Low-magnesium medium induces epileptiform activity in mouse olfactory bulb slices

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Igelström KM, Shirley CH, Heyward PM. Low-magnesium medium induces epileptiform activity in mouse olfactory bulb slices. J Neurophysiol 106: 2593–2605, 2011. First published August 10, 2011; doi:10.1152/jn.00601.2011.—Magnesium-free medium can be used in brain slice studies to enhance glutamate receptor function, but this manipulation causes seizure-like activity in many cortical areas. The rodent olfactory bulb (OB) slice is a popular preparation, and potentially ictogenic ionic conditions have often been used to study odor processing. We studied low Mg2+-induced epileptiform discharges in mouse OB slices using extracellular and whole cell electrophysiological recordings. Low-Mg2+ medium induced two distinct types of epileptiform activity: an intraglomerular delta-frequency oscillation resembling slow sniff-induced activity and minute-long seizure-like events (SLEs) consisting of large negative-going field potentials accompanied by sustained depolarization of output neurons. SLEs were dependent on N-methyl-D-aspartate receptors and sodium currents and were facilitated by GABA \(_A\) receptor blockers (Schwartzkroin and Prince 1978), 4-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptors, and \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionate. The events were initiated in the glomerular layer and propagated laterally through the external plexiform layer at a slow time scale. Our findings confirm that low-Mg2+ medium should be used with caution in OB slices. Furthermore, the SLEs resembled the so-called slow direct current (DC) shift of clinical and experimental seizures, which has recently been recognized as being of great clinical importance. The OB slice may therefore provide a robust and unique in vitro model of acute seizures in which mechanisms of epileptiform DC shifts can be studied in isolation from fast oscillations.

High potassium; persistent sodium current; slow DC shift; tonic phase; ion diffusion

SYNCHRONOUS OSCILLATIONS in the central nervous system are important in both health and disease and can range from very slow (0.001–1 Hz; Drew et al. 2008) to ultrafast (>250 Hz; Bragin et al. 1999). Hypersynchronous population activity is a hallmark of epileptiform activity, and understanding the mechanisms of seizure generation (ictogenesis) is a major goal in epilepsy research. In the intact brain, seizure discharges generally propagate across large regions, making it difficult to isolate mechanisms underlying seizure initiation, synchronization, and termination. It is therefore necessary to use a variety of reduced systems, from small neuronal networks in silico to brain slices models in vitro. Brief (<1 s) epileptiform events resembling interictal spikes can be readily induced in slices of hippocampus or cortex by using medium containing GABA\(_A\) receptor blockers (Schwartzkroin and Prince 1978), 4-aminopyridine (Rutecki et al. 1987), high K\(^+\) (Tancredi and Avoli 1987), or low Mg\(^{2+}\) (Tancredi et al. 1990). It has, however, proven much more difficult to induce and maintain prolonged seizure-like events (SLEs) in vitro. Although ictonic-clonic-like SLEs have been observed in slices of hippocampus (Anderson et al. 1986; Fellin et al. 2006; Traynelis and Dingledine 1988) and entorhinal cortex (Dreier and Heinemann 1991; Lucke et al. 1995), such reports are rare compared with the many studies on interictal bursts.

The olfactory bulb (OB) is an archetypical cortical structure consisting of hundreds of odor-specific functional columns, each comprising one glomerulus and the output neurons (mitral cells, MCs) affiliated with it (Chen and Shepherd 2005). The OB has long been known to generate various types of oscillations in vivo (Adrian 1942; Macrides and Chorover 1972), ranging from respiratory delta/theta frequencies (~1–12 Hz), through the beta range (15–30 Hz), to gamma oscillations (40–100 Hz) (Kay et al. 2009). Like other cortical structures with a capacity for oscillations, the OB is very seizure prone (Araki et al. 1995; Cain 1977; Fujiwara et al. 2010; McEvoy et al. 2002). The rodent OB slice (Fig. 1) is an attractive in vitro system, because it preserves a fully functional network including lateral inhibitory pathways (Augst et al. 2003). The OB slice has been widely used in studies of olfactory processing (e.g., Augst et al. 2003; Carlson et al. 2000; Hayar et al. 2004a; Shirley et al. 2010), and population oscillations at gamma and theta frequencies can be induced and studied in the slice (Friedman and Strowbridge 2003; Lagier et al. 2004; Schoppa and Westbrook 2001). Synchronized population output of glomerular columns is driven by theta bursting in external tufted cells, the frequency of which can be entrained by olfactory nerve stimulation at physiological sniff frequencies (De Saint Jan et al. 2009; Hayar et al. 2004a, 2004b). MCs respond to external tufted cell/olfactory nerve input with a long-lasting depolarization (LLD) that is synchronous in all MCs with apical dendrites in the same glomerulus (Carlson et al. 2000; De Saint Jan et al. 2009). LLDs are accompanied by negative local field potentials (LFPs) in glomeruli (Karnup et al. 2006) and have an early phase mediated by \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors and a late N-methyl-D-aspartate (NMDA) receptor-dependent component. Individual excitatory phases of delta/theta oscillations both in vitro and in vivo show LLD-like glutamate dependence (Cang and Isaacson 2003; Margrie and Schaefer 2003; Schoppa and Westbrook 2001), indicating that the glomerular amplification circuitry is responsible for both LLDs and respiratory oscillations.

OB slice studies can provide valuable information about the mechanisms of olfactory oscillations. As with all in vitro research, however, the use of artificial conditions requires critical evaluation of the physiological relevance of the findings. This may be a particular challenge in OB research.
because pathological oscillatory states of the bulb have rarely been studied. Several detailed electrophysiology studies on olfactory processing have used artificial cerebrospinal fluid (ACSF) with potentially ictogenic properties. For instance, delta oscillations in vitro were induced by high-intensity electrical stimulation, treatment with NMDA, or application of 12 mM KCl (Schoppa and Westbrook 2001). Furthermore, ultrafast oscillations in the OB (0.03–0.1 Hz) were only observed under low-Mg$^{2+}$ conditions (Puopolo and Belluzi 2001), and several classical studies investigating NMDA receptor neurotransmission or lateral excitation in the OB have used low-Mg$^{2+}$ medium (e.g., Aroniadou-Anderjaska et al. 1999b; Christie and Westbrook 2006; Didier et al. 2001; Isaacson 1999). Although these valid experimental strategies have usually been accompanied by adequate discussion of their limitations, little is known of the possible ictogenic effects of these manipulations.

In the present study, we recorded LFP activity in mouse OB slices in the absence of extracellular Mg$^{2+}$, a manipulation often used in OB studies but known to cause ictal-like activity in hippocampal, entorhinal, and neocortical slices (Anderson et al. 1986; Aram and Lodge 1988; Dreier and Heinemann 1991). We found that low-Mg$^{2+}$ conditions induced two types of epileptiform activity in the OB slice: (1) delta activity confined within glomerular columns and (2) widely synchronized, recurrent, minute-long tonic SLEs that occurred with remarkable reproducibility and could be maintained for hours. In addition to emphasizing the potential issues involved in using ictogenic medium, these findings indicate that the OB slice provides an unusually robust in vitro model of tonic seizures.

