tolerance in respect of food additives really means is that deliberate addition to the carcinogenic burden already upon us should be avoided where this is at all feasible."

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12. The beverages used were typical of products commercially available in Sweden. The orange juice had been imported in closed bottles from the United States. The sample had a specific weight of about 1.04 g/cm^3 and contained about 13 percent dry matter. (It could be ascertained that the orange juice had not been previously treated with DEP.) The white wine was a Turkish wine with an alcohol content of 12 to 14 percent (by volume) which had been imported in bulk and bottled in Sweden. (It could be ascertained that the wine had not been treated with DEP after its arrival in Sweden.) The beer was a Swedish product having a wort content of about 8.5 percent and an alcohol content of less than 2.8 percent. It is illegal to treat beer with DEP in Sweden.
13. The ^{3}H-labeled DEP (supplied through the courtesy of Dr. B. Öberg) was diluted 500 times with inactive DEP; the result was a sample with a specific activity of 22 μc/mmole which was used in the investigation reported here. The synthesis of the ^{3}H-labeled DEP is described in: B. Öberg, *Eur. J. Biochem.* **19**, 496 (1971).
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26 July 1971; revised 25 August 1971

Single Neuron Activity in Cat Gigantocellular Tegmental Field: Selectivity of Discharge in Desynchronized Sleep

Abstract. *Ratios of discharge rates in desynchronized sleep to those in waking and synchronized sleep of gigantocellular neurons are five to ten times higher than are those of neurons in adjacent tegmental fields and 25 to 30 times higher than in other brain sites. This marked concentration of activity in desynchronized sleep is compatible with an active role of gigantocellular neurons in the generation of this sleep phase.*

Evidence from lesion and stimulation studies implicates pontine brainstem structures as important in the generation of desynchronized sleep (D) (*1*). However, boundaries of the critical region (or regions) and identity of the controlling neural elements remain vague because, apart from being unphysiological, lesions and stimulation can produce their effects through action on fibers arising elsewhere as well as through action on somata intrinsic to the area studied. More precise and more physiological information about the locus and nature of neural elements generating activity characteristic of this phase of sleep can be sought through recordings of single cells.

Neurons that are a part of a central system actively controlling D sleep should show increases in discharge rates during this behavioral state which are greater than those of presumed "follower" neurons, such as those in cerebral and cerebellar cortices (*2*). We thus suggest that the degree to which activity is concentrated in D sleep, or the *selectivity* of firing in this state can be taken as one important criterion for testing the hypothesis that a given group of cells is at or near the origin of the increased neuronal activity during D sleep. The selectivity of discharge during D can be quantified through the ratio of D discharge rates to those of waking (W) and synchronized sleep (S).

The large neurons of the pontine reticular formation in the gigantocellular tegmental fields (FTG) are, on the basis of anatomical considerations, possible candidates for this controlling role. Brodal (*3*) has presented evidence that more than one-half of the 3000 to 4000 giant cells in the cat FTG send ascending axons beyond midbrain, and more than one-half send descending axons into spinal cord. Included in these estimates are those cells that have both ascending and descending axon branches. These direct and widespread connections to regions outside the brainstem suggest that these cells could effect the ubiquitous and marked changes in brain and spinal neuronal activity that occur during D sleep. The FTG corresponds to the nucleus reticularis pontis oralis *and* nucleus reticularis pontis caudalis, structures that the studies cited above (*1*) have implicated in the control of D sleep.

In our study we use previous observations (*2*) of the selectivity of discharge of cerebral and cerebellar cortical neurons and we analyze new measurements of the discharge rates of neurons in several brainstem sites. The results are compatible, in terms of the selectivity criterion, with a controlling role for FTG neurons in the generation of events during D sleep.

