Arousal of cerebral cortex electroencephalogram consequent to high-frequency stimulation of ventral medullary reticular formation

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We have theorized that large neurons in the ventral and medial reticular formation of the medulla are critical for both autonomic and cortical arousal. To test this theory, we anesthetized rats with urethane, lowered concentric bipolar stimulating electrodes into the medullary reticular formation, and implanted electroencephalogram (EEG) and ECG recording electrodes. We stimulated in the medulla with pulse frequencies ranging from 50 to 300 Hz while recording cortical EEG and ECG. These female rats were ovariectomized, and one subgroup was administered estradiol. Electrical stimulation at either 200 or 300 Hz among the large medullary reticular neurons in nucleus paragigantocellularis (PGi) caused a significant reduction in the portion of the EEG power spectrum represented by δ -waves (0.1–4 Hz) and θ -waves (4.1–8 Hz). Correspondingly, there were increases in γ -wave power (22–50 Hz), especially when using 300 Hz. Stimulation at ≤ 100 Hz produced fewer changes in cortical EEG. The major features of these results were not significantly different according to estradiol treatment. In contrast, estradiol administration significantly lowered ECG. Electrical stimulation in PGi increased ECG in vehicle control animals, but decreased it in estradiol-treated animals. Regarding the EEG, we conclude that, even in anesthetized animals, stimulation of PGi reticular neurons can increase cortical arousal and that high frequencies of stimulation amplify this effect.

cardiac | sex behavior | lordosis | autonomic

A classic set of findings related increases in CNS arousal to increased activity in certain parts of the reticular formation of the brainstem (1, 2). We hypothesized that the most primitive neurons contributing to this effect are found in the medullary reticular formation, large neurons derived from the Mauthner neurons found in the medulla of fish (3). Some of these neurons have large cell bodies and bifurcating axons that allow them to send information both ascending toward the midbrain, with possible import for cortical arousal, and descending toward the spinal cord, with possible import for autonomic arousal (4–7).

Large reticular neurons in nucleus paragigantocellularis (PGi) have been drawn into the signaling of stimuli of sexual importance. Their ascending (8, 9) and descending (10) afferents could subserve the function of regulating sexual reflexes as well as other behaviors. Lesions of PGi disrupt lordosis behavior (11) and disinhibit ejaculatory reflexes (12, 13). Stimulation of this medullary region activates the deep back muscles, whose contractions comprise lordosis in the female (14, 15). Ascending afferents affecting this neuronal group's electrical activity might represent sexually arousing conditions, which is of import for hypothalamic and preoptic neurons that regulate female and male sexual behaviors, respectively (16, 17). In terms of descending inputs to this reticular area, Loyd and Murphy (18) reported that projections descending from the midbrain to this reticular area are sexually dimorphic, and the degree of activation of such neurons by morphine is, similarly, sexually dimorphic (19).

Thus, we hypothesize that neurons in nucleus PGi might, as a consequence of their electrical activity, arouse the forebrain and parts of the autonomic nervous system and, further, that their

activation might have particular importance for the initiation of sexual behaviors. Yet Lai and Siegel (20, 21) reported atonia in certain muscles as a consequence of neurochemical stimulation of the medial medullary reticular formation. To explore these issues, we studied the effects of electrical stimulation of these large reticular neurons in the PGi of rats. The full electroencephalogram (EEG) spectrum was analyzed and divided into its classical components: the slowest waves (δ), θ , α , β , and the highest frequency waves (γ). We used ovariectomized female rats treated with either the vehicle control or estradiol to test for possible hormonal effects.

Results

EEG. Electrical stimulation of PGi (on target) had an arousing effect on the cortical EEG, as defined by a decrease in the relative power density in the δ -frequency range, with a corresponding increase in β - and γ -frequency ranges (Figs. 1 and 2*B*). In oil-treated animals, electrical stimulation at higher stimulating frequencies (200 and 300 Hz) significantly increased β - and γ -power densities ($n = 9$; $P < 0.05$), although it tended to decrease δ -waveform power ($n = 9$; $P = 0.06$), compared with prestimulation (Table 1). Electrical stimulation at the lower frequency of 100 Hz increased only the γ -waveform relative power density ($n = 9$; $P < 0.05$).

Similarly, in estradiol-treated animals, electrical stimulation at higher stimulating frequencies of 200 and 300 Hz consistently increased β - and γ -waveforms, an increase highly significant compared with prestimulation values ($n = 10$; $P < 0.01$) (Table 1). Furthermore, electrical stimulation significantly decreased δ -waveform power density at 300 Hz ($n = 10$; $P < 0.01$), and a trend approached significance at 200 Hz ($n = 10$; $P = 0.054$).

