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Research Report

Investigation into the role of gap junction modulation of intracortical connectivity in mouse neocortical brain slices

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ABSTRACT

General anesthetics are hypothesized to cause unconsciousness by interrupting communication pathways within the cerebral cortex. A correlate of this has been demonstrated in mouse neocortical slices, where anesthetics disrupt the spread of population field potential activity—resulting in a "decoupling" of activity recorded across spatial locations within the slice. In this study we investigated whether this decoupling can be explained by gap junction blockade, with a particular focus on the connexin36 (Cx36) subtype. Baseline, coupled seizure-like event (SLE) activity was recorded from two extracellular electrodes in slices perfused with no-magnesium artificial cerebrospinal fluid (aCSF). The connexin36 gap junction blocker mefloquine (25 μM) failed to decouple SLE activity in wild-type mice (median(range) decoupling rate of 0.70(0.03–3.00)%, not significantly different from controls). Slices from Cx36 knock-out mice exhibited coupled SLE activity under baseline conditions and readily decoupled when exposed to the general anesthetic etomidate. The general gap junction blocker carbenoxolone (CBX, 100 μM) strongly decoupled SLE activity compared to controls in wild-type mice (2.7(0.1–42.5) % compared to 0.03(0.0–0.5)%, $p=0.0001$). Taken together, the results show that Cx36 gap junction blockade does not cause decoupling of intracortical population activity, but the involvement of other gap junction subtypes cannot be ruled out.

1. Introduction

The biological basis of consciousness remains one of the most important unsolved mysteries in science. The practice of anaesthesia, which involves the controlled switching on and off of consciousness, provides a unique, accessible and perhaps under-utilized research tool for exploring this most complex of brain functions.

One explanation is that anesthetics disrupt consciousness by interrupting brain functional connectivity, preventing

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integration of information. Support for this hypothesis has been growing through an increasing number of *in vivo* EEG-based studies (Imas et al., 2006; Mhuircheartaigh et al., 2010; Ku et al., 2011; Schrouff et al., 2011; Liang et al., 2012). However, an *in vitro* model lending itself to mechanistic testing under controlled conditions has been lacking. Recently, we demonstrated in neocortical mouse brain slices that etomidate and ketamine disintegrate the spread of population field potential activity—resulting in a "decoupling" of activity recorded at spatially separated locations within the slice (Voss et al., 2012). The striking similarity of these findings to those reported by Ferrarelli and colleagues (Ferrarelli et al., 2010) in human subjects suggests this model could be further utilized to investigate underlying mechanisms of anesthetic disruption of cortical information processing.

In this study we investigated gap junction blockade as a possible explanation for anesthetic decoupling in neocortical slices. Functional connectivity in the brain is promoted by gap junctions (Nagy et al., 2004; Peng et al., 2012), allowing the contacting cells to exchange ions and small molecules linking them both metabolically and electrically (Deans et al., 2001; Hormuzdi et al., 2004). Many anesthetics have been shown to block gap junctions (Johnston et al., 1980; Bernardini et al., 1984; Mantz et al., 1993) and a genetic absence of connexin36 (Cx36) gap junctions facilitates anesthetic loss of consciousness (Jacobson et al., 2011). Thus, there is a strong case for mechanistic links between anesthetic gap junction blockade, decoupling and unconsciousness. In this study we focused on Cx36 because of the functional link between this gap junction subtype and anesthesia (Jacobson et al., 2011).

Using the neocortical slice no-magnesium seizure-like event (SLE) model, we investigated the effect of pharmacological blockade (mefloquine) and genetic ablation (Cx36 knock-out mice) of Cx36 gap junctions on SLE coupling. The aim was to determine whether blockade of Cx36 gap junction-linked neuronal networks could disrupt the spread of SLE activity in similar fashion to anesthetic drugs, thereby providing a mechanistic explanation for anesthetic decoupling.

