Glutamatergic Excitatory Responses of Anterior Cingulate Neurons to Stimulation of the Mediodorsal Thalamus and Their Regulation by GABA: An *in vivo* Iontophoretic Study

John Gigg,[1] Aiko M. Tan,[1] and David M. Finch[1,2]

[1] Brain Research Institute and [2] Department of Neurology, Reed Neurological Research Center, University of California, Los Angeles, California 90024-1761

Anatomical and physiological studies in the rat have shown projections from the medial dorsal thalamus to the anterior cingulate cortex. We used multibarrel iontophoresis to identify the neurotransmitter used in this thalamic projection. Extracellular responses were recorded from 165 cingulate neurons in anesthetized rats after electrical stimulation of the medial dorsal thalamus and vicinity. Forty-four of these cells (27%) showed an excitatory response to thalamic stimulation. In a further 40 cells that showed no baseline excitation, iontophoresis of the $GABA_A$ antagonist bicuculline methiodide revealed excitatory responses. The $GABA_B$ antagonist CGP-35348 attenuated longer-latency inhibition in 5 of 10 cells. In 23 of 49 (47%) of the above cells, AMPA antagonist iontophoresis (either CNQX or DNQX) selectively decreased the excitatory response to thalamic stimulation. The NMDA antagonist 3[(*R*)-2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid had no such effect. These data suggest that the thalamic projection to anterior cingulate cortex is glutamatergic, acting principally via AMPA receptors, and that the response of cingulate neurons to thalamic stimulation is regulated by GABA acting at both $GABA_A$ and $GABA_B$ receptors.

The medial dorsal (MD) thalamus is involved in memory processes (for review, see Squire, 1987) and plays a major role in one model of schizophrenia (Stevens, 1989; Carlsson and Carlsson, 1990; Wachtel and Turski, 1990). The projection from MD thalamus to the cingulate cortex appears to be excitatory: stimulation of the MD thalamus evokes single-spike responses in cingulate cortical neurons of rat, rabbit, and cat (Canedo, 1982; Ferron et al., 1984; Sikes and DeFrance, 1985). This projection has been studied intensely at the anatomical level (Leonard, 1969; Domesick, 1972; Beckstead, 1979; Giguere and Goldman-Rakic, 1988; Freedman and Cassell, 1991), but the identity of the neurotransmitter in this pathway remains unresolved Several lines of evidence have suggested that the transmitter is glutamate. Ottersen and Storm-Mathisen (1984) demonstrated glutamate-like immunoreactivity in neurons of the thalamus and cerebral cortex. However, these authors stated that this technique would have labeled glutamate in both the "transmitter pool" as well as the "metabolic pool." Perhaps the most convincing biochemical evidence for glutamate as a thalamocortical transmitter comes from a study demonstrating the uptake and retrograde transport of D-^{3}H-aspartate in thalamocortical neurons (Ottersen et al., 1983); these transport processes appear to be restricted to neurons that utilize glutamate and/or aspartate as transmitters (Streit, 1980; Cuenod et al., 1982). A similar technique has also been used by Robinson and Beart (1988) to suggest that the projection from the parataenial thalamic nucleus to nucleus accumbens uses glutamate and/or aspartate for neurotransmission. Also, Herrling et al. (1990) have provided electrophysiological evidence to suggest that glutamate is the transmitter in projections from the ventrolateral thalamus to cortex: intracellular recordings from pyramidal tract neurons in the cat show that EPSPs produced through ventrolateral thalamic stimulation are sensitive to both NMDA and non-NMDA antagonists.

In contrast to the above observations, an immunohistochemical investigation of glutaminase-containing neurons in MD thalamus has shown that neurons in this nucleus exhibit very little signal (Kaneko and Mizuno, 1988). Accordingly, there remains some doubt regarding the transmitter used in projections from the MD thalamus to the anterior cingulate cortex. To investigate this problem further, we recorded extracellular neuronal activity in cingulate neurons

Cerebral Cortex Nov/Dec 1992;2:477-484; 1047-3211/92/$4.00

during stimulation of MD and vicinity. These recordings were made with multibarreled electrodes so that responses to stimulation could be measured in the presence of iontophoresed glutamate antagonists. As cingulate neurons show a marked period of inhibition after MD stimulation (Ferron et al., 1984), we also iontophoresed antagonists to both $GABA_A$ and $GABA_B$ receptors to test whether this inhibition was GABAergic.

Materials and Methods

Experiments were performed on 34 adult male Sprague–Dawley rats. The anesthetic, surgical, and recording procedures were carried out as described previously (Mello et al., 1992a,b). Briefly, rats were anesthetized (chloral hydrate, 400 mg/kg, i.p.) and placed in a stereotaxic frame. Supplementary injections of chloral hydrate were given as required (0.2–0.4 ml, i.p.).

Twisted, bipolar stimulating electrodes (150-μm-diameter, stainless steel wire) were placed according to the stereotaxic atlas of Paxinos and Watson (1986). The following thalamic (and surrounding) structures were stimulated: mediodorsal thalamic nucleus, mediodorsal lateral thalamic nucleus, mediodorsal medial thalamic nucleus, parataenial thalamic nucleus, anterodorsal thalamic nucleus, and lateral habenular nucleus. Electrical stimuli were presented as two-pulse trains at either 0.14 Hz or 0.4 Hz, photically isolated from ground. Pulses were 0.2 msec in duration, 2.5 msec apart, and 500 μA in intensity.

Recordings were made using a "piggy back" multibarrel electrode assembly. The manufacture of these electrodes has been described in detail elsewhere (Mello et al , 1992b). In brief, electrodes were composed of a single recording pipette (2 μm tip diameter, filled with either 1 M NaCl or K-citrate in saturated fast green) attached to a seven-barreled iontophoresis pipette (approximately 30 μm total tip diameter). These electrodes were glued together using cyanoacrylate adhesive so that the tip of the recording pipette extended about 60 μm beyond that of the iontophoresis assembly.

