Paradoxical Phase of Sleep: Its Artificial Induction in the Cat by Sodium Butyrate

Abstract. *In intact cats and cats with the mesencephalon transected the intravenous administration of sodium butyrate and related compounds (0.3 to 3 millimoles per kilogram) induced the so-called paradoxical phase of the sleep, as indicated by electroencephalograms, electromyograms, heart rate, respiration, and eye movement. This phase appeared after a 3- to 5-minute period of sleep characterized by slow waves, and continued for 4 to 15 minutes.*

With the use of polygraphic recordings of eye movements and of electrical potentials from the brain (EEG) and muscles (EMG), one may classify the naturally occurring sleep of the cat into two stages. In one stage the sleep is characterized by slow waves and spindle-shaped bursts in the neocortical EEG and by slight tonic activity in the neck EMG; in the others the sleep is characterized by low-voltage fast waves in the EEG, no activity in the neck EMG, and jerky movements of the eyes. Since the neocortical EEG of the first stage is hardly differentiated from that of the awake state although the animal is behaviorally asleep, it is often designated as the paradoxical phase of sleep.

Several attempts have been made to induce this paradoxical state. There have been several reports that electrical stimulation of the reticular formation, posterior hypothalamus, and other nervous structures induces the state successfully (*1–4*). In these reports it is noteworthy that the paradoxical state was evoked after a relatively long period of latency, more than several minutes, and it is difficult to find reproducible causal relations between the stimulus and the evoked sleep state, or to find any relation between the intensity of the stimulus and the magnitude of the response. The administration of short-chain fatty acids to animals can also produce a reversible unconscious state similar to physiological sleep (*5–7*).

We have examined the relation between short-chain fatty acids and paradoxical sleep. In 20 intact cats with electrodes implanted in various areas of the brain and neck muscle, sodium butyrate and related compounds were given intravenously through an implanted polyethylene tube (1 mm diameter) inserted previously into a cephalic or saphenous vein. Eye movement, respiratory rhythm, and heart rate were recorded simultaneously. In experiments of short duration 45 cats decerebrated at the precollicular level (mesencephalic cats) were used. Acids were buffered at *p*H 7.4 to 7.6 with sodium hydroxide.

Figure 1 shows the sequential changes in the EEG, EMG, electrocardiogram (EKG), eye movements, and respiratory movements after the administration of sodium *n*-butyrate solution (1.5 m*M*/kg) to an intact cat. Figure 1*A* shows the polygraphic record before administration, when the animal was awake. After 30 to 60 seconds, as shown in Fig. 1*B*, the pattern of the EEG from the neocortex changed from low-voltage fast waves to the slow-wave pattern with spindle-shaped bursts. Three minutes later as shown in Fig. 1*C*, when the animal was behaviorally silent, the EEG pattern changed from slow waves and spindles to the low-voltage fast-wave pattern, with the activity of the neck muscle almost disappearing, jerky eye movements appearing, and the heart-rate increasing. These phenomena may be identical to those which occur during natural sleep. This state continued for 4 to 15 minutes, and after this the animal returned polygraphically to the awake state. The same amount of sodium butyrate (1.5 m*M*/kg) was given repeatedly seven times to the same cat at intervals of about 1 hour. In every trial after 1 to 7 minutes the EEG changed from the control awake

