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COVARIATION OF PUPILLARY AND AUDITORY CORTICAL ACTIVITY IN RATS UNDER ISOFLURANE ANESTHESIA

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Abstract—Very slow fluctuations of spontaneous activities significantly influence not only behavioral performance in a conscious state, but also neural activities in an unconscious state. Covariation of pupil and cortical activities may lend important insights into the state-dependent modulation of stimulus encoding, yet this phenomenon has received little attention, especially with regard to non-visual cortices. In the present study, we investigated co-fluctuation of pupil size and neural activity in the auditory cortex of rats under isoflurane anesthesia. Pupil fluctuation consisted of longitudinal irregular shifts, and 1-min cyclic modulations. Both spontaneous and auditory-evoked potentials (AEPs) covaried with the longitudinal fluctuation of pupil size, but not with the 1-min cycle. Pupil size exhibited a positive correlation with spontaneous activity and negative correlation with AEP amplitude, particularly when the pupil size was beyond the normal range. Stimulus-specific adaptation characterized using an oddball paradigm was less dependent on pupil size than AEP. In contrast to the cortical activity, heart rate covaried with pupil size with the 1-min oscillatory component, but not the non-oscillatory component. Furthermore, light exposure induced the pupil reflex through the autonomic system, but did not modify cortical activity, indicating that autonomic activity was not causing the cortical modulation. These results together suggest that cortical activities spontaneously covary with pupillary activity through central cholinergic modulation that triggers sympathetic nerve activation. Such a state-dependent property may be a confounding factor in cortical electrophysiology studies.

Key words: auditory cortex, pupil, isoflurane, spontaneous activity, auditory-evoked potential (AEP), stimulus-specific adaptation (SSA).

INTRODUCTION

Spontaneous neural activities in EEG and fMRI BOLD signals commonly exhibit very slow fluctuations, on the order of seconds and minutes, which are significantly correlated with perception threshold (Ress and Heeger, 2003; Pessoa and Padmala, 2005; Monto et al., 2008), cognitive performance (Wagner et al., 1998; Pessoa et al., 2002; Otten et al., 2006), and motor execution (Fox et al., 2007). Spontaneous fluctuations are also observed in non-conscious states. For example, urethane anesthesia is characterized by cyclic and sleep-like spontaneous alternation of brain states (Clement et al., 2008; Blasiak et al., 2013). Moderately deep anesthesia commonly leads to alternating high amplitude bursts and silent periods, a phenomenon called burst-suppression (Swank, 1949; Steriade et al., 1994). Furthermore, during both sleep (Massimini et al., 2003; Vanhatalo et al., 2004) and anesthesia (Arieli et al., 1996), the ongoing spontaneous state significantly influences sensory-evoked responses. Thus, these infra-slow spontaneous fluctuations should be taken into account in physiological measurements.

The very slow spontaneous fluctuation in the brain is likely mediated by cholinergic and noradrenergic modulation. Historically, noradrenaline has been believed to mediate arousal (Jouvet, 1969; Berridge and Waterhouse, 2003). However, cholinergic inputs also induce EEG activation, or cortical desynchronization, which leads to sensory gating and plasticity (Sato et al., 1987; Metherate et al., 1992; Bakin and Weinberger, 1996; Edeline, 2003; Grunwald et al., 2003). More recent studies have demonstrated that both cholinergic and noradrenergic input play important roles in brain-state-dependent gain modulation and task efficiency (Aston-Jones and Cohen, 2005; Metherate, 2011; Pinto et al., 2013; Polack et al., 2013; Fu et al., 2014; Reimer et al., 2014).

Because of the role of sympathetic and parasympathetic innervation in pupillary dilation (Yoshitomi et al., 1985; Ishizaka et al., 1998), pupillometry has been widely used for more than five decades to index the state of the brain (Hess and Polt, 1960, 1964; Lowenstein et al., 1963; Kahneman and Beatty, 1966). Pupil size also covaries with visual-evoked potentials (VEP) in a non-monotonic manner, in part because the pupil regulates the quantity of light entering the eye, and partly because of the specific brain state during which the pupil is being measured (Martins et al., 2003; Salim et al., 2010; Kuipers and Thierry, 2013). This

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Abbreviations: AAS, ascending activating system; AEPs, auditory-evoked potentials; ECoG, electrocorticogram; SSA, stimulus-specific adaptation; VEP, visual-evoked potentials.

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multifunctional view was confirmed in a recent study that demonstrated synchronicity between the infra-slow fluctuation of pupil size and cyclic alternations of the electrocorticogram (ECoG) in the visual cortex under urethane anesthesia (Blasiak et al., 2013). Thus, covariation of pupillary and cortical activities may provide important insights into the state-dependent modulation of stimulus encoding. However, this potentially lucrative line of research has received little attention to date, especially with regard to the non-visual cortices.