Extracellular single-unit activity was accepted for analysis only if action potential amplitudes were clearly distinguishable from noise. Unit activity was amplified (10×) and recorded on VCR tape. Peristimulus time histograms (PSTHs) were computed both on and off line using LABVIEW 2.0 software (National Instruments) running on a Macintosh IIcx computer. As necessary, accuracy of PSTH bins in the vicinity of the stimulus artifact was ensured by hand-counting spikes. The verification of recording and stimulating sites was carried out as described previously (White et al., 1990).

During recording, cells were exposed to various iontophoresed drugs (Neurophore BH-2 iontophoresis system, Medical Systems Corp.). All drugs were dissolved in 0.9% NaCl. Drug barrels were back-filled with combinations of the following: the $GABA_A$ receptor antagonist bicuculline methiodide (BMI; 5 mM, pH 3; Sigma); the $GABA_B$ receptor antagonist 2-hydroxy-saclofen (20 mM, pH 3; Tocris); the $GABA_B$ receptor antagonist phaclofen (10 mM, pH 3; Tocris); NMDA (50 mM, pH 9.5; Tocris); the $GABA_B$ antagonist CGP-35348 (10 mM, pH 3.0; kindly provided by Drs. L. Maître and H. Kaufman, Ciba-Geigy Ltd.); L-glutamate (0.5 M, pH 8; Sigma); the glutamatergic AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione [CNQX; 5 mM, pH 9; dissolved in 1% dimethyl sulfoxide (DMSO); Tocris]; the AMPA receptor antagonist 6,7-dinitroquinoxalene-2,3-dione (DNQX; 5 mM, pH 9; dissolved in 1% DMSO; Tocris); the glutamatergic NMDA receptor antagonist 3-[(*R*)-2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid (CPP; 10 mM, pH 8; Tocris); and DMSO (1%; Sigma). The central barrel of the iontophoresis electrode was filled with physiological (0.9%) NaCl for current balancing. Drug effects were deemed positive if responses demonstrated a 50% or greater change from baseline.

It was felt unlikely that the iontophoresis effects we observed were simply artifacts of pH or current: drugs iontophoresed with a similar pH, identical current polarity, and similar current intensities (e.g., bicuculline and CGP-35348; DNQX and CPP) produced very different results, making pH or current effects unlikely. In addition, 1 or more minutes of iontophoresis were often necessary for drug effects to become apparent (and drug effects lasted 1 or more minutes after the end of iontophoresis), which also argues against current effects.

Results

Baseline Responses

PSTHs were gained from 165 cells in 34 rats. Baseline neuronal responses could be divided into three categories: (1) no clear response (50% of cells) due in part to the low firing rate of the cell being recorded, (2) inhibition (23%) that often lasted >100 msec, and (3) excitation (27%) followed almost invariably by inhibition. Excitatory responses usually consisted of one spike with latencies ranging from 10 to 20 msec (mean, 11.3 msec).

Effects of AMPA Antagonists on Baseline Responses

In 8 of 13 cells tested (62%), excitatory responses evoked by thalamic stimulation were sensitive to the glutamatergic AMPA antagonists CNQX and DNQX. The stimulation and recording sites that showed a decrease in excitatory response during iontophoresis of an AMPA antagonist (either CNQX or DNQX) are shown in Figure 1. The recording sites were primarily in anterior cingulate areas 24 and 25, with some in area 32 of prefrontal cortex. Excitatory responses could clearly be seen "riding" on the rising phase of the evoked field potential (Fig. 2). Figure 3*A* shows an instance in which thalamic stimulation produced excitation in a cingulate neuron. This excitation was antagonized by DNQX (Fig. 3*B*). There were no obvious differences in efficacy between DNQX and CNQX in attenuating excitatory responses.

Because it was necessary to dissolve AMPA antagonists in DMSO, control experiments were performed

