Role of NMDA, Non-NMDA, and GABA Receptors in Signal Propagation in the Amygdala Formation

C. Wang, W. A. Wilson, and S. D. Moore. Role of NMDA, non-NMDA, and GABA receptors in signal propagation in the amygdala formation. *J Neurophysiol* 86: 1422–1429, 2001. Although the synaptic physiology of the amygdala has been studied with single neuron recordings, the properties of the networks between the various nuclei have resisted characterization because of the limitations of field recording in a neuronally diffuse structure. We addressed this issue in the rat amygdala complex in vitro by using a photodiode array coupled with a voltage-sensitive dye. Low-intensity single pulse stimulation of the lateral amygdala nucleus produced a complex multiphasic potential. This signal propagated to the basolateral nucleus and the amygdalostriatal transition zone but not to the central nucleus. The local potential, which depended on both synaptic responses and activation of voltage-dependent ion channels, was reduced in amplitude by the non-N-methyl-D-aspartate (non-NMDA) glutamate receptor antagonist 6,7-dinitroquinoxaline (DNQX) and reduced to a lesser extent by the NMDA glutamate receptor antagonist d-2-amino-5-phosphonovaleric acid (d-APV). We next characterized the less complex signals that propagated to more distal regions with or without the addition of the GABA receptor antagonist bicuculline (BIC). BIC alone greatly increased the signal propagation and permitted activation of previously silent areas within the amygdala. DNQX blocked signal propagation to amygdala regions outside of La, even in the presence of BIC, whereas d-APV had minimal effects on these distal signals. These data represent several novel findings: the characterization of the multi-component potential near the site of stimulation, the gating of signal propagation within the amygdala by GABAergic inhibition, the critical role of non-NMDA receptor-mediated depolarization in signal propagation, and the lack of a role for NMDA receptors in maintaining propagation.

**INTRODUCTION**

The amygdaloid formation consists of a group of temporal lobe nuclei that are involved in multiple functions including memory, emotion, attention, and autonomic control (Davis 1992). Animal models of experimental fear and anxiety have indicated a critical role for the amygdala in the acquisition and expression of fear-conditioned behaviors (Davis 1992; Davis et al. 1994). The rat amygdala has essentially the same extrinsic and intrinsic connections as in the human and nonhuman primate (Davis et al. 1994; McDonald 1998) and has served as a model for detailing physiological mechanisms underlying functions served by the human amygdala.

The amygdala has extensive reciprocal intranuclear and internuclear connections (Davis et al. 1994; Rainnie et al. 1993). The synaptic physiology of the basolateral amygdala has been previously characterized using intracellular recordings (Rainnie et al. 1991a,b). Individual neurons in the basolateral complex receive input from multiple sites; excitatory postsynaptic potentials (EPSPs) can be elicited in pyramidal neurons by stimulation of stria terminalis, external capsule, or lateral amygdala (Chapman et al. 1990; Rainnie et al. 1991a,b). These EPSPs consist of a fast component, mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors, and a slow component mediated by N-methyl-D-aspartate (NMDA) receptors (Calton et al. 2000; Rainnie et al. 1991a). Weisskopf and LeDoux (1999). The EPSPs are generally followed by a fast, GABA<sub>A</sub> receptor–mediated inhibitory postsynaptic potential (IPSP), or by a combined fast IPSP and slow (possibly GABA<sub>A</sub>-mediated) IPSP (Rainnie et al. 1991b; Washburn and Moises 1992). In disinhibited preparations, synaptic excitation may also elicit calcium spikes mediated by voltage-dependent Ca<sup>2+</sup> channels (Calton et al. 2000). Synaptic responses may also be evoked in central nucleus neurons by stimulation of the basolateral complex (Nose et al. 1991). These responses have been characterized as complex, multi-component EPSPs and IPSPs. The compound EPSP has components mediated by both NMDA and non-NMDA excitatory amino acid receptors. Several distinct IPSPs have been characterized as mediated by GABA<sub>A</sub> receptors, GABA<sub>B</sub> receptors (Rainnie et al. 1991b), GABA<sub>C</sub>-like receptors (Delaney and Sah 1999), and possibly glycine receptors (Nose et al. 1991).

