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# GRADED DEFRAGMENTATION OF CORTICAL NEURONAL FIRING DURING RECOVERY OF CONSCIOUSNESS IN RATS

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**Abstract—State-dependent neuronal firing patterns reflect changes in ongoing information processing and cortical function. A disruption of neuronal coordination has been suggested as the neural correlate of anesthesia. Here, we studied the temporal correlation patterns of ongoing spike activity, during a stepwise reduction of the volatile anesthetic desflurane, in the cerebral cortex of freely moving rats. We hypothesized that the recovery of consciousness from general anesthesia is accompanied by specific changes in the spatiotemporal pattern and correlation of neuronal activity. Sixty-four contact microelectrode arrays were chronically implanted in the primary visual cortex (contacts spanning 1.4-mm depth and 1.4-mm width) for recording of extracellular unit activity at four steady-state levels of anesthesia (8–2% desflurane) and wakefulness. Recovery of consciousness was defined as the regaining of the righting reflex (near 4%). High-intensity firing (HI) periods were segmented using a threshold (200-ms) representing the minimum in the neurons' bimodal interspike interval histogram under anesthesia. We found that the HI periods were highly fragmented in deep anesthesia and gradually transformed to a near-continuous firing pattern at wakefulness. As the anesthetic was withdrawn, HI periods became longer and increasingly correlated among the units both locally and across remote recording sites. Paradoxically, in 4 of 8 animals, HI correlation was also high at the deepest level of anesthesia (8%) when local field potentials (LFP) were burst-suppressed. We conclude that recovery from desflurane anesthesia is accompanied by a graded defragmentation of neuronal activity in the cerebral cortex. Hypersynchrony during deep anesthesia is an exception that occurs only with LFP burst suppression.**


**Key words: consciousness, anesthesia, correlation, synchrony, burst suppression.**

## INTRODUCTION

Spatiotemporal patterns of neuronal activity are strongly influenced by altered states of consciousness such as anesthesia, sleep and wakefulness. Anesthetic agents suppress firing rate (Detsch et al., 2002; Hudetz et al., 2009; Villeneuve et al., 2009), synaptic connectivity (Vizuete et al., 2012), and rhythms of population activity in neuronal networks (Hudetz et al., 2011; Lewis et al., 2012) These changes are thought to impair information processing and consciousness (Alkire et al., 2008; Lee et al., 2009; Brown et al., 2010).

A recent study in human patients demonstrated rapid spatial and temporal fragmentation of neuronal activity during propofol-induced unconsciousness (Lewis et al., 2012). Likewise, in rodents anesthetized with urethane, neurons tend to fire in synchronous bursts in a pattern different from normal ongoing activity in wakefulness (Erchova et al., 2002). The alternation of neuronal spiking periods under urethane and ketamine/xylazine anesthesia associated with shifts in bistable membrane potential and slow electrocortical oscillations have also been known as UP/DOWN states (Kasanetz et al., 2002; Clement et al., 2008; Destexhe, 2009). Similar behavior has been observed in natural slow-wave sleep (Destexhe et al., 2007).

Most earlier studies were made in relatively deep anesthesia and thus little is known about the spatiotemporal properties of neuronal firing at shallower and graded anesthesia levels, particularly those associated with loss and return of consciousness. A few recent investigations examined the graded effect of isoflurane on spontaneous or stimulus-evoked local field potential and unit activity in the cerebral cortex of rodents and ferrets. Only one of these studies included measurements in unrestrained wakeful animals (Sellers et al., 2013).

To learn more about the neuronal dynamics during the transition between anesthetized and wakeful states, here we set out to investigate the graded effect of anesthesia on cortical unit activity in chronically instrumented, unrestrained rats. The primary focus of the study was the state-dependent change in temporal correlation of high-frequency firing periods similar, although not identical, to UP/DOWN or ON/OFF states. We chose to investigate the effect of anesthesia during emergence,

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*Abbreviations:* BSR, burst suppression ratio; CCG, cross-correlogram; HI, high-intensity firing; ISI, interspike interval; K-S, Kolmogorov–Smirnov test; LFP, local field potentials; LO, low-intensity firing; MUA, multiunit activity; RM-ANOVA, repeated measures analysis of variance; T-K, Tukey–Kramer multiple-comparison test.

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that is, during a stepwise reduction of the anesthetic dose from deep to light levels and finally, to wakefulness. To aid this protocol, we chose to use desflurane – a modern, clinically used anesthetic that has the property of rapid equilibration and ease of control at steady-state condition. The range of anesthetic depths was chosen to include those closely associated with the recovery of consciousness – a phenomenon whose neuronal correlates are of significant interest.

## EXPERIMENTAL PROCEDURES

The experimental procedures and protocols were reviewed and approved by the Institutional Animal Care and Use Committee. All procedures conformed to the *Guiding Principles in the Care and Use of Animals* of the American Physiologic Society and were in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Academy Press, Washington, DC, 1996). All efforts were made to minimize discomfort and the number of animals used.

