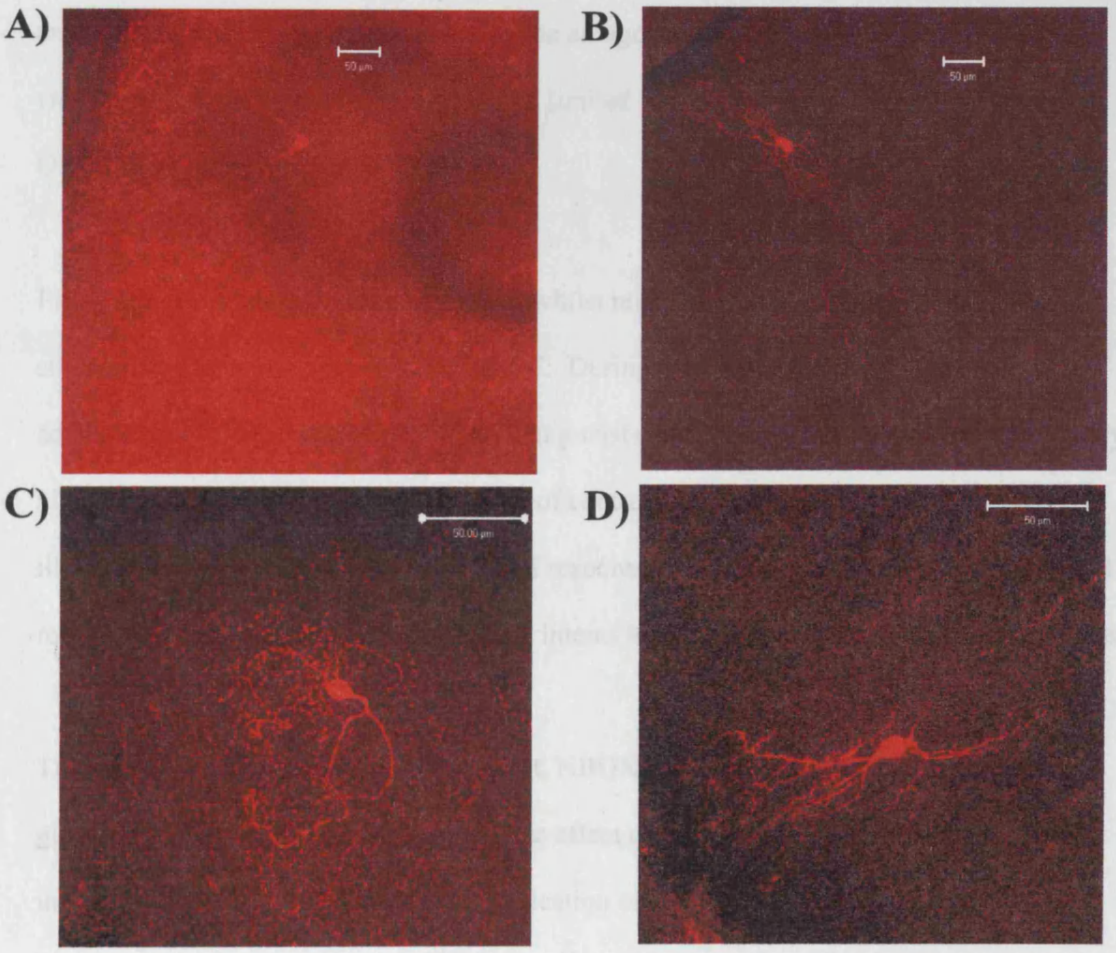

Figure 25. A representative selection of confocal images of biocytin- filled SSC neuronss recorded from *in vitro* slice preparations. The dorsal surface of the SC lies to the top left corner of each image. **A)** putative wide-field projection neuron. **B)** putative narrow-field projection neuron. **C)** putative stellate GABAergic interneuron. **D)** putative horizontal GABAergic interneuron.

9.3.3 Antagonist Experiments

The initial *in vivo* experiments all employed the GluR5 antagonist to reveal a function of the receptor subunit in the SSC and LGN. For this reason it was logical to apply a GluR5 antagonist during *in vitro* experiments. The antagonist LY382884 was not used in the *in vitro* experiments, however, because of its limited supply, therefore, the newer compound, UBP296 was administered in its place.

Firstly the GluR5 antagonist was applied whilst recording EPSC responses evoked by stimulating the optic tract input to the SSC. During recording 10μM UBP296 was administered in the presence of GABA antagonists but did not effect responses significantly after a ten minute perfusion (88.7±2.60% of control, n=3) (figure 26A). Figure 26B illustrates that, although some rundown of response is apparent, EPSC amplitude remained relatively unchanged throughout the experiments when results were pooled from three cells.

The less selective AMPA/KAR antagonist, NBQX was also applied in the presence of glutamate antagonists. This was to test the effect of the non-selective KAR block on inhibitory transmission. As with the application of 10μM UBP296, a 10 minute perfusion of 10μM NBQX did not significantly affect the amplitude of locally evoked IPSC responses (140.5±25.79% of control, n=4) (figure 27).

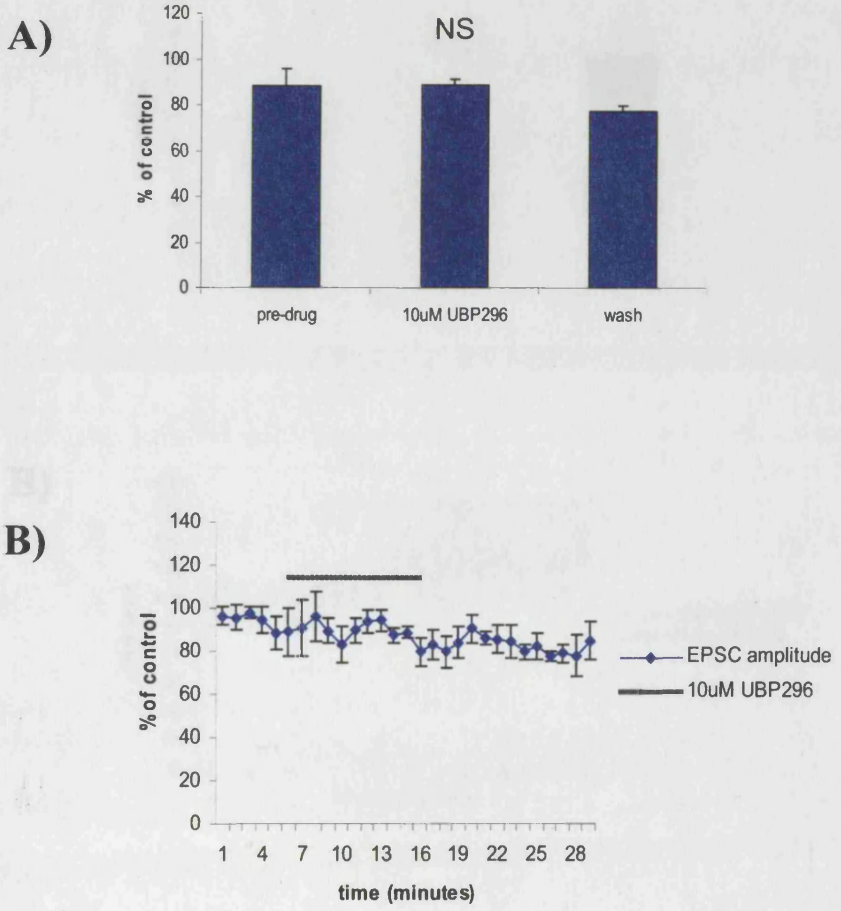

Figure 26. Effect of 10uM UBP296 on EPSC amplitude. **A)** Histogram to show that 10uM UBP296 does not significantly affect EPSC amplitude (88.7±2.60%, n=3). **B)** Plot of mean EPSC amplitude over time. Each point represents the average of 3 experiments in which six evoked responses were averaged and the EPSC amplitude was calculated and normalised to an initial value. Results are displayed as mean±sem. Student paired t-test, NS=not significant.

