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Research report

Wakefulness-promoting role of the inferior colliculus

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HIGHLIGHTS

- The following results suggest that the inferior colliculus (IC) promotes wakefulness.
- Electrical and chemical stimulation of the IC produces EEG and hippocampal activation.
- Electrical and chemical stimulation of the IC produces an increase in the heart rate.
- The firing rate of the IC neurons increases in correlation with EEG activation.
- Bilateral inhibition of the IC increases the time spent in sleep.

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ABSTRACT

The inferior colliculus (IC) is a mesencephalic auditory nucleus involved in several functions including the analysis of the frequency and intensity of sounds as well as sound localization. In addition to auditory processes, the IC controls the expression of defensive responses. The objective of the present study was to test the hypothesis that the IC contributes to the maintenance of wakefulness. For this purpose, several experimental approaches were performed in urethane-anesthetized guinea pigs. Electrical or chemical stimulation of the IC resulted in electroencephalographic (EEG) desynchronization, theta rhythm in the hippocampus and an increase in heart rate; all of these effects suggest an arousal reaction. Furthermore, by means of extracellular unit recordings, we determined that most IC neurons increased their spontaneous and tone-evoked responses in association with EEG desynchronization. We also studied the effect on sleep and wakefulness of bilateral acute inhibition of the IC by microinjections of muscimol (a $GABA_A$ agonist), as well as the effect of bilateral IC lesions in chronically-instrumented (drug-free) guinea pigs. Acute (via muscimol microinjections), but not chronic (via electrolytic lesions) inhibition of the IC decreased wakefulness., We conclude that the IC plays an active role in the maintenance of wakefulness. Further, we propose that this nucleus may mediate arousal responses induced by biologically significant sounds.

1. Introduction

Wakefulness (W) is characterized by awareness of external and internal stimuli, low amplitude and high frequency electroencephalographic (EEG) activity, as well as activation of the somatomotor and autonomic (sympathetic) systems [1]. Active W is also accompanied by a characteristic theta rhythm (4–9 Hz) in the electrogram of the hippocampus (Hipp) [2]. In contrast, quiescent behavior and oscillations of high amplitude and low frequency in the EEG and Hipp characterize slow wave sleep (SWS). Most general anesthetics (propofol, barbiturates, isofluorane, etc.) also produce similar EEG patterns [3–5]. In contrast, urethane anesthesia preserves a dual pattern of electrical activity; a W-like high frequency EEG accompanied by theta waves in the Hipp, and slow delta oscillations in the EEG and Hipp that are similar to the patterns that occur during SWS [6–10]. Urethane anesthesia has been used by several group to study various aspects of waking and sleep behaviors [9,11–13].

There is a profound interaction between sleep and auditory functions; sound stimuli not only are able to wake up sleeping humans or animals, but could modulate the characteristics of sleep behaviors [14–17]. Auditory cues, which are potent guides for behaviors during wakefulness, gain priority in situations such as

Abbreviations: CF, characteristic frequency; ECG, electrocardiogram; EEG, electroencephalogram; EMG, electromyogram; EXP, experimental approaches; IC, inferior colliculus; ICc, central nucleus of the inferior colliculus; i.p., intraperitoneal; HR, heart rate; Hipp, electrogram of the hippocampus; SPL, sound pressure level; SWS, slow wave sleep; W, wakefulness.

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fight, flight or other survival behaviors. During these behaviors, the auditory system assigns priorities to certain sounds and sound combinations. In addition, it is considered that audition is the only sensory system remaining active while asleep, at least in microosmatic animals [18].

The inferior colliculus (IC) is the key site of convergence for numerous parallel and serial pathways within the auditory system, as well as a crossroad of auditory afferent and efferent pathways [18,19]. This nucleus is involved in intensity, duration and frequency discrimination of sounds as well as the localization of sound sources in space [20–24]. In addition, IC neurons specialize in processing specific calls or sounds that contain tonal patterns. In this regard, neurons of the torus semicircularis, homolog to the IC in the frog, differentially respond to con-specific mating calls [25], and IC neurons of guinea pigs and bats are especially reactive to species-specific patterns of vocalization [26–28].