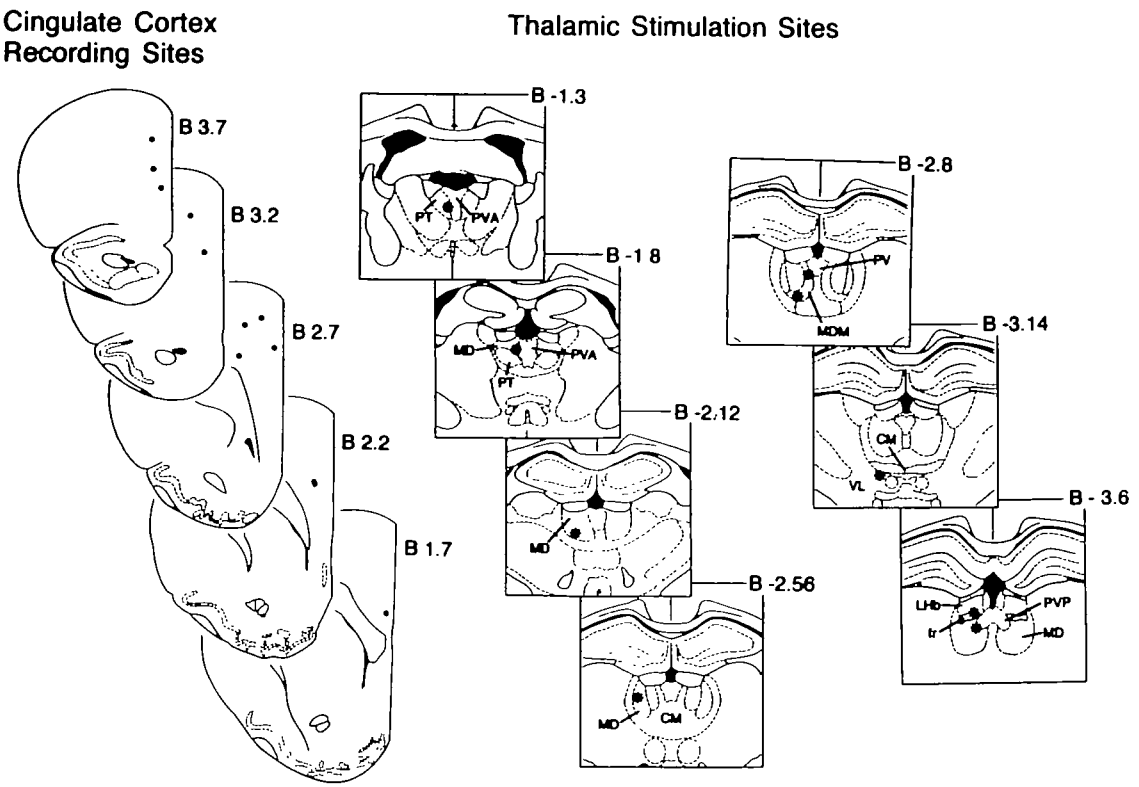


Figure 1. Cingulate cortex recording sites (*solid circles, left*) that exhibited baseline excitatory responses to thalamic stimulation (*asterisks, right*). Each of these excitatory responses was attenuated by the AMPA antagonists CNQX or DNQX. These sections are based upon the atlas by Paxinos and Watson (1986) The level of each section is indicated in millimeters with respect to bregma. *CM*, central medial thalamic nucleus, *fr*, fasciculus retroflexus, *LHb*, lateral habenular nucleus, *MDM*, mediodorsal thalamic nucleus, medial part; *PT*, parataenial thalamic nucleus; *PVA*, paraventricular thalamic nucleus, anterior part; *PVP*, paraventricular thalamic nucleus, posterior part; *VL*, ventrolateral thalamic nucleus.

to ensure that the iontophoresis of DMSO alone produced no antagonistic effects. Figure 4 shows the responses of one cingulate neuron to both DMSO and DNQX. Baseline responses of this cell to stimulation of the lateral mediodorsal thalamus consisted of a burst of spikes. This cell showed few spontaneous spikes. Figure 4, *B* and *C*, shows that while DMSO had no effect upon the excitatory response of the cell, DNQX (dissolved in DMSO) markedly reduced excitation: note that in this instance DMSO and DNQX were ejected with similar currents and had the same pH. As shown in Figure 4*D*, this effect of DNQX reversed almost completely.

In contrast to the results observed with CNQX and DNQX, no clear effect was observed with the NMDA antagonist CPP. However, cells in cingulate cortex did respond to iontophoresed NMDA with increased firing rates (data not shown).

Effects of GABA Antagonists on Baseline Responses

Iontophoresis of the $GABA_A$ antagonist BMI revealed excitatory responses in 39 out of 67 cells (58%) and also increased baseline excitatory responses (data not shown). BMI iontophoresis also increased the spontaneous firing rate in 70 out of 83 neurons (84%). the mean increase was from 2.87 ± 0.39 to 7.12 ± 0.73 spikes per second (mean ± SEM). However, BMI-revealed excitation could occur in the absence of a change in spontaneous discharge rate.

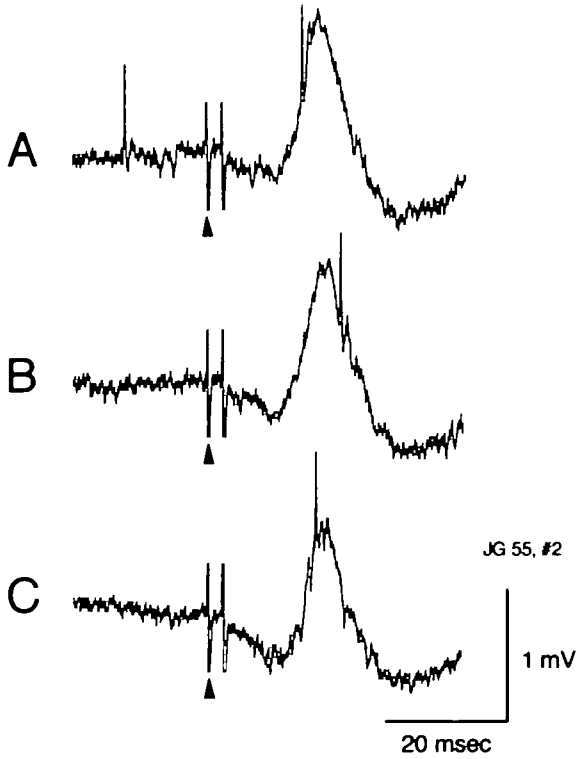


Figure 2. Excitatory responses (*A–C*) recorded from a neuron in cingulate cortex after MD stimulation. Note that excitatory spikes are associated with the evoked field potential (filter, 10–3 kHz). This excitatory response was attenuated by DNQX iontophoresis (data not shown). Stimulation parameters for this and following figures: electrical stimuli were presented as two-pulse trains at 0.4 Hz, photically isolated from ground; pulses were 0.2 msec in duration, 2.5 msec apart, and 500 μA in intensity.