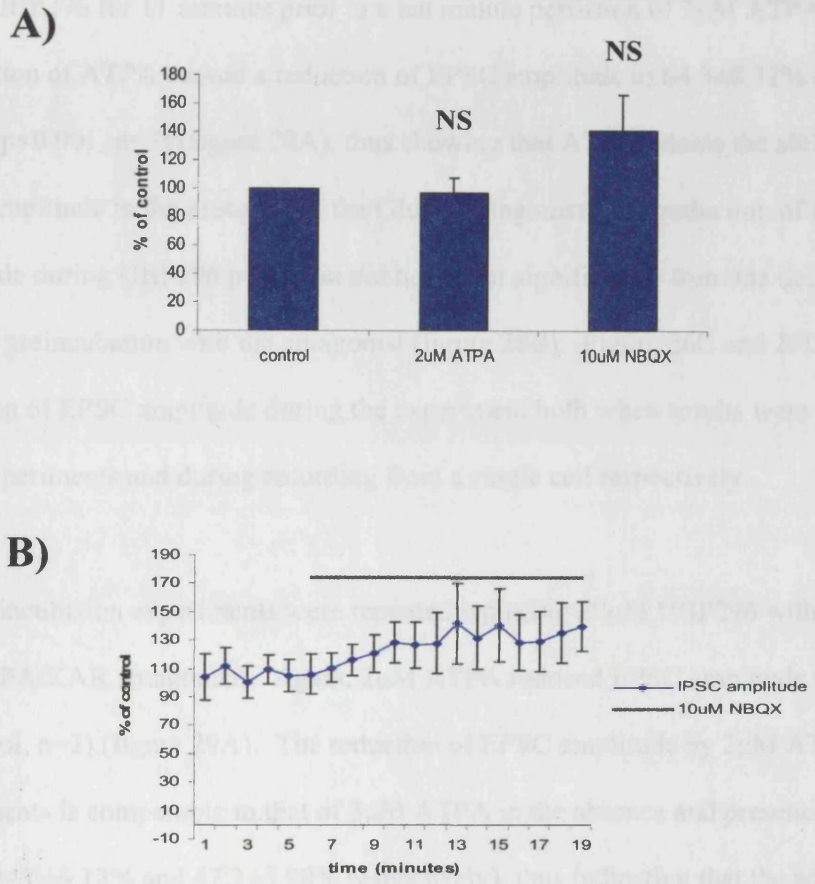

Figure 27. Affect of 10uM NBQX on IPSC amplitude. **A)** 10uM NBQX does not cause a significant change in IPSC amplitude. **B)** Plot of IPSC amplitude over time. The plot shows that IPSC amplitude remains unchanged with the application of 10uM NBQX. Each point represents an average of six evoked responses. Data is displayed as mean±sem, n=4. Student paired t-test, NS=not significant.

Because UBP296 had no effect when applied alone, experiments were undertaken to attempt to reverse the action of ATPA on EPSC amplitude. Slices were preincubated with 20μM UBP296 for 11 minutes prior to a ten minute perfusion of 2μM ATPA. The application of ATPA caused a reduction of EPSC amplitude to 64.9±8.32% of control values ($p<0.001$, n=3) (figure 28A), thus showing that ATPA retains the ability to reduce EPSC amplitude in the presence of the GluR5 antagonist. The reduction of EPSC amplitude during UBP296 perfusion did not differ significantly from the decrease observed without preincubation with the antagonist (figure 28B). Figure 26C and 26D illustrate the reduction of EPSC amplitude during the experiment both when results were pooled from three experiments and during recording from a single cell respectively.

The preincubation experiments were repeated replacing 20μM UBP296 with 10μM NBQX (an AMPA/KAR antagonist). Again, 2μM ATPA reduced EPSC amplitude (49.1±4.52% of control, n=2) (figure 29A). The reduction of EPSC amplitude by 2μM ATPA in these experiments is comparable to that of 3μM ATPA in the absence and presence of AMPAR block (44.6±6.12% and 47.2±5.90% respectively), thus indicating that the addition of 10μM NBQX does not affect the action of ATPA (figure 29B).

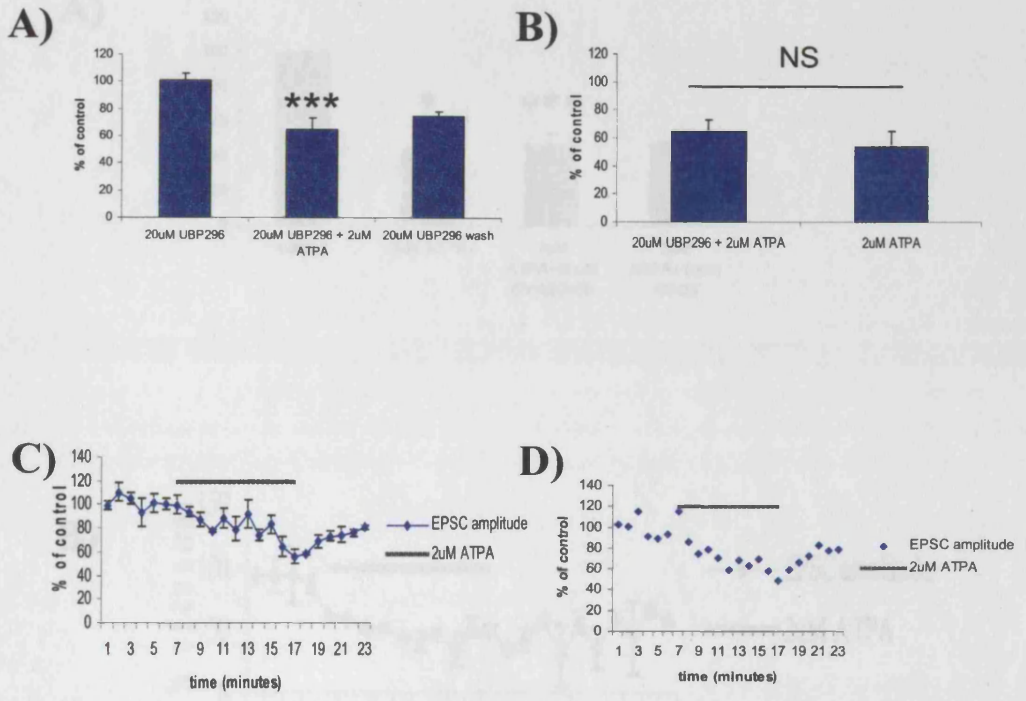

Figure 28. Effect of 20uM UBP296 on the reduction of EPSC amplitude by 2uM ATPA. **A)** The histogram shows that 2uM ATPA retains the ability to reduce EPSC amplitude. **B)** 2uM ATPA in the presence of 20uM UBP296 reduces EPSC amplitude. The magnitude of this reduction is not significantly different to the reduction observed in the absence of the antagonist. **C)** Plot of EPSC amplitude over time. **D)** Data from a single cell showing the effect of 20uM UBP296 on EPSC amplitude. Each point represents the average of six evoked responses where the EPSC amplitude was calculated and normalised to an initial value. Results are displayed as mean±sem. Student paired t-test, ***<0.001, NS=not significant.