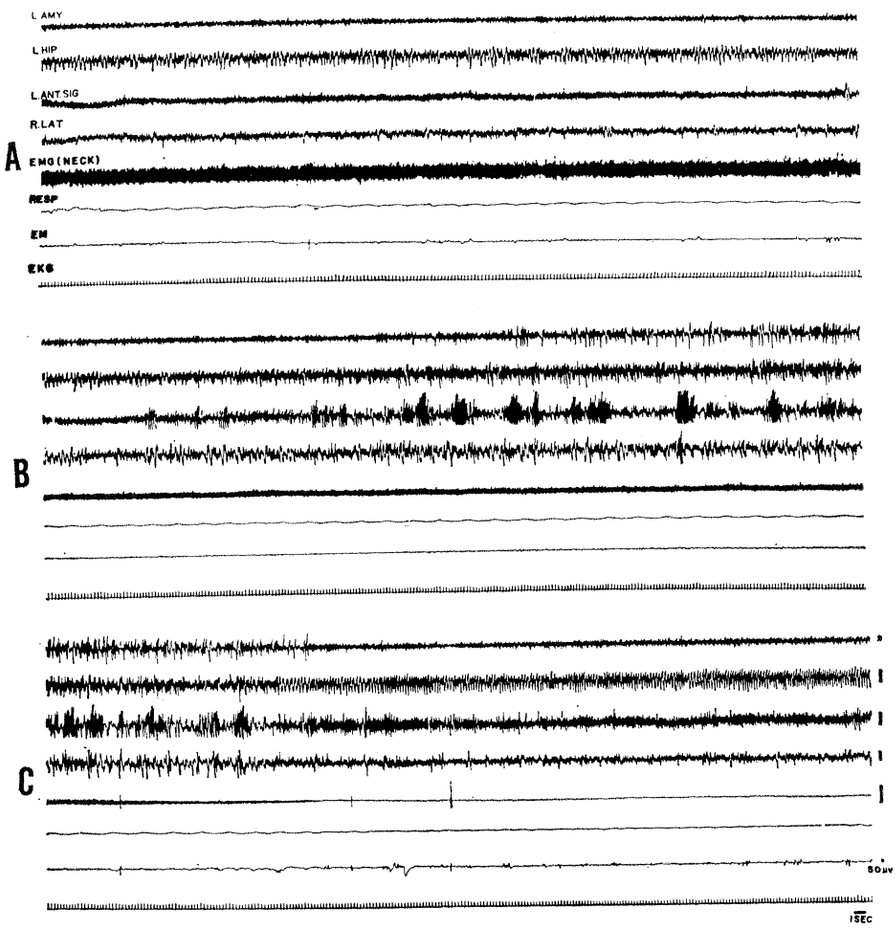

Fig. 1. Polygraphic recordings from the intact cat in the awake state (*A*), 1 minute after the injection of sodium normal butyrate solution (1.5 m*M*/kg) (*B*), and 3 minutes after injection (*C*). In each recording, from top to bottom, EEG from left amygdala (*L.AMY*), from left hippocampus (*L.HIP*), from left anterior sigmoid gyrus (*L.ANT.SIG*), from right lateral gyrus (*R.LAT*), neck muscle activity (*EMG*), respiratory rate (*RESP*), eye movement (*EM*), heart rate (*EKG*).

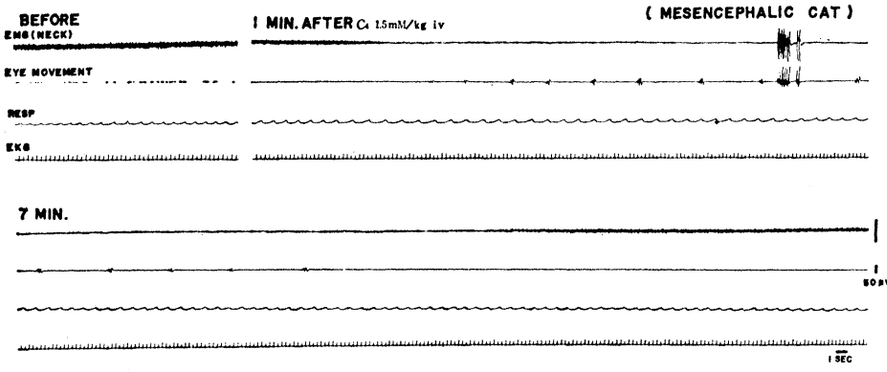

Fig. 2. The paradoxical phase of sleep in the mesencephalic cat. The control state is at top left, and the state after 1 minute is at top right. The state after 7 minutes is at the bottom. The activity shown by the EMG disappears and eye movements appear after 1 minute.

state to the slow-wave and spindle-burst pattern for 1 to 2 minutes, and the paradoxical phase followed for several to 10 minutes. In untreated cats, the spontaneous paradoxical phase occurred repeatedly for 6 to 10 minutes at intervals of 2 to 2.5 hours.

When the concentration of sodium butyrate was between 0.5 mM/kg and 1 mM/kg, the paradoxical phase of sleep was not observed, and the slow-wave phase continued for less than 15 minutes. If a more concentrated solution (4 to 5 mM/kg) was administered, appearance of the paradoxical phase of sleep was delayed, appearing for a short time of 0.5 to 2 minutes after a prolonged period of slow-wave sleep for 40 to 60 minutes.