In addition to auditory functions, the IC exerts a tonic pattern of control of the expression of defensive responses; electrical or chemical stimulation of this nucleus generates typical fear responses characterized by an increase in arousal as well as freezing and/or flight behaviors [29,30]. Microinjections of $GABA_A$ agonists into the IC increases the latency and decreases the frequency of learned switch-off responses to electrical stimulation of the IC [31]. Moreover, the microinjection of NMDA receptor agonists into the IC elicits complex defense reactions [32]. As part of defensive behaviors, the IC would be expected to increase the level of arousal; in fact, electrical or chemical (glutamate) stimulation of the IC elicits EEG activation [11,33].

Based on the preceding background, we hypothesized that the IC has an intrinsic capacity to promote W. In order to test this hypothesis, in urethane anesthetized guinea pigs we determined: (1) whether electrical and chemical stimulation of the IC induces signs of arousal such as EEG desychronization, hippocampal theta and an increase in the heart rate (HR) and, (2) if IC units increase their firing rate in conjunction with the EEG desynchronization. In addition, in behaving (drug-free) chronically-instrumented guinea pigs, we examined the effects on W following the inhibition or lesion of the IC.

2. Materials and methods

Twenty-eight adult male guinea pigs (Cavia porcellus), weighing 450–550 g, were used after having been determined to be in good health by veterinarians of the institution. All experimental procedures were conducted in accordance with the Uruguayan Animal Care Law (No 18611) and the "Guide to the care and use of laboratory animals" (8th edition, National Academy Press, Washington D.C., 2010), and were approved by the Institutional Animal Care and Use Committee. The minimal number of animals that were necessary to produce reliable scientific data was employed, and adequate measures were taken to minimize pain, discomfort or stress of these animals.

Five experimental paradigms (EXP) were utilized in anesthetized (acute experiments; EXP 1, 2 and 3) and in behaving (drug-free) animals (chronic experiments; EXP 4 and 5). The number of animals utilized in EXPs 1–5 were: 7, 4, 8, 5, and 5, respectively. All surgical and experimental procedures have been described in detail in previous studies [34–39].

2.1. Surgical procedures

2.1.1. Acute experiments

Guinea pigs were anesthetized with urethane (1.2–1.5 g/kg, i.p.) and placed in a stereotaxic apparatus. The skull was exposed and stainless steel screw electrodes were placed over the frontal and occipital cortices to record the EEG. A bipolar nichrome electrode was placed to record Hipp activity (Anterior 6, Lateral 2, Height 5; according to [40], zero is the interauricular line). Small holes (2–3 mm in diameter) were drilled bilaterally in the skull in order to provide access to the IC (A 0–1, L 0.5–2.5, H 4.5–5.5 mm) for electrical (Exp 1) and chemical (Exp 2) stimulation as well as for unit recordings (Exp 3) of the central nucleus of the IC (ICc), which is the core subdivision for auditory functions [41]. In addition, two electrodes were placed in precordial regions to monitor the electrocardiogram (ECG).

For Exp 1 and 3, a short polyethylene tube (4 mm diameter) was cemented in middle ears; the other end of the tube was connected to earphones in order to form a "closed" acoustic delivery system [36].

2.1.2. Chronic experiments

Guinea pigs were chronically implanted with electrodes to monitor sleep and W. Following anesthesia (pentobarbital, 35 mg/kg, i.p.), the animals were placed in a stereotaxic frame, the skull was exposed, and stainless steel screw electrodes were fixed over the frontal and occipital cortices to record the EEG. A bipolar nichrome electrode was placed to record Hipp activity, and a pair of nichrome electrodes was inserted into the neck muscles to record the electromyogram (EMG). All recording electrodes were then soldered to a nine-pin connector. The connector and a pair of parallel screw-threaded bars, which were designed to hold the animal in a stereotaxic position during experimental sessions (only for the Exp 4), were cemented to the skull with dental acrylic. In addition, small holes (2–3 mm in diameter) were drilled bilaterally in the calvarium to provide access to the IC for drug microinjections (EXP 4); these holes were covered with sterile bone wax between experiments. Two days before the first microinjection session, the dura matter was removed under local lidocaine anesthesia. After the completion of all surgical procedures, analgesics were administered systemically and antibiotics were applied topically to the scalp.