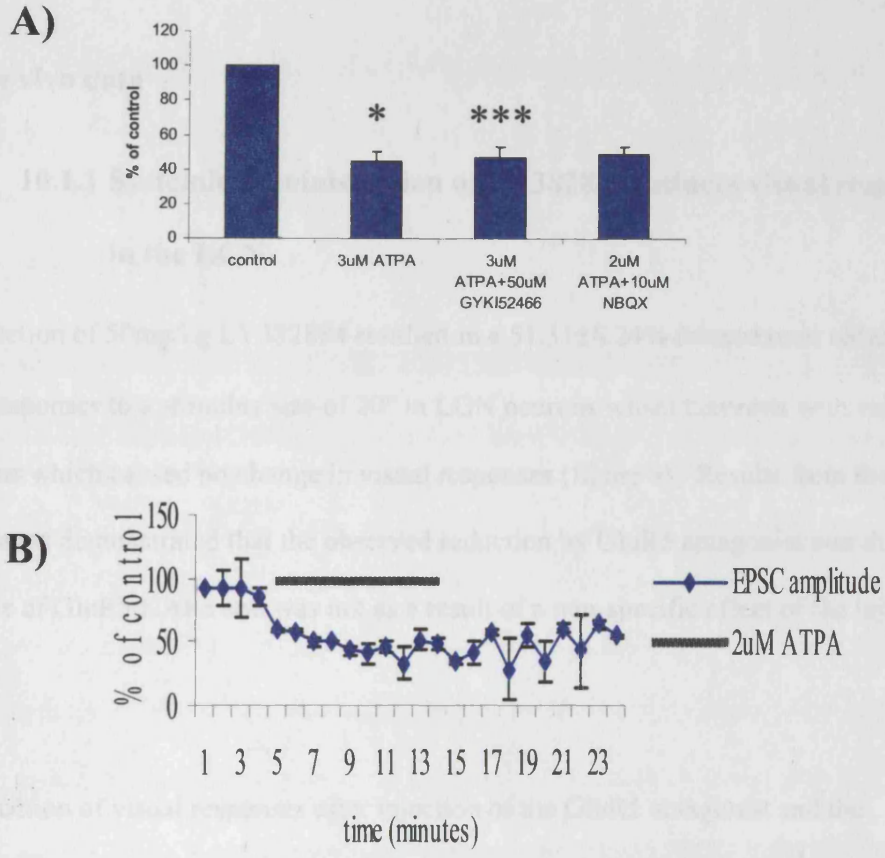

Figure 29. The effect of NBQX on EPSC amplitude reduction by ATPA. **A)** The histogram shows that NBQX does not reverse ATPA reduction of EPSC amplitude which decreases to levels seen during 3uM ATPA and ATPA + GYKI52466 application (49.1±4.52%, n=2). **B)** Plot of mean EPSC amplitude over time. Each point represents the average of 2 experiments in which six evoked responses were averaged and the EPSC amplitude was calculated and normalised to an initial value. Results are displayed as mean±sem. Student paired t-test, *<0.05, ***<0.001.

10 Discussion

The data presented here provide the first functional evidence for kainate receptors in the rodent subcortical visual system.

10.1 In vivo data

10.1.1 Systemic administration of LY382884 reduces visual responses in the LGN

The injection of 50mg/kg LY382884 resulted in a 51.31±8.24% (mean±sem) reduction of visual responses to a stimulus size of 20° in LGN neurons which contrasts with vehicle injections which caused no change in visual responses (figure 9). Results from these experiments demonstrated that the observed reduction by GluR5 antagonist was due to blockade of GluR5 KARs and was not as a result of a non-specific effect of the injection vehicle.

The reduction of visual responses after injection of the GluR5 antagonist and the subsequent recovery, suggests that the drug is acting at GluR5 KARs within the visual system. These KARs could also be located in neuronal networks influencing LGN relay cell responses. It is not possible to localise the drug effect on GluR5 receptors to any one part of the visual system during systemic administration as the compound would affect GluR5 receptors throughout the brain. In addition, an explanation of possible mechanisms of action is not possible as GluR5 receptors have been associated with both the modulation of GABAergic transmission (Clarke *et al.*, 1997;Cossart *et al.*, 1998;Frerking *et al.*,

1998;Min *et al.*, 1999;Rodriguez-Moreno *et al.*, 1997;Semyanov & Kullmann, 2001), glutamatergic transmission (Rodriguez-Moreno & Sihra;Lauri *et al.*, 2005;Kamiya & Ozawa, 1998) and postsynaptic EPSP generation (Swanson *et al.*, 1996).

Therefore, systemic administration of a GluR5-selective concentration of LY382884 (50mg/kg) establishes that KARs are involved in visual processing and effect LGN responses to visual stimuli (both spots of different size and contrast: figures 7 and 8), but does not reveal the mechanism by which these affects are achieved.

10.1.2 Systemic injection of LY382884 does not affect responses in the optic chiasm

Injection of GluR5 antagonist reduces visual responses recorded from the LGN, however, no change was observed in responses recorded from the optic chiasm (figure 9). This suggests that GluR5 KARs are involved in visual processing downstream of the eye. These data do not, however, indicate the precise location of the receptors in the visual pathway, but provide some evidence that GluR5 receptors are able to influence visual processing within the LGN. The results do not eliminate the possibility that the antagonist is unable to cross the blood-retinal barrier (BRB) and, therefore, incapable of affecting receptors present in the retina. The inability to pass the BRB gains further support from evidence that KAR function has been demonstrated in the retina (DeVries & Schwartz, 1999;Brandstatter *et al.*, 1997); data suggest that AMPARs mediate transmission via b2 bipolar cells and KARs mediate transmission from photoreceptors via b3 and b7 bipolar cells, all of which are 'OFF' bipolar cells (DeVries, 2000). Further experiments to clarify

this issue could include the current method of systemic administration coupled with high-performance liquid chromatography (HPLC) analysis of retinal tissue to detect drug presence. Alternatively, tritiated compound could be administered and identified using either scintillation counting of retinal extracts or autoradiography of retinal sections. The latter technique may also provide information on drug binding sites.

Constraints of time and compound availability (especially given the large doses required by this approach) limited the systemic administration studies, which could also have been conducted in the SSC. However, iontophoresis enabled a more detailed characterisation of KAR function in the structures of the visual pathway.

10.1.3 In vivo iontophoresis of LY382884 produces heterogeneous effects in the SSC

Microiontophoresis enables drug delivery directly to the neuronal environment from which recordings are made. Such an approach allows the *in vivo* characterisation of cellular responses to drug application within a very limited area, ideally affecting specific receptors in the immediate vicinity of the cell under investigation.

Iontophoretic recordings were attempted in both the LGN and SSC, although greater success was achieved in the SSC. Lack of success in the LGN may have been due to the deeper location of the LGN in the brain; LGN recordings were less stable, whereas the more superficial location of the SSC may have led to improved recording stability.

Within the group of observations from this set of experiments, it was clear that there were varied responses to the iontophoresis of the GluR5 antagonist LY382884; responses were seen to increase, decrease or remain antagonist-resistant.

These findings suggest that GluR5 is involved in modulation of both visual responses and response habituation in the SSC.

These data represent the first demonstration that GluR5 is physiologically functional in the SSC. However, iontophoresis does not provide evidence for the location of these receptors or their mechanism of action.

Although iontophoresis permits drug delivery to a more specific location within the CNS than systemic administration, there are a number of limitations associated with this technique. Whilst iontophoresis allows the application of a drug to the immediate cellular environment, this does not guarantee drug access to the receptor under investigation. A number of factors must be considered, such as drug access to the recorded cell, cellular geometry (whether the cell and it's dendrites are compact or widespread) and receptor location on or around the recorded cell (Stone, 1985). These factors mean that the exact placement of the electrode with respect to the cell soma and dendritic arbour may affect the result obtained if the receptors are differentially located on the cell.

Many cells of the SSC have large somas and an extensive dendritic arbour (fig 25A and B) (Hilbig *et al.*, 2000), and these factors could account for the heterogeneous results obtained.

For example, were GluR5 to be found on the dendrites and the electrode placed near the soma, little effect of the antagonist would be observed whilst recording from a cell with an extensive dendritic arbour. However, if GluR5 is located near the soma (or the recording is made from a smaller cell) an effect would be seen (fig 25C and D).