We examined components of the evoked responses in the amygdala using voltage-sensitive dye imaging with a photodiode array. This relatively new technique has been successfully utilized to characterize spatiotemporal aspects of evoked and spontaneous activity in neocortex (Tsau et al. 1999; Wu et al. 1999b), olfactory bulb (Keller et al. 1998), piriform cortex (Demir et al. 1998), and thalamocortical pathways (Laaris et al. 2000). As the sampling rate of the diodes is considerably faster than that of charge-coupled device (CCD) cameras, it may be used to record rapid electrophysiological events in brain slices stained with voltage-sensitive dyes. The resulting composite images retain a high degree of spatial resolution (Lowe 1999; Wu et al. 1999a). These two features of the photodiode array make it feasible to study the signal propagation along network pathways.

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We used this system to characterize the signal propagation and synaptic pharmacology of the in vitro amygdala formation. We found that the response proximal to the stimulation site was composed of both synaptic and nonsynaptic elements that could be identified pharmacologically. In addition, we found that the distal responses contained almost entirely synaptically mediated components. We were able to simultaneously record the temporal phases of the synaptic responses and the anatomic distribution of these same responses. We then characterized the effects of neurotransmitter blockade on the synaptic responses and thus were able to uncover key aspects of the function of the amygdala network.

**METHODS**

Coronal brain slices (500 μm) were prepared from 21- to 35-day-old male Sprague-Dawley rats using a vibratome (Series 1000; Technical Products International, St. Louis, MO). Rats were decapitated under halothane anesthesia, and the brains were quickly removed. Coronal slices containing the amygdala formation were identified and incubated in standard artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 10 dextrose, 26 NaHCO3, 2 KCl, 1.25 KH2PO4, 2 CaCl2, and 1 MgSO4, equilibrated with 95% O2-5% CO2.

After at least 1 h of incubation, slices were transferred into separate incubation chamber containing ACSF and the voltage-sensitive dye JPW1131 (also known as RH479; from Dr. L. Loew, University of Connecticut, Farmington, CT) at 0.02 mg/ml for 40–60 min. The stained slices were then transferred to the recording chamber and perfused (3 ml/min) with dye-free ACSF at 29°C for at least 30 min before recording to wash out unbound dye. The optical signal was obtained with a Zeiss microscope (Axioskop 2FS; Carl Zeiss) using a water immersion lens (×10), coupled with a 464 hexagonal photodiode array (WuTech Instruments). Light from a HAL halogen lamp (12 V 100 W) was passed through a 705 ± 50 nm filter (D705/50; Chroma Technology) to the slice where it was focused. The image of the slice was then projected to the 464-element photodiode array through a ×10 water immersion objective lens and a zoom port. The zoom was set at about 0.83 so that the photodiode array maximally covered the objective field. With this setting, each diode received light from a 80 × 80 μm2 area of the objective field. The objective plane was also calibrated with a CCD camera (SensiCam; Cooke) and each pixel received 3.125 × 3.125 μm2 area of light (with a ×10 objective lens). The double calibration allowed location of a specified area on the slice to a corresponding diode. The output from each diode was then amplified, multiplexed, digitized, and stored in the computer (Wu et al. 1999a).

Single stimuli were delivered through a tungsten microelectrode (0.02 in. × 5 μM, 8 Degree; A-M System, Carlsborg, WA) placed in the lateral nucleus of amygdala. The intensity of stimulation was 70–100 μA/200 μs. Glass microelectrodes were made from TW150F-4 glass (thin wall glass with filament, 1.5 mm diam; World Precision Instruments) using a micropipette puller (P-87; Sutter Instrument). These electrodes were filled with NaCl (150 mM; 3–5 MΩ) and were used to monitor field potentials to monitor viability of the slice. The signal from the microelectrode was amplified with an Axopatch 200B amplifier (Axon Instruments) and then stored concurrently with the optical images.

