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The role of metabotropic glutamate receptors in the thalamocortical circuit during spontaneous epileptic activity

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This is to certify that the thesis prepared by Joy Vashisht Sharma entitled "The Role of Metabotropic Glutamate Receptors in the Thalamocortical Circuit During Spontaneous Epileptic Activity" has been approved by his committee as satisfactory completion of the thesis requirement for the degree of Master of Science.

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The role of metabotropic glutamate receptors in the thalamocortical circuit during spontaneous epileptic activity

A thesis submitted in partial fulfillment of the requirement for the degree of Master of Science at Virginia Commonwealth University

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List of Abbreviations

(1S, 3R)-ACPD	trans-(1S,3R)-1-amino-1,3-cyclopentanedicarboxylic acid
°C	degrees Celsius
μM	micromolar
μm	micron
(S)-MCPG	(S)-α-methyl-4-carboxyphenylglycine
AC	alternate current
Ach	acethylcholine
ACSF	artificial cerebrospinal fluid
CO_2	carbon dioxide
EEG	electroencephalograph
GA	generalized absence
gK^-	K^- ion conductance
GTC	generalized tonic-clonic
Hz	hertz
IEI	inter-event interval
I_{k-leak}	potassium leak current
LGN	lateral geniculate nucleus
LTS	low-threshold calcium spike
MΩ	mega ohm
mA	microamperes
Mg^{2-}	Magnesium ion
mGluR	metabotropic glutamate receptors
mm	millimeters
ms	milliseconds
n	sample size
Na^-	sodium ion
NE	noradrenaline
nM	nanomolar
NMDA	N-methyl-D-aspartate
nRT	nucleus reticularis thalami
O_2	oxygen gas
PI	phosphoinositide
REM	rapid eye movement

RMP	resting membrane potential
SWD	spike wave discharges
TC	thalamocortical
T-current	low-threshold calcium current
TTX	tetrodotoxin

ABSTRACT

THE ROLE OF METABOTROPIC GLUTAMATE RECEPTORS IN THE THALAMOCORTICAL CIRCUIT DURING SPONTANEOUS EPILEPTIC ACTIVITY

Joy Vashisht Sharma, B.S.

A thesis submitted in partial fulfillment of the requirement for the degree of Master of Science at Virginia Commonwealth University

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Spontaneous epileptic activity resembling spike wave discharges (SWD) characteristic of Generalized Absence (GA) epilepsy was induced in rat thalamocortical (TC) slices by incubating the slices in low-Mg^{2-} artificial cerebrospinal fluid (ACSF). Extracellular field potentials were recorded in the cortex and thalamus to determine the effects of the broad spectrum metabotropic glutamate receptor (mGluR) agonist, trans-(1S, 3R)-1-Amino-1, 3-cyclopentanedicarboxylic acid (ACPD). ACPD elicited concentration dependent effects on the duration of SWD. At 1 μM ACPD (n=3) there were no significant changes in duration of activity. At 20 μM, 11 of 13 slices displayed a decrease in duration of activity (mean = 40.47 ± 5.07). In all cases, 200 μM ACPD

(n=6) transformed SWD into single spike activity. Furthermore, the broad spectrum mGluR antagonist, (S)-α-Methyl-4-carboxyphenlglycine ((S)-MCPG), when pre-applied to TC slices at 500 μM followed by co-application with 200 μM ACPD (n=4), prevented SWD from switching into single spike activity.

To determine the site of action of the mGluR agonist, experiments involving regional perfusion of 200 μM ACPD to the cortex (n=3) and thalamus (n=4) were conducted. mGluR activation in the cortex was unable to block SWD, while perfusion of the mGluR agonist to the thalamus transformed SWD into single spikes on all occasions. mGluRs may provide an alternative therapeutic target for the pharmacological treatment of GA epilepsy.

INTRODUCTION

Classification and Definition of Epilepsy

Statistics show that at least one percent of the population either currently has or will develop epilepsy during their lifetime. The word epilepsy is derived from the Greek word *epilambanein* meaning "to seize" or "to attack" (Gross, 1992) and is clinically defined as the occurrence of recurrent spontaneous seizures. Seizures are the physical manifestation of an excessive and synchronous discharge of a hyperexcitable population of neurons in the brain. The type of seizure depends largely on the area of the brain which emits the abnormal electrical discharge. When the abnormal electrical discharge is confined to a particular focus of the brain, the seizure is classified as a *partial seizure*. If the firing originates in both hemispheres of the brain, the seizure is classified as a *generalized seizure*. Partial seizures can also become generalized if the discharge originating from the focus is strong enough to spread and recruit both hemispheres of the brain. This type of activity is termed secondary generalization.

Generalized epilepsy is subclassified into the generalized tonic-clonic (GTC) type and the generalized absence (GA) type. These differ in terms of clinical manifestation and etiology. GTC seizures consist of an initial tonic phase, in which the body becomes rigid and

the individual loses consciousness and may fall. This is followed by a clonic phase, in which the body develops strong jerking movements.

GA epilepsy, on the other hand, is a transient loss of consciousness with an abrupt onset and offset which is electrophysiologically characterized by electroencephalographic (EEG) recordings displaying bilateral symmetrical spike and wave discharges (SWD) at a frequency of 3-4 Hz (Gloor & Fariello, 1988). These seizures are typically non-convulsive, prominent in children (age four to adolescence) and may go unnoticed. This disorder interrupts perception, cognition, memory and renders the patient temporarily unresponsive to stimuli (Holmes, 1987). GA seizure events are also pharmacologically unique. The anti-epileptics ethosuximide and trimethadione, are effective in blocking GA seizures but are ineffective for other seizure types. On the other hand, the anti-epileptics phenytoin and carbamazepine which are effective in the treatment of GTC seizures actually exacerbate GA seizures. Evidence from animal models implicates the thalamocortical (TC) circuit in the generation of GA seizures (Prince & Farrell, 1969; Avoli & Gloor, 1982; Avoli et al., 1983; Gloor and Fariello, 1988).

Thalamocortical Circuit

Sensory and motor information is relayed to and from the cortex via thalamic nuclei. In addition to acting as a relay station, the thalamus also serves as a site for the gating of information as well as subcortical processing (Steriade & Llinas, 1988). The TC circuit

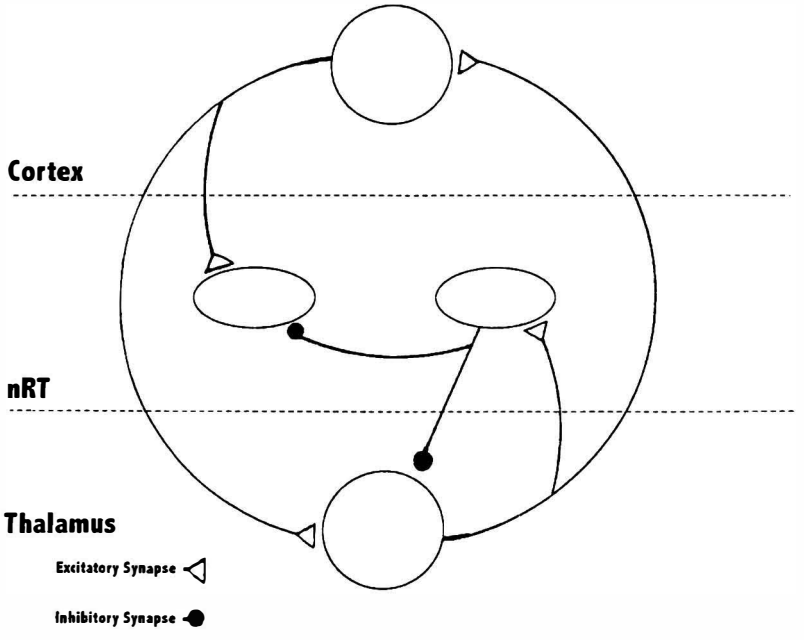
consists of thalamic nuclei which are specific to particular sensory modalities and which project primarily to their respective specific sensory cortices (Jones, 1985). The sensory cortices have reciprocal excitatory connections to the thalamic nuclei from which they receive their input. A thin sheet of GABAergic neurons, known as the nucleus reticularis thalami (nRT) are located between the cortex and thalamus adjacent to the thalamic nuclei. Excitatory axon collaterals from thalamic relay neurons and cortical pyramidal cells project to nRT neurons which in turn send inhibitory connections to the thalamic neurons from which they receive collateral excitation (Fig. 1).