The present study hypothesizes that such covariation of the pupil and cortical activities is commonly observed irrespective of both the anesthetic agent and the stimulus modality. We analyzed ECoG data from the auditory cortex to test whether and how the spontaneous and auditory-evoked activities under isoflurane anesthesia covaried with the pupil size.

EXPERIMENTAL PROCEDURES

This study was carried out in strict accordance with ''Guiding Principles for the Care and Use of Animals in the Field of Physiological Science,'' published by the Japanese Physiological Society. The experimental protocol was approved by the Committee on the Ethics of Animal Experiments at the Research Center for Advanced Science and Technology, the University of Tokyo (Permit Number: RAC130107). All surgeries were performed under isoflurane anesthesia, and every effort was made to minimize suffering. After experiments, animals were euthanized with an overdose of pentobarbital sodium (160 mg/kg, i.p.).

The size of the left pupil and neural activity in the right auditory cortex were monitored simultaneously throughout the measurement period, with the animal enclosed in a dark, sound attenuating chamber (AMC-4015; O'Hara & Co. Ltd., Tokyo, Japan). Surgeries were carried out as previously reported in order to measure cortical activity epipially with a surface microelectrode array (Takahashi et al., 2003, 2005; Shiramatsu et al., 2013).

ANIMAL PREPARATION

Six male Wistar rats, postnatal age 8.7 ± 0.2 weeks (mean ± standard deviation (s.d.)), with a body weight of 265 ± 12 g, were used. Rats were anesthetized with isoflurane in an air carrier (3% at induction and 1–2% for maintenance), and held in place with a custom-made head-holder. A heating blanket was used to maintain body temperature at approximately 37 °C. Initially, the skin was incised under local xylocaine anesthesia (0.3–0.5 ml). A ground needle electrode was subcutaneously inserted into the right forepaw. A small craniotomy was made near the bregma to embed a 0.5-mm thick integrated circuit socket as a reference electrode, making electrical contact with the dura mater. The right temporal muscle, cranium, and dura overlying the auditory cortex were surgically removed, and the exposed cortical surface was perfused with saline to prevent desiccation. Cisternal cerebrospinal fluid drainage was performed to minimize cerebral edema, then the right (ipsilateral to the exposed cortex) eardrum was ruptured and waxed, to ensure unilateral sound input from the contralateral ear. Respiratory rate, heart rate, and hind-paw withdrawal reflexes were monitored throughout the experiment. Unlike our previous experiments, atropine sulfate was not administered because of its mydriatic effect.

Monitoring of the pupil

The left pupil was observed through a surgical microscope (Nagashima Medical Instruments Co. Ltd.; SN-100, Tokyo, Japan) and recorded by a CCD camera (Watec Co. Ltd.; WAT-910HX, Tokyo, Japan). The microscope was located 8 cm in front of the left eye. In order to monitor the pupil in a dark room without inducing its light reflex, a custom-made array of infrared LEDs was used to illuminate the left eye. During the monitoring, a thin stainless wire was used to keep the eyelids apart. A small drop of saline was applied to the pupil every 60 min to prevent desiccation. Pupil images were acquired every 3 s, and the size of the pupil estimated with a public domain image processing program (National Institutes of Health; ImageJ). In order to pool data across animals, pupil size was converted into a *z*-score when needed. The *z*-score was derived from the mean and s.d. of pupil size throughout the experimental day.

Surface microelectrode recording

A surface microelectrode array was used to map either spontaneous or auditory-evoked potentials (AEPs) over the auditory cortex (Takahashi et al., 2003, 2005; Shiramatsu et al., 2013). The microelectrode array was made on a flexible polyimide substrate to conform to the curvature of the cortical surface, with a 10×7 grid of recording sites within an area of $4.5 \times 3.0\ mm^2$. Each recording site was $80 \times 80\ \mu m^2$, and the electrode impedance was approximately 400 kΩ under 1-kHz, 0.1-V sinusoidal waves. The dense mapping of AEPs using surface microelectrode arrays revealed the tonotopic organization and delineation of auditory fields in the auditory cortex (see Fig. 5(c)).

ECoG signals were obtained with an amplification gain of 1000, digital filter bandpass of 0.3–500 Hz, and sampling frequency of 1 kHz (Cyberkinetics Inc., Tokyo, Japan; Cerebus Data Acquisition System). Spontaneous activity was characterized at the recording site where a click elicited the largest AEP (see Fig. 5(c)). For each test stimulus, AEP amplitude was quantified at the recording site where the largest AEP was elicited.

Acoustic stimulus

A speaker (10TH800, Matsushita Electric Industrial Co. Ltd., Osaka, Japan) was positioned 10 cm from the contralateral ear (see Animal Preparation). Test stimuli were calibrated at the pinna with a 1/4-inch microphone (Brüel & Kjær, 4939) and spectrum analyzer (Ono Sokki