Data acquisition and analysis was performed using NeuroPlex software (RedShirt Imaging, LLC) on a Pentium PC. A vibration isolation system (250WS-1; Minus-k Technology) was used to minimize the vibration noise.

After at least 30 min of washing of the stained slice in the recording chamber, a 2-s data acquisition (at a sample rate of 0.944 ms per frame) was triggered manually every 5 min. The stimulus was delivered during the acquisition with a 0.5-s delay. Averaged responses from a 240 × 240 μm2 area (3 by 3 diodes) close to the stimulating electrode was constantly observed until the responses were stabilized for half an hour before applying any drug. With this recording method, the responses remained stable for at least 2 h. Once the responses were stabilized for 30 min (6 recordings), the drugs were washed in for 30 min (6 recordings) followed by a 60-min wash out (12 recordings). The stabilized recordings from each condition (or the last 6 from wash out) were then averaged for comparison. An averaged response of six diodes immediately adjacent to the stimulating electrode (within the lateral nucleus of the amygdala complex) was used for analysis of temporal responses. With the evoked response, the very tip of the stimulating electrode generated an artifact revealed by a single diode that had a significantly larger response than other diodes (Fig. 1B). This diode was excluded from analysis but was used as an indicator for anatomically correlating diodes within specific areas of the slice.

The response of voltage-sensitive dye (JPW1131) has millisecondsod and a linear dependence on voltage change within ±100 mV (Lowe 1999) with a specific light absorption at 705 ± 50 nm. The optical imaging was made of pseudocolor intensity scaling in which the warm color corresponded to depolarization. A fixed scale was used to compare the images between the control and drug application. The term “fixed scale” refers to scaling signals of all diodes to a set maximum and minimum value, as opposed to “variable scale” in which the response of each diode is relative to its own individual maximum and minimum of response. The variable scale is best used for displaying the dynamic spreading of the peak signal; the fixed scale is best used for demonstrating the pattern of the spreading and relative intensity of the signal in different regions. In addition, fixed scale is used so that the changes before and after drug can be determined. Data are expressed as means ± SE.

All pharmacological agents were obtained from Sigma, except for d-2-amino-5-phosphonovaleric acid (d-APV; Acros) and TTX (Calbiochem, La Jolla, CA). Pharmacological agents were prepared from stock solutions and dissolved in ACSF before use. All stock solutions were made using distilled water except for 6,7-dinitroquinoxaline (DNQX), nifedipine [with dimethyl sulfoxide (DSMO)], and dl-threo-beta-hydroxyaspartic acid (THA; with 300 mM of NaOH).

**RESULTS**

**Characterization of the evoked signal in the lateral amygdala**

Results are reported from a total of 45 slices. To examine the network properties of the amygdala complex (Fig. 1A), we stimulated the lateral nucleus (La) and observed the propagation of the signal toward the basolateral nucleus (BL) and the amygdalostriatal transition zone (ASTr; Fig. 1C). Under normal conditions, the evoked signal did not propagate to the central nucleus (Ce). These results are consistent with the anatomical findings of direct connections from La to AStr but not to the nucleus (Ce). Figure 1B shows the diode array with each short line representing a 2-s trace from a single diode following a single stimulus. Since the dye binds to all cell membranes and changes its absorbance (at 705 ± 50 nm) according to membrane potential, the signal we observed represented integrative changes in membrane potential from both neuronal and nonneuronal elements. The diode located directly over the tip of the stimulating electrode always produced a markedly larger signal than the other diodes (Fig. 1B) and served as a reference point to locate different portions of the slice.