The finding that changes in visual response and response habituation were not correlated may suggest that these effects are mediated via different mechanisms. Potential mechanisms by which these results may have arisen are discussed in more detail below.

10.1.3.a Postsynaptic GluR5 kainate receptors

The results observed from *in vivo* iontophoresis could be explained by a postsynaptic GluR5 location. Evidence for postsynaptic KARs comes from a number of sources.
In some instances KAR-mediated EPSCs are negligible in single evoked responses and are only observed during high frequency stimulation (Castillo *et al.*, 1997;Cossart *et al.*, 1998; Frerking *et al.*, 1998;Li & Rogawski, 1998). However, a small KAR-mediated component in recorded EPSCs has been produced in response to single stimuli at sensory inputs into the spinal cord (Li *et al.*, 1999), in somatosensory layer IV cortical neurons (Kidd & Isaac, 1999) and cerebellar Golgi cells (Bureau *et al.*, 2000). This evidence along with the observation that KARs contribute to EPSCs in CA1 interneurons (Cossart *et al.*, 1998;Frerking *et al.*, 1998) suggests that KARs could be located at postsynaptic sites on interneurons within the SSC. Here they may contribute to the generation of EPSCs and subsequent GABA release onto SSC neurons. The blockade of these receptors could, therefore, lead to an indirect increase in visual response or a reduction in response

habituation due to the disinhibition of GABAergic transmission. A postsynaptic location at the RGC-SSC neuron, however, may result in the reduction of visual responses during GluR5 block.

KAR-mediated EPSC/Ps appear to be slower and smaller than AMPAR-mediated responses during both agonist application and synaptically released glutamate (Castillo *et al.*, 1997;Cossart *et al.*, 1998). For this reason it has been suggested that postsynaptic KARs may play a role in the integration of excitatory input to enhance synaptic response of postsynaptic neurons (Frerking and Ohliger-Frerking, 2002).

The location and involvement of KARs in postsynaptic transmission in other areas of the CNS may suggest a similar scenario occurs in the SSC, with the heterogeneous responses obtained being a product of the limitations of the iontophoretic technique as discussed above.

10.1.3.b Modulation of presynaptic neurotransmitter release by GluR5

The results obtained from *in vivo* iontophoresis could be the result of presynaptic GluR5 modulating glutamate or GABA release.

Glutamate release

There is convincing evidence that GluR5-containing receptor assemblies are mainly concerned with the modulation of input into the brain rather than mediating synaptic transmission (Clarke *et al.*, 1997;Cossart *et al.*, 1998;Frerking *et al.*, 1998;Min *et al.*,

1999;Rodriguez-Moreno *et al.*, 1997;Semyanov & Kullmann, 2001). Furthermore, KAR involvement in the bi-directional modulation of excitatory synapses has been illustrated at MF-CA3 synapses in the hippocampus possibly via an autoreceptor that senses synaptically released glutamate (Lauri *et al.*, 2001; Schmitz *et al.*, 2001; Contractor *et al.*, 2000).

Investigations into the underlying mechanisms of the bi-directional quality of KAR-mediated responses have led to the following suggestions. The activation of KA-autoreceptors may cause an afterdepolarisation resulting in a transient increase in intracellular Ca^{2+} and thus increasing glutamate release probability with subsequent stimuli (Kamiya *et al.*, 2002). No link, however, has yet been found between these two events.

In contrast, the reduction of glutamate release may be due to presynaptic KARs. The activation of these receptors has been shown to reduce transmitter release via the reduction of presynaptic Ca^{2+} influx (Kamiya & Ozawa, 2000). The reduction of the Ca^{2+} conductance may come about from the inactivation of axonal Na^{+} channels, shunting of the presynaptic membrane or as a result of Ca^{2+} channel inactivation by membrane depolarisation (Kamiya & Ozawa, 2000).

This may explain the observation of both increases and decreases in visual response and habituation depending upon the receptor location at the synapses affected by the ejected antagonist. If the antagonist affects an autoreceptor this may lead to reduction of glutamate release and frequency-dependent facilitation, whereas the block of presynaptic receptors may well result in the enhancement of synaptically released glutamate.

Another possibility could be that KARs, which gate cation channels, would cause a reduction in neurotransmitter release if a KAR-mediated presynaptic depolarisation caused the inactivation of K^+-channels. This may result in the subthreshold depolarisation of the neuron terminal resulting in the enhancement of neurotransmitter release. Therefore, antagonism of presynaptic KAR activation would again result in the reduction of KAR-mediated glutamate release, thereby reducing frequency-dependent facilitation.

In addition, increases in visual response during drug ejection may be due to the reduction of glutamate release at the retinal ganglion cell (RGC)–interneuron synapse, which would result in the reduction of GABAergic transmission at the interneuron-SSC neuron synapse. The converse result (i.e. a reduction of visual response) could also be due to antagonism of KARs reducing glutamatergic transmission at the RGC-SSC neuron synapse. Also the reduction of glutamatergic transmission at this synapse may cause a reduction in habituation. This would be owing to the disinhibition of the SSC neuron as a result of decreased activation of inhibitory interneurons.

Both the reduction in visual response and increase in habituation may be attributed to a rise in glutamate release; blocking presynaptic KARs could relieve the inhibition of glutamatergic transmission that they may induce at the RGC-interneuron synapse. Such an action may produce a decrease in visual response due to the greater stimulation of GABAergic transmission at the interneuron-SSC neuron synapse. The effect on this pathway may also result in the observed increase in habituation. However, blocking GluR5

at the RGC-SSC neuron synapse may also increase glutamatergic transmission to produce an increase in visual response.

GABA release

Previous work has shown that GluR5 is involved in the inhibition of IPSCs (Clarke *et al.*, 1997; Clarke & Collingridge, 2004). It was thought that this reduction of IPSPs was a presynaptic phenomenon via an indirect mechanism, i.e. an increase in GABA release causing the activation of a presynaptic $GABA_B$ autoreceptor and the activation and subsequent desensitization of a postsynaptic $GABA_AR$ (Frerking *et al.*, 1999). This would result in the shunting of the postsynaptic cell (Frerking *et al.*, 1999). However, KA has been shown to reduce IPSPs via a GABA-independent mechanism (Fisher & Alger, 1984). In addition, the GluR5 agonist, ATPA, reduces IPSPs in the presence of either $GABA_AR$ or $GABA_BR$ antagonists (Clarke *et al.*, 1997; Clarke & Collingridge, 2004). This suggests that the activation of KARs results in the direct reduction of GABAergic transmission and not via the modulation of GABAergic transmission by synaptically released GABA (Clarke *et al.*, 1997; Clarke & Collingridge, 2004). This evidence also indicates that the activation of KARs may cause the reduction of GABA release via a heterosynaptic pathway or by direct actions on presynaptic terminals.

Further work has indeed suggested the existence of two populations of KARs at hippocampal interneurons the activation of which may be dependent on different glutamate concentrations (Rodrigeuz-Moreno *et al.*, 2000). The study proposes that low glutamate concentrations activate presynaptic KARs which reduce GABA release, whereas

somatodendritic KARs (Devries & Schwartz, 1999) may increase interneuronal firing (Rodrigeuz-Moreno *et al.*, 2000; Fisher & Alger, 1984).

Thus the action of the GluR5 antagonist on SSC neuron activity may be dependent on which population of KARs are blocked. For this reason it is possible that the GluR5 antagonist, LY382884, may increase GABAergic transmission by acting at presynaptic KARs at the interneuron-SSC neuron synapse and result in the reduction of visual response. An increase in habituation may also be the result of an increased GABAergic transmission at the interneuron-SSC neuron synapse.