There was no significant difference in the effects of stimulation between oil- and estrogen-treated groups.

We observed a frequency of stimulation effect in both oil- and estrogen-treated animals. In oil-treated animals, the result of a stimulation frequency of 100 Hz was significantly different from both 200 and 300 Hz for δ -waveform and only from 200 Hz for γ -waveform ($n = 10$; $P < 0.05$). In estrogen-treated animals, stimulation at 100 Hz had effects significantly different from 200 and 300 Hz across EEG waveforms ($n = 10$; $P < 0.05$).

Controls against unanticipated spread of current spread (e.g., to the pyramidal tract) were 3-fold: (i) use of concentric bipolar electrodes, (ii) off-target controls not giving the same results, and (iii) no observed movement, as would have followed from pyramidal tract stimulation.

A small number of trials in which animals were anesthetized

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Abbreviations: EEG, electroencephalogram; PGi, paragigantocellularis.

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Fig. 1. Electrical stimulation of PGI caused EEG of a urethane-anesthetized rat to change from higher amplitude, lower frequencies to low-amplitude, higher frequency activity. Off-target stimulation did not consistently have this effect. Stimulation was at 300 Hz (pulses per second) for 30 sec.

with Nembutal also resulted in the demonstration of an EEG-activating effect of PGI stimulation (data not shown). Thus, the previous results are not peculiar to urethane anesthesia.

A representative histological analysis for a stimulation point in PGI is shown in Fig. 3. Not every point of stimulation in the

Table 1. Prestimulation (control period) relative power densities for three stimulation frequencies and EEG domains

Stimulation frequency, Hz	OVX + Oil			OVX + E2		
	Relative power density, Hz			Relative power density, Hz		
	δ	β	γ	δ	β	γ
300	83.43	2.40	2.54	85.45	1.68	1.83
200	84.31	2.21	2.27	84.12	1.66	1.42
100	84.12	2.21	2.24	85.92	1.69	1.81

medullary reticular formation yielded this result. Overall, regardless of treatment and stimulating frequency, electrical stimulation that missed PGI (off target; Fig. 2A) occasionally activated the EEG, but did not have a consistent effect on EEG relative power densities.

ECG. Comparisons of prestimulation data revealed an estrogen treatment effect: a significant decrease in heart rate in comparison with oil-treated animals before electrical stimulation of the medulla ($P < 0.0001$) (Fig. 4).

Unlike the EEG, electrical stimulation dorsal to PGI (off target) affected ECG arousal responses in oil-treated animals, whereas no effect was observed in estrogen-treated animals regardless of stimulating frequency (Fig. 4A). In oil-treated animals, electrical stimulation at 300 Hz increased heart rate transiently (estrogen, $n = 11$; oil control, $n = 8$; $P < 0.05$). The

Fig. 2. PGI stimulation altered the EEG. Electrical stimulation in PGI (B, on target) decreased relative power density in the lowest frequency range (δ) and increased it in the highest frequency ranges (β and γ) regardless of whether the animal was pretreated with estradiol benzoate or not (oil control). The y axis is plotted as the change in relative power density percentage, with 100% representing the sum of the power densities of all EEG frequencies. Effects were strongest at 300 Hz (pulses per second). There were not consistent effects when the electrode missed PGI (A, off target). Relative power density in the α - and θ -ranges did not change significantly.

Fig. 3. Histological confirmation of a PGI injection site (arrow), with cresyl violet staining and with reference to the rat brain atlas of Paxinos and Watson (26). Only animals with histological confirmation of PGI stimulation were used in statistical analyses. IV, fourth ventricle; io, inferior olive.

ECG response was more robust with 200 and 100 Hz stimulating frequencies ($P < 0.01$ and $P < 0.001$, respectively).

Electrical stimulation of PGI (on target) affected ECG arousal responses for both oil- and estrogen-treated animals, but surprisingly those two groups of animals trended in opposite directions (Fig. 4B). A frequency of stimulation effect was observed in oil-treated animals, where 100-Hz stimulation is significantly different from 300 Hz during the first and last 5 sec of stimulation ($n = 8$; $P < 0.05$).

After estrogen pretreatment, electrical stimulation of PGI strikingly decreased heart rate during the first 5 sec of stimulation ($n = 11$; $P < 0.001$). Stimulation at 300 Hz was the most effective. Habituation of the effect was observed in that the size of the decrease declined during stimulation as shown in Fig. 4B by the last 5 sec of stimulation (dur2').

