We were able to follow the phenomenon of complete reflex suppression which was observed during quiet and active sleep more closely by plotting the % occurrence of the reflex, minute by minute, during consecutive cycles of sleep and wakefulness (Figure 2). In this aspect of our analysis no marked variations were noted during short episodes of the alert or drowsy states. In quiet sleep, however, and especially during those episodes which terminated in active sleep, a gradual reduction in the % occurrence of the reflex response was observed. As clearly indicated in both Figures 1 and 2, there was almost complete suppression of the masseteric reflex during active sleep. With suprathreshold stimuli during active sleep, we frequently observed an additional phasic reduction in the reflex amplitude concomitant with bursts of rapid eye movements.

In summary, as an initial step in the analysis of the functional significance of central inhibitory systems during behavioral states, we investigated the spontaneous fluctuations of a brain-stem reflex during sleep and wakefulness. We observed a gradual, but statistically significant, decrease in the amplitude of the reflex response as the animal passed from the alert state through the drowsy and quiet sleep states, and into active sleep. During active sleep the reflex response was almost completely abolished. The studies on spinal reflexes similarly report reflex depression during active sleep and during bursts of rapid eye movements, but note only slight or no change during quiet sleep when compared with wakefulness[1-6]. There are a number of possible explanations for this discrepancy: (1) the techniques of reflex excitation and recording have differed slightly, (2) in the present study a liminally-induced test reflex was used, (3) we analyzed and treated statistically a large population of reflex amplitudes, and (4) previous studies on spinal reflexes did not differentiate between the drowsy and quiet sleep states[13,14].

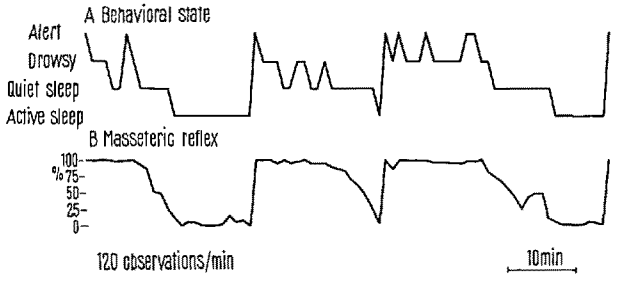

Fig. 2. This figure shows the % occurrence of the masseteric reflex (B) during consecutive sleep cycles (A). In this analysis the reflex responses were counted as either present or absent. Note the gradual decrease in the % response during the periods of quiet sleep preceding active sleep.

Zusammenfassung. In frei beweglichen Katzen wurden Veränderungen des Reflexes zum Musculus massetericus während des Schlafes und im Wachzustand untersucht. Eine graduelle Abnahme der Amplitude des Reflexes wurde beobachtet, wenn die Tiere vom Wachzustand über eine «schläfrige Phase» (drowsy state) in die sogenannte «ruhige Schlafphase» (quiet sleep) gelangten. Während des paradoxalen Schlafes (active sleep) waren die Amplituden der Reflexpotentiale hochgradig herabgesetzt.

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31 July 1967.

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[14] This work has received bibliographic aid from the UCLA Brain Information Service which is a part of the National Information Network of the NINDB, and supported under Contract No. PH-43-66-59.

The Effects of Primary Afferent Depolarization on Excitability Fluctuations of Ia Terminals within the Motor Nucleus

We have recently reported[1] that stimulation of group I muscle and of low-threshold cutaneous afferents can reduce the fluctuations of successive monosynaptic reflexes elicited by constant afferent volleys. The time course of this effect and its sensitivity to picrotoxin suggested that the paths leading to primary afferent depolarization (PAD) were involved in variability reduction. It was then proposed that variability of the monosynaptic reflex resulted mainly from membrane potential fluctuations of the Ia afferent terminals. This would affect the amount of transmitter substance released by each presynaptic knob[2] and/or the number of afferent terminals invaded by the presynaptic impulse[3]. To test this possibility further we studied the effects of conditioning afferent volleys on the excitability fluctuations of Ia afferent terminals within the motoneuronal nucleus.