**METHODS**

**OB slice preparation.** Swiss outbred male mice ages 18–35 days were used, and all experiments were approved by the University of Otago Animal Ethics Committee. Animals were killed by decapitation, and horizontal OB slices (350 μm) were prepared as previously described (Heyward et al. 2001). Briefly, the bones surrounding the OBs were removed and the brain was immediately exposed to ice-cold ACSF containing (in mM) 120 NaCl, 3 KCl, 1.3 CaCl$_2$, 1.3 MgCl$_2$, 25 NaHCO$_3$, 10 glucose, and 5 N,N-bis(2-hydroxyethyl)-2-aminoethane-sulfonic acid, pH 7.2 when saturated with 95% O$_2$-5% CO$_2$. Both OBs were dissected out, and their ventral side was glued to the stage of a vibrating microtome (Vibratome Series 1000; The Vibratome, St. Louis, MO; or Leica VT1000S). Three horizontal slices were obtained from each bulb. For experiments using microcuts, slices were transferred to an ice-cold Sylgard-coated dish and a sapphire blade (World Precision Instruments, Sarasota, FL) was used to make a fine cut through a specific layer under a dissecting microscope (Shirley et al. 2010). Slices were allowed to recover in a 30°C holding bath for 1 h before experiments began. After the recovery period, slices were stored in the holding bath at room temperature and transferred to the recording chamber one at a time.

**Ictogenic ACSF.** Ictogenic ACSF was made by excluding added MgSO$_4$ from the ACSF and raising the KCl concentration to 5 mM (0-Mg/HK). Some experiments also used medium lacking added Mg$^{2+}$ (0 Mg) with standard K$^+$ (3 mM) or medium with standard Mg$^{2+}$ (1.3 mM) and high K$^+$ (HK; 5 mM KCl). Picrotoxin (PTX; 100 μM) was included in the 0-Mg/HK medium for some experiments using microcuts (see Fig. 4C).

**Electrophysiology.** Brain slices were submerged in a two-sided perfusion chamber (Heyward 2010) with an ACSF flow rate of 2–3 ml/min. Bathing medium was pumped to the bath by a peristaltic pump using tubing of low gas permeability (Sanipure-60; Cole Parmer). The temperature was maintained at 30°C (TC-BIP; Cell Microcontrol). For experiments using direct visualization of neurons, the perfusion bath was mounted on a fixed-stage microscope (BX51; Olympus, Tokyo, Japan). MC somas were identified using a ×40 water-dipping objective (NA 0.8; Olympus) and near-infrared differential interference contrast (NIR-DIC) videomicroscopy (wavelength 850 ± 10 nM). In other experiments, slices were viewed under a ×4 dry objective using videomicroscopy with incident oblique illumination by a fiber optic cable submerged in the recording bath. In some experiments, an interface chamber of our own design was used for field recordings. This was fabricated using fused filament fabrication with a RepRap printer (www.reprap.org) by Able Innovation Sweden (www.ableinnovation.com). For recording, slices were placed in the interface chamber and maintained at 30°C in an ACSF flow rate of 2–2.5 ml/min with warmed humidified 95% O$_2$-5% CO$_2$ gas continuously flowing across the top of the slice.

Recording pipettes were pulled from filamented borosilicate glass on a Sutter P97 puller (Sutter Instruments, Novato, CA). Single-unit activity was recorded using pipettes with a resistance of 5–15 MΩ when filled with ACSF. LFP recordings used pipettes with a resistance of 3–5 MΩ when filled with ACSF. Pipettes for whole cell recording had a resistance of 4–8 MΩ when filled with solution containing (in mM) 125 K-glucuronate, 2 MgCl$_2$, 10 HEPES, 2 Mg-ATP; 0.2 Na$_2$GTP, 1 NaCl, and 0.2 EGTA.

For single-unit studies, signals were recorded using an AM Systems model 2400 amplifier, filtered at 5 kHz, and digitized at 10 kHz. For dual-site single-unit recordings, an Axoprobe 1-A amplifier (Axon Instruments) was used. We made dual-site LFP recordings and combined single-unit and LFP recordings using model 750 and model M701 microprobe amplifiers (World Precision Instruments). Field recordings were filtered at 1 kHz and digitized at 10 kHz. All extracellular recordings used WinEDR (University of Strathclyde, Glasgow, UK) for data acquisition. Whole cell recordings were filtered at 5 kHz and digitized at 10 kHz and were performed using either the “blind” technique or with direct visualization using standard NIR-DIC imaging. Blind recordings were made using the AM Systems model 2400 amplifier and WinEDR as acquisition software. Whole cell recording using NIR-DIC was performed using a Multiclamp 700A amplifier. Digidata 1322A acquisition system, and pClamp 9.2 software (Molecular Devices, Sunnyvale, CA). Seal resistances were routinely >1 GΩ, and voltage recordings were not corrected for a calculated junction potential of 9–10 mV.

**Chemicals.** The following agents were used for bath application: (2R)-amino-5-phosphono-pentanoic acid (AP5; Sigma), (RS)-baclofen (Tocris), CGP 55845 hydrochloride (Tocris), 5,7-dichlorokynurenic acid (dCK; Tocris), 6,7-dinitroquinoxaline-2,3-dione (DNQX; Tocris), (+)-MK-801 maleate (Tocris), dl-threo-β-benzylloxyaspartic

![Fig. 1. The olfactory bulb (OB) slice. Mitral cell (MC) somas are organized in the anatomically well-defined mitral cell layer (MCL), and their apical dendritic tufts reside within glomeruli, where they receive input from the olfactory nerve (ON). MCs receive GABAergic feedback within glomeruli and from granule cell (GC) dendrites in the external plexiform layer (EPL). In addition, lateral inhibition is achieved by long-range glutamatergic projections by short-axon cells in the glomerular layer (GL), which activate interneurons in distant glomeruli. The granule cell layer (GCL) contains the GC bodies, and the EPL contains MC lateral dendrites and GC dendrites.](http://jn.physiology.org/)
acetylcholine (ACh, Tocris), glycine (Sigma), 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine hydrochloride (GYKI 52466; Sigma), muscimol (Sigma), phentolamine (Sigma), PTX (Sigma), SR-95531 (gabazine; Sigma), strychnine (Sigma), and YM298198 hydrochloride (Tocris).