Comparisons of Changes in EEG and ECG. In PGI, in most cases ($n = 16$ rats), stimulation at 100, 200, or 300 Hz yielded significant changes in both ECG and EEG. In the remaining 11 animals, stimulation caused significant changes in the EEG, but not in the ECG. Never was there an instance in which stimulation caused an ECG change but no EEG change.

Discussion

The main finding of this study was that electrical stimulation of a small region of the medullary reticular formation, PGI, was able to activate the cortical EEG of anesthetized rats. This result

occurred in a region of the medulla where cells have been discovered whose effective stimuli cover more than one sensory modality and whose receptive fields are large (22, 23) (E. M. Martin, C. Pavlides, and D.W.P., unpublished data). Further, large nerve cells in this region include some with long bifurcating axons, one branch ascending (of possible importance for cortical arousal) and the other branch descending (of possible importance for autonomic arousal) (4–7) (E. M. Martin and D.W.P., unpublished data). Therefore, the stimulation site in the present work may excite neurons that are crucial for the activation of behavior.

These neurons may be of special importance for sexual arousal. Their neuroanatomical connections and physiology as studied so far suggest that they help convey signals from the genitalia to the forebrain and that they influence vertebral muscle motoneurons important for sexual behavior. We suggest theoretically that their CNS-arousing functions, including those explored in these experiments, are especially important for the initiation of courtship and copulatory behaviors. Possible sex differences in the types of results presented earlier remain to be explored.

The activity of the medullary reticular neurons we stimulated also could be involved in the regulation of CNS arousal serving other nonsexual functions. Although classical views emphasize that the lowest frequencies of EEG waves, in the δ -range, are associated with sleep and the highest frequencies are associated with alertness and attention in the waking state, some findings hint at further complexity. Posner and colleagues (24) reported that different networks that carry out the functions of alerting, orienting, and executive control of attention are associated with distinct sets of oscillatory activities operative over distinct time courses (e.g., the alerting network demonstrating decreases in θ -, α - and β -band activities 200–450 msec after a warning signal).

We note that on-target stimulation in PGI was not affected by estradiol treatment. Therefore, it could be inferred that either estrogens do not impact the neural pathway from medullary reticular formation to cortex or the high frequency of stimulation, potentially supraphysiological, overwhelmed hormone effects that otherwise would have been detected.

However, there was a strong and surprising effect of estradiol on the ECG. The effect of electrical stimulation after estradiol treatment was opposite that in the control condition. One interpretation is that estradiol permitted neurons in PGI to act as would have been predicted for nerve cells in the classically recognized cardiac depressor region (25). This result is never-

Fig. 4. Stimulation in PGI caused different types of changes in ECG (heart rate) in estradiol benzoate (EB) compared with vehicle (oil) control ovariectomized female rats. Open symbols, oil controls; filled symbols, EB treated. Prestimulation values (pre) are compared with the first 5 sec of stimulation (dur1), the final 5 sec (dur2), and poststimulation values (post). Electrical stimulation was at 100, 200, or 300 Hz (pulses per second). (A) Medullary reticular stimulation outside PGI (off target). (B) Electrical stimulation in PGI (on target).

theless surprising because the EEG activation reported in this article, as well as the fact that neurons in this region are more active in association with the activation of behavior (E. M. Martin, C. Pavlides, and D.W.P., unpublished data), would portend an increase in heart rate upon their stimulation, rather than a decrease. Another unpredicted result is that EEG changes were often more sensitive to PGI stimulation than ECG changes. Presumably that could mean that the strength of ascending connections of the stimulated neurons was greater than the strength of their descending connections.

Potential caveats regarding the interpretations of these results include the fact that we may have been stimulating fibers of passage. Additionally, we note that Siegel and colleagues (20, 21) reported muscular atonia after medial medullary stimulation. From their reports, we assume that their stimulating electrodes were dorsal, medial, and anterior to ours, and that such a difference in placements could account for the differences in results.

Future work will analyze biophysical properties of these neurons by using patch-clamp techniques and will search for behavioral correlations of their electrical activity by using recordings from unanesthetized animals.

Methods

Subjects. Twenty-eight ovariectomized female rats were individually housed in a 12-h light/12-h dark cycle (700–1900 lights on) with laboratory chow and water ad libitum for 1 week before the experiment. Forty-eight hours before electrical stimulation, rats were treated with either 10 μ g/0.1 ml estradiol benzoate dissolved in sesame oil, injected s.c., or 0.1 ml of the vehicle. All animal procedures and surgeries used in this study were performed in accordance with the Animal Welfare Act and the Department of Health and Human Services and are approved by The Rockefeller University's Animal Care and Use Committee.