The TC network exists in two main functional states which are dependent on the prevailing behavioral state of the brain (Steriade & Llinas, 1988). During the awake state and REM sleep, the circuit is in a tonic firing mode, relaying synaptic inputs to the cortex in a highly linear fashion. However, during slow wave sleep, activity in the circuit is switched to a rhythmic burst firing mode, altering transmission to the cortex. Rhythmic burst firing during slow wave sleep, otherwise known as sleep spindles, is a normal phenomenon occurring at a frequency of 7-14 Hz (in humans). However, at lower frequencies (3-4 Hz) it is considered pathological as in the case of GA epilepsy (Gloor & Fariello, 1988). Therefore, considerable focus has been placed on understanding how the TC circuit can generate and support rhythmic bursting activity and what regulates the functional state of this network.

The architecture of the reciprocal excitatory and inhibitory connections of the TC circuit, in conjunction with a low-threshold

Figure 1. Schematic of synapses important in thalamocortical rhythm generation. The thalamus sends excitatory projections to the cortex and receives excitatory feedback projections. Each of these pathways (thalamocortical and corticthalamic) sends an excitatory collateral projection to the GABAergic nucleus reticularis thalami (nRT). nRT in turn sends inhibitory projections onto thalamic neurons from which it receives collateral excitation, as well as reciprocal inhibitory synapses within nRT.

Figure 1


Cortex
nRT
Thalamus
Excitatory Synapse
Inhibitory Synapse

calcium current (T-current), predisposes the system to support phasic bursting activity (Steriade & Llinas, 1988; Coulter et al., 1989; McCormick, 1992). Thalamic relay neurons are known to generate three types of rhythmic activity: Intrinsic rhythmic bursting (0.5-3 Hz); sleep spindles (7-14 Hz); and absence seizures (3-4 Hz) (McCormick, 1992). While the slow rhythmic burst firing (0.5-3 Hz) has been demonstrated to be an intrinsic property of thalamic relay neurons, sleep spindles and absence seizures arise from interaction between thalamic relay neurons, nRT cells and cortical cells within the TC circuit (McCormick, 1992).

The T-current is critical to the generation of bursting activity and while it is not limited to thalamic relay neurons, it appears to be prominent in these cells (Coulter et al., 1989). At the resting membrane potential (RMP) of thalamic cells (-65 to -70 mV) the T-current is inactive. To de-inactivate this current, thalamic cells must be hyperpolarized below the RMP. This occurs *in vivo* in response to inhibitory postsynaptic potentials from GABAergic nRT neurons. Hyperpolariztion de-inactivates the T-current, generating a low-threshold calcium spike (LTS). The calcium influx in turn depolarizes the cell past the threshold for sodium-dependant action potentials, leading to bursting activity.

The transition from rhythmic burst firing in slow wave sleep to tonic firing activity during waking or REM sleep is achieved via depolarization of the RMP of thalamic relay neurons by 10-20 mV (Hirsch et al., 1983; Steriade & Llinas 1988). Depolarization inhibits

neuronal burst firing through inactivation of the T-current, although synaptic potentials are still able to elicit single or trains of action potentials. Activation of ascending brainstem and hypothalamic inputs to thalamic neurons has long been assumed to underlie these changes in thalamic excitability and the transition of rhythmic oscillations from burst to tonic firing mode (Uhlrich et al., 1990). These afferent projections to thalamic relay neurons use the neurotransmitters acethylcholine (Ach) and noradrenaline (NE). Increased release of Ach and NE causes a slow depolarization of thalamic relay neurons via block of a potassium leak current ($I_{k\text{-}leak}$) (McCormick & Prince 1987, 1988; McCormick 1992). This slow depolarization of the RMP inactivates the T-current, while depolarizing the RMP toward single-spike firing threshold facilitating the transfer of incoming action potentials to the cerebral cortex. Although the vast majority of studies implicate these ascending activating systems from the brainstem and hypothalamus in controlling thalamic excitability, the major input to thalamic relay cells is from layer VI cells of the cerebral cortex (Montero, 1991). This suggests that this feedback pathway may also be important in TC activity.

Corticothalamic connections modulate synaptic processes and transmission of afferent inputs through the thalamus and are particularly critical in the generation of SWD of GA seizures. Although spindle discharges can occur in decorticate animals, SWD of GA seizures require functional connectivity between the cortex and thalamus (McCormick, 1992; Vergnes and Marescaux, 1992; Coulter & Lee,

1993). Physiological, pharmacological, and immunocytochemical data have demonstrated that corticothalamic projections are glutamatergic, acting mainly via the N-methyl-D-aspartate (NMDA) and metabotropic glutamate receptors (mGluR) (Fonnum, 1981; Deschenes & Hu, 1990; Martin et al., 1992; McCormick & von Krosigk, 1992; Eaton & Salt, 1996).

McCormick and von Krosigk (1992) have shown that high frequency stimulation (typically 50 Hz) of the corticothalamic projections results in prolonged excitatory postsynaptic potentials in thalamic relay cells due to a reduced K^- conductance (gK^-). This reduction in gK^- is mediated through the activation of mGluRs. This results in the depolarization of the RMP of thalamic relay neurons just as the activation of ascending projections from the brainstem and hypothalamus. Both can block the rebound burst firing that is present during slow wave sleep and GA epilepsy. They can also promote tonic firing, a state of thalamic activity associated with enhanced sensory transmission and arousal.

Metabotropic Glutamate Receptors (mGluRs)

Unlike ionotropic glutamate receptors, which depolarize neurons via entry of cations through their channels, mGluRs mediate their effects through GTP binding proteins. At least eight mGluRs have been identified which can be placed into three groups, Group I, II, and III, on the basis of sequence homology, pharmacology, and activation of intracellular signaling systems (Nakanishi, 1992). Group I includes

mGluR1, mGluR5, and their splice variants. These receptors stimulate phospholipase C and phosphoinositide (PI) hydrolysis. In hippocampal CA1 and CA3 pyramidal cells activation of Group I mGluRs results in a decrease in gK^- (Charpak et al., 1990; Shirasaki et al., 1994). However, it is not clear whether this is mediated by direct coupling of the G-protein to a K^- channel or is secondary to activation of PI hydrolysis. Group II consists of mGluR2 and mGluR3 and Group III consists of mGluR4, mGluR6, mGluR7, and mGluR8. These two groups are negatively coupled to adenylyl cyclase and are most likely located at presynaptic terminals. They act as autoreceptors to reduce glutamate release (Salt & Eaton, 1995, 1996).