Alternatively KAR activation resulting in GABAergic transmission facilitation (Cossart *et al.*, 2001; Mulle *et al.*, 2000) may be due to the activation of somatodendritic receptors (Devries & Schwartz, 1999; Kidd & Isaac, 1999). It is therefore, possible that blocking these KARs would reduce facilitation of GABAergic transmission at the interneuron-SSC neuron synapse. The resulting reduction of GABA release may subsequently lead to an increase in visual response. A reduction of habituation may also be due to the reduction of GABAergic transmission at the interneuron-relay cell synapse.

10.1.4 ATPA response

ATPA was applied iontophoretically with the aim of selectively activating GluR5. The subsequent application of the antagonist LY382884 would then be expected to cause a reduction in this ATPA response. It is, therefore, surprising that LY382884 failed to reduce ATPA-evoked responses, however, it is also acknowledged that ATPA is able to activate

AMPARs (Clarke *et al.*, 1997). In light of this information, the ATPA-evoked response is in all probability mediated by AMPARs.

The failure to reduce or block neuronal response to ATPA could be the result of KARs already being deactivated due to their rapid deactivation kinetics to ATPA and the subsequent activation of AMPARs (Chittajallu *et al.*, 1999). Alternatively the responses to ATPA ejection may have been solely mediated by AMPARs; as the exact concentration of drug ejected is not possible to determine, ATPA may have been ejected at a high enough concentration to activate AMPARs.

Experiments where no difference between control and experimental conditions were observed may lead to the suggestion that GluR5 receptors are not involved in direct synaptic transmission between the RGCs and cells of the SC. This could indeed be the case, however, other possibilities exist; a lack of effect of LY382884 on habituation and visual response may be explained by a lack of GluR5 subunits or functional GluR5-containing receptors influencing the cells recorded or any GluR5 subunits present may be located too far from the electrode to be affected by the antagonist.

The lack of antagonist effect on AMPA and NMDA responses demonstrates that LY382884 ejection currents were not great enough to cause a non-specific effect on all ionotropic receptors; higher ejection currents were previously shown to do so (data not shown).

10.2 *In vitro* slice recording

To further characterise the location and possible mechanism of action of GluR5 in the SSC, whole-cell voltage clamp experiments were conducted in SSC slices.

Whilst isolating the structure from its native environment, and potentially removing the majority of connections from other brain regions, this approach is particularly advantageous for detailed characterisation of receptor function and local circuitry. This would therefore enable the question of pre- or postsynaptic GluR5 location to be addressed, because it removes the variability which is due to heterosynaptic activation.

10.2.1 Isolation of direct excitatory input and local inhibitory input into the SSC

As it is well known that SSC function is strongly influenced by extensive inhibitory circuitry involving local GABAergic interneurons (Mize, 1992), it was important to dissociate the inhibitory input from the excitatory input. This enabled the study of the relative contribution of GluR5 to both excitatory input and inhibitory circuits within the SSC.

To isolate the excitatory component of the cellular response within the SSC, $GABA_A$ and $GABA_B$ antagonists were included in the perfusion medium to abolish inhibitory input (figure 15A). This resulted in an isolated EPSC, which reflected stimulated input from the optic tract. Local inhibitory input was isolated by addition of a cocktail of AMPAR,

NMDAR and mGluR antagonists (figure 15B). This resulted in an isolated IPSC response, reflecting the contribution of local inhibitory interneurons.

10.2.2 ATPA reduces EPSC amplitude in dose-dependent manner

Initial studies of GluR5 in tissue slices used a low concentration of ATPA (1μM) as this has been shown to be GluR5-selective in the CA3 region of the hippocampus (Clarke *et al.*, 1997). However, administration of such a low concentration resulted in no observed effect on EPSC amplitude. It was therefore important to further investigate if this was a dose-dependent phenomenon. Application of 10μM ATPA resulted in a reduction of EPSC, but also resulted in a postsynaptic inward current. As previously described, KARs can be located postsynaptically (Castillo *et al.*, 1997). Therefore, it was prudent to investigate whether the postsynaptic inward current was due to the activation of postsynaptic KARs. In addition, ATPA has been shown to activate AMPARs (Clarke *et al.*, 1997), therefore differentiating between the generation of inward current and EPSC reduction was vital, to determine whether these effects were mediated by the same or different receptor groups.

In order to determine which receptor groups may be involved a five point dose-response curve was constructed (figure 17B). The effect upon inward current was also evaluated (figure 18). From this data it was apparent that higher doses of ATPA (5-10μM) were responsible for the generation of postsynaptic inward current. Although the non-competitive antagonist GYKI52466 may be used as a non-specific AMPAR/KAR antagonist (Palmer & Lodge, 1993; Donevan & Rogawski, 1993; Zorumski *et al.*, 1993), the compound can be used at concentrations selective for AMPARs, allowing investigation

of KAR-mediated activity. Therefore, to exclude AMPAR activity, it was essential to include GYKI52466 in the perfusate. Inclusion of GYKI52466 abolished the inward current, demonstrating that this effect caused by ATPA was mediated by AMPARs. These findings are in agreement with previous studies in the CA1 and CA3 region of the hippocampus, where ATPA reduces excitatory synaptic transmission which is not associated with the generation of an inward current (Vignes *et al.*, 1998). Also in agreement with the current data the application of ATPA concentrations higher than 2μM resulted in an inward current (Vignes *et al.*, 1998).

In view of the fact that ATPA was originally synthesized as a selective agonist for AMPARs (Lauridsen *et al.*, 1985), it is perhaps not surprising that ATPA displays activity at AMPARs. What is unexpected is the concentration at which ATPA appears to activate AMPARs in this study. In HEK293 expression systems a >6000-fold selectivity for KARs over AMPARs is observed (Clarke *et al.*, 1997). In contrast, in this study using a native tissue preparation, effects of ATPA at AMPARs required only a 2.5-fold increase in ATPA concentration.

10.2.3 ATPA reduces EPSC amplitude both with and without AMPAR block by GYKI52466

3μM ATPA reduces EPSC amplitude by a similar percentage both in the absence and presence of GYKI52466 in the perfusate medium. The reduction of responses during AMPAR block indicates that the lower concentrations of 2 and 3μM ATPA reduce EPSC amplitude solely by the activation of GluR5 KARs (figure 20).

In the CA1 region of the hippocampus the activation of GluR5 appears to directly suppress inhibitory transmission (Clarke *et al.*, 1997). However, research both in this and other areas of the central nervous system have revealed that the activation of KARs and GluR5 subunits in particular is able to reduce excitatory input. KARs appear to mediate a variety of actions including a presynaptic reduction of excitatory transmission within the hippocampus.

The activation of presynaptic KARs with the application of a higher dose of kainate (3μM) is able to cause a reduction of excitatory transmission at the mossy fibre-CA3 (MF-CA3) synapse (Kamiya & Ozawa, 2000). The decrease in excitation is possibly the result of depolarisation of the presynaptic axon or terminal causing the inactivation of Na^+-channels which leads to the block of action potential conduction and Ca^{2+} influx (Kamiya & Ozawa, 2000). It was later shown that the presynaptic inhibition of excitatory transmission was GluR6-dependent (Contractor *et al.*, 2000), however, the use of ATPA in a subsequent study, indicated that GluR5 subunits may indeed contribute to the depression of excitatory transmission at the MF-CA3 synapse and in the CA1 region of the hippocampus (Vignes *et al.*, 1998).

10.2.4 ATPA reversibly reduces EPSC amplitude

Application of 2μM ATPA reduces EPSC amplitude (at a concentration that does not generate an AMPA-mediated inward current) (figure 18A), and as such this KAR-specific concentration was used in subsequent experiments.