A typical local response in the amygdala following stimulation is shown in Fig. 2A. The trace was produced by averaging the response of the six diodes surrounding, but exclu-
These three components were all wavelength specific (Fig. 2). The optical response included three components: a fast depolarizing peak (phase 1), a valley (phase 2), and a slow depolarizing peak (phase 3). Each facet of the hexagon measures 1 mm. La, lateral amygdala nucleus; BL, basolateral amygdala nucleus; Ce, central amygdala nucleus; AStr, amygdalostriatal transition zone; CTX, neocortex. The shadow of the stimulating electrode is seen extending into La, and a faint shadow of the glass micropipette recording electrode is seen extending into the AStr. B: tracings of the individual photodiodes within the hexagonal array. Each short trace represents a 2-s optical response acquired from a single diode. The diode positioned over the stimulus electrode can be identified in the bottom right quadrant by the large deflection. C: a photodiode imaging frame with an overlay of the outline of the amygdala nuclei to demonstrate the range of the evoked signal at a 25-ms latency (last frame of D). D: pseudocolor frames from photodiode arrays showing the intensity of optical signal in different regions of the amygdala formation during the evoked response. Stimulation was delivered to La (1st frame) and the signal subsequently propagated toward BL and AStr. Each successive frame is separated by 2.5 ms. Depolarization is represented by warm color, while hyperpolarization is represented by cold color. Fixed scale was used for pseudocolorization in which every response trace was relative to a fixed scale; this technique best revealed the spatial distribution of the signal propagation. The depolarization generally decayed to baseline within about 200 ms. Signal intensity beyond the scale was represented by the limits of the scale, i.e., the red color represents the intensity that great than or equal to the upper limit of the scale.

Fig. 1. Propagation of evoked signal in the amygdala formation revealed by photodiode array. A: section of the rat brain slice containing amygdala formation. The position of the hexagonal photodiode array over the slice is indicated by dotted boundaries. Each diode over the stimulus electrode, demonstrating lack of "cross-talk" between diodes.

The characteristics of the evoked optical signal were first examined using the local (La) response (Fig. 1A). Phase 1 was composed of two kinetically distinguishable components (solid arrow, Fig. 3, A and B). The phase 1 fast component was presumably a fast sodium potential, since the peak was not changed by DNQX (20 μM), bicuculline (BIC; 20 μM), or removal of Ca2+ from the medium, but it was blocked completely by TTX (0.5 μM; Figs. 3 and 4; n = 3 slices). The slower component of phase 1 was reduced by application of DNQX by 31 ± 6%, and addition of BIC to the DNQX perfused slice had little effect on this phase (Fig. 3A; n = 4 slices; Fig. 3A). However, d-APV did not change the slope or amplitude of the phase 1 slow component even in the presence of BIC (n = 5 slices; in Fig. 3B, arrowhead indicates the peak of the phase 1 slow component). Therefore we concluded that the phase 1 slow component was mainly composed of a synaptically activated AMPA receptor–mediated potential. Note that BIC alone increased both the slope and amplitude of the phase 1 slow component (Fig. 3B), indicating that BIC facilitated neurotransmitter release.

As DNQX greatly deepened the phase 2 valley (Fig. 3A), the AMPA receptor–mediated potential was also a significant component of this phase. GABA A inhibition also played an important role in forming the phase 2 valley as BIC suppressed the valley (Figs. 3B and 6, A and C). The inhibition by d-APV started at phase 2 (Fig. 3B), indicating a small composition of the NMDA component in this phase. Therefore phase 2 was
primarily a combination of rapid decay of AMPA potentials, GABAergic inhibition, and subsequent activation of phase 3.

Phase 3 was less sensitive to DNQX (Fig. 3A), and the residual potential after DNQX was only observed in diodes proximal to the stimulation site (within 400 \mu m), suggesting that it was composed of nonsynaptically activated components. DNQX inhibition of phase 3 was only 13 \pm 2\%, significantly smaller compared with inhibition of phase 1 (n = 5, P < 0.01; Fig. 7). On the other hand, n-APV inhibited phase 3 by 17 \pm 3\% but had no significant effect on phase 1 (n = 5; Fig. 7). To determine the composition of phase 3, we applied several calcium channel blockers including nifedipine (100 \mu M, an L-type channel blocker), Ni^{2+} (50 \mu M, a T-type channel blocker), agatoxin-TK (50 nM, a P/Q-type channel blocker), \omega-conotoxin MVIIA (100 nM, a N-type channel blocker) in the presence of DNQX, n-APV, and BIC (n = 3 slices). Phase 3 was sensitive to nickel and \omega-conotoxin MVIIA (Fig. 4), while nifedipine and agatoxin had little effect (data not shown). After addition of Ca^{2+}-free medium, we observed a calcium-independent component remaining in phase 3. This component was blocked by TTX (0.5 \mu M), indicating a slow sodium-dependent potential in phase 3.

