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Seizure-like thalamocortical rhythms initiate in the deep layers of the cortex in a co-culture model

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ABSTRACT

The oscillatory rhythms underlying many physiological and pathological states, including absence seizures, require both the thalamus and cortices for full expression. A co-culture preparation combining cortical and thalamic explants provides a unique model for investigating how such oscillations initiate and spread. Here we investigated the dynamics of synchronized thalamocortical activity by simultaneous measurement of field-potential recordings and rapid imaging of Ca^{2+} transients by fluorescence methods. Spontaneous sustained hypersynchronized "seizure-like" oscillations required reciprocal cortico-thalamocortical connections. Isolated cortical explants can independently develop brief discharges, while thalamic explants alone were unable to do so. Rapid imaging of Ca^{2+} transients demonstrated deep-layer cortical initiation of oscillatory network activity in both connected and isolated explants. Further, cortical explants derived from a rat model of genetic absence epilepsy showed increased bursting duration consistent with an excitable cortex. We propose that thalamocortical oscillatory network activity initiates in deep layers of the cortex with reciprocal thalamic interconnections enabling sustained hyper-synchronization.

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Introduction

Strong reciprocal connections between the thalamus and cortex are responsible for generating rhythmic oscillatory neuronal network activity. These thalamocortical oscillations are known to have important neurophysiological functions that include sensor–motor information integration and modulation of sleep (Andolina et al., 2007; Iyengar et al., 2007; Pinault and Deschenes, 1992; Razak et al., 2009). Aberrant thalamocortical oscillations in this network are also responsible for pathological conditions, of which the best characterised are absence seizures (Avoli and Gloor, 1982; Blumenfeld and McCormick, 2000; Kostopoulos et al., 1981; Pinault, 2003; Steriade and Contreras, 1995). The thalamus and the cortex are both essential for initiation and propagation of these epileptic thalamocortical oscillations (Vergnes and Marescaux, 1992). Despite the well-recognised interactions between these brain structures the specific roles that each play in initiation, evolution and maintenance of pathophysiological oscillatory network activity are still unclear.

In vivo electrophysiological analysis in animal models of absence epilepsy suggests the spike-and-wave discharges (SWD), that are a hallmark of such seizures, are initiated in the cortex and secondarily engage the thalamus (Meeren et al., 2002, 2001; Pinault, 2003; Polack et al., 2007). However, a major limitation of *in vivo* electrophysiology studies is relatively poor spatial sampling due to the limited number of electrodes that can be used at once. While studies using functional magnetic resonance imaging have attempted to explore this issue, this approach does not have high temporal resolution and is an indirect measure of neuronal activity (McKeeff et al., 2007). Rapid imaging technologies now enable higher spatial and temporal resolution of neuronal activity (Berger et al., 2007; Derdikman et al., 2003). These techniques are limited *in vivo* to cortical surface recordings or to *in vitro* studies that can encompass deeper brain structures.

Here we use an *in vitro* thalamocortical co-culture preparation (Caeser et al., 1989; Lotto and Price, 1994; Molnar and Blakemore, 1991; Rennie et al., 1994; Yamamoto et al., 1989) that is amenable to fast imaging, to investigate temporal and spatial aspects of oscillatory thalamocortical network activity. This has two major advantages in the context of this study. First, the neuronal circuitry that is being measured is contained within the sampled field enabling us to rule out initiation or influences of other neuronal populations. Second, organotypic cultures lose the opaque dead cell layer often seen in acute slices thereby increasing the signal-to-noise. The results demonstrate that sustained network events were initiated in the deep layers of the cortex and secondarily spread to involve the thalamus and more widespread cortical areas. This activity was dependent on the reciprocal interconnectivity of the thalamus and cortex. Further, cortex-only explants

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from a genetic model of absence epilepsy (GAERS) exhibited significantly more hyperexcitable activity compared to non-epileptic control explants. This demonstration of how oscillatory thalamocortical network activity develops and evolves in the co-culture model may give insight into how this occurs *in vivo* in physiological and pathophysiological settings, and in particular the importance of the inter-relationship between the cortex and the thalamus.

Methods

Organotypic slice culture

Twenty-four Genetic Absence Epilepsy Rat from Strasberg (GAERS) and 26 non-epileptic control NEC pups were used to prepare co-cultures. All experiments were approved by the University of Melbourne Animal Experimentation Ethics Committee. P0–P3 rats were anaesthetised by halothane vapour and decapitated into ice. The cortex and thalamus were isolated aseptically in cold Gey's balanced salt solution with 6.5 mg/mL glucose. Cortices were cut coronally with a McIlwain tissue chopper at a thickness of 300 μm. Thalamic sections were cut at 400 μm. After cutting, brain slices were stored for 15 min in ice-cold GBSS. Cortical and thalamic slices were then selected and placed next to each other (within 1 mm) on a Millicell culture insert (Millipore). Cultures were maintained at 37 °C in 5% CO_2 for 14 days, fed twice-weekly with 1.2 mL of medium consisting of 50% (V/V) MEM, 25% (V/V) Earles balanced salt solution, 25% V/V heat-inactivated horse serum containing 6.5% (W/V)glucose and 2% (V/V) B27 supplement.