In situ hybridization studies show prominent expression of mGluR1 within the thalamus (Fotuhi et al., 1993). Martin et al. (1992) have located mGluR1 on the dendrites of thalamic relay neurons and nRT cells. mGluR3 mRNA is highly expressed in nRT cells and Salt and Eaton (1995, 1996) have suggested that these receptors may function presynaptically on the terminals of GABAergic nRT cells projecting onto thalamic relay neurons. The disinhibitory actions of specific mGluR agonists is blocked by application of the broad spectrum mGluR antagonist (S)-α-Methyl-4-carboxyphenylglycine ((S)-MCPG; active enantiomer).

Activation of mGluR1 on thalamic relay neurons results in a decreased gK^-. This leads to a prolonged depolarization which in turn inactivates the T-current and switches the circuit from burst phase to tonic firing phase. Application of the non-specific mGluR agonist

trans-(1S, 3R)-1-amino-1, 3-cyclopentanedicarboxylic acid (ACPD; active on Group I and II) onto the TC circuit should block burst firing through its action on thalamic relay neurons and cortical cells. nRT neurons possess mGluR1 on their dendrites and presynaptic Group II mGluRs on their terminals which inhibit GABA release. ACPD has opposing effects on these cells. Although nRT cells are slowly depolarized by mGluR1, activation of their presynaptic mGluR results in decreased neurotransmitter release. Activation of some cortical pyramidal cells, such as the burst generating neurons of layer V by ACPD results in a prolonged depolarization (Wang & McCormick; 1991). In burst generating neurons, prolonged depolarization results in a shift from rhythmic burst firing mode to single spike activity.

TC Rhythm Generation: Low Magnesium Model

Connections between the cortex and thalamus can be preserved in an *in vitro* brain slice preparation from rodents using the slice angle described by Agmon and Connors (1991). In these TC slices, incubation with low-Mg^{2-} results in rhythmic burst firing. This rhythmic burst firing is due to the release of glutamate following unblocking of the NMDA receptor by Mg^{2-} (Coulter & Lee, 1993). Spontaneous TC rhythms in this *in vitro* model are of comparable frequency, duration, anatomical distribution, and pharmacology to *in vivo* SWDs in rodent genetic models of GA epilepsy (Coulter & Lee, 1993; Zhang et al., 1996). Rodent sleep spindles are 10-15 Hz in frequency and SWDs occur at a frequency of 5-10 Hz in genetic models

of GA epilepsy (Buzsaki, 1991). This is the first model to preserve TC rhythm generation *in vitro*. Therefore it represents a valuable model to study the physiology and pharmacology of GA epilepsy (Coulter & Lee, 1993).

Central Hypothesis of Thesis

Previous studies have demonstrated the effects of ACPD on *individual* thalamic relay neurons, nRT, and cortical cells. However, the effects of the broad based mGluR agonist on the intact TC circuit have not been characterized. It is hypothesized here that the activation of mGluRs by ACPD in the TC circuit will block epileptic burst firing activity.

MATERIALS AND METHODS

Slice Preparation

Male Sprague-Dawley rats (50-70 days postnatal) were anesthetized with halothane. Animals were then decapitated, and the brain removed and placed into chilled (4°C), oxygenated (95% O_2/5% CO_2) dissection medium. Dissection medium was composed of artificial cerebrospinal fluid (ACSF), with the NaCl replaced isoosmotically with sucrose. Sucrose was used to prevent acute Na^+-dependent toxicity resulting from the dissection process (Rasmussen & Aghajanian, 1990).

Thalamocortical slices were cut using the slice angle described by Agmon and Conners (1991). The cerebellum was blocked off and the brain was placed on a 10° ramp, rostral side facing down and rotated 55° from a parasagital plane. At this angle a vertical cut was made through the tissue with the tissue rostral to the cut discarded. The remaining tissue was glued to the stage of a vibratome (Lancer 1000, TPI, St. Louis, MO) with the cut surface down. An agar block was glued behind the brain for stability and then the brain was immersed in cold sucrose based ACSF. The first few slices were removed and discarded. When the approximate location of the thalamocortical slices was reached, 450 μm slices were collected. To confirm thalamocortical connections, slices were viewed under a microscope at the time of

dissection. Two to three slices per rat showed axonal bundles from the thalamus traversing the striatum up to the cortex (Fig. 2). These slices were transferred to an incubator (32°C) and submerged in oxygenated ACSF that contained no added Mg^{2+}. The ACSF was composed of (in mM): NaCl, 130; KCl, 3; NaH_2PO_4, 1.25; CaCl, 2; $NaHCO_3$, 26; and glucose, 10. Slices were incubated in low-Mg^{2+} ACSF medium for ≥ 2 hours.

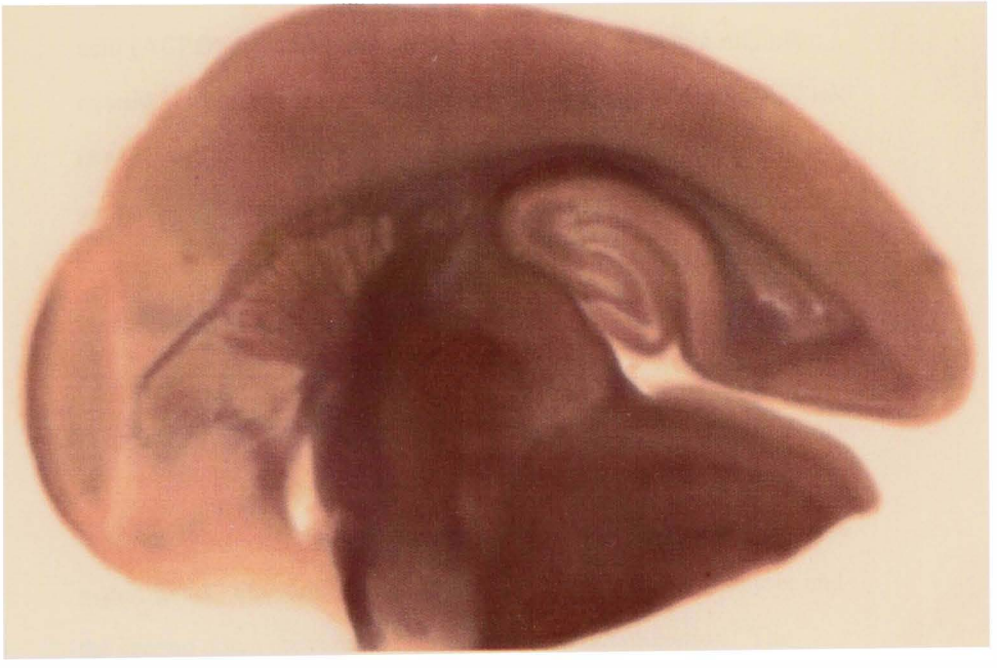
Extracellular Field Potential Recording

Prior to recording, slices were transferred to an interface chamber, where they were perfused with warmed (35°C) low-Mg^{2+} ACSF at 1 ml/min. Differential AC-coupled extracellular recordings at a gain of 1000 were conducted using 2 MΩ insulated tungsten electrodes (bandpass filtered at 10 - 5000 Hz). Recording electrodes were placed in the thalamus and cortex. Data was displayed during experiments on a four channel chart recorder (AstroMed Dash IV, Warwick, RI) and amplified signals were digitized (Digital Data Recorder, VR-10B, Instrutech Corp., Elmont NY) and recorded onto videotape for later analysis.