**Data analysis.** Cross-correlation analysis was done using Clampfit. For cross-correlation of field SLEs, DC LFP recordings were down-sampled to 200 Hz. For analysis of delta activity, traces were band-pass filtered (0.25–4 Hz). To allow for cross-correlation analysis of the slow depolarization in whole cell recordings independent of action potential spiking, membrane potential was averaged over time by downsampling to 1 Hz after low-pass filtering at 2 Hz. Power spectral analysis of delta activity was done on 5 min of band-pass filtered (0.25–4 Hz) recording, using the automatic fast Fourier transform (FFT; Hamming window) in Clampfit. Burst analysis was done on single-unit recording traces on visually identified periods of delta bursting. Duration was quantified automatically by burst analysis in Clampfit (minimum 5 spikes/burst, Poisson surprise <5 rejected), and the dominant burst frequency was determined from the peaks of autocorrelation histograms of “time of peak data” (50-ms bins). Afterhyperpolarization (AHP) amplitudes during 0-Mg/HK washin were determined by calculating the voltage difference between the AHP peak and the resting membrane potential. For this purpose, the resting potential was defined as the average of the AHP peak and action potential threshold (beginning of rising phase) in standard ACSF. For Fig. 3C, the graphical representation of action potential firing frequency over time was obtained by FFT smoothing of a rate histogram (5-s bins). Statistical tests were paired t-tests unless otherwise stated, and a significance level of 0.05 was used for all tests. Data are means ± SE.

**RESULTS**

**Evolution of epileptiform activity in 0-Mg/HK.** Irregular, spontaneous negative fields could be recorded from glomeruli under control conditions, as described by others (e.g., Karnup et al. 2006) (n = 12/13 slices, Fig. 2A, average frequency 0.5 ± 0.08 Hz). MCs at rest fired either continuously or with irregular bursts (Fig. 2A, bottom trace). When ionogenic 0-Mg/HK medium was washed in, spontaneous fields transformed into a delta-frequency oscillation (0.5–2 Hz) (Fig. 2B). Delta oscillations began within 2–4 min of washin and showed a gradual increase in signal power over the first minutes of treatment (Fig. 2C, top trace). The delta oscillation was not synchronous across glomerular columns (cross-correlation coefficient (CC) = 0.04 ± 0.02, n = 4; Fig. 2C), consistent with the known lack of excitatory interglomerular coupling in mammals (Chen and Shepherd 2005). During this phase, MCs showed a stereotypical ~10-mV membrane potential oscillation and delta burst pattern, readily seen in extracellular single-unit and whole cell current-clamp recordings (Fig. 2B, bottom trace). Onset of delta bursting in MCs was preceded by ~1 min of a gradually increasing action potential firing rate, and intraburst frequency then increased linearly over time for 1–4 min (range of R2 values = 0.4044–0.9291, median R2 value = 0.8075, n = 13). Early-onset delta activity occurred in 85% of MCs in response to 0-Mg/HK (n = 134). It was facilitated by the increasing extracellular K+ concentration ([K+]o), since it occurred more frequently in HK medium (75% of MC, n = 72) than in 0-Mg medium (41% of MC, n = 81). Delta oscillations were weak or absent in field recordings from the external plexiform layer (EPL; n = 7 slices).

After this initial period of increasing delta activity, the glomerular oscillation was interrupted by the onset of recurrent minute-long negative-going DC shifts (SLEs), which occurred simultaneously in glomeruli located 500 μm apart ([K+]o = 0.67 ± 0.08, n = 4 slices). During the SLEs, varying degrees of glomerular oscillations were seen. Figure 2D shows an example of one SLE recorded from two different glomeruli, with one glomerulus showing electrical flattening (top trace) and the other showing an increase in delta activity during the event (bottom trace). There was no obvious fast population spike or wave activity during SLEs, but power spectral analysis revealed an increase in signal power at high (100–200 Hz) frequencies (n = 4). Smooth synchronous SLEs without delta oscillations could be recorded from the deeper layers [EPL, mitral cell layer (MCL), and internal plexiform layer (IPL)]. The events were regular and recurrent at 0.012 ± 0.005 Hz (n = 54, example in Fig. 2E). They never ceased spontaneously in the presence of 0-Mg/HK medium, and washout of

![Fig. 2. Epileptiform activity in the OB slice. A: spontaneous field potentials recorded from the GL in normal artificial cerebrospinal fluid (ACSF; interface chamber) and an example of MC current-clamp recording under control conditions (submersion chamber). B: after a few minutes in Mg²⁺-free, high-K⁺ (0-Mg/HK) medium, glomerular fields transformed into a delta oscillation accompanied by a stereotypical MC burst pattern. C: bandpass-filtered trace showing the gradual increase in delta power during 0-Mg/HK washin (top). A lack of cross-correlation (CC) is shown between dual-site field potential recordings (FP1 and FP2) from 2 glomeruli 500 μm apart (bottom; average in black, individual recordings in gray). D: example of a seizure-like event (SLE) recorded from 2 glomeruli, showing the synchronous direct current (DC) shift and varying degrees of delta activity. The inset shows the average CC function in black and that of individual dual-site recordings in gray. E: example of the long-term stability of recurrent SLEs.](http://jn.physiology.org/Download)
ictogenic medium led to complete cessation of SLEs within 5–10 min.

**Sustained depolarization of output neurons during SLEs.** Current-clamp recordings showed that SLEs were accompanied by a sustained depolarization in MCs that lasted for the duration of the event (n = 68). The amplitude of the depolarization was highly consistent between SLEs in individual cells but varied greatly between cells, ranging from a slight depolarization with limited effects on action potential amplitude (Fig. 3A1) to massive depolarizing plateaus that resulted in strong spike inactivation (Fig. 3A2). Consistent with observations in LFP recordings, the rise of the depolarization was gradual, and the membrane potential reached its maximum after seconds to tens of seconds. The onset of the sustained depolarization was sometimes preceded by a short period of delta-frequency bursting, and intense activity consistent with synaptic events was seen immediately before onset (Fig. 3A2, single asterisks). At the peak of the depolarization, there was either chaotic membrane potential activity at depolarized potentials (Fig. 3A2, double asterisks) or variable patterns of membrane voltage oscillations. The most striking pattern of MC activity during this phase was an ~20-mV oscillation at 20–25 Hz, during which each depolarization was crowned by two to four action potentials with gradually declining amplitude (Fig. 3A1, double asterisks). Recovery of membrane potential toward baseline was accompanied by the gradual recovery of spike amplitude and often delta to subdelta oscillations/bursts at gradually declining interburst frequency. After the cessation of firing, MCs showed an afterhyperpolarization, the amplitude of which also varied between cells. Dual whole cell recording of pairs of MC located up to 500 μm apart confirmed that the slow sustained depolarization was synchronous across the slice (CC = 0.57 ± 0.07, n = 7). Simultaneous SLEs were also recorded consistently from MCs located >500 μm apart using dual single-unit recording (n = 42/45 pairs).