Extracellular field potential recordings

Co-cultures and their supporting membranes were transferred to a recording bath where they were continually superfused with oxygenated (95%O_2/5%CO_2) artificial cerebrospinal fluid (ACSF) maintained at 30–32 °C with a TC-324B temperature controller (Warner Instruments, Hamden, CT). Except where noted, the ACSF contained (in mM): 120 NaCl, 3 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 1.2 $NaHPO_4$, 23 $NaHCO_3$, 11 glucose. Fluorescence imaging and acquisition of Ca^{2+} transients were performed on an Olympus BX51WI microscope using an XL Fluor 4X/340 NA 0.28 objective (Olympus, Japan). Simultaneously, field recordings were acquired from the cortex and thalamus regions using a MiniDigi 1A two-channel acquisition system (Axon Instruments, CA, USA). Custom-made bipolar 0.025 mm tungsten (Goodfellow, UK) electrodes were used with an A-M Systems 3000 AC/DC amplifier (A-M Systems, WA, USA), Gain = 1 K, High Pass = 1 Hz, Low pass = 0.1 kHz; electrographs were recorded using AxoScope9 software (Axon Instruments, CA, USA) sampling at 500 Hz. Mg^{2+}-free ACSF perfusate, identical in composition to ACSF except for equimolar $CaCl_2$ substitution for $MgCl_2$, was used to promote synchronous firing. Cultures were surgically separated by running a scalpel between cortical and thalamic parts of the co-cultures.

Single-cell recordings

Thalamic neurons were impaled with sharp microelectrodes (80–150 MX, with filament) filled with 0.5 mM Oregon Green 488 BAPTA-1 (Molecular Probes, Oregon) in 200 mM potassium acetate, and back filled with 3 M potassium acetate. The indicator was injected into the cell by applying hyperpolarizing current (0.05–0.1 nA) for 5–20 min via an Axoclamp 2B amplifier (Axon Instruments, California). Slices were viewed through an upright microscope using a 60× NA 0.9 water immersion objective (Olympus, Japan) and an Olympus Fluoview FV300 confocal laser scanhead.

High-speed fluorescence imaging

The Ca^{2+}-sensitive dye Fluo-5FAM (Molecular Probes, Eugene, OR) was dissolved in DMSO to a final concentration of 1 mM. Slice cultures were exposed to 1.25 μM (2.5 μL in 2 mL of ACSF) in the recording bath for 15–20 min then perfused in normal ACSF for 15 min prior to optical recording. Cultures were viewed (as described above) with images captured using a fast CCD camera (80 × 80 pixels) recording at 500 Hz frame rate with 10× camera amplifier gain (NeuroCCD; RedShirtImaging, Fairfield, CT). Epifluorescence illumination was provided by a Lambda LA 175 W xenon arc lamp driven by a stable power supply (Sutter, Novato, CA). Optical recordings were taken concurrently with field potential recordings for 10 s (5000 frames). Post-image analysis was performed on specific structures of the organotypic slices by defining regions of interest (ROI). Average fluorescence changes, relative to resting light intensity ($\Delta F/F$), were calculated for each region using NeuroPlex software supplied by RedShirtImaging (Fairfield, CT, USA).

Data analysis

To quantify the cycle frequency of the oscillatory activity recorded in the cultures, inter-burst interval (IBI) was used. This was defined as the interval between the negative peaks of two adjacent events in a discharge of oscillatory activity, or the interval between the first event in a cluster when bursts occurred in clusters, similar to published methods (Adams et al., 2009; Reid et al., 2008). Burst duration was measured as time between the first and last negative peaks in a burst while burst complexity was represented by the number of spikes in that period. Data were presented as mean ± standard error of the mean (SEM) also displayed by the bars on figures, with a significance level set at $p < 0.05$. Differences in means were tested by a two-tailed t-test or, when more than two means were compared, a one-way ANOVA was performed. χ^2 analysis was used to test difference in dichotomous variables.

Results

Thalamocortical co-cultures re-establish functional interconnections

After 2 weeks in culture the thalamic and cortical explants had spread and grown together (Fig. 1B). The thalamic explant consistently spread more widely and thinly than the cortical explant. To determine whether axons from the thalamus had invaded the cortex, individual neurons were injected with Oregon Green BAPTA. Dye-filled neurons from the thalamus extended into the middle of the cortical explant (Fig. 1A, $n = 3$) indicating that axonal connections were established between the thalamic and the cortical co-cultures. Dye-filled neurons were stimulated in the thalamus and evoked potentials recorded in the cortex, and vice-versa (data not shown) indicating functional connectivity between the explants.

Thalamocortical co-cultures generate synchronized oscillatory burst firing discharges lead by the cortical explants

Field potential recordings were made in the thalamus and cortex under Mg^{2+}-free ACSF conditions to establish if oscillatory network activity could be generated by the co-cultures. Synchronized oscillatory burst firing was rarely recorded simultaneously in both the thalamus and cortex. From a total of 81 co-cultures, we were able to record such events in co-cultures from 3 non-epileptic controls (NEC) and one GAERS rat (Fig. 2A, B) with a final efficiency of 5%. This low efficiency is similar to that reported by other groups using co-cultures but does demonstrate that thalamocortical oscillatory activity can develop in these co-cultures (Anderson and Price, 2002; Bolz et al., 1992; Molnar and Blakemore, 1991). However, no

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