Experimental Protocol

Agonists and antagonists for mGluR's were bath applied. A minimum 30-min control period of stable activity was recorded, followed by a 20-min period of drug exposure, and then a wash for ≥ 30-minutes. Trans-(1S, 3R)-1-Amino-1, 3-cyclopentanedicarboxylic

Figure 2. Photograph of a rat thalamocortical slice. Note the continuous axonal bundles traversing the striatum interconnecting the cortex and thalamus.



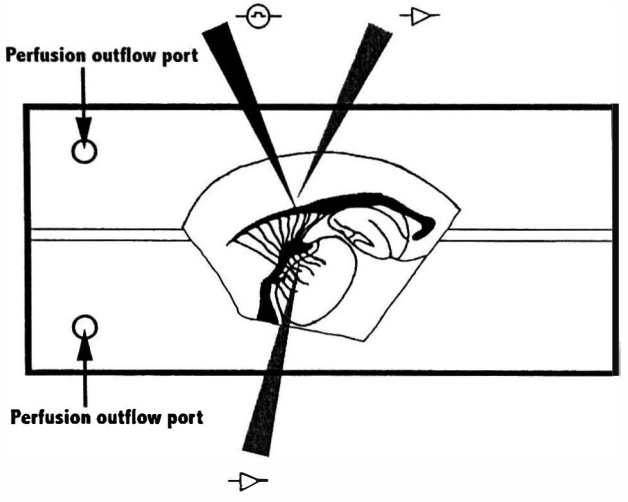
acid (ACPD) the active enantiomer of the broad spectrum mGluR agonist (Group I & II) was applied to TC slices at 1 μM (n=3), 20 μM (n=13), and 200 μM (n=6). (S)-MCPG, a broad based mGluR antagonist (Group I & II) was bath applied at 500 μM (n=4) alone and co-applied with 200 μM ACPD to determine the effects of these drugs on spontaneous rhythmic bursting in the TC circuit.

Regional Perfusion Experiments

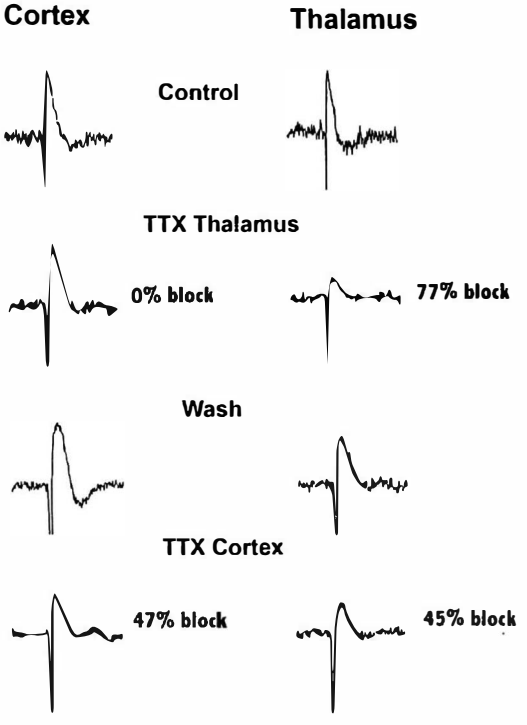
The interface recording chamber (Hass Chamber, Medical Systems Inc., New York, NY) was modified to support dual perfusion experiments. One drug flowed down the left side of the chamber and the second down the right without any cross contamination. The apparatus consisted of a thin piece of plexi-glass divided by teflon tubing (0.008" diameter) glued (silicone based glue) to the middle that separated the two perfusion mediums. A small opening was drilled at one end of each chamber and aligned with the perfusion outflow ports. This apparatus was designed to fit on top of the existing brain slice chamber (Fig. 3A). TC slices were trimmed at the time of dissection so they could be placed on this apparatus with the cortex on one side of the tubing and the thalamus on the other. To validate separation of cortical and thalamic perfusing mediums electrical stimuli pulses (100 μs in duration, 200-600 μA) were delivered via a pair of insulated tungsten electrodes with their tips glued approximately 150-200 mm apart. Bipolar stimuli where delivered in the white matter of the

Figure 3. Regional perfusion apparatus and validation. **A:** Schematic of regional perfusion experiment apparatus, indicating the position of the stimulating and recording electrodes for traces in B. Note the two separate perfusion outflow ports and the trimming of the thalamocortical slice so it could be placed on the apparatus with the cortex on one side of the tubing and the thalamus on the other. **B:** Extracellularly recorded traces in normal Mg^{2+}-containing medium illustrating the response of both cortex and thalamus to stimulation as shown in A. *Control*. Note response in both cortex and thalamus to stimulation. *TTX Thalamus*. Note large decrease in thalamic response but no change in cortical response. *Wash*. Note recovery of thalamic response after wash. *TTX Cortex*. Note large decrease in both cortical and thalamic response.

A.


Perfusion outflow port
Perfusion outflow port

B.


Cortex
Thalamus
Control
TTX Thalamus
0% block
77% block
Wash
TTX Cortex
47% block
45% block

somatosensory cortex to activate cortical and thalamic neurons (Fig. 3B). The thalamus was then perfused with 1 μM tetrodotoxin (TTX). Stimulation demonstrated a block of the thalamic but not cortical response. After ≈30 minute wash the thalamic response was recovered. The cortex was then perfused with TTX and both thalamic and cortical responses to stimulation were abolished. To determine the location of ACPD action 200μM ACPD was selectively perfused in the cortex (n=3) or thalamus (n=4) during extracellular field potential recordings.

RESULTS

Although cortical and thalamic burst activity is tightly coupled, cortical bursts were used to analyze the data because these signals are stronger due to higher cell density and laminar distribution of neurons (Coulter & Lee, 1993). The duration measure also reflected the duration of thalamic burst complexes, since cortical and thalamic activity is tightly coupled. Average duration and inter-event time interval (IEI) of bursting events were quantified for the last five events prior to agonist/antagonist solution change and compared to the last five events during drug application. Following washout of the drug, measurements were taken as described above.

Bath application of the broad spectrum mGluR agonist, ACPD to TC slices demonstrated concentration dependent effects on the duration of spontaneous epileptic activity (Fig. 4). All results are reported as mean percent change (± standard error) in activity unless otherwise stated. At 1 μM concentration ACPD (n=3) was ineffective at blocking SWD (Fig 5A). However, small non-significant decreases were seen in the duration of bursting activity (-0.6 ± 3.2) and inter-event interval (IEI, -0.8 ± 1.3).

At 200 μM concentration ACPD (n=6) transformed SWD into single spike activity. The duration of SWD ranged from 3.8 to 17.2

Figure 4. Concentration dependent effects of ACPD on duration of spike wave discharges.

