The contingent negative variation and the excitability of the spinal monosynaptic reflex

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SUMMARY A method is described which permits simultaneous measurement of changes in slow electroencephalographic potentials (Contingent Negative Variation—CNV) and the excitability of the spinal monosynaptic arc (H reflex) during the foreperiod of a simple reaction time experiment. Data from 11 normal subjects show that during the development of the CNV there is an augmentation of the amplitude of the H-reflex. It is suggested that the two phenomena are controlled by a common subcortical structure.

The execution of a planned motor action or decision for which the subject has been forewarned is preceded by a slow negative electroencephalographic (EEG) change known as the Contingent Negative Variation (CNV), (Walter et al., 1964). It is recorded from practically all normal persons when a stimulus ($S_1$) indicates to the subject that another stimulus ($S_2$) will appear after a fixed interval of one or a few seconds at which time a certain action has to be done, such as pressing a switch. The execution of this action is usually accompanied by the sharp termination of the CNV. The CNV waveform, its take-off, amplitude, and cut-off vary with the psychological state of the subject and are different in patients with psychiatric and neurological disorders (McCallum and Knott, 1973). Changes of autonomic variables occur during the foreperiod when the CNV develops (Papakostopoulos, 1973) and there is an increase of muscle tone even in limbs not involved in the motor action (Freeman, 1934; Davis, 1940). Tension in muscles not directly involved in a particular motor action is also a well-known concomitant of the Jendrassik manoeuvre but different writers have contradictory opinions about its nature (Buller and Dornhorst, 1957; Gassel and Diamantopoulos, 1964). This phenomenon has been investigated using the H reflex (Hoffmann, 1918; Magladery and McDougal, 1950; Magladery et al., 1952), which can be considered as the equivalent in man of monosynaptic reflexes in experimental animals (Lloyd, 1943). The H-reflex has also been used as a measure of monosynaptic reflex excitability during changes of attention and mental activity (Paillard, 1955; Bathien, 1971; Coquy and Coulmance, 1971) and before and during voluntary movement (Requin, 1969; Gottlieb et al., 1970; Pierrot-Deseilligny et al., 1971). EEG data have not been included in these studies and the anticipatory behaviour of the subjects to various stimuli has not always been objectively controlled, although instructions to be avoided have been given by some authors (Gottlieb et al., 1970).

The relationship between the CNV and this spinal monosynaptic reflex has been determined by evoking the H-reflex at various times during the interstimulus and intertrial intervals.

METHOD

Eleven subjects, paid volunteers, 20–25 years of age—five males and six females—without signs or symptoms of neuromuscular or any other disorder, were studied. They were not informed of the purpose of the investigation. The subjects lay in a prone position with their feet over the end of a bed. The knees were kept flexed at about 20° by a pillow placed under the ankles. The subjects were told to relax and were helped by adjusting the position of the upper part of the bed. They were asked to place their arms at the level of their head which was turned slightly to the right.

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FIG. 1. On the left typical soleus EMG responses from two subjects (P.O. and P.S.) to progressively increasing stimulation of the posterior tibial nerve. The H response increased up to a certain level and then declined with the appearance of the M response. The curves on the right give the course of the two responses for the two subjects. The arrow indicates the stimulus intensity which was used throughout the experiment. P.O.: — — — M, — — — H. P.S.: — — — M, — — — H. Horizontal scale in mV.

THE H REFLEX Bipolar surface stimulating electrodes (DISA 13K62), each 6 mm diameter separated by 25 mm between centres, were used. The stimuli, applied at the right posterior tibial nerve in the popliteal fossa, had a duration of 0.5 msec and were generated by a DISA Ministim stimulator triggered by a PDP 12 computer. The stimulating electrode was held in position by a manipulator fixed to the bed. Silver/silver chloride recording electrodes 4 cm apart were placed on the soleus muscle in a midline plane and were fixed in position by collodion. Before their application the skin was treated with sandpaper and alcohol. The impedance measured at 40 Hz was less than 4 KΩ. A strip of lead placed between stimulating and recording electrodes acted as an earth and was connected to the earth of the EEG
FIG. 2. Schematic timing diagram of stimuli presentation. The CNV is elicited during the 2 sec interval between two stimuli S₁ and S₂. Sets of these stimuli (A–J) are presented with variable intertrial interval and constitute a sequence the duration of which is 4 min 46.5 sec. Long vertical lines represent the occurrence of the stimulus for the H response. During trials A, C, F, H, and I this occurs at various times during the S₁–S₂ interval (during CNV); in B, D, E, F, and G the stimulus for the H response occurs during the intertrial interval (out of CNV). The completion of a cycle is automatically followed by another identical one. The trigger pulse for starting EEG data storage precedes S₁ by 1 sec.