### Electrode implantation

Experiments were performed on eight adult (260–440 gm) male Sprague–Dawley rats (Harlan Laboratories, Madison, WI). All animals were kept on a reversed light–dark cycle in dedicated rooms of the Animal Resource Center for at least one week prior to physiological experiments. On the day of the aseptic surgery, the rat was anesthetized using isoflurane (Abbot Laboratories, Chicago, IL) in an anesthesia box. The animal's head was then secured in a rat stereotaxic apparatus (Model 900, Kopf Instruments, Tujunga, CA) and a gas anesthesia adaptor (Stoelting Co., Wood Dale, IL) was placed over the snout to continue anesthesia at ~2.0% isoflurane. Body temperature was rectally monitored and maintained at 37 °C via an electric heating pad (TC-1000, CWE Inc., Ardmore, PA). The antibiotic, Enrofloxacin (10 mg/kg s.c.), was administered prior to surgery onset. The dorsal surface of the head was prepared for sterile surgery with betadine and alcohol. Bupivicaine, a local anesthetic, was injected under the skin prior to surgery. A midline incision was then made and the skin was laterally reflected to expose the cranium. Connective tissue was gently scraped and any bleeding was cauterized. A multishank, 64-contact microelectrode array (Neuronexus Technologies, Ann Harbor, MI; 5-mm length, 200-μm electrode spacing, 200-μm shank spacing) was chronically implanted within V1 (7.0 mm posterior, 3.0–3.5 mm lateral, relative to bregma), spanning the entire depth of the cortex (Fig. 1A).

To implant the microelectrode array, a craniotomy of rectangular shape of approximately 2 × 4 mm was prepared using a low-speed, compressed air-driven dental drill and bur No. FG 1 (Sullivan/Schein Dental, Melville, NY). The exposed dura mater was then resected and the electrode array inserted using a micromanipulator. The electrode was subsequently advanced at increments of 10 μm to a depth of approximately 2.1 mm below the brain surface. To secure the neural probe, the perimeter was covered with silicon gel (Kwik-Sil, World Precision Instruments, Sarasota, FL). A reference wire, attached to the neural probe, was wrapped around a cranial steel screw located between bregma and lambda in the opposite hemisphere (~4.0 mm posterior, ~2.0 mm lateral, relative to bregma). Additional sterilized stainless steel screws (MX-080-2, #0-80 × 1/8″, Components Supply Co. Inc., Fort Meade, FL) were used to secure the electrode to the cranium. The whole assembly was embedded with Cerebond (MyNeurolab, Saint Louis, MO), a nontoxic skull fixture adhesive, such that the connectors protruded from the skull fixture adhesive cap. Carprofren (5 mg/kg s.c. once daily) and Enrofloxacin (10 mg/kg s.c. once daily) were administered for 2 and 7 days, respectively. The animal was then observed for 7–10 days for any infection or other complications.

### Experimental protocol

On the day of the experiment, the rat was placed in a cylindrical anesthesia chamber ventilated with a heated, humidified gas mixture of 30% $O_2$, balance $N_2$. Inspired $O_2$ and anesthetic gas concentrations were continuously monitored (POET IQ2, Criticare Systems, Waukesha, WI). Since monitoring accuracy is 0.1%, an indication of the target or target ±0.1% concentration was accepted. Body temperature was rectally monitored and maintained at 37 °C. The room was then darkened and the rat was allowed to freely move around in the box for approximately one hour to accommodate to the environment. Under 8% desflurane anesthesia, the headstage (64-channel zif-clip, Tucker-Davis Technologies, Alachua, FL) was connected to the implanted electrode array with a connecting wire bundle that terminated on the preamplifier outside the anesthesia chamber. Extracellular potentials were recorded using a 64-channel neural acquisition system (Cerebus, Blackrock Microsystems, Salt Lake City, UT) and analyzed for ongoing unit activity and local field potentials (LFP). For unit activity, the signal was analog-filtered at 250–7500 Hz, digitally sampled at 30 kHz and auto-thresholded using a root mean square multiplier of −6.25. For LFP, the signal was analog-filtered at 0.3–500 Hz and digitally sampled at 1 kHz. Ten minutes of ongoing activity was sequentially recorded under 8%, 6%, 4%, and 2% desflurane anesthesia and at wakefulness (0%). An equilibration time of 15–20 min was allowed before recording in each condition. In one experiment at 2% desflurane, the headstage was accidently disconnected; therefore data in this condition were not obtained.

### Spike sorting

Extracellular unit activity from each electrode contact was sorted into individual units using the public domain offline spike sorter PowerNAP (OSTG Inc., Fremont, CA); examples are illustrated in Fig. 1B. This software applies principal component analysis (PCA) along with various clustering methods for sorting (Fee et al., 1996;