FIG. 2. Characteristics of the optical signal from the evoked response. A: the top trace represents a typical multiphasic optical response after single pulse stimulation of La, taken from averaged response of the 6 diodes adjacent to the tip of the stimulating electrode (but excluding the central diode containing the stimulus artifact). Three phases were identified as indicated by the numbered arrows: a fast depolarization peak (phase 1), a valley (phase 2), and a slow depolarization peak (phase 3). The optical response was wavelength specific (705 \pm 50 nm) as indicated by the complete loss of signal (same slice) in the bottom trace. B: top trace shows the same averaged optical response (different slice from A) with standard artificial cerebrospinal fluid (ACSF). Bottom trace shows complete loss of evoked signal after superfusion of ACSF containing TTX (0.5 \mu M). No optical artifacts were ever observed immediately adjacent to the tip of stimulating electrode after TTX superfusion (n = 3 slices) or with nonspecific wavelength filters.

FIG. 3. Properties of phase 1 and 2 of the evoked responses. A: all traces were obtained from averaging the responses of 6 diodes around the tip of the stimulating electrodes at La. Control trace shows phase 1 (the fast depolarizing peak) composed of 2 kinetically distinct components: the fast component (p1_fast, solid arrow) and the slow component (p1_slow, open arrow). 6,7-Dinitroquinoxaline (DNQX; 20 \mu M) inhibited the slow component without affecting the fast component (n = 6 slices). DNQX also inhibited phase 3. Adding bicuculline (BIC; 20 \mu M) to DNQX did not change the fast and slow components of phase 1 but caused an increase in the rate of rising of phase 3 (p3) (n = 3 slices). B: BIC enhanced the rise and amplitude of the slow component but did not alter the initial fast component of phase 1 (n = 5 slices). Solid arrow indicates the peak of the fast component (p1_fast), and open arrowhead indicates the peak of the slower component (p1_slow). BIC also enhanced the depolarization during phase 2 and 3, which eliminated the valley in the control trace. Adding n-APV (50 \mu M) to BIC did not change the kinetics or the peak of phase 1, with the inhibitory effect beginning at phase 2 but becoming more pronounced during phase 3 (n = 5 slices). Adding DNQX inhibited the slower component of phase 1 and deepened the valley, making an obvious phase 2 (n = 5 slices). DNQX in the presence of BIC also inhibited phase 3.

FIG. 4. Properties of phase 3 of the evoked responses. Traces show summed effects of various receptor and ion channel antagonists (n = 3 slices). Trace a: combined DNQX, n-APV, and BIC. Trace b: addition of Ni^{2+} (50 \mu M). Trace c: addition of agatoxin-TK (20 nM) and \omega-conotoxin MVIIA (100 nM). Trace d: removal of Ca^{2+} from superfusion medium. Trace e: TTX (0.5 \mu M). None of the above treatments affected the fast component of phase 1 except TTX; n = 3 slices.
We also attempted to remove the contribution from active glutamate reuptake to the phase 3 depolarization using THA (500 mM, reportedly an inhibitor of glutamate uptake following trains of stimulation) (Momose-Sato et al. 1998; Tong and Jahr 1994). However, we found that THA appeared to affect the signal in a complex manner. In control conditions, it depressed both phase 1 and 3 (data not shown), which is consistent with a previous finding that THA inhibited glutamate transmission in the amygdala (Wang et al. 1995). However, based on the observation that there was little response left in BL after treatment with DNQX and D-APV (Fig. 6), we did not consider that glutamate reuptake significantly contributed to the residual signal. Adding BIC into slices perfused with DNQX significantly enhanced phase 3 over its level in the presence of DNQX alone (Figs. 3A and 7), indicating that GABA blockade facilitates activation of voltage-dependent channels.