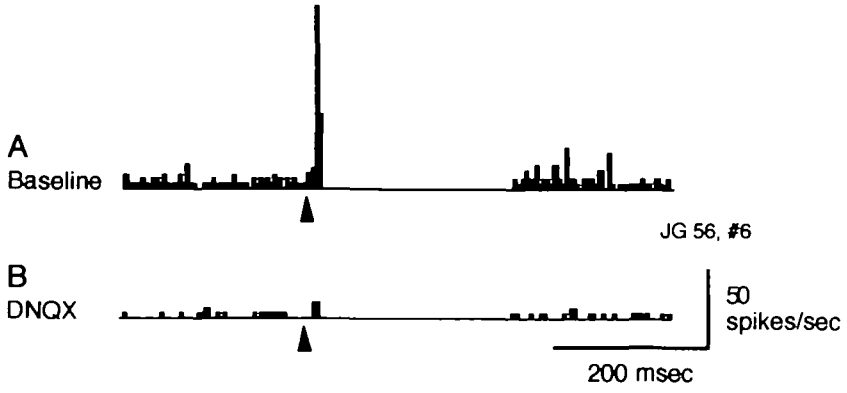


Figure 3. Effect of DNQX on evoked excitation. *A*, Baseline response of cingulate neuron to MD thalamic stimulation. *B*, Response after iontophoresis of DNQX (−200 nA, 2 min continuous current) shows attenuated excitatory response. PSTHs are composed of 46 and 50 sweeps, respectively. Bin width in this and succeeding figures is 5 msec. *Arrowheads* in this and other figures represent stimulus onset.

An example of BMI-revealed excitation is shown in Figure 5. During baseline stimulation, the cell showed no clear excitatory response (Fig. 5*A*). However, after BMI iontophoresis, a large excitatory response was induced (Fig. 5*C*). The nature of the response changed from a single spike during baseline responses (Fig. 5*B*) to a burst of spikes after BMI iontophoresis (Fig. 5*D*). The long-duration inhibition in Figure 5*A* appears to be longer than that in Figure 5*C*; however, BMI had no effect upon long-duration inhibition (see below). This suggests that the inhibitory period in Figure 5*A* was "masked" by the low firing rate of the cell during baseline stimulation.

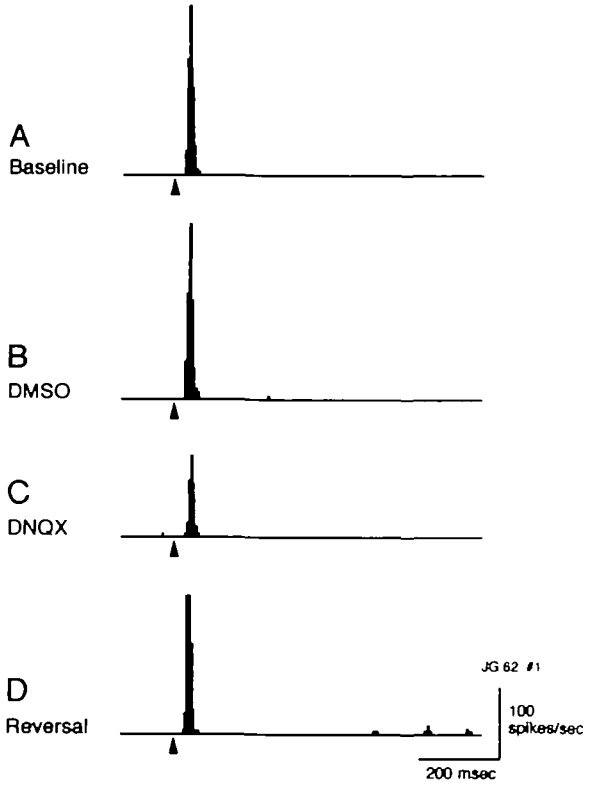


Figure 4. Effects of DMSO and DNQX on evoked excitation. *A*, Baseline response of cingulate neuron to lateral mediodorsal thalamic stimulation. Note lack of spontaneous firing. Response to stimulation was a variable latency burst of three or four spikes. *B*, Vehicle control. DMSO ejection has no effect upon excitation (−200 nA, 2 min continuous current). *C*, Response after DNQX iontophoresis (−200 nA, 2 min continuous current) shows pronounced decrease in excitation. *D*, Reversal. Excitatory response recovers to near baseline level 9 min after DNQX ejection was terminated. PSTHs *A–D* are composed of 47, 41, 40, and 47 sweeps, respectively.

The majority of cells responded to MD stimulation with a prolonged period of inhibition. The late inhibitory period was unaffected by iontophoresis of the $GABA_B$ antagonists phaclofen and 2-hydroxy-saclofen (stimulation at 0.14 Hz in both cases). In 5 out of 10 cases, however, late inhibition was attenuated through iontophoresis of the more potent $GABA_B$ antagonist CGP-35348. Iontophoresis of CGP-35348 increased the spontaneous firing rate slightly in 9 out of 10 of these cells from 9.2 ± 2.11 to 12.2 ± 1.56 spikes per second (mean ± SD). The response of one cell to CGP-35348 iontophoresis can be seen in Figure 6, which also shows, for comparison, the response of another cell to BMI iontophoresis. The baseline response of the cell in Figure 6*A1* shows a clear, prolonged inhibitory phase. The iontophoresis of BMI (Fig. 6*A2*), onto this cell revealed an excitatory response and increased the spontaneous firing of the cell without affecting the later period of inhibition. the broken line running from *A1* to *A2* in Figure 6 clearly shows that the end of inhibition occurred at the same time after stimulation under both baseline and BMI conditions. Figure 6*B1* shows the baseline response of a different cell to MD thalamic stimulation. a small excitatory response followed by prolonged inhibition. Note that this inhibition was similar in time course to that for the cell in Figure 6*A*. However, in contrast to the effect from BMI iontophoresis, ejection of CGP-35348 (Fig 6*B2*) markedly reduced the later inhibitory period (compare broken line runninng from *B1* to *B2* in Fig. 6) with only a small increase in excitation. In one instance, CGP-35348 did reveal a large excitatory response. The latency of this CGP-revealed response was longer (by about 5 msec) than that revealed in the same cell through BMI iontophoresis.