In EXP 5, bipolar electrodes were also implanted bilaterally into the IC in order to record local field potentials and perform electrolytic lesions.

2.2. Experimental procedures

2.2.1. Exp 1. Electrical stimulation of the IC

The EEG, Hipp activity, and ECG were amplified (×1000), filtered (0.1–100 Hz), acquired (512 Hz, 2^{16} bits) and processed with Spike 2 software (CED, Cambridge, UK).

Sound stimuli consisted of white noise (100 ms in duration), which was used to produce local field IC potentials (TDT waveform generator). Sound intensity ranged from 30 to 90 dB Sound Pressure Level (SPL). Sound stimuli were delivered contralateral to the recorded IC using Bayer DT48 earphones attached to the middle ear tubes; the earphones were calibrated by means of a decibel-meter (Radio Shack).

In order to identify the site of electrical stimulation, the IC local field potential evoked by white noise was localized (Fig. 1A). Cathodal stimulation was performed using monopolar (Tungsten) electrodes with tips of ≈50 μm (the anode was located subcutaneously). In preliminary experiments bipolar concentric electrodes were employed with comparable effects. The IC was stimulated throughout its dorsal-ventral extent (with 500 μm steps). Square pulses (0.2 ms at 100 Hz) were delivered during 0.2–20 s at an intensity of 20–800 μA. In order to identify the sites of stimulation, at the end of each experiment the IC was marked with DC anodic current (1–5 mA for a period of 10–20 s) (Fig. 1B).

2.2.2. Exp 2. Chemical stimulation of the IC was performed in 4 animals

The effect of chemical stimulation of the IC was determined using unilateral microinjections of bicuculline 5 mM ($GABA_A$ antagonist, Sigma–Aldrich, St. Louis, MO, USA) in 0.2 μl ($n = 2$). Control microinjections with vehicle (saline) were also carried out in 2 animals. In all the cases, microinjections began within 5 min after stabilization of the recordings.

Each microinjection was conducted during a period of 2 min using a 2 μl Hamilton syringe (Hamilton, Reno, NV, USA) that was hermetically connected to a syringe needle (tip diameter approximately 200 μm). Only one microinjection was performed in each animal. The microinjection site was marked by microinjection of 0.2 μl of Chicago Sky Blue (2%) at the end of each experiment.

2.2.3. Exp 3. Recordings of IC units under urethane anesthesia

Standard IC extracellular neuronal recordings were obtained by micropipettes (5–50 MΩ) filled with NaCl 2 M and Chicago Sky-Blue 2%. The signals were amplified, filtered (300–5000 Hz), and then acquired (20 kHz) and processed with Spike-2 software. At the end of each successful recording track, Chicago Sky-Blue was iontophoretically applied; following the histological processing of the brain, the recording site was reconstructed.

Sound stimuli consisted of white noise, which was used to search for IC units, and tone bursts (100 ms, 5 ms rise-decay time) which were delivered at the neuronal characteristic frequency (CF). Sound stimuli were delivered contralateral, ipsilateral or binaural at an intensity that ranged from 30 to 90 dB SPL.

2.2.4. Exp 4. Inhibition of the IC in chronic drug-free animals

All chronic animals were housed individually in a temperature-controlled room (22 ± 1 °C) in a soundproof chamber with a 12 h light/dark cycle (lights-on at 06.00 am); food and water were provided ad libitum (the same conditions were used for Exp 5).

Seven days after surgery, the animals were adapted for a period of four hours per day for 7–10 days to the recording conditions. In each experiment, the animals' head was firmly held in a stereotaxic position and body movements were restricted in a hammock-like bag.

Recording sessions were performed during the day, since there are no significant differences in the temporal (day/night) distribution of sleep and wakefulness in guinea pigs [37]. During the recording sessions, EEG, Hipp and EMG recordings were acquired and stored in a PC for analysis using Spike 2 software.

The microinjection procedure was similar to that in Exp 2. Muscimol hydrobromide, a $GABA_A$ agonist (25 mM, Sigma–Aldrich, St. Louis, MO, USA), was dissolved in saline (NaCl 0,9%) immediately before use. Muscimol or saline (0.2 μl) were