10.2.5 ATPA causes an increase in paired pulse ratio

To investigate the location of the GluR5 receptor which causes the reduction in EPSC amplitude, a paired-pulse protocol was applied. By administering paired stimuli, a difference between primary and secondary responses is indicative of a change in release probability from the presynaptic terminal, indicating the activation of a presynaptic receptor. In contrast, no change in paired-pulse ratio would lead to the conclusion that the reduction in EPSC is due to a postsynaptic receptor. In this instance the protocol indicated that the activation of GluR5 receptors in the SGS caused an increase in paired-pulse ratio (figure 22), thus increasing the paired-pulse facilitation observed prior to ATPA perfusion.

It has been postulated that paired-pulse facilitation may occur at synapes when some Ca^{2+} binding sites associated with the release machinery are already occupied. This would reduce the requirement for further Ca^{2+} binding and also increase binding site affinity for Ca^{2+} at the same time (Thomson, 2000). Ca^{2+} entry may depend upon the following conditions: 1) the depolarisation characteristics which differentially activate populations of Ca^{2+} channels; 2) Synaptotagmin expression which functions as a possible Ca^{2+} sensor and primes release mechanisms during the initial presynaptic action potential, thus increasing the pool of vesicles ready for release by subsequent action potentials; 3) Fusion of these vesicles may be dependent upon the Ca^{2+} channels activated during depolarisation of the terminal.

It is possible that the activation of presynaptic GluR5 receptors may lead to enhanced Ca^{2+} entry into the synaptic terminal through activated channels. Thus the first depolarisation of the terminal during GluR5 activation may permit raised levels of Ca^{2+} entry leading to an enlarged pool of vesicles available and primed for exocytosis.

10.2.6 Locally evoked IPSCs remain resistant to ATPA administration

The administration of antagonists to all GluRs except KARs produces a locally-driven, isolated IPSC recorded in SSC neurons (figure 15B). This response provides a useful tool to determine possible KAR involvement in the modulation of inhibitory input to the SSC.

There is strong evidence to suggest that KARs (Rodriguez-Moreno *et al.*, 1997;Cossart *et al.*, 2001) and more specifically GluR5 receptors (Clarke *et al.*, 1997;Braga *et al.*, 2003) can be located on GABAergic interneurons and at GABAergic terminals to modulate interneuron activation and modulate GABAergic transmission. However, it is clear from the results observed with local stimulation of IPSCs that KARs are not located at sites on inhibitory interneurons in the SSC. The lack of ATPA-mediated effect on IPSC amplitude (figure 23) indicates that KARs do not influence local GABAergic transmission in the SSC.

10.2.7 ATPA reversibly reduces compound EPSC/IPSC responses

It is known that retinal ganglion cells projecting to the SSC are able to produce excitatory responses followed by a $GABA_A$-mediated inhibitory response in the SGS neurons (Lo *et al.*, 1998). Figure 24A illustrates the compound response recorded in SGS cells. The

application of ATPA to the compound EPSC/IPSC causes a reduction of both components (figure 24B and C). These results provide evidence that not only are GluR5 receptors located on direct excitatory retinal input to SGS neurons, they also may indirectly effect inhibitory input. The previous set of experiments demonstrates that GluR5 receptors are not involved in inhibitory transmission (see above), therefore, the observations from this later investigation strongly suggest that GluR5 receptors are present on excitatory input into the local inhibitory circuit.

10.2.8 Location of KARs determined by *in vitro* patch-clamp experiments

Taking into account the results from *in vitro* EPSC and compound EPSC/IPSC recordings it may be suggested that GluR5 is located presynaptically on excitatory retinal terminals (figure 30). Firstly the change in paired-pulse ratio indicates an alteration of vesicle release probability in the presynaptic terminal. Secondly it was observed that both the EPSC and IPSC components of the compound response were reduced during GluR5 agonist application. This would indicate that GluR5 receptors not only reduce excitatory transmission at inputs which drive responses in SSC neurons, but these receptors are also able to reduce glutamatergic transmission which drives inhibitory circuits within the SSC. Therefore, it may be reasonable to propose that GluR5 is primarily involved in the presynaptic modulation of glutamatergic transmission via the retinal input to the SSC.

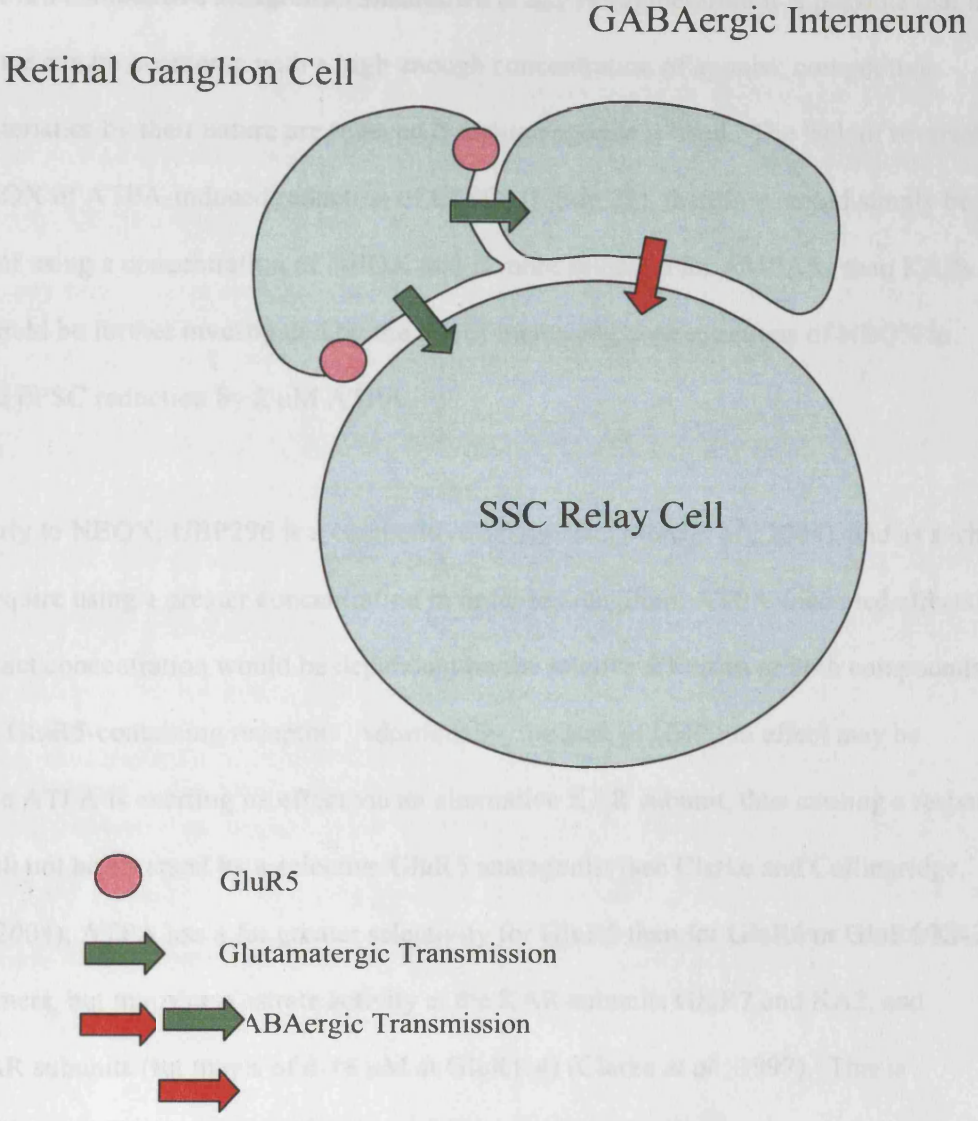

Figure 30. The representation of a serial synapse in the SSC. An increase in paired-pulse ratio seen during ATPA application indicates a change in neurotransmitter release probability. Therefore, one can conclude that GluR5-containing receptors can be localised to a presynaptic site at excitatory retinal input into the SSC.