Methods. The experiments were performed in 10 cats made spinal (at the first cervical level) under ether anaesthesia and with head circulation occluded. All animals were immobilized with gallamine triethiodide (Flaxedil) and maintained on artificial respiration. The lumbosacral spinal cord was exposed and the right S1 and L7 ventral roots sectioned. The lateral (GL) and medial (GM) gastrocnemius, as well as the plantaris flexor digitorum and hallucis longus (PL-FDHL) and common peroneal (P) nerves on the right side were sectioned and their central ends prepared either for stimulation or recording. Neural responses as well as stimulating current pulses were electronically integrated and their mean areas ($\bar{A}$) and corresponding variances (σ^2) continuously calculated with an analogue computer[1].

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Results. A constant stimulating pulse (0.01–0.1 msec, 1–15 V) was applied through a tungsten microelectrode placed within the GL or GM motor nucleus[4]. This produced 2 distinct responses in the S1 and L7 ventral roots. The early response (labelled MD in Figure 1A) arises from direct activation of the motoneurones or their axons and the delayed response from their monosynaptic activation[5]. The Ia fibres terminating on GL and GM motoneurones are also stimulated and their antidromic responses can be recorded from the peripheral nerves[6] (GL in Figure 1A).

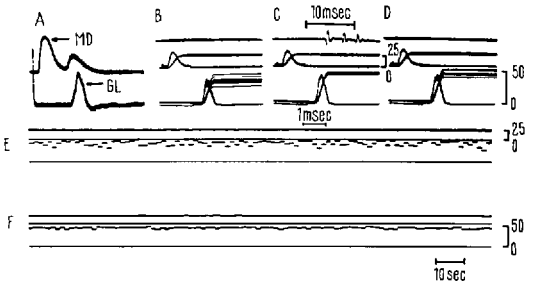

Fig. 1. (A) S1 ventral root (upper traces) and GL antidromic responses (lower traces) produced by single shock stimulation (0.1 msec, 10 V) of the GL motor nucleus. (B), (C) and (D) show, from above downwards, the conditioning afferent volley recorded from the dorsal root entry zone, MD responses and their corresponding areas and GL responses with their areas. Rows (E) and (F) show, from above downwards, successive areas of MD responses and their baseline, areas of GL responses and corresponding baseline. Test stimulus strength was of 4 V in (B), (C) and (F) and of 4.2 V in (D) and (E). In (C) and (F) 3 shocks 1.5 T to PL-FDHL preceded the test stimulus by 35 msec. The GL fibres activated in (D) had a threshold ranging between 1.0 and 1.12 T. Maximal activation of group I fibres was achieved with stimuli 2.1 T. For a series of 355 successive responses, partly illustrated in row (E), the mean and standard deviation of the MD and GL responses were 24.25 $\pm$ 0.89 and 49.10 $\pm$ 5.47 respectively. Similarly, for a run of 444 conditioned responses (row F) the MD and GL responses gave 21.04 $\pm$ 0.89 and 46.48 $\pm$ 2.40. Temperature of exposed cord, 37 °C.

Successive MD responses evoked by pulses of constant strength were remarkably constant while the antidromic GL responses showed considerable fluctuations (Figures 1B, D and E). The rapid fluctuations of antidromic responses of Ia fibres do not seem to result from variations in the stimulating pulses. The stimulating current was fairly constant ($\pm$ 3–5% change in 10 min) and its correlation with the antidromic responses was low (0.009 up to 0.152).

The behaviour of the MD and GL responses elicited by graded stimulation resembles that of the monosynaptic reflex[1]. As the mean responses increased, the variance of the MD (Figure 2A, open triangles) and of the antidromic GL responses (Figure 2B, open circles) increased up to a maximum and then declined. The variation coefficient (standard deviation/mean response) of the MD responses was smaller than that of the GL responses throughout the whole range explored (Figure 2D).