Surgery. Rats were anesthetized with 1.2 g/kg urethane i.p., followed by ECG electrode implantation and craniotomy. In preliminary experiments, a small number of rats were anesthetized with 40–45 mg/kg Nembutal i.p. In all experiments, a single ECG wire (no. 101R7T; Medwire, Mt. Vernon, NY) was inserted into the left pectoral muscles to record ECG activity. After the rat was mounted onto the stereotaxic frame (Kopf Instruments, Tujunga, CA), EEG activity was recorded with skull screws (coordinates: with bregma and λ leveled, 2 mm posterior to bregma, 3 mm left vs. 3 mm anterior to bregma, 2 mm left) as a stimulating concentric bipolar electrode (SNEX-100 $\times$ 25 mm; FHC, Bowdoinham, ME) was stereotaxically advanced toward PGI nucleus of the medullary reticular formation (expected coordinates 11.8 mm posterior to bregma, 1.7 mm left, depth 10.5 mm). Each animal was stimulated at millimeter intervals starting at 1 mm above the expected target (PGI).

Stimulation Parameters. The following range of parameters was determined from a pilot study that gave optimal cortical and autonomic arousal responses. Pulse frequencies ranged from 50 to 300 Hz, with a pulse duration setting at 0.1 msec and an amplitude setting at 1.8 V. The optimal response was defined as the greatest change in relative power density of cortical EEG

waves and an increase in ECG frequency response to electrical stimulation compared with prestimulation. Frequencies of <100 Hz were ineffective and, therefore, not included in the full experimental protocol. Stimulation duration ranged from 30 to 90 sec to determine how cortical and autonomic arousal levels were affected through time. Higher frequencies (e.g., 300 Hz) were used for a shorter duration (30 sec), whereas lower frequencies (e.g., 100 Hz) were used for a longer period (90 sec), calculated in such a manner as to keep the total number of pulses the same (9,000 pulses). Stimulation with lower frequencies (i.e., 5–50 Hz) and longer durations of stimulation (i.e., 3–10 min) also were used. However, changes in EEG and ECG were minimal to undetectable, and the results were not included in this report.

Recordings of both EEG and ECG were done directly, online, by using the ADInstruments' PowerLab unit (Castle Hill, Australia). EEG was calculated according to the relative power densities of the five frequency domains listed previously. This calculation considers the entire set of amplitudes of waves in the EEG as 100%, does the Fourier analysis, and, as a result, measures the share of the 100% energy because of each EEG frequency domain.

Histology. At the end of the stimulation procedure, lesions were made with an electrolytic current of 5 μ A for 15 sec through the stimulating concentric bipolar electrode. Animals were perfused transcardially with 0.9% saline followed by 4% paraformaldehyde. The brainstem tissue containing the lesion site was removed and stored overnight in 4% paraformaldehyde, followed by 30% sucrose solution. Lesion sites were sectioned to 50 μ m thick, stained with cresyl violet, and reconstructed under a Bausch and Lomb (Rochester, NY) projection microscope, with reference to the atlas of Paxinos and Watson (26). On-target points were in PGI, and off-target points were medial or dorsal.

Spectral Analysis. The PowerLab system (ADInstruments) allowed real-time data collection, amplification, and filtration of signals and simultaneously displayed EEG and ECG electrical activities in separate channels. The EEG was Fast Fourier-transformed, and the spectral data were used to calculate the relative power density. The EEG spectrum was divided into the following frequency bands: δ (0.3–4 Hz), θ (4–8 Hz), α (8–12 Hz), β (12–22 Hz), and γ (22–50 Hz). The ECG frequency (heart rate) was automatically calculated by PowerLab and exported to Excel for analysis. The EEG data presented are from 30-sec epochs that compared the differences in relative power density between prestimulation and stimulation to determine stimulation efficacy. The ECG data presented are from 5-sec epochs that compared heart rate in beats per minute across time points to determine stimulation efficacy and also whether heart rates habituated under stimulation. Epochs were obtained from the last 5 sec prestimulation, first 5 sec with stimulation, last 5 sec with stimulation, and first 5 sec poststimulation.

Only animals with histological placement confirmed were used in statistical analysis. The means $\pm$ SEM of EEG and ECG responses were analyzed, with Bonferroni-corrected paired *t* tests after ANOVAs with repeated measures as appropriate. Significance was defined as *P* < 0.05 (two-tailed).

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