Under pentobarbital anesthesia, four cats were implanted with electrodes for recording electrographic data [electrooculogram (EOG), parietal electroencephalogram (EEG), transcortical EEG, and nuchal electromyogram (EMG)] by methods previously described (*4*). In addition, a steel plate was fixed to the frontal sinus; this plate had threaded bolts extending from it that were screwed to a metal bar at the time of recording; this was done to minimize the head-on-neck movements that are a major source of instability in brainstem unit recordings. Extracellular single unit activity was recorded from glass-insulated platinum-iridium microelectrodes that were controlled by an Evarts-type micromanipulator (*5*). The cylinder for holding the micromanipulator was positioned over the vermian cortex of the cerebellum and mounted on the bone at an angle of 30° from the vertical. The anterior-posterior position of the cylinder center was varied to give a window on the brainstem that included, rostrally, the upper pons and, caudally, the upper medulla. The

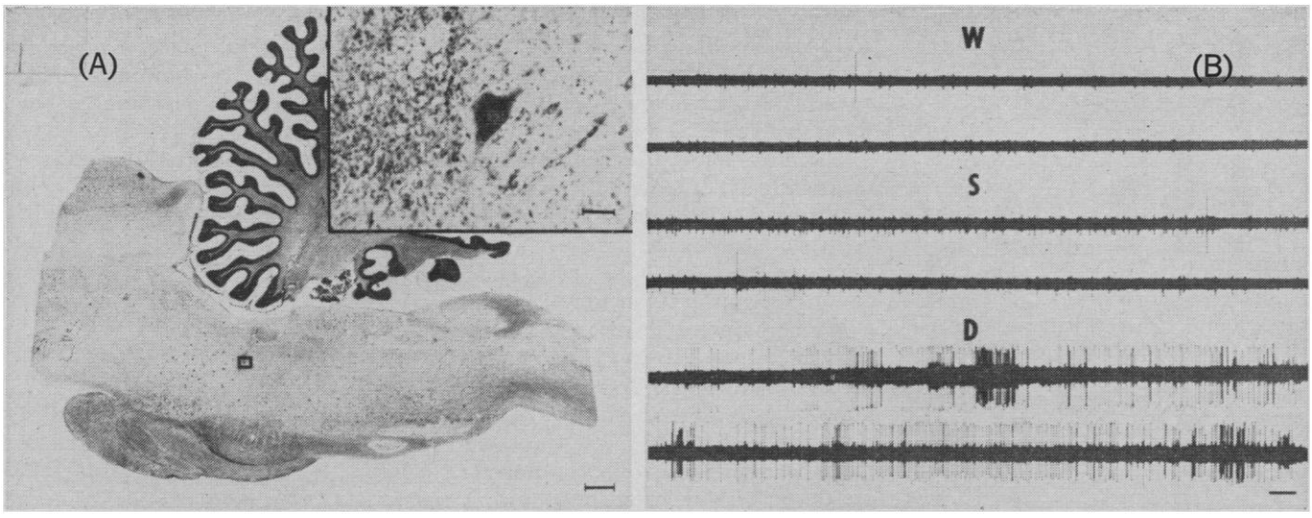

Fig. 1. (A) Photomicrograph of a sagittal section of the brain showing a microelectrode tract descending through the cerebellum into the gigantocellular pontine reticular formation (scale, 1 mm). Inset, a microlesion near a "giant cell" in the FTG (scale, 50 μm). (B) Activity of a neuron recorded during W, S, and D at the site of the microlesion in (A) (scale, 0.1 second). There is a marked increase in discharge rate during D. In the 5 seconds of record shown for each state, there is one discharge in W, two in S, and about 350 in D.

effective lateral extent of this window was 3 mm to either side of midline.

Experiments were begun 1 week after implantation procedures; the unanesthetized cats were placed in a small box with the head held rigid by means of the bolts described above. Adaptation to the restraint condition was achieved in one or two preliminary sessions. The night before adaptation and recording sessions, cats were placed in a slowly moving wheel (< 1 rev/min) for about 12 hours. After this partial sleep deprivation the cats tolerated restraint and generated electrographically normal sleep cycles throughout the recording periods.