Effects of AMPA Antagonists on BMI-revealed Excitation

AMPA antagonists attenuated the BMI-revealed excitatory response in 15 of 36 cells tested (42%). Figure

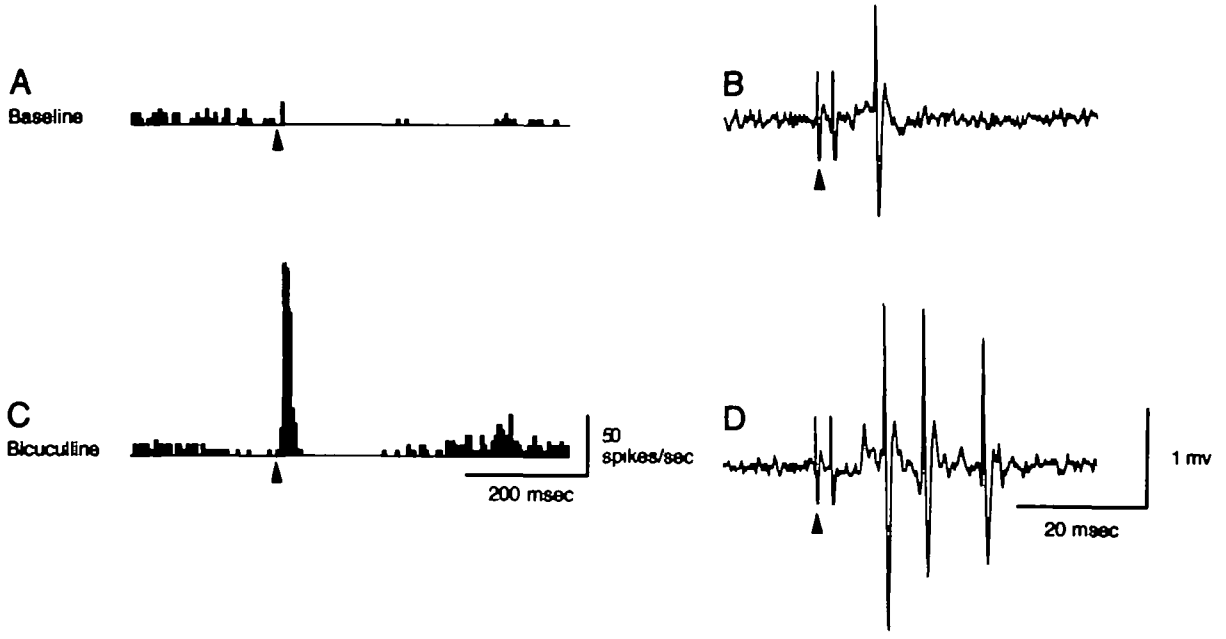
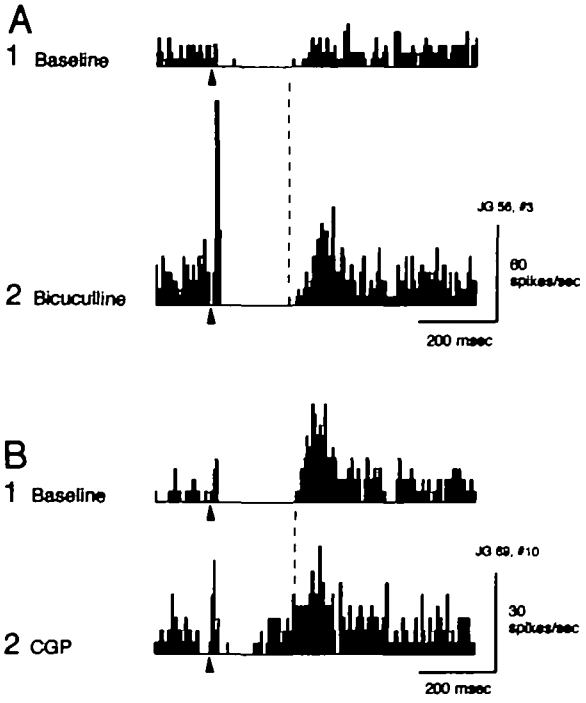


Figure 5. Excitatory response revealed by iontophoresis of BMI. *A*, Baseline response of cingulate neuron to MD thalamic stimulation. *B*, Single sweep to show occasional excitatory spike to baseline stimulation. *C*, BMI iontophoresis (+50 nA, 2 min) revealed a large excitatory response. *D*, Evoked response just after BMI iontophoresis is now a burst of spikes of decreasing amplitude. Note that BMI did not affect inhibitory period after excitation. This cell also exhibited a response recovery after BMI (data not shown). PSTHs in *A* and *C* are composed of 50 sweeps

7 shows an instance in which DNQX iontophoresis antagonized BMI-revealed excitation. Figure 7*A* shows the baseline response of the cell. Note that the cell was almost "silent" during this baseline period. Iontophoresis of BMI (Fig. 7*B*) produced an increase in spontaneous rate and revealed an excitatory response. DNQX antagonized this excitatory response without either affecting the spontaneous firing rate of the cell or late inhibition (Fig. 7*C*) After cessation of DNQX iontophoresis, several minutes passed before recovery of the excitatory response (Fig. 7*D*)



Candidate Inhibitory Cells

During recording, four candidate inhibitory neurons were encountered (2% of all cells; no reliable iontophoretic data were obtained) These cells showed distinctive evoked bursts that are thought to reflect activity of inhibitory cells (McCormick and Prince, 1985; McCormick et al., 1985). Responses from one of these candidate inhibitory cells can be seen in Figure 8: note the high spontaneous firing rate and the large excitatory response (Fig 8*A*). As can be seen from the associated spike train (Fig. 8*B*), this excitatory response consisted of a prolonged burst of short-duration action potentials that exhibited little frequency adaptation.