Effects of DNQX, D-APV, and BIC on the propagation of the signal

Stimulation of La generated optical signals that propagated to BL and AStr (Figs. 1C and 5B). The traces of BL responses were generated by averaging a cluster of 7 diodes at BL centered 0.5 mm away from the tip of the stimulating electrode. Applying DNQX (20 μM) caused a significant block of the signal spreading toward both BL and AStr (Fig. 5B), and the optical signal was limited to La. This residual distal trace consisted of a fast sodium spike and slow potential as shown by the optical traces (Fig. 5A). In comparison to traces from the La local response, traces from distal areas appeared to be less complex, as the phases (see above) were much less distinct. All effects of DNQX, D-APV, and BIC on the traces recorded in AStr were qualitatively similar to those of BL (data not shown).

On the other hand, D-APV (50 μM) did not significantly change the area of spreading but caused a small reduction of the optical signal in phase 3 (Fig. 5, C and D). Comparing the reduction caused by D-APV in local (La) and distal (BL) with or without BIC, we found that NMDA component did not play a significant role in gating the signal propagation (Figs. 5D and 6D).

Addition of BIC (20 μM) greatly enhanced both the intensity of responses (Fig. 6, A and C) and the area of propagation (Fig. 6, B and D) to involve areas that were previously silent, in particular portions of the central amygdala nucleus. Addition of DNQX still significantly blocked the propagation and reduced the intensity of the response (Fig. 6, A and B), while D-APV still did not block signal propagation and only caused a reduction in the late (phase 3) depolarization (Fig. 6, C and D).

Analysis of peak responses showed that the percentage inhibition by DNQX in BL (81 ± 3%) was significantly greater than in La (31 ± 6%; n = 5; P < 0.01; Fig. 7). In contrast, inhibition of responses by D-APV was similar in La (17 ± 3% for phase 3 of La) and BL (15 ± 1%; n = 5; Fig. 7). In the presence of BIC, the enhancement of the peak responses was 28 ± 5% for phase 1 of La and 88 ± 10% for BL peak, demonstrating a significantly greater effect in the distal region (BL; n = 5; P < 0.01; Fig. 7). In the presence of BIC, the peak

![Figure 5](http://jn.physiology.org/)

**FIG. 5.** Effects of DNQX and D-APV on the optical responses and signal propagation. A: DNQX (20 μM) inhibited the depolarization of the evoked response locally in La and distally in BL. The traces are from 1 preparation. The inhibition was consistently more pronounced in phase 1 and 2 (n = 5 slices). B: consecutive pseudocolor frames showing the signal propagation from La toward BL and AStr. The total area of propagation was not reduced by D-APV, but the intensity of the optical signal was decreased at the late stage of the evoked response. Frames were separated by 15 ms, and fixed scale was used. C: optical traces of the evoked responses at La and BL before and after addition of D-APV (50 μM) in another slice. The inhibition by D-APV was more dominant in phase 3, while phase 1 in La was not changed by D-APV. Stimulus intensity increased to 250 μA (from 70 μA in A and B) to enhance N-methyl-D-aspartate (NMDA) receptor–mediated component. D: pseudocolor frames demonstrating the signal propagation from La to BL and AStr. The total area of propagation was not reduced by D-APV, but the intensity of the optical signal was decreased at the late stage of the evoked response. Frames were separated by 15 ms, and fixed scale was used.
of phase 1 was measured at the inflection of the initial response as indicated by the open arrowhead in Fig. 3B. This determination of the peak of the evoked response was used to minimize possible contamination by epileptiform activity during the late phase of the response in the presence of BIC.