Initially negative action potentials with durations of more than 0.5 msec and no notches were assumed to arise from somata rather than from fibers. When the signal-to-noise ratio was greater than 2:1, the action potentials were converted to uniform pulses by a variable threshold Schmitt trigger and one-shot pulse generator. Cathode ray oscilloscope monitoring was done constantly to assure the accuracy of transformation of unit activity to uniform pulses. These pulses, the unit activity, and the electrographic data were recorded for at least 1 minute in each behavioral state on an FM tape recorder. In addition to the taped data, a continuous record of the electrographic data and a record of the unit activity were obtained on a Grass ink-writing polygraph. Criteria for the behavioral states of W, S, and D have been defined (*4*). When a neuron did not discharge during a sample period, we maintained the micromanipulator in the same position until we observed an action potential of the same form and amplitude again before considering the silent period as valid. A PDP-12 computer was used to determine the intervals between discharges of the nerve cell to the nearest 0.1 msec; the sequential record of the interspike interval durations formed the basis for statistical analysis.

At the end of experiments, cats were anesthetized, and the brains were perfused. Serial sagittal sections of 25 μm were stained with a combined fiber and nuclear stain; the locations of the recording sites were determined from the readings obtained on the micromanipulator scale and from the site of microlesions made during recording sessions (10 to 15 μa, 12 seconds), and from the electrode tracts. Figure 1A shows a descent and a microlesion. Anatomical definition of lesion sites was that used in the Berman atlas (*6*). Of 287 neurons observed, 198 were recorded during three behavioral states, and 69 of these fulfilled our criteria of a 2:1 signal-to-noise ratio throughout the sample periods. Of the 69 neurons, 34 were localized in the FTG, 13 in tegmental fields adjacent to FTG, 14 in the tegmental reticular nucleus (of Bechterev), and 8 in the pontine gray matter.

At recording sites localized later to the FTG, neuron activity was characterized by high amplitude action potentials with large, isolated electrical fields; signal to background noise ratios were

Table 1. Summary statistics for discharge rates (impulse per second) of 34 FTG neurons. The Wilcoxon matched-pairs signed-ranks test (*8*) shows the means of the discharge rates of the 34 neurons in D to be significantly higher than those in W and S (one-tail test, $P < .00003$).

Statistic	Discharge rates (impulse per second) by behavioral state		
	W	S	D
Geometric mean	0.105	0.203	10.380
Median	0.802	0.591	13.084
Arithmetic mean	7.592	5.411	19.050

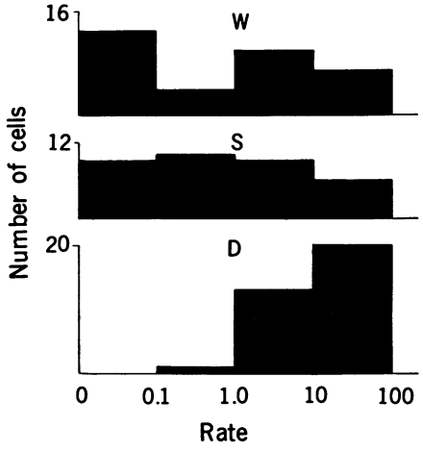

Fig. 2. Mean discharge rates (impulse per second) of the 34 FTG neurons during W, S, and D. There is a shift of the population of neurons toward higher mean discharge rates during D sleep.

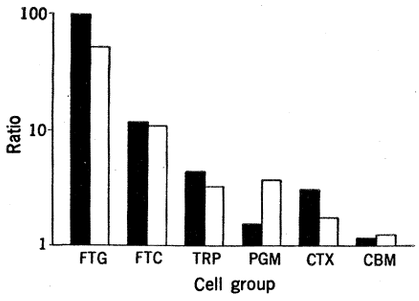

Fig. 3. Ratio of geometric mean discharge rates in D sleep to those in W (filled bars) and to those in S (open bars) for neurons observed in FTG, the tegmental fields adajacent to FTG (*FTC*), the tegmental reticular nucleus (*TRP*), pontine gray matter (*PGM*), posterolateral neocortex (*CTX*), and cerebellar Purkinje cell simple spikes (*CBM*).