10.2.9 Antagonist Pharmacology

NBQX is a competitive antagonist (Sheardown *et al.*, 1990) therefore it is possible that its influence can be overcome with a high enough concentration of agonist; competitive characteristics by their nature are reduced if enough agonist is used. The lack of reversal by NBQX of ATPA-induced reduction of EPSCs (figure 29), therefore, could simply be a result of using a concentration of NBQX that is more selective for AMPARs than KARs. This could be further investigated by the use of increasing concentrations of NBQX to reverse EPSC reduction by 2 μM ATPA.

Similarly to NBQX, UBP296 is a competitive antagonist (More *et al.*, 2004), and as such may require using a greater concentration in order to antagonise ATPA-mediated effects. The exact concentration would be dependent on the relative affinities of both compounds for the GluR5-containing receptor. Additionally, the lack of UBP296 effect may be because ATPA is exerting its effect via an alternative KAR subunit, thus causing a response that will not be reversed by a selective GluR5 anatagonist (see Clarke and Collingridge, 2002, 2004); ATPA has a far greater selectivity for GluR5 than for GluR6 or GluR6/KA2 heteromers, but may demonstrate activity at the KAR subunits GluR7 and KA2, and AMPAR subunits (K_i values of 6-14 μM at GluR1-4) (Clarke *et al.*, 1997). This is possibility should not be excluded and could be investigated using a combination of antagonists if and when they become available; the administration of an antagonist with a greater selectivity for the GluR7 or KA2 subunits along with the GluR5 antagonist may well reverse the effect thus indicating that the reduction of excitatory transmission is mediated by GluR7 or KA2. Thus far, however, there are no compounds which specifically

interact with GluR7 or KA2 as neither of these subunits are able to form functional homomeric receptors (Ozawa *et al.*, 1998).

Consequently, the pharmacology involved in this investigation may preclude GluR6-mediated activation, nevertheless, GluR7 or KA2 activation must still be considered. It is known that GluR7 subunits can form functional receptor assemblies (Schiffer *et al.*, 1997; Dingledine *et al.*, 1999) and have a higher affinity for ATPA than GluR6 subunits in recombinant expression systems (Clarke *et al.*, 1997). In addition, binding studies demonstrate that ATPA has a similar affinity for both GluR7 and AMPAR subunits (Clarke *et al.*, 1997). Although affinity for GluR5 subunits has been calculated as being 1000-fold lower than for AMPARs (Clarke *et al.*, 1997), it appears that this may not be the case in the SSC. The current investigation suggests that in the SSC slice preparation ATPA may have a greater affinity for AMPARs than that previously determined, therefore, it should not be assumed that affinity of ATPA for GluR7 subunits would be similar to reported values. In light of this, it would be prudent to further investigate KAR subunit configuration in SCC neurons perhaps using the combined approach of whole-cell voltage clamp recording and subsequent PCR investigation of subunit expression (Lambolez *et al.*, 1992).

10.2.10 Retinal input and recruitment of input

The *in vitro* data presented here strongly indicate the presence of functional, presynaptic KARs at retinal terminals in the SSC. Moreover, the role of these receptors appears to involve the reduction of glutamatergic transmission between RGC terminals and SSC neurons.

Using these findings, it is now possible to propose an explanation for the heterogeneous observations of the *in vivo* iontophoresis of GluR5 antagonist in the SSC. Firstly, data showing a reduction in visual response may be a result of the heterosynaptic increase of GABAergic transmission. This could occur as a result of blocking GluR5 receptors at retinal terminals which would increase glutamatergic transmission at the RGC-interneuron synapse and subsequently increase GABAergic transmission onto SSC neurons, causing a reduction in visual response.

Additionally a decrease in visual response due to antagonist ejection may be due to the increase in glutamatergic transmission from retinal input or descending cortical afferents of neighbouring receptive fields (RF2 in figure 31). Topographically mapped retinal afferents and cortical afferents from area 17/V1 of the visual cortex may be located at the same GABAergic interneuron (Rizzolatti *et al.*, 1974) which may influence centre-surround inhibition of an adjacent receptive field (RF) (Binns & Salt, 1997). Depending on whether neighbouring receptive fields are activated by the same stimulus and the antagonist has access to receptors at retinal or cortical afferents from these receptive fields it is possible that antagonist ejection may cause a reduction in visual response. This may occur because the antagonist causes an increase in glutamatergic transmission onto GABAergic interneurons mediating centre-surround inhibition thus increasing GABAergic input to the relay cell and decreasing excitation of the recorded cell.

The presence of topographically mapped cortical terminals at SSC neurons could also account for an increased visual response during antagonist administration. It is possible that GluR5 receptors may also be located at these excitatory terminals. The ejection of antagonist would cause an increase in excitatory transmission at these synapses and, therefore, an increase in visual response in the SSC neuron. An alternative and more straightforward explanation for the increases in visual response would be that they are due

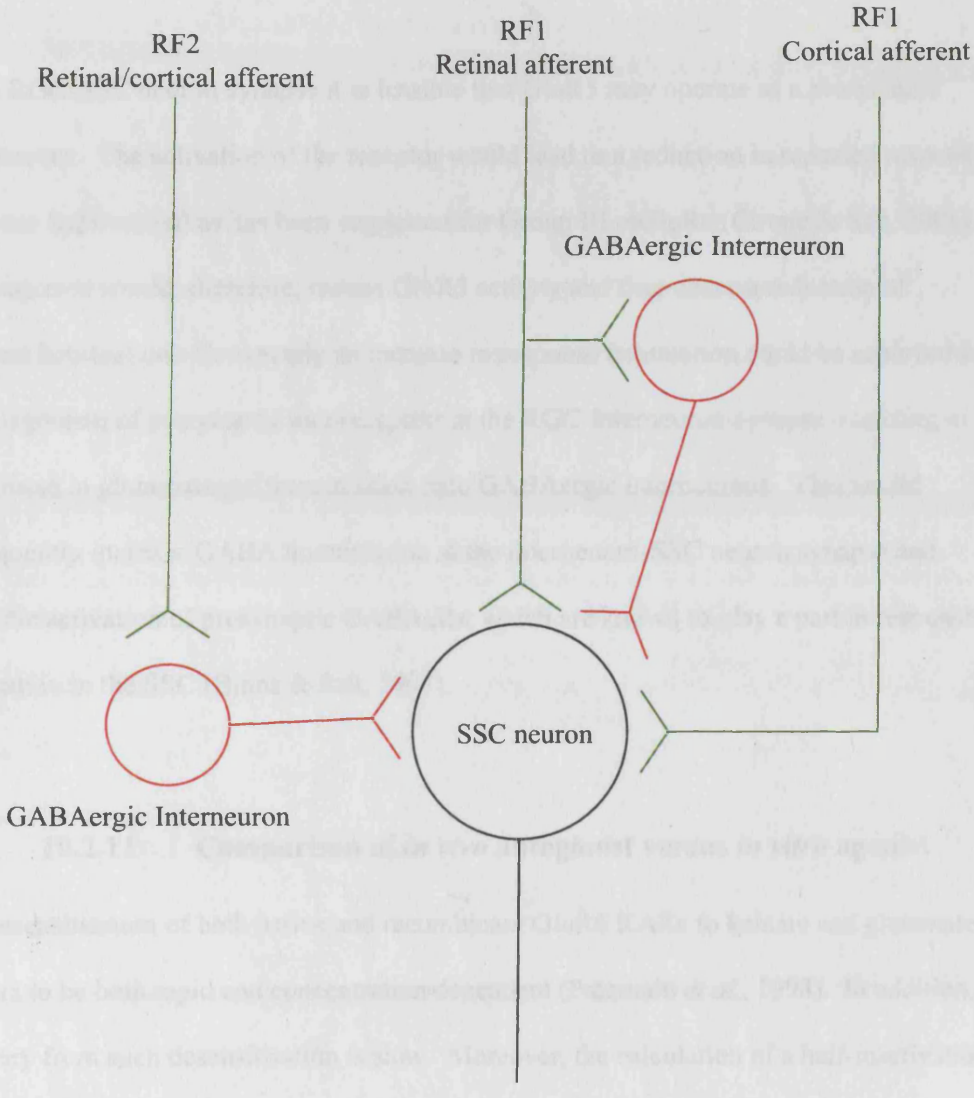

Figure 31. A circuit diagram illustrating the possible interactions between two adjacent receptive fields (RFs) in the SSC. Red represents GABAergic pathways, red represents glutamatergic pathways. Details of the network and consequences of activations are given in the text.

to the increase in glutamatergic transmission at the RGC-SSC neuron synapse during GluR5 antagonist ejection.