Figure 4

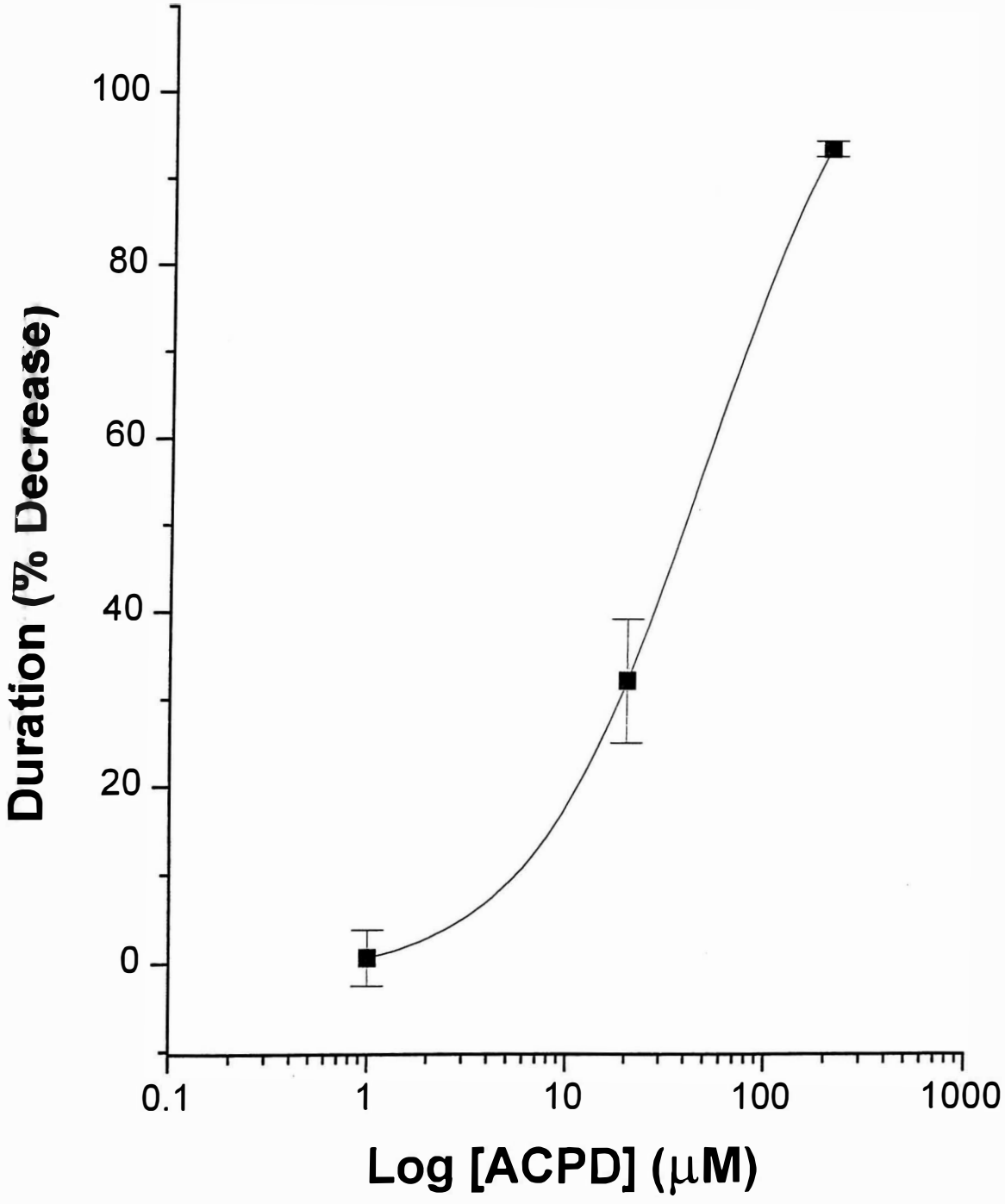
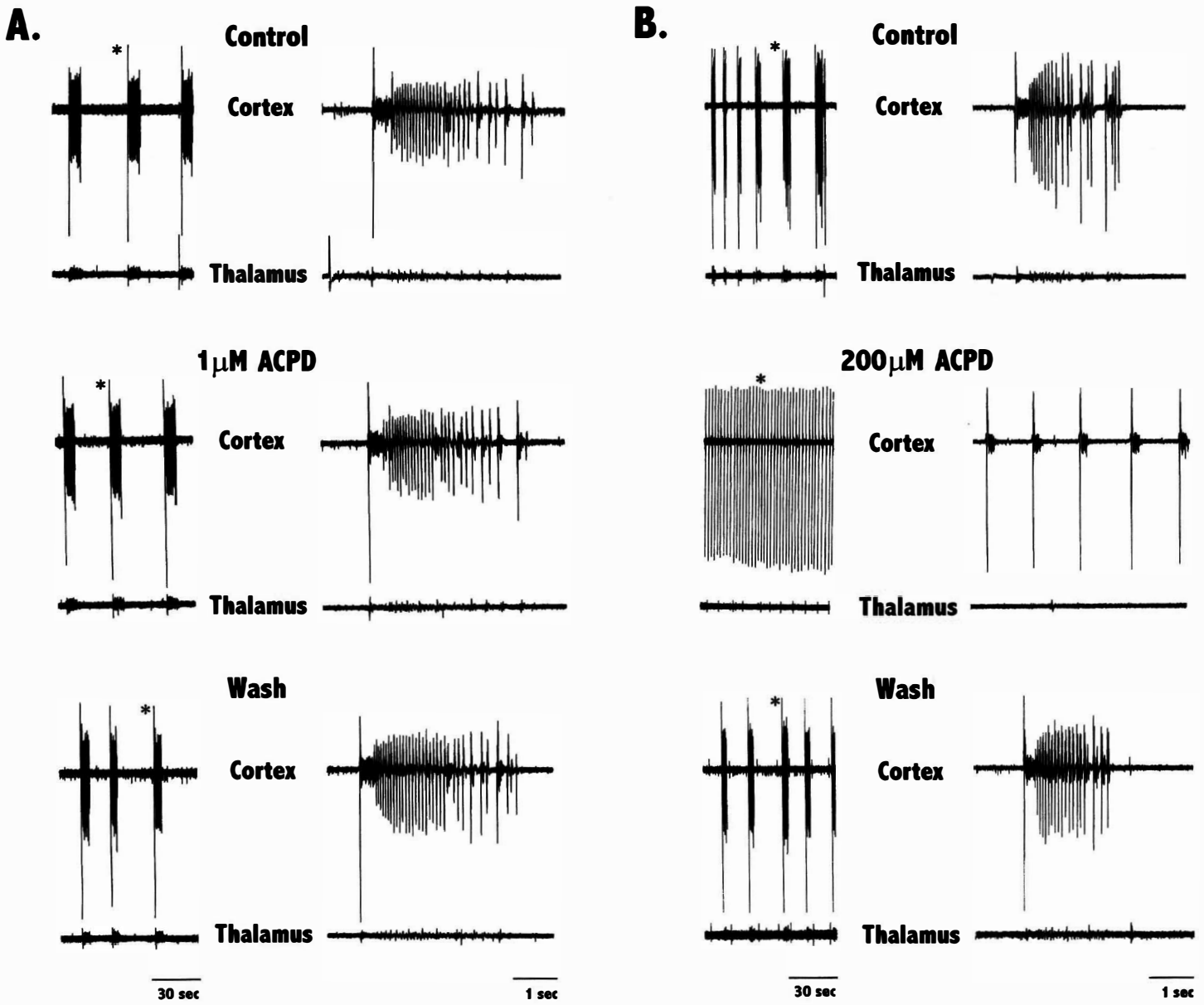


Figure 5. ACPD effects on spontaneous generalized spike wave discharges (SWD). **A:** Effects of 1 μM ACPD on SWD. *Control.* SWD in low-Mg^{2+} ACSF recorded in cortex and thalamus. In A and B, the asterisk in the slower sweep to the left is the discharge expanded on the right. *1 μM ACPD.* Effects of 1μM ACPD on these SWD. Note this concentration had no effects on SWD. **B:** Effects of 200 μM ACPD on SWD in a different thalamocortical slice. *Control.* SWD in low-Mg^{2+} ACSF recorded in the cortex and thalamus. *200 μM ACPD.* Effects of 200 μM ACPD on these SWD. Note activity switch to single spikes and decrease in inter-event time interval. *Wash.* Recovery from effects of ACPD. Note return of SWD and increase in inter-event time interval demonstrating reversible effects of ACPD.