2. Results

2.1. *Baseline coupled SLE activity*

Coupled SLE activity was reliably generated in wild-type and Cx36 knock-out slices by recording from two electrodes separated by approximately 5 mm (see detailed data below and Fig. 1); and was characterized by epileptiform population spikes (up to 1 mV in amplitude) recorded in both channels with a time lag of less than one second (Fig. 2a).

2.2. *Effect of Cx36 gap junction blockade on SLE coupling*

A low rate of SLE decoupling was observed during mefloquine exposure (median (range) 0.70(0.03–3.00)%), but this was not significantly different from the control value of 0.35(0.0–18.0)% ($p=0.1508$) (Fig. 3a). In Cx36 knock-out animals, coupled SLE activity was achieved in all slices ($n=5$, 2 animals) with an electrode separation of 3.8(1.7–5.2) mm. The similarity in electrode separation between wild-type (see below) and knock-out

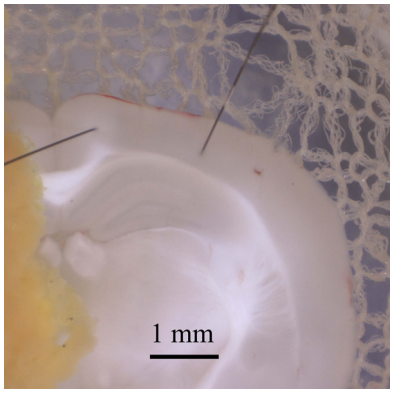

Fig. 1 – Photograph from one example showing the final electrode recording positions.

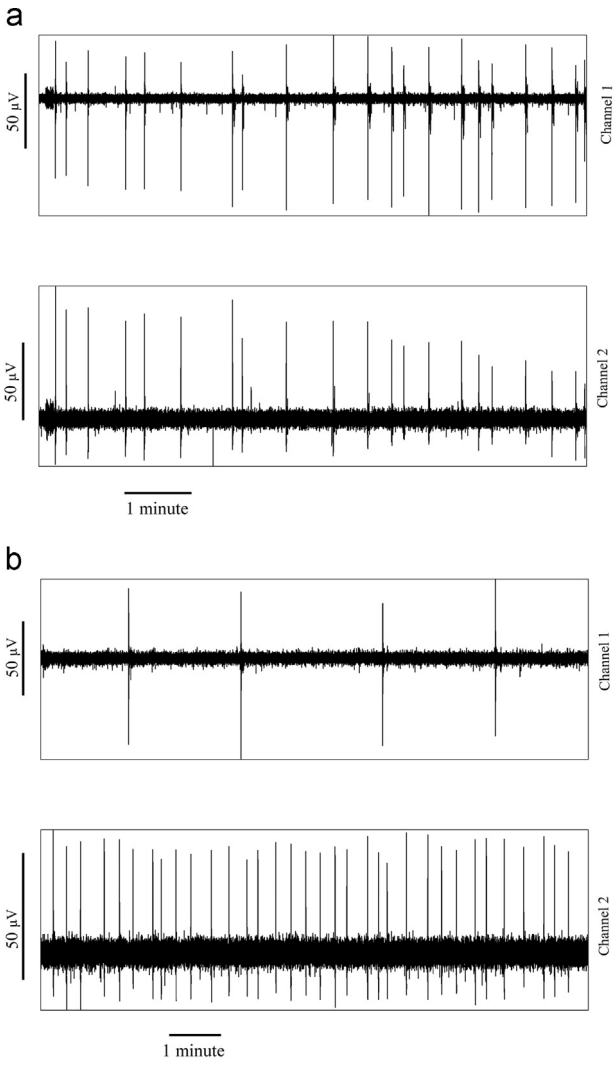

Fig. 2 – Examples from one slice showing (a) coupled activity between the two recording channels during baseline recording and (b) decoupled activity between the same two channels during carbenoxolone perfusion.

slices confirmed that SLE activity in the latter was similarly coupled under baseline recording conditions. Importantly, etomidate (24 μM) caused SLE activity to decouple in 4 of 5