**Fig. 3. MC recordings.** A: whole cell recording of SLEs in 2 representative MCs showing the minute-long depolarization and variable firing patterns accompanying SLEs. In A1, action potential (AP) firing rate reached a peak within the first few seconds of the events (single asterisks), and complex burst activity at 20–25 Hz often developed at depolarized potentials (double asterisks). In A2, an example of another MC is shown during an SLE, displaying a greater depolarization than the cell in A1, increased synaptic noise before onset (single asterisks), and chaotic membrane potential activity instead of the 20- to 25-Hz rhythm (double asterisks). B: progressive loss of afterhyperpolarizations (AHPs) during 0-Mg/HK washin. The whole cell recording of a MC shows spontaneous long-lasting depolarization (LLDs) at rest (B1) and individual APs with AHPs (B1, bottom). Progressive depolarization of membrane potential began after a few minutes of washin and was accompanied by a reduction in AHP amplitude (B2). At SLE onset, AHPs were entirely absent (B3). C: gradual decrease in AHP amplitude over time. AHP amplitudes are plotted for all APs as a function of time, showing a progressive decrease in AHP amplitude before SLE onset (top). The black line shows AP firing frequency over time (smoothed). The bottom traces show current-clamp recordings of a MC under control conditions (gray) and during onset of hyperexcitability in 0-Mg/HK (black), showing an increased AP firing frequency and smaller AHPs.
Progressive loss of AHPs before SLE onset. The tonic-like seizure morphology and slow synchronization of OB events resembled SLEs recorded under low-Ca\(^{2+}\) conditions in the CA1 (Konnerth et al. 1986). In the low-Ca\(^{2+}\) model, seizure onset is preceded by a gradual loss of AHPs in pyramidal neurons, eventually leading to a sustained depolarization (SLE) accompanied by a slowly spreading potassium wave (Haas and Jefferys 1984; Jefferys and Haas 1982; Konnerth et al. 1986; Yaari et al. 1986). AHP amplitudes (relative to resting membrane potential) were quantified in MC whole cell recordings \((n = 8)\) during 0-Mg/HK washin. Action potentials in MCs showed stable AHP amplitudes under control conditions and during the first 2–3 min of washin (Fig. 3B1). The onset of hyperexcitability was characterized by slight membrane depolarization, a gradual increase in action potential firing frequency with or without delta bursting, and a progressive reduction in AHP amplitude (Fig. 3, B2 and C). At the onset and peak of the SLE, MCs lost their AHPs and instead showed varying degrees of afterdepolarizations, in the most extreme cases leading to beta oscillations (Fig. 3A1). SLE phases were accompanied by cyclical changes in AHP amplitude, with a decrease in amplitude at onset and a recovery toward the end of the event (Fig. 3C, \(n = 8\)). These observations are consistent with cyclical changes in extracellular K\(^{+}\) levels, affecting the driving force for currents mediating AHPs.

SLEs originate in glomeruli. Olfactory glomeruli normally provide powerful odor-specific amplification of sensory input (Chen and Shepherd 2005), and field bursts are driven by intrinsically bursting external tufted cells (De Saint Jan et al. 2009; Hayar et al. 2004a, 2004b). Consistent with a leading role of the glomerular layer (GL), SLEs in the EPL tended to lag behind those in the GL (Fig. 4A, left), with CC peaks at positive lag values \((n = 4/5\) slices, Fig. 4A, right). To test whether glomeruli are necessary for SLE generation, we made a microsurgical cut between the EPL and GL, removing the GL but preserving the EPL. To obtain a more informative measure of baseline activity than field recordings can provide, we recorded MC membrane potential in whole cell current clamp and looked for evidence of sustained depolarizations and periodic high-frequency firing, consistent with SLEs. Despite a clear excitatory response to ictogenic medium, none of the cells tested showed any evidence of seizure-like activity in the absence of glomeruli \((n = 4,\) Fig. 4B).

SLEs propagate through the EPL. The OB lacks interglomerular excitatory synaptic pathways that could activate distant MCs, but nonsynaptic lateral excitation could occur in the EPL or GL. To first test whether propagation was limited to one of these layers, microwounds were made through the olfactory nerve layer (ONL), GL, EPL, or the IPL (Augst et al. 2003; Shirley et al. 2010). Dual-site LFP recordings were made from the EPL on either side of the cut, to record the slow synchronous DC shift without contamination by delta activity. Cross-correlation analysis gave an average CC of 0.70 ± 0.079 in intact slices \((n = 9,\) Fig. 4C). Cuts through the GL \((n = 11)\) or the IPL \((n = 13)\) did not affect synchrony \((CC = 0.72 ± 0.056\) and \(0.57 ± 0.073,\) respectively; \(P > 0.05\) compared with control, \(F = 16.39,\) ANOVA/Bonferroni post hoc), but a cut through the EPL abolished synchrony \((CC = 0.13 ± 0.066;\) \(n = 12;\) \(P < 0.001;\) Fig. 4C). Visual inspection of traces revealed that the small peaks seen in the averaged CC for the slices in which the EPL was cut were the result of artifactual correlation between some traces, because of the similarity in SLE frequency or transient co-occurrence of one or more events.

In a similar experiment, single-unit recordings were made of action potentials in pairs of MCs located 500–1,000 \(\mu m\) apart

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**Fig. 4.** SLEs originate in glomeruli and propagate through the EPL. A: example of a dual-site recording from the GL and EPL, revealing a lag between the glomerular SLE and the event in the EPL. The CC function (average in black, individual recordings in gray) shows a peak offset from 0 in 4/5 recordings, indicating a leading role of glomeruli. B: firing rate histogram from a MC, showing peaks corresponding to SLEs in the intact slice and an inability to generate SLEs when the GL has been surgically removed. C: CC functions of SLEs after microcuts through different layers, showing disruption of synchrony after a cut through the EPL (dark gray). Graph at right shows the average CC ± SE for the different cuts. **\(P < 0.01.\)**

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on either side of cuts through the ONL, GL, or EPL. Blinded qualitative assessment of synchrony was done on coded data traces, with synchrony defined as obvious coexpression of repetitive SLEs in the two cells. In slices with a cut through the GL, 4/5 cell pairs were classified as synchronous, and in slices with an ONL cut, 5/5 were synchronous. In contrast, 0/5 of slices with the EPL cut were classified as synchronous (data not shown). Together, these findings exclude the possibility that SLEs propagate via lateral excitatory pathways in the GL or via MC axon collaterals in the IPL.

Plausible propagation pathways in the EPL include glutamate spillover (Aroniadou-Anderjaska et al. 1999b; Isaacson 1999), excitatory effects of GABA released from granule cells (GCs), and lateral potassium diffusion (Lian et al. 2001; Park and Durand 2006; Yaari et al. 1986). The time scale of SLE propagation in 0-Mg/HK was tested by performing GL field recordings with two electrodes located 500 μm apart. SLE onset was difficult to define in DC recordings because of the gradual rise of the field potential, but cross-correlation analysis showed that the peaks in the cross-correlograms were significantly offset from zero, with an average delay of 4.5 ± 1.1 s ($P < 0.05$, 1-sample $t$-test, $n = 4$ slices). Such slow propagation would be most consistent with a nonsynaptic mechanism of spread, such as potassium diffusion (Durand et al. 2010) or, possibly, astrocytic calcium waves (Dudek 2002; Kumaria et al. 2008). Because ion diffusion and glial function are difficult to manipulate unequivocally in brain slices (Agulhon et al. 2008; Durand et al. 2010; Fellin and Hayden 2005), we tested the effects of manipulations of GABA$_A$ receptor function and glutamate spillover.