Discussion

The projection from MD thalamus to cingulate cortex in the rat has been shown to be excitatory (Ferron et

←

Figure 6. Effect of CGP-35348 upon long-duration evoked inhibition. *A* shows the lack of effect of BMI upon the late phase of inhibition. *A1*, Baseline response of cingulate neuron to stimulation of lateral MD thalamus. Note pronounced inhibitory period following stimulation. *A2*, Response of same cell after BMI iontophoresis (+50 nA, 5 min continuous ejection) showed evoked burst, again followed by prolonged inhibition. The *broken line* running between *A1* and *A2* demonstrates that this inhibitory period was of the same duration during these stimulus conditions. *B* shows that the principal effect of CGP-35348 was to attenuate late inhibition. *B1*, Baseline response of cingulate neuron to stimulation of MD thalamus (different cell from that in *A*). Stimulation of MD thalamus produced a small evoked response followed by prolonged inhibition. The time course of this inhibition was similar to that for the cell in *A*. *B2*, Response after ejection of CGP-35348 (+400 nA, 3 min continuous ejection). The duration of the inhibitory period was shortened, and there was a modest increase in excitation. The excitatory response in both *B1* and *B2* was a single spike. PSTHs *A1*, *A2*, *B1*, and *B2* are composed of 47, 42, 60, and 60 sweeps, respectively.

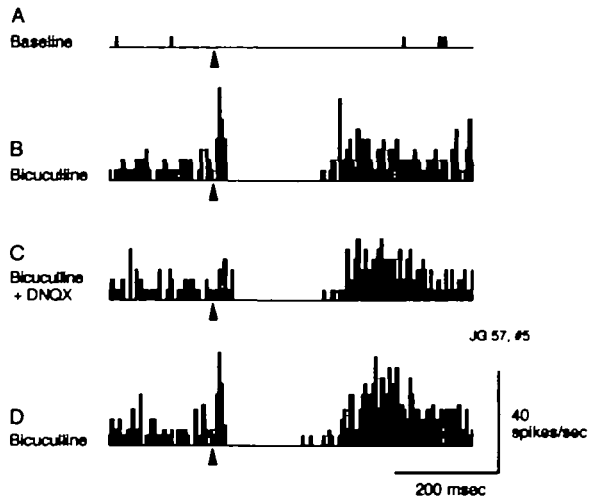


Figure 7. Effect of DNQX on BMI-evoked excitation. *A*, Baseline response of cingulate neuron to MD thalamic stimulation. Note low spontaneous discharge rate and lack of excitatory response. *B*, BMI iontophoresis (+50 nA, 2 min) induced both a large evoked response and also an increase in spontaneous rate. *C*, DNQX iontophoresis (−300 nA, 2 min continuous current) attenuated the excitatory response without affecting the spontaneous firing rate (BMI iontophoresis constant throughout). *D*, Recovery of excitatory response 7 min after cessation of DNQX iontophoresis. Each PSTH is composed of 50 sweeps.

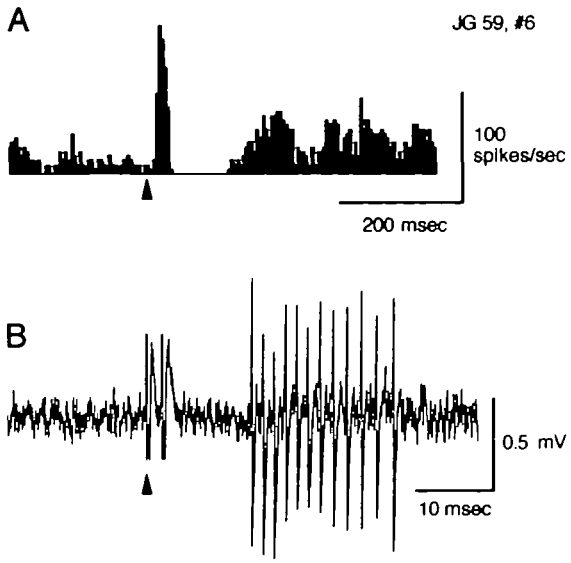


Figure 8. Candidate inhibitory neuron. *A*, Response of candidate inhibitory neuron to MD thalamic stimulation. Note the large excitatory response, high spontaneous discharge rate, and conspicuous postexcitatory period of inhibition. PSTH is composed of 50 sweeps. *B*, Associated evoked spike train. Note that the response to thalamic stimulation consisted of a prolonged burst of short-duration, high-frequency spikes with little frequency adaptation.

al., 1984). The present results suggest that the neurotransmitter in this excitatory projection is glutamate, which acts postsynaptically at the AMPA glutamate-receptor subtype. These results are largely in agreement with previous studies that have suggested the existence of glutamatergic thalamic projections (Ottersen et al., 1983; Ottersen and Storm-Mathisen, 1984; Robinson and Beart, 1988; Albin et al., 1989; Herrling et al., 1990; LeDoux and Farb, 1991; but see Kaneko and Mizuno, 1988, who observed few glutaminase-containing cells in the MD nucleus). Although the short-latency excitatory responses presented here could be seen riding on the earliest part of the evoked field potential, we cannot be certain that all of these spikes were produced through monosynaptic activation. Sikes and DeFrance (1985) recorded short-latency responses of cingulate neurons to MD thalamic stimulation in the rabbit and suggested that these responses may have been produced through recurrent (i.e., intra-cingulate) excitation. Therefore, it is possible that some of the short-latency spikes we observed were produced through similar recurrent connections

Cingulate neurons responded to NMDA iontophoresis with increased spontaneous firing rate; however, the NMDA receptor antagonist CPP appeared to be ineffectual in attenuating cingulate excitatory responses to stimulation of MD thalamus. This suggests that although NMDA receptors are present on cingulate neurons, their activation is not essential for excitatory transmission in this pathway. However, there are some important caveats to this. It is important to note that this assertion is based solely upon data from extracellular unit recordings. Herrling et al. (1990) have shown in the cat that NMDA receptors do play a role in the generation of EPSPs at the intracellular level in the pathway from ventrolateral thalamus to cortex. This strongly suggests that a similar NMDA component may indeed be present in the cingulate EPSP response to activity of MD thalamic afferents. Such activation of NMDA receptors in the MD thalamic pathway to cingulate cortex may become apparent under conditions of stimulation different from those used here (e.g., with paired-pulse stimulation). Also, we cannot be certain from our results that CPP actually reached those synapses responsible for the generation of excitatory responses.