The latency to peak was also examined. The latency to peak was 5.5 ± 0.2 ms for phase 1 of La and 10.6 ± 0.3 ms for BL. Given the distance between the two measured areas was about 400 μm, an estimated velocity of 78.4 ± 6.9 mm/s (n = 5) was obtained for the evoked signal transmission. Because the peaks indicated the maximum synaptic activation, the velocity reflected the propagation of synaptic transmission. When the synaptic transmission was blocked with DNQX, the latency to peak was 2.5 ± 0.2 ms for phase 1 of La and 4.0 ± 0.2 ms for BL, respectively, an approximate velocity of 266.7 ± 61.6 mm/s (n = 5). Such a velocity reflected nonsynaptic conduction through the neural fibers, which is considerably faster than the velocity for synaptic transmission. BIC and D-APV had no significant effects on the latencies (or the velocity) of signal propagation.

**DISCUSSION**

Clinical studies suggest that the amygdala is necessary for processing various types of emotional information (Adolphs et al. 1994, 1998; Huguenin and Chauvel 1993; LaBar et al. 1998). This idea is supported by animal models of experimental anxiety that indicate a critical role for the amygdala in the acquisition and expression of fear-conditioned behaviors (Davis 1992; Davis et al. 1994; Fanselow and LeDoux 1999). In addition, other workers have emphasized the importance of the amygdala in memory and attentional processes (Gallagher and Holland 1994; McGaugh et al. 1993), which, along with emotional processing and autonomic regulation, may demonstrate a continuum of functionality within the temporal lobe. The amygdala subserves these functions through a unique set of external connections and intrinsic networks (Pitkanen et al. 1997).

Previous studies using visualization of voltage-sensitive dyes with a photodiode array have focused on evoked and spontaneous activity in neocortex (Tsu et al. 1999; Wu et al. 1999b), olfactory bulb (Keller et al. 1998), piriform cortex (Demir et al. 1998), and thalamocortical pathways (Laaris et al. 2000). In neocortex, spread of epileptiform activity was visualized following superfusion with either Mg²⁺-free medium or normal medium containing BIC (Tsu et al. 1999; Wu et al. 1999b). These studies indicated that populations of neocortical neurons form dynamic ensembles that propagate waves of excitation that are distinct from epileptiform activity. In the above studies, the spatiotemporal aspects of the activities could be examined with much greater resolution than with conventional field electrode recording. A previous study using optical imaging showed that signal propagated to the medial and central amygdala nuclei following stria terminalis stimulation.
effects of D-APV on the phase 3 of La and peak of the BL responses inhibited phase 3 but not phase 1 of the La response (n = 1428 C. WANG, W. A. WILSON, AND S. D. MOORE). The local optical signal produced by electrical stimulation in the in vitro amygdala preparation. First, our data indicate that the local optical signal produced by electrical stimulation in the La contains multiple physiological components that may be isolated pharmacologically. A significant portion of this signal was dependent on synaptic transmission and included responses to both excitatory and inhibitory neurotransmitters. However, in the region near the stimulating electrode, the stimulus also produced a large signal that was independent of synaptic depolarization mediated by AMPA receptors. These voltage-dependent cation channels in the vicinity of the stimulus electrode contribute significantly to the total depolarization at the site of origin of the efferent fibers and may regulate patterns of efferent fiber spike activity. However, addition of DNQX reduced the amplitude of all phases of the optical signal, including this late depolarization, suggesting that local activation of voltage-dependent channels is dependent in part on excitatory synaptic activity.

Second, the signals propagated to areas away from the stimulus site appeared to be almost entirely dependent on synaptic activity, and specifically AMPA receptor–mediated depolarization. The AMPA receptor antagonist DNQX could effectively block the signal to all target areas outside the La, and this effect persisted when the preparation was disinhibited with BIC. However, regardless of whether BIC was present, D-APV had minimal effect on the propagation of the signal, suggesting that NMDA receptors have little role in mediating the extent of synaptic activation.