Stimulation of group I afferents of PL-FDHL increased the GL antidromic responses (Figures 1C and 2C, circles). It is generally accepted that this effect results from PAD[6]. The excitability increase was 114.2%, measured as the ratio of non-conditioned (Figure 1D) to conditioned test stimulus strengths (Figure 1C) necessary to produce the same antidromic response. Although the mean responses were the same in both cases (within 9%), fluctuations of the GL responses were strikingly reduced by the conditioning afferent volley (Figures 1C and F). The variation coefficient was reduced from 0.111 to 0.051. This effect

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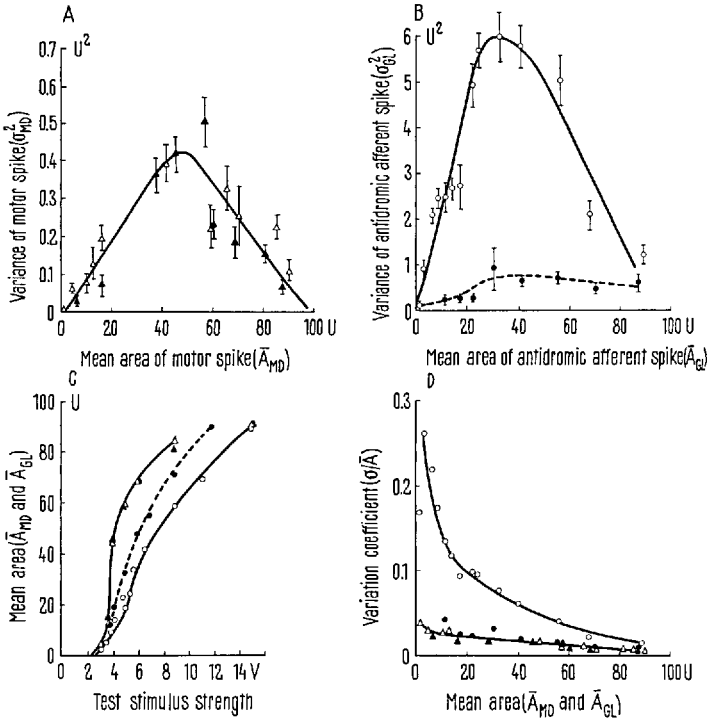

Fig. 2. (A) and (B) Variance vs. mean area curves for the MD (triangles) and GL responses (circles) produced by stimuli of increasing strengths applied to the GL motor nucleus. (C) Mean area vs. test stimulus strength and (D) variation coefficient vs. mean area curves constructed from the data shown in (A) and (B). Each point is the average of the raw data obtained by point-plotting the computed values every 7.5 sec during 5 min at a fixed stimulus strength. Open symbols, control measurements. Closed symbols, during application of 3 shocks at 300/sec 1.5 T to PL/FDHL, 35 msec before test stimulus. Control and conditioned points were obtained alternately. The vertical bars in (A) and (B) give 2 standard deviations of the raw data. Temperature of the exposed cord, 36.5–37 °C. U, arbitrary area units for the mean; U^2, corresponding units for variance.

was observed throughout most of the explored range of responses (Figures 2B and D). The time course of variability reduction resembled that of the excitability increase of Ia afferent terminals[6]: onset 4–10 msec, peak 30–40 msec and gradual decay up to 100 msec. Variability reduction was also induced by stimulation of the P nerve (with stimulus strengths 1.2–2 T, i.e. times threshold of the most excitable fibres in the nerve).

With stimulus strengths to PL-FDHL smaller than 1.5 T there was facilitation of the MD responses. Onset was about 5 msec, peak at 10 msec and gradual decay up to 30–40 msec after the conditioning stimulus. With greater conditioning stimulus strengths early depression (between 2–5 msec) and facilitation lasting up to 100 msec were frequently observed. In the conditions under which Figures 1 and 2 were obtained, the conditioning volley had a negligible effect on the mean MD responses (Figures 1B, C and 2C). The variance (Figure 2A) and variation coefficient curves (Figure 2D) also remained unaffected.