as high as 14:1. In view of the unusually large spike amplitudes, most of the neurons recorded in the FTG are likely to have been "giant cells"; thus the observations described are likely to have been made on those cells. Most FTG neurons either showed very low discharge rates (< 1 impulse per second) or were silent during W and S, while during D discharge rates increased to large multiples of those during W and S (Figs. 1B and 2). To determine the ratio of D discharge rates to those in W and S, we calculated first the geometric means of discharge rates in W, S, and D for the population of 34 FTG neurons. Then we calculated the medians and arithmetic means for the same data (Table 1). All of the measures are much higher in D than in W or S. Use of the geometric mean, as contrasted with the median or arithmetic mean, preserves the ratio scale for computation of population ratios of discharge rates (*7*). For FTG neurons these ratios were D/W, 98.5, and D/S, 51.1; in other words, the group geometric mean discharge rate of the 34 FTG neurons in D was 98.5 times that in W and 51.1 times that in S.

Such concentration of neuronal activity in D (when compared with W and S) has not been found in any other brain site. The magnitude of the ratios for FTG neurons is compared with that of neurons observed at other sites (Fig. 3). Of particular importance in terms of the selectivity criterion is the progressive decrease of the ratios at recording sites increasingly distant from the FTG; the ratios are five to ten times lower in adjacent tegmental reticular structures, while at distant recording sites (posterolateral neocortex and Purkinje cells in cerebellar vermis) and in the tegmental reticular nucleus and pontine gray (sites thought to have strong cerebellar connections), ratios are 25 to 30 times lower than in FTG.

The data from FTG neurons satisfy the selectivity criterion discussed earlier better than any other group of cells yet encountered. The large ratios of discharge rates in D to those in W and in S seen in FTG neurons are what might be expected in recordings from neurons whose activity is most intimately related to the events of D. The lower ratios in adjacent reticular regions demonstrate that rate increases of this magnitude during D are not a property of all pontine reticular cells; rather, there appears to be a decreasing gradient of activity ratios with recording sites increasingly distant from FTG. Our findings are therefore consistent with the hypothesis that neurons in the FTG may be involved in the generation of D sleep phenomena.

Our data are suggestive of, rather than proof of, a critical role for FTG neurons in generating desynchronized sleep phenomena. It is possible that the FTG neurons are themselves "follower" neurons under the control of other, still unrecorded cells in the brain. We believe that the method of recording and the approach to data analysis described will permit assessment of this alternative. By these methods, additional physiological evidence bearing on the central control question can be derived from an analysis of the temporal relationship of FTG neuronal activity to the tonic and phasic events of D sleep. More definitive tests of pacemaker activity in neurons such as these by intracellular recording techniques may also be possible.

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Central and Peripheral Control of Gill Movements in Aplysia

Abstract. *Two types of gill contraction in* Aplysia *were used to study the relation of peripheral and central pathways in controlling behavioral responses in a mollusk. A weak or moderate tactile stimulus to the mantle elicits gill contraction (gill-withdrawal reflex) as a component of a more extensive withdrawal response; a stimulus applied directly to the gill elicits a localized response of the gill pinnule (pinnule response). Central pathways through the abdominal ganglion are both necessary and sufficient for the gill-withdrawal reflex, and motor neuron L7 makes direct connections with gill muscles, without engaging the peripheral plexus. Peripheral pathways are necessary and sufficient for the pinnule response. As a result of the independence of peripheral and central pathways, habituation by repeated tactile stimulation of one pathway does not affect the responsiveness of the other pathway.*

The nervous system of higher animals varies in the proportions of central ganglionic masses and peripheral nerve plexuses. In vertebrates, peripheral nerve plexuses are restricted to certain viscera, such as stomach and intestine, but in higher invertebrates, nerve plexuses (*1*) are often more extensive, innervating skin and muscles of locomotion. For example, mollusks and annelids have well-developed central nervous systems, yet peripheral path-