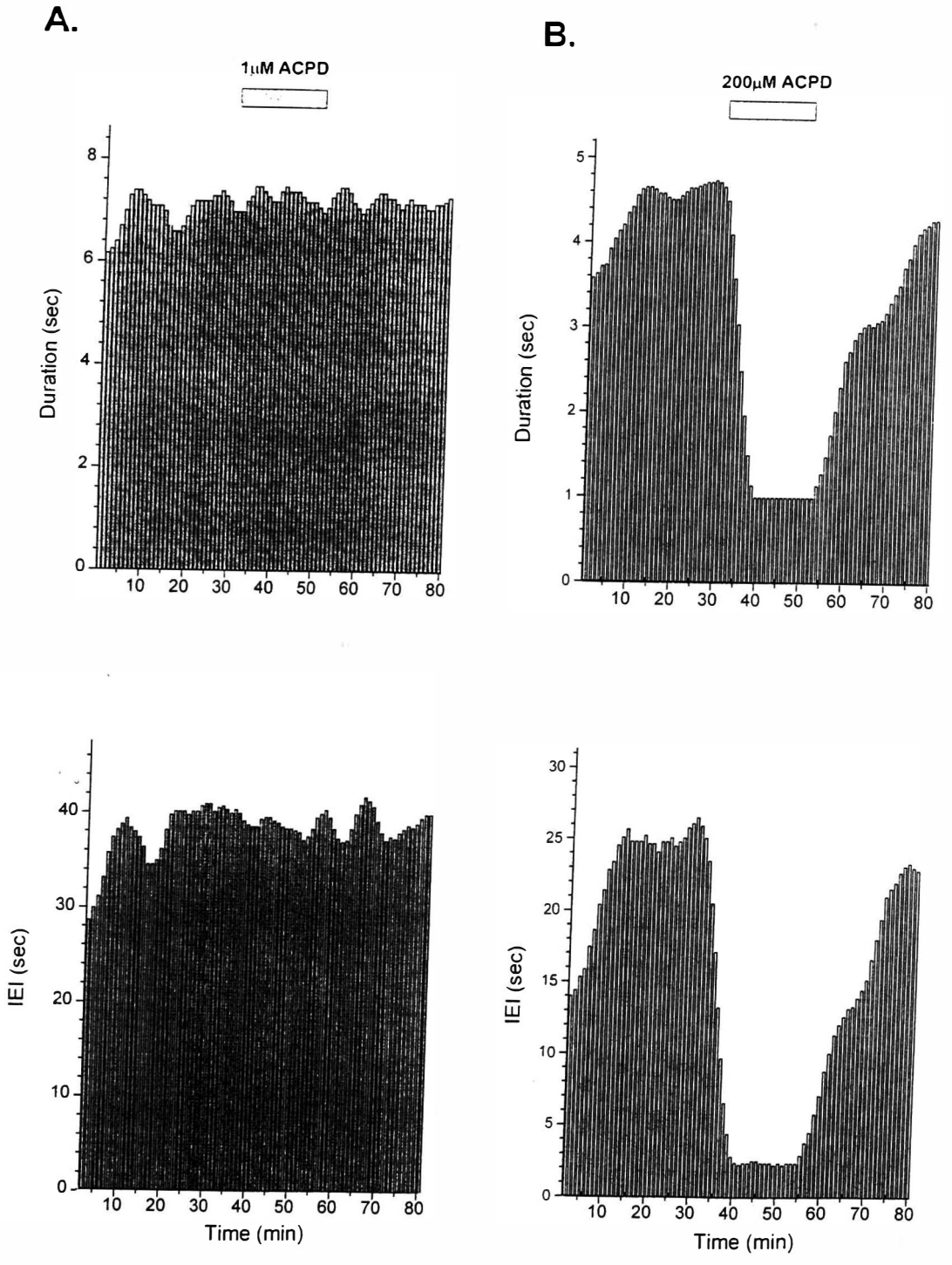

A.
Control
Cortex
Thalamus
1μM ACPD
Cortex
Thalamus
Wash
Cortex
Thalamus
30 sec
1 sec
B.
Control
Cortex
Thalamus
200μM ACPD
Cortex
Thalamus
Wash
Cortex
Thalamus
30 sec
1 sec

seconds in these six slices. In all slices activity switched from rhythmic burst firing to single spikes (Fig. 5B), which returned to SWD following the wash out of ACPD (range was 17.4 to 92.1%). On one occasion 200 μM ACPD was re-applied after wash and the burst firing switched to single spikes. Change in duration and IEI was -93.7 ± 0.9 and -87.8 ± 4.1, respectively. In general, for low-Mg^{2-} induced epileptiform activity there is a reciprocal relationship between duration of discharges and time interval between bursting (Fig. 6; Aram & Lodge, 1988; Zhang et al., 1996).

At 20 μM concentration, ACPD (n=13) predominantly decreased SWD through a reduction in the duration and a reciprocally coupled increase in the discharge frequency. Eleven of 13 slices displayed a 40.5 ± 5.1 decrease in duration of activity with IEI decreasing 52.4 ± 3.5. The remaining two slices exhibited a 13.8 ± 8.8 increase in burst duration with a 17.5 ± 26.3 increase in IEI. Again wash out of drug effects varied, in two slices duration of activity was exacerbated compared to control by 21.5 and 24.5%. In the remaining nine slices, wash ranged from 57.9 to 100% of control levels.

In order to confirm that the observed effects were in fact mediated by activation of mGluR, the broad spectrum mGluR antagonist, (S)-MCPG was pre-applied and subsequently followed by co-application with 200 μM ACPD. Direct co-application of 200 μM ACPD with 500 μM (S)-MCPG (n=1) did not prevent SWD from being transformed into single spikes (not shown). Co-application of the agonist and antagonist following twenty minute bath perfusion of 500