Similar changes in the paradoxical phase of sleep were also induced by sodium butyrate in the mesencephalic cat, while the mesencephalic cats not injected with sodium butyrate showed no sign of the paradoxical phase. As illustrated in Fig. 2, 1.5 to 3 minutes after administration the neck-muscle activity disappeared for 4 to 15 minutes and returned to the control amount.

At this time, irregularity of the heart rate and respiratory movements, and jerky movements of the eyes and facial muscles characteristic of the paradoxical phase of sleep in the intact cat, were observed. These facts indicate that the paradoxical phase is induced in the mesencephalic cat by the butyrate. If the butyrate (1.5 mM/kg) was administered at intervals of 40 to 50 minutes to the same cat, the paradoxical phase was always produced for 4 to 15 minutes, with a delay of 1.5 to 2 minutes. Even after seven or eight injections no sign of desensitization was noticed. Compared with the intact state, the time at which the activity of the neck muscle disappeared in the EMG or at which other signs appeared was relatively constant within a narrow range (1.5 to 2 minutes).

If a small amount of sodium butyrate (0.15 mM kg^{-1} min^{-1}) was given continuously for 20 to 30 minutes in the mesencephalic cat, the duration of this phase could be prolonged for 40 to 70 minutes. The application of saline as a control solution (1 to 3 ml/kg) under the same experimental conditions could not induce the paradoxical phase of sleep in the intact or the mesencephalic cats.

The concentrations of sodium butyrate and related compounds which could reproducibly induce the paradoxical phase of sleep in the cat by intravenous application were as follows; sodium n-butyrate (1 to 3 mM/kg), sodium isobutyrate (1 to 3 mM/kg), sodium isovalerate (1 to 2 mM/kg), sodium n-caproate (0.3 to 0.5 mM/kg), sodium γ-hydroxybutyrate (1 to 1.5 mM/kg), sodium γ-butyrolactone (0.5 to 1 mM/kg), sodium α-hydroxyisobutyrate (1 to 1.5 mM/kg). Sodium propionate (1 to 2 mM/kg), sodium acetoacetate (1 mM/kg), and β-hydroxybutyrate (1 to 1.5 mM/kg) were not effective in producing the paradoxical phase of sleep.

From polygraphic observations in the intact and mesencephalic cat, it is concluded that sodium butyrate and related compounds are capable of inducing the paradoxical phase of sleep after a state of appropriate duration characterized by slow waves and spindle-shaped bursts. The manner in which the paradoxical phase of sleep is produced by these substances is not resolved.

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References

1. G. F. Rossi, E. Favale, T. Hara, A. Giussani, G. Sacco, *Arch. Ital. Biol.* **99**, 270 (1961).
2. M. Jouvet, *ibid.* **100**, 152 (1962).
3. E. Grastyān, in *The Central Nervous System and Behavior*, M. A. B. Brazier, Ed. (Josiah Macy, Jr. Foundation, New York, 1955), p. 119.
4. K. Yamamoto and R. Kido, *No To Shinkei* **13**, 887 (1961).
5. F. E. Samson, N. Dahl, D. R. Dahl, *J. Clin. Invest.* **35**, 1291 (1956).
6. B. Holmquist and D. H. Ingvar, *Experientia* **13**, 331 (1957).
7. R. P. White and F. E. Samson, *Am. J. Physiol.* **186**, 271 (1956).

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Köllner Effect and Suppression of the View of an Eye

Abstract. *Köllner's discovery of binasal hemianopia as an early stage in binocular color rivalry is extended to the study of the subsequent suppression of the color seen by an eye. The Köllner effect is one of the five two-color configurations that occur initially, and through which total suppression develops by the visible expansion of one of the colors. However, suppression sometimes occurs without a two-color phase.*

When corresponding areas of the two retinas of a subject are exposed to different colors there may be binocular color mixture. When this does not occur, there is binocular color rivalry, characterized by the alternation of suppression of the view of the left and the right eye. The popular theory is that the brain employs suppression "as a psychic means of escape from conflict" (*1*). It is not known how "the brain" accomplishes suppression, nor how suppression of the view of one eye occurs during binocular color rivalry.

Köllner first reported that the colors