The response of neurons in cingulate cortex to thalamic stimulation appears to be regulated by GABAergic inhibition. Iontophoresis of the $GABA_A$ receptor antagonist BMI revealed excitatory responses that were sensitive to the iontophoresis of AMPA antagonists. BMI also increased the spontaneous discharge rate of neurons but had no effect upon longer-latency phases of inhibition.

Although AMPA antagonists were successful in attenuating excitatory responses, their efficacy may have been dependent upon whether excitatory responses were present during baseline or were revealed by BMI. Sixty-two percent of baseline excitatory responses were attenuated, whereas only 42% of BMI-revealed responses were attenuated. There may be several reasons for this. First, the disparity may simply be due to sampling error Second, the BMI-revealed excitatory drive may have been too "strong" to be easily overcome by AMPA antagonists. Some of the inputs to the cell being recorded will be from a large local population of neurons that are also being driven by thalamic stimulation. These inputs may also be disinhibited through BMI iontophoresis, thereby producing an increase in excitatory drive to the cell. In many cases this drive may be too large to be overcome by AMPA antagonists, hence a lower AMPA antagonist

success rate in attenuating BMI-revealed excitation. In support of this second suggestion, we observed that during some instances of BMI iontophoresis, evoked burst responses could be seen that were coincident with large negative deflections in field potential. Ferron et al. (1984) have reported similar deflections in response to MD stimulation. These negative deflections in field potential may reflect the synchronous activity of a number of neurons.

Although neocortical neurons respond to the $GABA_B$ agonist baclofen with long-duration hyperpolarization (El-Beheiry and Puil, 1990), iontophoresis of the $GABA_B$ antagonists saclofen and phaclofen had no effect upon either short-latency or long-latency inhibition. The $GABA_B$ antagonist CGP-35348, however, was effectual in (1) reducing late inhibition, (2) increasing spontaneous firing rate, and (3) increasing excitation in two cases. One reason for the success of CGP-35348 might be its relatively high potency: CGP-35348 appears to be some 30 times more potent than phaclofen in the antagonism of L-baclofen (Olpe et al., 1990). Although Olpe et al. (1990) did not compare the activity of CGP-35348 against that of 2-hydroxy-saclofen, it is not unreasonable to assume that CGP-35348 is the more potent as 2-hydroxy-saclofen exhibits only 10 times the potency of phaclofen (Kerr et al., 1988). These observations regarding the effect of BMI and CGP-35348 suggest that neurons in cingulate cortex exhibit both short- and long-latency GABAergic inhibitory components. The former is of short duration and reflects the activation of $GABA_A$ receptors, while the latter is of much longer duration and is mediated by the activation of $GABA_B$ receptors.

A neuronal circuit model that can account for these results contains glutamatergic thalamic efferents that excite cingulate pyramidal cells and inhibitory neurons. This circuit allows for monosynaptic excitation and bisynaptic (feedforward) inhibition of cingulate pyramidal cells. Feedback inhibition could also be present. The results suggest a glutamatergic input to cingulate pyramidal cells that acts primarily via AMPA receptors. We have no pharmacological data regarding the thalamic excitation of inhibitory cells. In this model, inhibitory neurons are GABAergic, show evoked bursts of spikes, inhibit a large population of principal cells, and provide inhibition to at least some other inhibitory neurons (e.g., Fig. 8*A* shows inhibition of a candidate inhibitory neuron). This inhibition occurs through activation of $GABA_A$ and $GABA_B$ receptors. Inhibitory neurons may be of different types: one may terminate solely on $GABA_A$ receptors, whereas the other may terminate solely on $GABA_B$ receptors. Such segregation of inhibitory cells into distinct types appears to exist in both the amygdala and ventral tegmental area and may be a general feature of mammalian nervous systems (Sugita et al., 1992).

The MD thalamus appears to be an important structure for learning and memory formation (Mishkin, 1982; Aggelton and Mishkin, 1983; Friedman et al., 1990; for review, see Squire, 1987). As the phenomenon of long-term potentiation (LTP) has been closely associated with memory formation (Teyler and DiScenna, 1984, 1987), perhaps LTP is expressed in this thalamocortical projection. Iriki et al. (1991) have induced LTP in neocortex through coactivation of thalamic and cortical inputs onto single cells. We have observed excitatory responses to both thalamic and hippocampal stimulation in single cingulate neurons (J. Gigg, A. M. Tan, and D. M. Finch, unpublished observations), which suggests that LTP may also be produced in the cingulate cortex through coactivation of convergent thalamic and hippocampal afferents.

In summary, the present results suggest that the projection from MD thalamus to anterior cingulate cortex is glutamatergic. The processing of this thalamic input appears to be regulated by GABAergic inhibition.

Notes

This work was supported by NIH Grant NS 16721.

Correspondence should be addressed to John Gigg, 73-364 Center for Health Sciences, Brain Research Institute, University of California, Los Angeles, CA 90024-1761.

References

Aggelton JP, Mishkin M (1983) Memory impairments following restricted medial thalamic lesions in the monkey. Exp Brain Res 53:199–209

Albin RL, Aldridge JW, Young AB, Gilman S (1989) Feline subthalamic nucleus neurons contain glutamate-like but not GABA-like or glycine-like immunoreactivity. Brain Res 491:185–188

Beckstead RM (1979) An autoradiographic examination of corticocortical and subcortical projections of the mediodorsal-projection (prefrontal) cortex in the rat. J Comp Neurol 184:43–62.

Canedo A (1982) Subcortical influences upon prefrontal cortex of the cat. Brain Res 232:449–454.