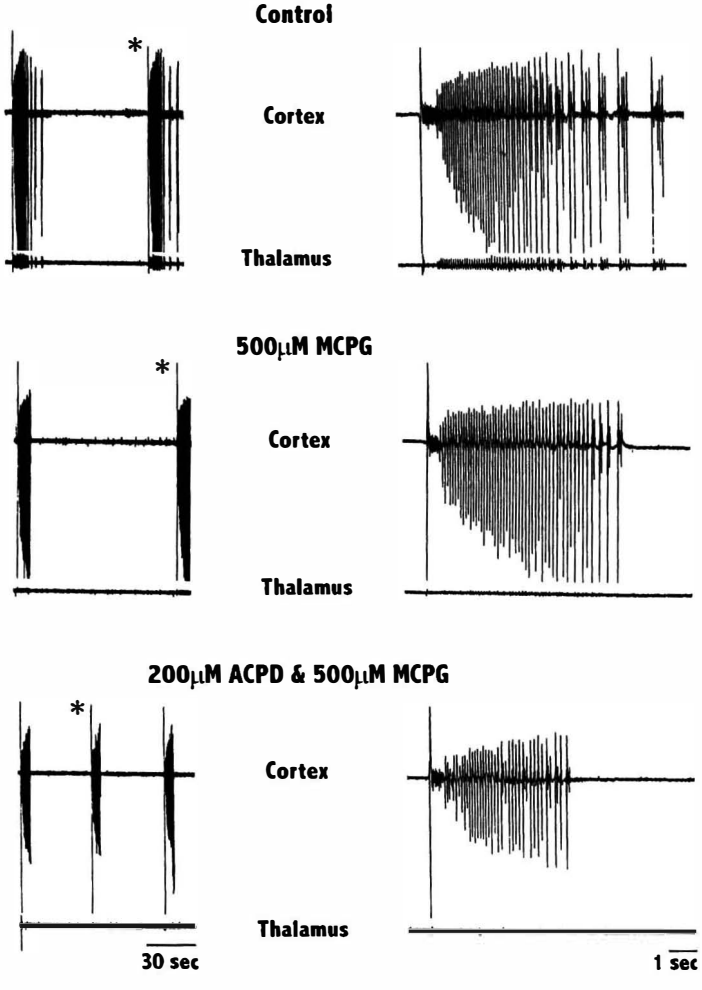
Figure 6. Histograms quantifying the effects from Fig. 5. **A:** Effects of 1 μM ACPD (1 μM, applied during the bar over the top graph) on the duration (top graph) and inter-event time interval (IEI, bottom graph) of SWD. Note that 1 μM ACPD had non-significant effects on duration and IEI. **B:** Effects of 200 μM ACPD (200 μM, applied during the bar over the top graph) on duration (top graph) and IEI (bottom graph) of SWD. Note that 200 μM ACPD markedly decreased the duration and IEI. Also note that these effects were reversible.


A.
1μM ACPD
Duration (sec)
0
2
4
6
8
10 20 30 40 50 60 70 80
B.
200μM ACPD
Duration (sec)
0
1
2
3
4
5
10 20 30 40 50 60 70 80
IEI (sec)
0
10
20
30
40
10 20 30 40 50 60 70 80
Time (min)
IEI (sec)
0
5
10
15
20
25
30
10 20 30 40 50 60 70 80
Time (min)

μM (S)-MCPG alone (n=3) prevented SWD from switching into single spike activity. A small decrease in duration of bursting, -38.6 ± 7.4 and IEI -54.5 ± 6.8 was noted. At 500 μM concentration of (S)-MCPG by itself had no apparent effects on SWD. In two experiments pre-application of 500 μM (S)-MCPG followed by co-application of 200 μM ACPD with 500 μM (S)-MCPG blocked the effects of ACPD. In these same slices the co-application was followed by re-application of the ACPD alone, which again switched rhythmic burst firing to single spike discharges indicating that the effects of ACPD are recovered after MCPG block. Furthermore, upon washout of ACPD, single spike discharges returned to SWD (Fig. 7).

To determine the site of action of ACPD, regionally selective perfusion of 200 μM ACPD to the cortex (n=3) and thalamus (n=4) was conducted (Fig. 8). mGluR activation in the cortex was unable to block SWDs. On two occasions there was a small decrease in duration of bursting, 11.7 ± 2.2 and on another occasion there was a 13.3% increase in activity duration. The two slices that exhibited a decrease in bursting duration also displayed an increase in burst amplitude (18%), while there were no apparent amplitude alterations in the slice exhibiting increase in duration burst. However, in all cases regional perfusion of 200 μM ACPD to the thalamus in TC slices (n=4) switched SWD to single spikes. On two occasions 200 μM ACPD was initially perfused to the cortex and subsequently to the thalamus followed by wash. Again the wash demonstrated reversible effects in the thalamus.

Figure 7. MCPG blocks effects of ACPD. *Control*. SWD in low-Mg^{2+} ACSF recorded in cortex and thalamus. Asterisk in slower sweep to the left is the discharge expanded at the right. *500 μM MCPG*. Effects of 500 μM MCPG on these SWD. Note the antagonist has non-significant effects. *200 μM ACPD & 500 μM MCPG*. Effects of co-application of 200 μM ACPD with 500 μM MCPG. Note MCPGs block of ACPD effects. *200 μM ACPD*. Effects of 200 μM ACPD. Note the recovered effects of ACPD upon application. *Wash*. Effects of washout of ACPD. Note the reversible effects of ACPD.


Control
Cortex
Thalamus
500μM MCPG
Cortex
Thalamus
200μM ACPD & 500μM MCPG
Cortex
Thalamus
30 sec
1 sec

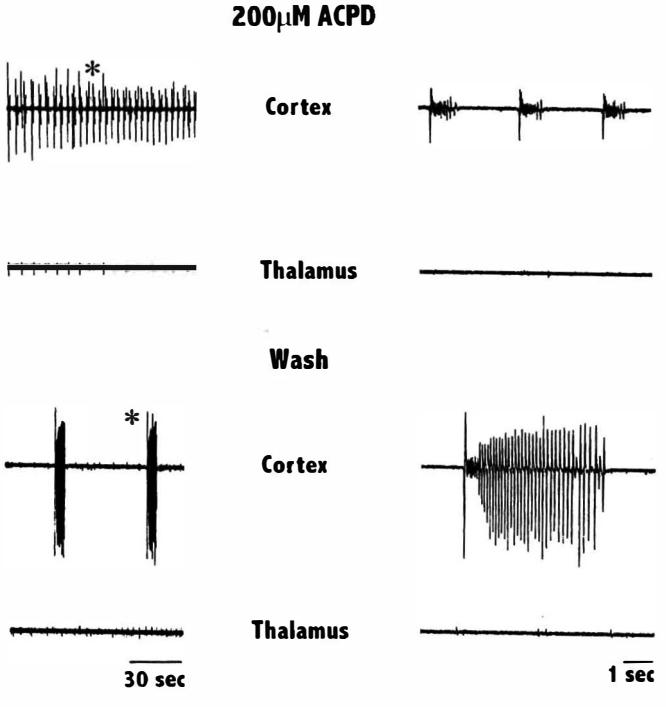
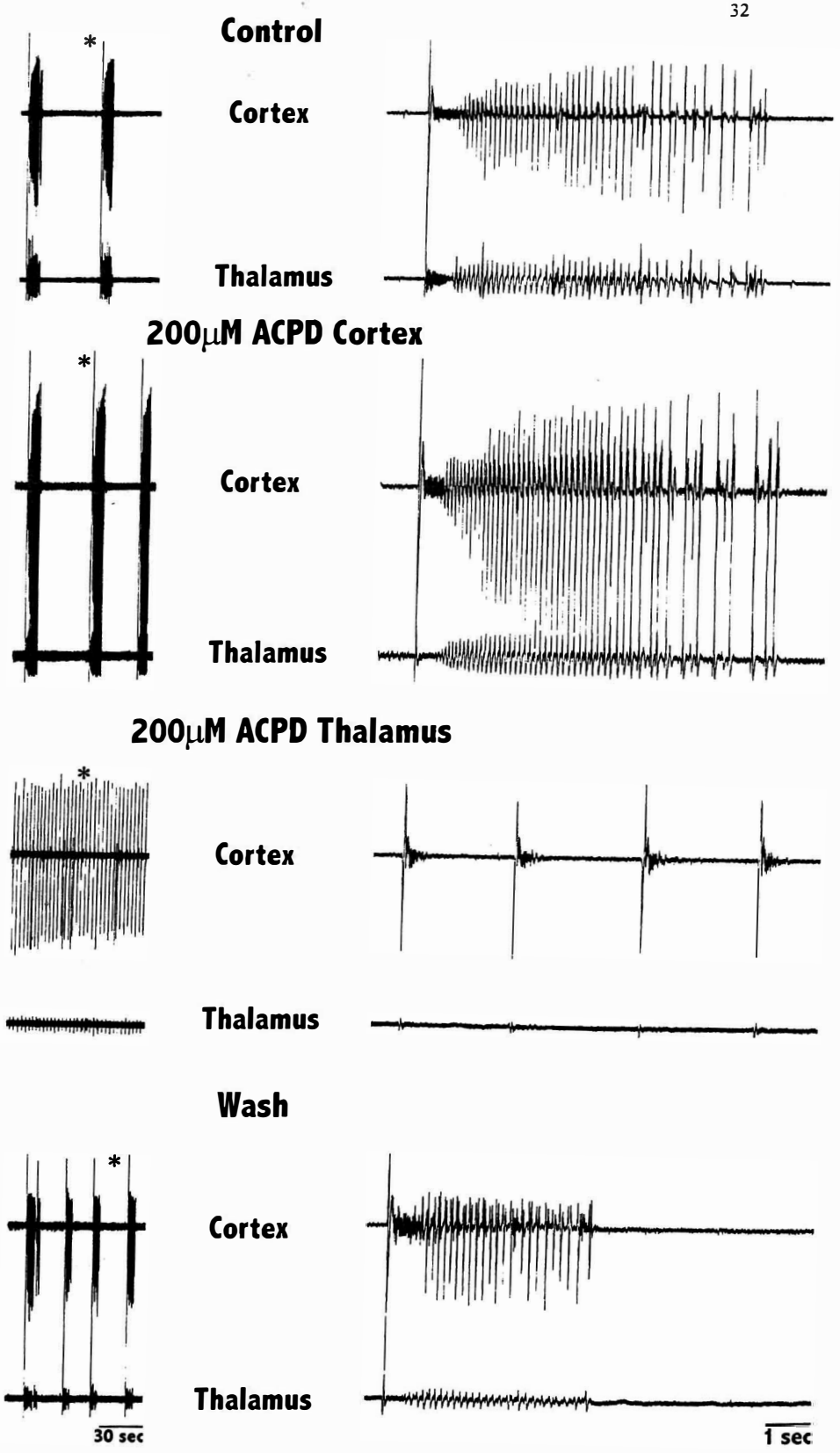