Glutamate spillover in the EPL is not responsible for SLE propagation, and glutamate reuptake is not needed for SLE termination. If cyclic changes in extrasynaptic glutamate levels underlie the widespread depolarization and its propagation, efficient postictal glutamate reuptake would be required before the initiation of the next cycle. Blockade of glutamate transport would therefore be expected to disrupt stably recurrent SLEs. In normal ACSF, glutamate uptake blockade with DL-TBOA (30 μM) did not cause SLEs but rapidly induced large delta oscillations in glomeruli, with an increase in spectral power in the delta range (0.25–4 Hz) from 1.85 ± 1.46 to 20.1 ± 14.3 μV$^2$/Hz ($n = 5$, $P < 0.05$, Wilcoxon test; Fig. 5A). To test whether or not glutamate uptake was necessary for SLE termination, we added DL-TBOA during ongoing SLE expression in intact ACSF. This caused a more rapid rate of rise of negative-going DC shifts and a marked increase in delta frequency power, from 1.46 ± 0.48 μV$^2$/Hz during SLE expression to 62.8 ± 24.0 μV$^2$/Hz after DL-TBOA addition ($n = 8$, $P < 0.05$, Wilcoxon test; Fig. 5B). The strong delta activity prevented quantification of SLE properties from LFP recordings. MC current-clamp recordings, however, revealed greater depolarizations during SLEs in DL-TBOA (20 μM, $P < 0.05$, $n = 5$ cells), whereas SLE termination was unaffected (Fig. 5C). These findings indicate that glutamate reuptake regulates the severity of epileptiform activity. However, the failure of DL-TBOA to block SLE initiation or termination suggests that cyclical changes in glutamate levels are not solely responsible for SLE propagation.

Excitatory GABA is not necessary for SLE initiation or spread, and inhibitory GABA is not necessary for SLE termination. Another possible pathway for SLE propagation may be the GC-MC-GC dendro-dendritic synapses. Tetanus-evoked SLEs in hippocampal slices bathed in low Mg$^{2+}$ are dependent on GABA$_A$ receptor-dependent depolarization (Fujiwara-Tsukamoto et al. 2006; Kohling et al. 2000), and excitatory responses to GABA may also play roles in high-K$^+$ seizure models, at least in immature animals (Dzhala and Staley 2003; Dzhala et al. 2005; Isaev et al. 2007; Khazipov et al. 2004). To test the influence of GABA$_A$ receptors on SLEs, we added PTX (100 μM) to the 0-Mg/HK medium after induction of stable recurrent SLEs. Within minutes, typical SLEs, with slow DC rise times and weak delta oscillations, transformed into events with sudden onset and high-amplitude delta oscillations (Fig. 6A). The time to the negative peak of the SLE decreased from 24.8 ± 2.13 to 4.73 ± 1.78 s in the presence of PTX ($P < 0.05$, $n = 4$ slices; Fig. 6B, top), and the delta signal power increased in all glomeruli, from 3.84 ± 2.35 to 43.0 ± 15.8 μV$^2$/Hz ($P < 0.05$, $n = 8$ glomeruli, Wilcoxon test; Fig. 6B, bottom). The fast rise of the LFP was associated with a steeper depolarization in individual MCs ($n = 5$; 10 μM gabazine was used to block GABA$_A$ receptors in whole cell experiments; Fig. 6C). Fields in the EPL showed less delta activity than did those in the GL (absolute delta power 1.71 ± 0.45 μV$^2$/Hz, $n = 9$), indicating that this effect of PTX was mainly intraglomerular. Furthermore, there was no evidence of impaired ability to return to baseline after DC shifts, showing that inhibitory

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**Fig. 5. Effects of glutamate reuptake blockade on delta oscillations and SLEs.** A: transformation of spontaneous glomerular fields into delta oscillations in DL-threo-β-hydroxyaspartic acid (DL-TBOA) in normal ACSF (top). Graph at bottom left shows an example of the power spectra in ACSF and DL-TBOA, and graph at bottom right shows a significant increase (*$P < 0.05$) in absolute power in the delta range (0.25–4 Hz). B: example of a glomerular recording of SLEs showing an increase in the rate of rise and a marked increase in delta activity in the presence of DL-TBOA. The inset shows the averaged delta power in 0-Mg/HK in the absence and presence (*$P < 0.05$) of DL-TBOA. C: MCs recorded in current clamp showed a greater depolarization and more bursting in DL-TBOA.
feedback is not responsible for SLE termination. Further supporting a purely inhibitory role of GABA_A receptors during OB SLEs, the agonist muscimol (1 μM) gradually decreased SLE frequency (from 1.19 ± 0.19 to 0.49 ± 0.07 min⁻¹ after 15 min, P < 0.05) and eventually abolished SLEs and action potential firing.

The effect of dl-TBOA is not dependent on decreased GABA synthesis. GABAergic interneurons rely on neuronal glutamate transporters for the supply of glutamate needed for GABA synthesis (Danbolt 2001). Because the effects of PTX and dl-TBOA were very similar (Fig. 5B vs. Fig. 6A), we controlled for the possibility that dl-TBOA operates by functionally shutting off GABAergic feedback. For this experiment, the effects of dl-TBOA (30 μM) on delta activity and SLEs were tested in the presence of PTX. The most notable effect of dl-TBOA on glomerular fields in PTX + 0-Mg/HK was a transformation of the already strong delta activity into sawtoothlike patterns at delta frequencies (Fig. 7A), with a marked increase in delta power, from 51.4 ± 11.9 to 158.7 ± 21.6 μV²/Hz (n = 5, P < 0.05, Wilcoxon test; Fig. 7A, right). This activity was mainly intraglomerular, since recordings made from the EPL showed fewer oscillations (n = 5, Fig. 7B). SLEs in the EPL were of a significantly larger amplitude in the presence of dl-TBOA than in PTX alone (0.61 ± 0.22 vs. 1.15 ± 0.33 mV; n = 5, P < 0.01), but there was no evidence of an impaired ability of the LFP to return to baseline (Fig. 7B), confirming that neither GABA nor glutamate reuptake were necessary for SLE termination.