At the RGC-SSC neuron synapse it is feasible that GluR5 may operate as a presynaptic autoreceptor. The activation of the receptor would lead to a reduction in repeated responses (response habituation) as has been suggested for Group III mGluRs (Cirone & Salt, 2001). An antagonist would, therefore, reduce GluR5 activity and thus cause a reduction of response habituation. Conversely an increase in response habituation could be achieved by the antagonism of presynaptic autoreceptors at the RGC-interneuron synapse, resulting in an increase in glutamatergic transmission onto GABAergic interneurons. This would subsequently increase GABA transmission at the interneuorn-SSC neuron synapse and cause the activation of presynaptic $GABA_BRs$, which are known to play a part in response habituation in the SSC (Binns & Salt, 1997).

10.2.11 Comparison of *in vivo* antagonist versus *in vitro* agonist

The desensitisation of both native and recombinant GluR6 KARs to kainate and glutamate appears to be both rapid and concentration dependent (Paternain *et al.*, 1998). In addition, recovery from such desensitisation is slow. Moreover, the calculation of a half-inactivation value of 2.8μM coupled with *in vivo* extracellular glutamate concentrations of around 3μM (Lerma *et al.*, 1986), suggests that approximately 50% of KARs are desensitised in native tissue under physiological conditions. These data suggest that even small variations in glutamate concentration may alter the availability of KARs for activation; at times of exposure to higher concentrations of glutamate, such as during epileptiform activity or

ischemia (Attwell & Mobbs, 1994). This would have implications for the role of KARs in the modulation of neuronal excitability, such that at high glutamate concentrations KAR function may become compromised due to desensitisation thus curtailing normal KAR-mediated regulation of synaptic transmission.

The small overlap observed during activation-inactivation studies indicates the existence of a small 'window current' (Paternain *et al.*, 1998). This is a period of steady state receptor activation which is maintained providing the agonist concentration remains constant (Hille, 2001). The demonstration of this phenomenon for GluR6 activation-inactivation indicates that at physiological concentrations of ambient glutamate, it is entirely possible that a fraction of native KARs would remain activated. This would go some way to explain why in vivo iontophoresis of antagonist results in the alteration of responses, whereas *in vitro* preparations often appear to be antagonist-resistant, but antagonist may reverse agonist effects.

11 Conclusions

Taken as a whole the data presented here suggest that KARs are present in the rat SSC and, moreover, have a role in normal visual function.

Systemic application of GluR5 antagonist was found to produce a reduction in visual responses in the LGN. This reduction was independent of spot size or contrast of the stimulus, suggesting that GluR5 KARs do not contribute to the transmission of contrast sensitivity or centre surround information. Instead, GluR5s may play a gross modulatory role in responses recorded in the LGN. The possibility of a retinal GluR5 contribution to alterations in LGN responses was precluded, as recordings from the optic chiasm following systemic application of the antagonist remained unchanged.

Iontophoresis in the SSC demonstrated that GluR5 has a role in both visual processing and response habituation. However, due to the nature of microiontophoresis it was difficult to clearly classify the effects of the compound. Heterogeneous responses to antagonist application were observed in both visual responses and response habituation, and these effects were not seemingly correlated. The intrinsic circuitry of the SSC may be responsible for these varied results, with GluR5s located pre- or post-synaptically at glutamatergic or GABAergic terminals. Whilst GluR5s were clearly involved in visual processing in the SSC, their exact location and mechanism of action could not be determined.

Patch clamp studies using SSC slices further elucidated the role of GluR5 in synaptic transmission. Using an agonist at concentrations specific for the GluR5 subunit, a reduction

in excitatory transmission was observed for all neurons studied, in contrast to the heterogeneous responses seen *in vivo*. A paired-pulse protocol was used to determine pre- or post synaptic location. In all except one of these recordings an increase in paired-pulse ratio was observed following agonist application, strongly suggestive of a pre-synaptic location. Activation of GluR5 produced no effect on locally stimulated inhibitory responses, precluding an influence on GABAergic transmission in the SSC. However, stimulation of excitatory optic tract input could produce both excitatory and inhibitory responses in the same cell, indicative of direct excitation via glutamate and indirect inhibition via GABAergic interneurons. The GluR5 agonist reduced both components of this response, indicating that GluR5 is likely to be located on presynaptic retinal terminals contacting both excitatory projection neurons and inhibitory GABAergic interneurons.

In conclusion, this study is the first to demonstrate a role of GluR5 in visual processing in the rat SSC, and is suggestive of a role of GluR5 in the rat LGN.

12 Future Work

Future studies in this field should focus on the following objectives.

- Electron microscopy to confirm the presynaptic location of KARs in the rat SSC.
- *In vivo* iontophoresis and single-unit, extracellular recording in the LGN to characterise the involvement of KARs in this structure, with subsequent patch clamp studies to separate cortical and retinal input.
- Aspiration of cell contents following patch clamp recording, followed by single-cell PCR to confirm and identify the presence of KAR subunits (plus splice variants).
- HPLC on retinal tissue following intravenous application of LY382884 to determine whether the drug crosses the blood retinal barrier.
- Investigation into cellular mechanisms mediated by GluR5 in the SSC and LGN. Metabotropic mechanisms may be examined with the use of pertussis toxin (Gi/o inhibitor), staurosporine (a PKA/PKC inhibitor), or the more selective calphostin C (a PKC inhibitor). Ion channel involvement may be studied by manipulating Ca^{2+} or K^{+} concentrations.

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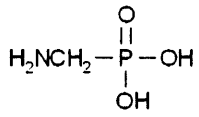
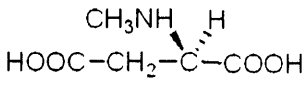
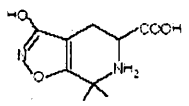
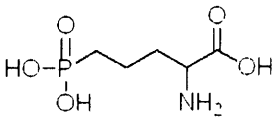
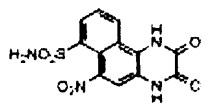
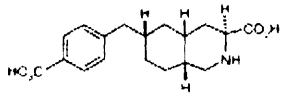
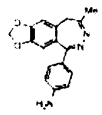
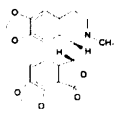
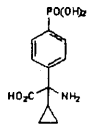
14 Appendix

Table 2. Pharmacological compounds used in the research

COMPOUND	SPECIFICITY	STRUCTURE
AMPA	AMPA agonist	
NMDA	NMDA agonist	
ATPA	GluR5 agonist	
AP-5	NMDA antagonist	

NBQX	AMPA/KAR antagonist	
LY382884	GluR5 antagonist	

DRUG	SPECIFICITY	STRUCTURE
GYKI 52466	AMPA antagonist	
Bicuculline	$GABA_A$ antagonist	
CPPG	Group III mGluR antagonist	
LY393053	Group I & II mGluR antagonist	

CPG55845	$GABA_B$ antagonist	