200μM ACPD
Cortex
Thalamus
Wash
Cortex
Thalamus
30 sec
1 sec

Figure 8. Effects of 200 μM ACPD on SWD during regionally selective perfusion. *Control*. SWD in low-Mg^{2+} ACSF recorded in cortex and thalamus. *200 μM ACPD Cortex*. Effects of 200 μM ACPD on these SWD during selective perfusion of the cortex. Note increase in amplitude of SWD in this slice. *200 μM ACPD Thalamus*. Effects of 200 μM ACPD on SWD during selective perfusion of the thalamus on the same TC slice. Note activity switch from rhythmic burst firing to single spikes. *Wash*. Note reversible effects of ACPD upon washout.


Control
Cortex
Thalamus
200μM ACPD Cortex
Cortex
Thalamus
200μM ACPD Thalamus
Cortex
Thalamus
Wash
Cortex
Thalamus
30 sec
1 sec

DISCUSSION

The present study employed extracellular field potential recordings to determine the effects of mGluR activation by ACPD on spontaneous rhythmic bursting in the intact TC circuit. ACPD was found to block GA seizures and transform SWD activity into tonic firing activity in the intact TC circuit in a concentration dependent manner. On all occasions, 200 μM ACPD switched TC activity to tonic discharges.

Alteration in activity resulting from application of ACPD was confirmed by the fact that the SWD activity returned upon washout of the mGluR agonist. Furthermore, application of the mGluR antagonist, (S)-MCPG was able to block the actions of ACPD. In this regard, pre-application of (S)-MCPG was required to block the effects of ACPD because it is a weak antagonist (Nakanishi, 1994; Conn & Pin 1997). Though the effects of wash were variable between slices, on average bursting activity was recovered to a greater extent in the 20 μM compared to 200 μM ACPD experiments. In addition, 20 μM ACPD was able to decrease the duration of bursting but was unable to switch the bursting to single spikes as was evident during 200 μM ACPD perfusion. These findings indicate that not all the mGluR were occupied/activated by the lower concentration of ACPD.

Regional perfusion experiments implicate the thalamus as the

predominant site of ACPD action. Although ACPD application to the cortex had some effects on the amplitude of SWDs, it was unable to switch rhythmic burst discharges to tonic firing in TC slices when applied to the cortex alone. ACPD activates both Group I and II mGluR so there are two possible mechanisms by which this agonist is able to limit sustained repetitive firing and transform SWD to tonic firing. First, activating Group I mGluR on thalamic relay neurons brings about a reduced gK^-, resulting in a slow depolarization of the RMP inactivating the T-current, therefore blocking SWD. The second mechanism by which rhythmic bursting is blocked by ACPD is activation of Group II mGluR on the presynaptic terminals of nRT neurons, resulting in decreased GABA release. Reduction of IPSCs on thalamic relay neurons may prevent the de-inactivation of the prominent T-current underlying bursting activity. Though the potency of ACPD is greater on Group II compared to Group I mGluRs (Conn & Pin, 1997) it was not possible to identify which Group or subtype of mGluR or whether both Group I *and* II mGluR were responsible for transforming SWD to single spikes. In future experiments using the regional perfusion apparatus, specific Group I or Group II agonists can be applied to the thalamus to elucidate which mGluR receptor subtype is responsible for altering the mode of TC circuit activity.

The present study supports the hypothesis that activation of mGluR's on thalamic neurons via corticothalamic projections is capable of controlling the firing mode of the TC circuit in a manner comparable to the ascending brainstem and hypothalamic connections. Therefore,

mGluRs may provide an alternative therapeutic target for the pharmacological treatment of GA epilepsy. *In vivo*, however, it is unlikely that both the ascending brainstem and the descending corticothalamic projections serve to control the firing mode of the TC circuit (Godwin et al., 1996). It has been proposed that corticothalamic projections play a modulatory role in the TC circuit (Koch, 1987; Godwin et al., 1996).

The ascending brainstem and hypothalamic projections are diffuse causing widespread activation of thalamic neurons (Salt & Eaton, 1995; Uhlrich et al., 1988). In the case of the lateral geniculate nucleus (LGN) it has been shown that the ascending connections project to the proximal one third of the dendritic tree of thalamic relay neurons (Koch, 1987). Therefore, these projections have global activation and more direct control of the RMP of thalamic cells (Godwin et al, 1996). Unlike the ascending projections the descending corticothalamic connections acting via mGluR activate specific thalamic neurons. In the case of the LGN, corticothalamic input is located on the distal two-thirds of the dendritic tree of thalamic relay neurons (Koch, 1987). Therefore, the proposed function of the corticothalamic projections (and thus mGluR) may not be to depolarize the soma of thalamic relay neurons altering the RMP, but rather to modulate the gain of incoming action potentials because of their selective and focal projections (Godwin et al., 1996). It has also been hypothesized that the corticothalamic projections serve as an "attentional searchlight". Meaning higher cortical areas are capable of detecting and amplifying

selective signals (Crick, 1984). Widespread activation, however, of most of the mGluRs on the focal corticothalamic projections may modulate the intrinsic mode of the TC circuit.

List of References

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Vita