GABAergic inhibition also regulated the extent of signal propagation, as seen by the effect of disinhibition with BIC. Under this condition, depolarization was observed in several previously silent areas of the amygdala, including portions of Ce. Such gating of inputs to Ce by GABAergic interneurons has previously been suggested on the basis of anatomic and electrophysiologic data (Royer et al. 1999). An interesting observation was that BIC produced a significantly larger enhancement of phase 3 or the late depolarizing response in distal areas. The enhancement of phase 3 by BIC is probably mediated by multiple mechanisms. First, BIC should potentiate glutamate release, thereby activating more glutamate receptors. Second, BIC may also block Ca\(^{2+}\)-dependent K\(^+\) channels (Khawaled et al. 1999), which may subsequently cause prolonged activation of the voltage-gated Ca\(^{2+}\) or Na\(^+\) channels that are major components of phase 3. In fact, our previous study demonstrated an increased Ca\(^{2+}\) conductance in the presence of BIC (Calton et al. 2000). Third, the evoked epileptoform burst activity in the presence of BIC (Gean and Chang 1991) may also contribute to phase 3. Therefore BIC, even in the presence of DNQX and D-APV, may enhance the voltage-gated components of phase 3 to an extent greater that the combined voltage-gated and NMDA components in the control condition (Fig. 6C). We also occasionally observed spontaneous, nonevoked synchronized activities in the presence of BIC. These spontaneous activities always arose from the lateral side of the BL (corresponding to the bottom left corner of the photodiode frame) and did not propagate to other areas of the amygdala. The amplitude was much smaller than that of the evoked response, and the duration was also shorter (about 150 ms) than that of the evoked response (beyond 300 ms).

Optical recordings of excitatory synaptic responses in the rat olfactory bulb appear qualitatively similar to our findings in the amygdala, as CNQX significantly attenuated the signal, while APV reduced a small residual component (Keller et al. 1998). However, in contrast to the amygdala, BIC had little effect on the optical signal, suggesting minimal endogenous inhibitory tone mediated by GABA\(_A\) receptors. In mouse barrel cortex, BIC increased the amplitude of the optical signal evoked by thalamic stimulation, without affecting the spatial propagation, while superfusion of Mg\(^{2+}\)-free medium produces an APV-sensitive spread of excitation to adjacent cortical areas (Laaris et al. 2000).

We observed that all residual components of the optical signal, including the initial fast transient, were eliminated by addition of TTX. Under these conditions, the only detectable signal was the stimulus artifact that was confined to the single diode overlaying the tip of the stimulating electrode. Thus current spread from the stimulus did not appear to be a factor in any component of the physiological signal, even from diodes immediately adjacent to the stimulus site. We have also shown that the optical signal was not contaminated by nonspecific artifacts, as filtering out the signal at the wavelength of the dye response completely eliminated the signal.

Our observations of transmitter-dependent activation of the basolateral complex is consistent with the data obtained from...
single neurons using intracellular recordings (Calton et al. 2000; Rainnie et al. 1991a,b; Washburn and Moises 1992) and appears to accurately reflect the time course of monosynaptic activation observed in those studies. However, we have also demonstrated the extent to which the spatial distribution of the synaptic response is dependent on glutamatergic and GABAergic transmission. We hypothesize that the small signal remaining after blockade of glutamatergic and GABAergic neurotransmission may be due to the depolarization of afferent fibers and terminals projecting into the BL, antidromic activation of neuronal elements in the BL, or due to a synaptic response produced by other transmitters in this pathway (e.g., neuropeptides).

We suggest that use of the photodiode array in conjunction with voltage-sensitive dyes should greatly enhance our understanding of network function in the amygdala. In the amygdala formation, we have so far demonstrated that intracellular signal propagation is determined by non-NMDA and GABA receptors, while NMDA receptors participate to a much lesser extent. Given the apparent critical role of the amygdala in generating fear and anxiety responses (Davis 1992; Davis et al. 1994), we speculate that this gating action may account for the potent anti-anxiety effect of drugs that facilitate GABA receptors—mediated activity (Davis et al. 1994; Sanders and Shekhar 1995). We also suggest that modulation of AMPA receptors may have similar anxiolytic effects.

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