Roles of NMDA and AMPA receptors in seizure-like activity and delta oscillations. SLE initiation requires excitatory mechanisms to overcome inhibitory (or shunting) membrane currents. In the low-Ca²⁺ seizure model, a gradual loss of AHPs and slowly rising [K⁺]₀ contribute to the network reaching threshold for the onset of sustained synchronous depolarizations (Konnerth et al. 1986; Yaari et al. 1986). In the OB, we observed a loss of AHPs preceding SLE onset but also a gradual increase in delta activity/burst firing. This suggests that not only changes in outward currents but also increased neurotransmitter release are likely to contribute. Furthermore, the leading role of the GL suggests that initiation of the sustained depolarization occurs within glomeruli, where both AMPA and NMDA receptor are expressed and involved in glomerular amplification. NMDA receptors play an important role in seizure-like activity in other 0-Mg slice models (e.g., Anderson et al. 1986; Gulyas-Kovacs et al. 2002). Preliminary experiments indicated that competitive antagonists of the glutamate- and glycine-binding sites of the NMDA receptor, AP5 (100–300 μM) and dCK (100 μM), respectively, failed to block SLEs in 0-Mg/HK. We hypothesized that extracellular glutamate may reach such high concentrations as to outcompete AP5. Similarly, τ-serine, which is abundant in the OB (Schell et al. 1995), may potentially be released in response to high glutamate levels (Wolosker 2006) and compete with added dCK. Consistent with this hypothesis, the NMDA receptor pore blocker MK-801 (25 μM) abolished SLEs when added during ongoing epileptiform activity (n = 5 slices, Fig. 8, b and c) and

Fig. 6. Control of SLE severity by GABA_A receptors. A: simultaneous field recordings from 2 glomeruli located 500 μm apart showing SLEs in 0-Mg/HK medium and after the addition of picrotoxin (PTX). B: the reduction of time to SLE peak in PTX (top) and the increase in delta power (bottom). *P < 0.05. C: example of a whole cell current-clamp MC recording of an SLE recorded in 0-Mg/HK and an SLE after addition of the GABA_A receptor blocker gabazine (GZ).
Before SLEs can be induced in normal [Mg^{2+}]_o. If this is true, exposing the slice to 0 Mg alone should cause SLEs dependent on NMDA receptors but not on AMPA receptors. In 0-Mg medium, all cells responded with phasic high-frequency firing consistent with SLEs, with 64% showing spike inactivation during events (n = 45 slices). Compared with HK and 0-Mg/HK conditions, SLE onset was slower and more variable (5–30 min), indicating that depolarization by HK is important for rapid SLE initiation. Consistent with a major role of NMDA receptor disinhibition, AP5 (n = 3) or dCK (n = 4) were sufficient to abolish SLEs in 0-Mg medium, whereas DNQX was ineffective (n = 3).

Blockade of 0-Mg/HK-induced SLEs with MK-801 unmasked continuous delta bursting in MCs (n = 5, Fig. 8c). This observation agrees with known properties of glomerular synchronous depolarizations (LLDs), which are AMPA receptor driven (Aroniadou-Anderjaska et al. 1999a; Carlson et al. 2000; De Saint Jan et al. 2009; Karnup et al. 2006; Schoppa and Westbrook 2002, 2001; Urban and Sakmann 2002). Indeed, subsequent addition of the AMPA/kainate antagonist GYKI 52466 (50 μM) abolished delta bursting in all cells (n = 5, Fig. 8, c and d).

To test whether or not AMPA receptor-driven glomerular activity may facilitate the development and maintenance of SLEs, we next added GYKI 52466 during ongoing SLE expression. The effects of this treatment were variable, with SLEs being abolished in 25% of slices (n = 2/8). When slices were preincubated with GYKI 52466 before 0-Mg/HK, no delta activity was seen (n = 4/4 slices), and SLEs were prevented in 50% of slices (n = 2/4). This indicates a facilitating, but not essential, role of AMPA receptors.

If the depolarizations underlying the glomerular delta rhythm are mediated by known properties of long-lasting synchronous depolarizations in glomeruli, individual field bursts should have a late NMDA receptor-mediated component (Aroniadou-Anderjaska et al. 1999a; Carlson et al. 2000; Karnup et al. 2006). To test this, delta bursting was induced, and AP5 (100 μM) was used to block NMDA receptors. This experiment was performed in HK medium, in the presence of Mg^{2+}. Under these conditions, the AMPA receptor blocker DNQX (20 μM) abolished delta bursting entirely in all cells tested (n = 8). Consistent with a late NMDA receptor component, treatment with AP5 caused a 53% decrease in the duration of spike bursts, from 464 ± 62 to 220 ± 57 ms (P < 0.05, n = 6/7 cells; in 1/7 cells, regular bursting was abolished). The shortening of burst trains was accompanied by an increase in interburst frequency, from 0.86 ± 0.12 to 1.53 ± 0.20 Hz (P < 0.05, n = 6), as observed by others (Schoppa and Westbrook 2001).

Together, the above observations show that SLEs were critically dependent on NMDA receptors and facilitated by AMPA receptors. The delta oscillation was AMPA receptor dependent, whereas NMDA receptors prolonged the depolarizing phase of the oscillation and thereby controlled the oscillation frequency.

Roles of other neurotransmitter receptors. Inhibitory feedback may also result from GABA acting at GABA<sub>R</sub> receptors or from glycine or taurine acting at strychnine-sensitive glycine receptors (GlyRRs). The mGlur receptor is also expressed in the OB and excites MCs (Heinbockel et al. 2004). We therefore tested for effects of antagonists of these receptors on SLE intensity...
(measured as SLE peak action potential firing rate) and SLE termination. The GABA$_B$ receptor antagonist CGP 55845 (5 µM) had no effect on SLE intensity (95.9 ± 12.7 vs. 82.5 ± 19.8 Hz, P > 0.05, n = 6) and did not prevent SLE termination. The alternative endogenous GlyR agonist, taurine, has an inhibitory action on MCs that is partially mediated by the GlyR (Igelström and Heyward 2008). The GlyR antagonist strychnine (10 µM), however, had no effect on SLE intensity or termination (52.3 ± 7.5 vs. 54.8 ± 7.1 Hz, P > 0.05, n = 6). To test whether or not the mGlu$_1$ receptor was critically involved in SLE expression, we added the blocker YM298198 (1 µM) after establishing recurrent SLEs in 0-Mg/HK. There was no appreciable change in SLE intensity (66 ± 6.9 Hz in 0-Mg/HK vs. 68 ± 13.5 Hz after YM298198 addition, P > 0.05, n = 4). The role of the mGlu$_1$ receptor in SLE development was tested by comparing the response to a control 0-Mg/HK treatment with the response to a subsequent treatment in the presence of YM298198 following a 10-min preincubation with the blocker. SLE induction was not affected by the presence of the mGlu$_1$ receptor blocker, and the SLE peak action potential frequency was not significantly altered (116.5 ± 22.3 vs. 108.5 ± 19.5 Hz, P > 0.05, n = 4).

**SLEs in the OB are sensitive to the classical anticonvulsant phenytoin.** The persistent sodium current ($I_{\text{NaP}}$) appears to contribute to sustained depolarizations in the low-Ca$^{2+}$ model (Bikson et al. 2003; Heinemann et al. 1985), and many antiepileptic drugs are $I_{\text{NaP}}$ blockers (Rogawski and Löscher 2004). $I_{\text{NaP}}$ would be expected to be active at the membrane potentials reached during SLEs and might therefore contribute to SLE initiation and maintenance. We tested the anticonvulsant effects of phenytoin (PHT), an $I_{\text{NaP}}$-blocking antiepileptic drug (Lampl et al. 1998; Segal and Douglas 1997). LFP recordings from the EPL showed that SLEs were readily abolished by PHT (50 µM) within 10 min (n = 4 slices). Single-unit recordings confirmed that high-frequency firing episodes corresponding to SLEs were replaced by continuous firing (n = 4 slices), and whole cell recordings showed that the sustained MC depolarizations were eliminated (n = 4 slices). At a low concentration (10 µM), the effect of PHT was slower and more variable (15–45 min) but reproducibly abolished SLEs (n = 4). Riluzole (10 µM), a preferential $I_{\text{NaP}}$ blocker (Urbani and Belluzzi 2000), had an identical effect on SLEs (n = 3, current-clamp recording). The Na$^+$ channel blocker TTX (100 nM) also rapidly abolished SLEs (n = 3, field potential recording).