KATZ and THESLEFF[7] have shown that the input resistance of muscle fibres is inversely related to their diameter. This situation seems to hold also for afferent fibres and motoneurones[8]. Since presynaptic terminals are presumably smaller than motoneurones, one would expect fluctuations of membrane potential to be greater in the former. However, fluctuations of individual elements are only reflected in population responses if their correlation is high[9]. It is therefore suggested that the interneurones ending on the Ia afferent terminals[6] are highly correlated in their spontaneous activities and are, presumably, the main source of variability of the monosynaptic reflex. Reduction of excitability fluctuations of Ia terminals by afferent volleys could be explained: (a) During the PAD produced by the conditioning afferent volleys the conductance of the Ia afferent terminals is probably increased[6]. This would reduce their input resistance and also the membrane potential fluctuations. (b) The long lasting depression that follows activation of the paths leading to PAD[6] would temporarily exclude them as variability sources. Experiments are now in progress to evaluate the 2 possibilities.

Résumé. Sur la préparation de chat spinal aigue, les réponses antidromiques des fibres afférentes Ia dans un noyeau de motoneurones montrent des fluctuations considérables réduites par des volées afférentes qui provoquent une dépolarisation des afférences primaires. Les réponses provoquées par activation directe des motoneurones sont très stables, et leurs fluctuations non affectées par la stimulation afférente. Ces faits suggèrent que la variabilité du réflexe monosynaptique est principalement due aux fluctuations du potentiel de membrane des parties terminales des fibres afférentes Ia.

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Decreased Thyroid Radioiodine Uptake after Diazoxide in Rats

Diazoxide (7-chloro-3-methyl-1, 2, 4-benzothiadiazine, 1, 1-dioxide) is a compound chemically related to thiazide diuretics. The change in the chemical structure, mainly the absence of the free sulfamyl group, results in considerably different biological effects: a high and rapid hypotensive action with a decrease of total peripheral vascular resistance, a reversal of the saluretic action of chlorothiazide in Na-retention and an increased diabetogenic effect. In this paper, the inhibitory influence of diazoxide on the uptake of radioiodine ^{131}I in the thyroid gland of rats is reported.

Method. The radioiodine ^{131}I (in the form of KI) in a dose of 0.2 μCi in 1 ml of physiological saline is injected i.p. to male Wistar rats weighing 220–260 g and fed standard laboratory diet (Larsen). Four h after application of the radioisotope, the animals are sacrificed by coal-gas. Immediately after killing, the thyroid glands are taken out, weighed on the torsion balance, put in the test tubes with 10% NaOH solution and homogenized by boiling. The radioactivity of these preparations is measured in the well-type scintillation counter Tesla. One tenth of the dose of radioiodine is measured likewise as a standard. The results are expressed in % of the dose of the isotope in 1 mg of the thyroid. Five mg of diazoxide (Hyperstat Schering) in the original solution are injected in the tail vein of the rat just prior to the application of the radioiodine. (We are grateful to Schering Comp., Bloomfield, New Jersey for kindly supplying the diazoxide.)

Results. The results are presented in the Table. In the control group of rats the average uptake of radioiodine is 0.65% of the dose in 1 mg of tissue, in 14 rats after the application of diazoxide only 0.49%. The variance of both groups of the values is not statistically different as evaluated by the F test ($p > 0.05$). The comparison of the mean values of both groups by the t test shows a statistically significant difference ($p < 0.01$). In a control experiment, we were unable to prove any decrease of the radioiodine uptake after i.v. injection of 1 ml of physiological

The effect of diazoxide on thyroid radioiodine uptake in rats

Group	No. of rats	^{131}I-uptake %d/mg (mean $\pm$ S.E.M.)
Controls	11	0.65 ± 0.04
Diazoxide	14	0.49 ± 0.04 ($p < 0.01$)