Carlsson M, Carlsson A (1990) Schizophrenia: a subcortical neurotransmitter imbalance syndrome? Schizophr Bull 16:425–432.

Cuenod M, Bagnoli P, Beaudet A, Rustioni A, Wiklund L, Streit P (1982) Transmitter-specific retrograde labelling of neurons. In: Cytochemical methods in neuroanatomy (Palay SL, Chan-Palay V, eds), pp 17–44 New York. Liss.

Domesick VB (1972) Thalamic relationships of the medial cortex in the rat. Brain Behav Evol 6:457–483.

El-Beheiry H, Puil E (1990) Unusual features of GABA responses in layer IV–V neurons of neocortex. Neurosci Lett 119:83–85.

Ferron A, Thierry AM, le Douarin C, Glowinski J (1984) Inhibitory influence of the mesocortical dopaminergic system on spontaneous activity or excitatory response induced from the thalamic mediodorsal nucleus in the rat medial prefrontal cortex. Brain Res 302:257–265.

Freedman LJ, Cassell MD (1991) Thalamic afferents of the rat infralimbic and lateral agranular cortices. Brain Res Bull 26:957–964

Friedman HR, Janas JD, Goldman-Rakic PS (1990) Enhancement of metabolic activity in the diencephalon of monkeys performing working memory task: a 2-deoxyglucose study in behaving rhesus monkeys. J Cogn Neurosci 2:18–31.

Giguere M, Goldman-Rakic PS (1988) The medial dorsal thalamic nucleus of the monkey also projects to the anterior cingulate cortex. J Comp Neurol 277:195–213.

Herrling PL, Meier CL, Salt TE, Seno N (1990) Involvement of NMDA and non-NMDA receptors in cortico-cortical and thalmo-cortical excitatory postsynaptic potentials in the anaesthetized cat. J Physiol (Lond) 425:89P.

Iriki A, Pavlides C, Keller A, Asanuma H (1991) Long-term

potentiation of thalamic input to the motor cortex induced by coactivation of thalamocortical afferents J Neurophysiol 65:1435–1441.
Kaneko T, Mizuno N (1988) Immunohistochemical study of glutaminase-containing neurons in the cerebral cortex and thalamus of the rat J Comp Neurol 267:590–602.
Kerr DIB, Ong J, Johnston GAR, Abbenante J, Prager RH (1988) 2-Hydroxy-saclofen: an improved antagonist at central and peripheral $GABA_B$ receptors. Neurosci Lett 92:92.
LeDoux JE, Farb CR (1991) Neurons of the acoustic thalamus that project to the amygdala contain glutamate Neurosci Lett 134:145–149.
Leonard CM (1969) The prefrontal cortex of the rat I. Cortical projections of the mediodorsal nucleus II. Efferent connections. Brain Res 12:321–343.
McCormick DA, Connors BW, Lightfall JW, Prince DA (1985) Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex. J Neurophysiol 54:782–806.
McCormick DA, Prince DA (1985) Two types of muscarinic response to acetylcholine in mammalian cortical neurons. Proc Natl Acad Sci USA 82:6344–6348.
Mello LEAM, Tan AM, Finch DM (1992a) Convergence of projections from the rat hippocampal formation, medial geniculate and basal forebrain onto single amygdaloid neurons: an *in vivo* extra- and intracellular study Brain Res 587:24–40.
Mello LEAM, Tan AM, Finch DM (1992b) GABAergic synaptic transmission in projections from the basal forebrain and hippocampal formation to the amygdala: an *in vivo* iontophoretic study. Brain Res 587:41–48.
Mishkin M (1982) A memory system in the monkey. Philos Trans R Soc Lond [Biol] 298:85–95.
Olpe H-R, Karlsson G, Pozza MF, Brugger F, Seinmann M, Riezen HV, Fagg G, Hall RG, Froestl W, Bittiger H (1990) CGP 35348: a centrally active blocker of $GABA_B$ receptors. Eur J Pharmacol 187:27–38.
Ottersen OP, Storm-Mathisen J (1984) Glutamate- and GABA-containing neurons in the mouse and rat brain, as demonstrated with a new immunocytochemical technique. J Comp Neurol 229:374–392.
Ottersen OP, Fischer BO, Storm-Mathisen J (1983) Retrograde transport of D-[^{3}H]aspartate in thalamocortical neurones. Neurosci Lett 42:19–24.
Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. New York: Academic.
Robinson TG, Beart PM (1988) Excitant amino acid projections from rat amygdala and thalamus to nucleus accumbens. Brain Res Bull 20:467–471.
Sikes RW, DeFrance JF (1985) Cingulate cortex response to electrical stimulation of the mediodorsal thalamic nucleus Exp Neurol 89:428–441.
Squire LR (1987) Memory and brain. New York: Oxford UP
Stevens JR (1989) The search for an anatomical basis of schizophrenia: review and update. In: Neurology and psychiatry: a meeting of minds (Muller J, ed), pp 64–87 Basel: Karger
Streit P (1980) Selective retrograde labelling indicating the transmitter of neuronal pathways. J Comp Neurol 191: 429–463.
Sugita S, Johnson SW, North RA (1992) Synaptic inputs to $GABA_A$ and $GABA_B$ receptors originate from discrete afferent neurons. Neurosci Lett 134:207–211
Teyler TJ, DiScenna P (1984) Long-term potentiation as a candidate mnemonic device. Brain Res Rev 7:15–28
Teyler TJ, DiScenna P (1987) Long-term potentiation. Annu Rev Neurosci 10:131–161.
Wachtel H, Turski L (1990) Glutamate. a new target in schizophrenia? Trends Pharmacol Sci 11:219–220.
White TD, Tan AM, Finch DM (1990) Functional reciprocal connections of the rat entorhinal cortex and subicular complex with the medial frontal cortex: an *in vivo* intracellular study. Brain Res 533:95–106.