**DISCUSSION**

We have reported that perfusion with ictogenic ACSF containing no extracellular Mg$^{2+}$ and moderately elevated K$^+$ robustly induces epileptiform activity in the OB slice. Two types of hypersynchronous activity were observed: 1) AMPA receptor-driven delta activity confined within glomerular columns and 2) NMDA receptor- and Na$^+$ current-dependent SLEs occurring simultaneously across the slice. We have characterized the basic pharmacology of the events and found them not to be critically dependent on GABA$_B$, glycine, or mGlu$_1$ receptors. SLEs could be induced with remarkable reproducibility in virtually 100% of slices. The implications of these findings for olfactory and epilepsy research are discussed below.

**Delta activity and olfactory oscillations.** Glomeruli in OB brain slices are known to possess an intrinsic capacity for the generation of spillover-mediated LLDs and delta frequency oscillations (e.g., Carlson et al. 2000; Schoppa and Westbrook 2001). The epileptiform delta oscillation was consistent with the thoroughly characterized network properties of olfactory glomeruli (Aroniadou-Anderjaska et al. 1999a; Cang and Isaacson 2003; Carey et al. 2009; Carlson et al. 2000; Christie and Westbrook 2006; Hayar et al. 2004a; Karnup et al. 2006; Margrie and Schaefer 2003; Schoppa and Westbrook 2002, 2001; Urban and Sakmann 2002; Yuan and Knopfel 2006). The observation that delta oscillations could be induced in normal [Mg$^{2+}$]$_o$ by raising [K$^+$]$_o$ from 3 to 5 mM indicates that this oscillation can occur near physiological K$^+$ levels. Induction of delta oscillations with 0-Mg/HK or TBOA may be useful for in vitro study of glomerular physiology, but it will be important to distinguish between physiological and potentially pathophysiological activity.

**The role of delta oscillations in SLE expression.** Despite the important role of glomeruli in SLE initiation, the glomerular delta oscillation did not seem to be directly responsible for SLE initiation. First, we found no temporal relationship between the delta oscillation and the different phases of the ultraslow oscillation underlying SLEs. Second, when the delta oscillation was blocked with AMPA receptor antagonists, SLEs could still occur. However, increased glutamate release during delta bursting is likely to facilitate SLE onset by contributing to general excitability. Under 0-Mg conditions in normal [K$^+$]$_o$, delta activity was reduced and onset latencies were increased, indirectly supporting a seizure-facilitating role of the delta oscillation.

SLEs resemble the slow DC shift of experimental and clinical seizures. Seizures in other low-Mg$^{2+}$ brain slice models typically show spike or wave activity of >1 Hz frequency, tightly synchronized over long distances. Such rapid synchrony is at least in part achieved by lateral recurrent excitatory connections, a network component that is lacking in the OB. Another component of seizures, however, is the so-called “slow DC shift” (Gunnit and Takahashi 1965; Hughes et al. 2005; Ikeda et al. 1999; Mahnke and Ward 1970; Mayanagi and Walker 1975; Miller et al. 2007; Vanhatalo et al. 2003), which has often been overlooked because of the common use of high-pass filtering in EEG and LFP recordings (Rodin and Modur 2008). Slow DC shifts are also the universal property of tonic SLEs in vitro and reflect rises in [K$^+$]$_o$ and sustained depolarization of neurons (Anderson et al. 1986; Aram and Lodge 1988; Dreier and Heinemann 1991; Gloveli et al. 1995; Jensen and Yaari 1997; Jones 1989; Kawaguchi 2001; Lasztkozi et al. 2004; Stanton et al. 1987; Wahlter et al. 1986; Wong and Prince 1990; Yaari et al. 1986). Although not extensively studied, DC shifts can propagate at a slow time scale distinct from that of the synchronization of the fast superimposed spikes (Trevelyan et al. 2007; Tsau et al. 1998; Wong and Prince 1990; Wu et al. 1999). Furthermore, the DC shift may invade distant regions before faster frequencies (Dreier and Heinemann 1991), indicating that the slow spread of potassium-mediated depolarization may be a major mechanism in seizure propagation. The recently acknowledged clinical role of slow EEG activity calls for novel in vitro models of ultraslow epileptiform phenomena. The low-Ca$^{2+}$ model is unique in providing the opportunity to study slow DC shifts in...
relative isolation from clonic activity, and our findings show for the first time that slow negative DC shifts can be the sole component of SLEs in a cortical brain network with intact synaptic transmission.

Recognition of the DC shift as a defining property of the tonic phase of seizures may allow better comparison between studies using different brain regions. The current findings suggest that in cortical networks lacking recurrent excitatory axon collaterals, epileptiform DC shifts can initiate and propagate without the need for faster coupling of oscillations. It is interesting to note that the tonic SLEs in the OB resembled those seen in the surgically isolated CA1 in low Mg\(^{2+}\) and high K\(^+\) and in the “chemically” isolated CA1 in low Ca\(^{2+}\) (Jensen and Yaari 1988; Konnerth et al. 1986; Lewis et al. 1990). There are striking similarities between the OB and the CA1, namely, the arrangement of output neurons in a single layer and a relative lack of axon collaterals, compared with the CA3 region and neocortex. This network arrangement would contribute to the relative inability of these areas to generate interictal and clonic activity. The pyramidal cell layer in the CA1 is thicker and more densely packed than the MCL in the OB, which may explain why ephaptically mediated fast frequencies can sometimes occur in the CA1 (Jefferys 1995; Taylor and Dudek 1982) but were not seen in the OB.

The OB as a novel model system for antiepileptic drug screening. Clinically effective \(I_{\text{NaP}}\)-blocking drugs were effective in blocking SLEs in the OB, as found in the hippocampal low-Ca\(^{2+}\) model (Heinemann et al. 1985; Hood et al. 1983; Snow et al. 1985). These drugs may act in part by blocking the initial amplification leading to regenerative K\(^+\) release and the tonic depolarization. The OB slice seizure model presented in this article may provide an in vitro system in which the epileptiform DC shift and its drug sensitivity can be studied without the need to block synaptic transmission with low-Ca\(^{2+}\) medium.

Antiepileptic drug screening strategies utilize whole animal models in early stages of screening and cultured neurons for mechanistic studies (Stables and Kuperberg 1997). The major disadvantage of this combination is that it only weakly links mechanistic data with in vivo anticonvulsant actions. Brain slice models of acute seizures can shorten the “experimental distance” between molecular targets and network events, but although interictal-like activity can be readily induced in brain slices (e.g., Tancredi et al. 1990), it has been considerably more difficult to develop reproducible models of prolonged spontaneous SLEs. Several studies have described SLEs in hippocampal or cortical slices bathed in low Mg\(^{2+}\) (Anderson et al. 1986; Aram and Lodge 1988; Drejer and Heinemann 1991), high K\(^+\) (Traynelis and Dingledine 1988), 4-aminopyrididine (Luhmann et al. 2000), low Ca\(^{2+}\) (Konnerth et al. 1986), or GABA\(_A\) receptor blockers (Karnup and Stelzer 2001). However, most studies using ictogenic conditions in hippocampus or cortex report only interictal-like activity, which has uncertain relevance to ictogenesis. It also appears that the available models have at least one of the following shortcomings: 1) SLEs occur only in a small proportion of slices (e.g., Traynelis and Dingledine 1988; Wong and Yamada 2001), 2) recurrent SLEs are not stable over time (e.g., Anderson et al. 1986; Drejer and Heinemann 1991), 3) SLEs take a long time to develop (e.g., Konnerth et al. 1986), or 4) SLEs only occur in slices from immature brains (e.g., Dzhala and Staley 2003). Furthermore, the complexity of neocortical and hippocampal networks makes it difficult to characterize and isolate different aspects of seizure generation. These problems may at least in part explain why brain slice models are not used routinely for antiepileptic drug screening. In contrast, SLEs in the OB slice showed rapid-onset, long-term stability and excellent reproducibility, properties that are highly desirable for high-throughput applications.

Mechanisms of SLE initiation and termination. The exact contribution of different neuronal elements to SLEs in the OB remains to be determined, and such studies may greatly benefit from in silico approaches. The requirement of the GL for SLE expression indicates a key role for glomerular amplification in SLE initiation. Such a role of glomeruli would also be consistent with the dependence on NMDA receptors, which are involved in glomerular amplification (Carlson et al. 2000; Schoppa and Westbrook 2001), and on \(I_{\text{NaP}}\) which generates external tufted cell bursting (Hayar et al. 2004b). Neural mechanisms of seizure termination are not well understood (Lado and Moshe 2008) and are difficult to address experimentally. We have established that negative feedback via GABA and glycine receptors does not contribute to termination and did not find evidence of a role of glutamate reuptake. Termination could theoretically be achieved by hyperpolarizing currents in individual neurons. A strong candidate is the sodium-activated potassium current \((I_{\text{KNa}})\), which provides up to 60% of the depolarization-evoked delayed outward current in MCs (Budelli et al. 2009) and has sufficiently slow kinetics to account for hyperpolarizations of tens of seconds after prolonged Na\(^+\) influx (Kim and McCormick 1998; Kubota and Saito 1991; Schwindt et al. 1989). The neuronal Na\(^+\)-K\(^+\)-ATPase may be involved in terminating SLEs through extrusion of Na\(^+\), removal of ictogenic extracellular K\(^+\), and by hyperpolarizing the membrane potential. Other possible mechanisms include seizure-induced acidosis (Xiong et al. 2000) and depolarization block of spike generation (Bragin et al. 1997).

Glutamate spillover vs. ion diffusion in SLE spread. The slow propagation of SLEs in the OB (in the order of seconds) would be consistent with regenerative potassium release over many seconds, followed by potassium clearance. Although we could not test this hypothesis directly in our current experimental system, the inability of manipulations of the other two possible propagation pathways in the EPL (glutamate spillover and excitatory GABA) to alter SLE expression indirectly supports a potassium-mediated mechanism. Recordings with K\(^+\)-sensitive electrodes and careful lesion studies as performed by Lian et al. (2001) may shed more light on this possibility. Slow astrocytic intracellular and intercellular calcium waves, associated with gliotransmission, have been suggested to play a role in epilepsy (Dudek 2002; Kumaria et al. 2008). Glial Ca\(^{2+}\) waves and gliotransmission might occur in the EPL (Kozlov et al. 2006), but the origin of such events, their extent within the glial syncytium in situ, and their potential involvement in modulating normal neuronal activity or seizure initiation and spread are controversial (Agulhon et al. 2008; Fellin and Hayden 2005; Hamilton and Attwell 2010). Recent imaging studies addressing this issue in vivo (Kuga et al. 2011) have suggested that interglial Ca\(^{2+}\) waves propagate widely (>500 μm) but serve to modulate cerebral blood flow in response to changes in neuronal circuit activity.
Implications for olfactory processing. Because the OB slice is deprived of its normal input from the olfactory epithelium and cortex, it cannot be inferred from this study that minute-long synchronous depolarizations can occur in vivo. Puopolo and Belluzzi (2001), who described O-Mg/bicuculline-induced events in OB slices from immature rats, suggested that long-lasting NMDA-dependent depolarizations may play a role in odor sensitivity and neuronal plasticity. However, neither the oscillations described in the above-cited study nor the SLEs described in this study appear to be compatible with normal physiological function. On the contrary, it seems plausible that if the olfactory cortex were presented with such sudden-onset high-frequency output from MCs, a cortical seizure could be triggered. Indeed, maximal electrical stimulation of MC axons for several seconds has been shown to initiate seizures in waking rabbits (Freeman 1986). The above argument does not, however, exclude the possibility that slowly propagating depolarizing waves may occur in the OB and be physiologically relevant. Optical recordings from the turtle OB in vivo have revealed a slow DC shift lasting several seconds, occurring across large areas of the bulb in response to odor (Lam et al. 2000). In that study, low-frequency noise necessitated the use of high-pass filtering, so the properties of this DC potential were not studied. It appeared, however, to begin in response to odor stimulation and spread from a single activated glomerulus across large parts of the bulb (see Fig. 4 in Lam et al. 2000). It is thus possible that odor activation of glomeruli may cause a propagating $\overline{K}^+$ wave, which enhances excitability and odor responsiveness across the OB. Such a mechanism of lateral excitation may operate alongside lateral inhibitory circuits (Aungst et al. 2003; Shirley et al. 2010). Differences in temporal properties of these different mechanisms may be important in shaping the dynamics of the odor response. For instance, odor responsiveness may be coupled to behavioral states by cortical control of inhibitory circuits in the EPL (Balu et al. 2007) and interglomerular inhibitory circuits may operate locally to enhance temporal and spatial odor discrimination (Shirley et al. 2010), whereas potassium diffusion might enhance broad-spectrum responsiveness in odor-rich environments.

Summary. We have found that the OB slice bathed in ictogenic medium expresses recurrent minute-long SLEs resembling the slow DC shift of experimental and clinical seizures. Our findings confirm that despite the limited clinical significance of the OB, great care is needed when conducting or interpreting studies using ictogenic conditions. In addition, the OB slice may provide an unusually robust model of acute seizures particularly suited for the study of nonsynaptic propagation mechanisms and pharmacological antiepileptic drug studies.

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