FIG. 3. Superimposed CNVs with the H response eliciting stimulus appearing and not appearing during the S₁–S₂ interval (N = 25). Vertical lines represent the average amplitude of the H response (N = 5) at the indicated point in time. These peak to peak values were obtained as shown in the H response (bottom right).
FIG. 4. The H responses from three subjects (L.M., P.S., and P.O.) at fixed times during CNV (Hd) out of CNV (Ho) and when no CNV eliciting task is performed (Hn). For Hd and Ho five traces are superimposed each being the average of five responses. For Hn the average wave form for 10 trials and the standard deviation are presented. The corresponding M responses are shown in 1, 2, and 3. Note the stability of the M response when present (in subjects P.S. and P.O. 1, 2, and 3). H responses during CNV (Hd) are larger than during the intertrial interval (Ho). In cases L.M. and P.O., Hn is smaller than Ho and Hd; in case P.S. it assumes a value in between.

recording system and the stimulator. The artefact from the electrical stimulus picked up by the scalp electrodes was very small. The pre-amplifiers had a bandwidth of 10 KHz (3 db down). The PDP 12 computer sampled and stored the output of the pre-amplifier at a rate of 5,000 data points/sec for 50 msec after the stimulus.

The H-reflex was displayed on a storage oscilloscope (Tektronix) close to the subject and on the computer oscilloscope together with the corresponding CNV. By superimposing the responses on the storage oscilloscope, the stability of successive H-reflexes and the directly evoked muscle responses (M) when present was monitored on-line. The choice of intensity of stimulation was determined by the need to elicit a stable H response while remaining less than
maximum where saturation effects might mask changes of H response. It was determined for each subject at the beginning of the recording. Figure 1 shows the recruiting curve for H and M responses for two subjects. The stimulus voltages shown by the arrow (51 V and 75 V for subjects P.O. and P.S. respectively) were used throughout their recordings. At this stimulus level, the M response is absent or small (less than 2% of the maximum response). When present, the M response acted as an indicator of electrode stability during the experiment. There was no difference in the results from subjects showing a small M response and of others who did not.

Generalized muscular tension was monitored by surface EMG electrodes affixed to the triceps surae and the tibialis anterior muscles of the stimulated leg and the flexor muscles of the relevant hand.

CONTINGENT NEGATIVE VARIATION Auditory stimuli, a click (S₁) and a tone (S₂), 2 sec apart, were used to elicit the CNV. The tone had a maximum duration of 1 sec but could be interrupted at any time by pressing a switch with the left thumb. The subjects were told that being forewarned by the click they had to press as quickly as possible and stop the tone (S₂). The duration of the tone was used as a measure of the subject's reaction time. The CNV was recorded from a vertex electrode to two electrodes on the mastoid processes joined together. Eye movement artefacts were eliminated by asking the subjects to keep their eyes open and fixed on a distal point. In addition, an electrode on the forehead was used to compensate for eye movements and blinks (McCallum and Walter, 1968). Both CNV and eye movements were recorded with time constants of 5 sec and an upper filter of 70 Hz from two channels of a 16-channel Elema Schonander electroencephalograph fitted with Universal Amplifiers. The output of the amplifiers was written on paper and sampled by the computer at 64 points/sec for 4 sec periods of time. To establish an accurate baseline the sampling always started 1 sec before S₁. In addition, the average value of the DC level immediately before the 4 sec epoch was automatically subtracted from all data values collected within the epoch.

TIMING The occurrence of the stimuli for eliciting the CNV and the H response was controlled by the PDP 12 computer. The time sequence of the stimuli is shown in Fig. 2. By interposing the stimuli for evocation of the H response at non-regular intervals among the non-regular CNV trials, the subject could not predict either the time of S₁ or if an H response would or would not be evoked during the period between S₁ and S₂. Five or more sequences, each lasting nearly 5 min, were presented to each subject.

OTHER VARIABLES In addition to the CNV, the evoked potentials to the electrical stimuli were recorded from a midline electrode 2 cm posterior to the vertex and from another electrode 3 cm to its left. These electrodes were also referred to common mastoid electrodes. Respiration, electrocardiogram, heart rate, and galvanic skin resistance of the palm of the right hand were also recorded. In addition, bilateral occipital intrinsic EEG activity was monitored. A further channel was used to monitor the stimuli.

PROCEDURE The subjects were given instructions about the significance of the two stimuli. They were told that the experiment was to measure their reaction time to the second stimulus (S₂) but premature reactions would be considered as mistakes and would require the repetition of the experiment.

After the experiment, all subjects were asked if they had discovered any way to predict the time of the next trial and if they had any difficulty throughout the experiment. The answers were negative from all subjects.

The CNV data and the corresponding H response were stored as individual trials during the experiment and processed later.

RESULTS

The CNV and the H reflex were obtained from all subjects tested.

The wave form of the H response was triphasic with the middle wave, a negative deflection, dominating the whole complex. The latency and amplitude of the H response and the maximum M response varied slightly from subject to subject as did the maximum H/M ratio. The M response had a mean latency of 13 ± 1·6 msec and maximum amplitude of 13·6 ± 2·9 mV. The mean latency of the H response was 36·6 ± 2·3 msec, maximum H amplitude (first positive to the negative peak) 10·2 ± 1·3 mV; the H/M ratio varied across subjects between 0·66 and 0·79, except one subject who had equal values for H and M. The time interval between positive peaks of the maximum H response was about 0·75 msec greater than that of the M response.

The amplitudes of H responses elicited during the development of the CNV and after its termination in one subject are shown as vertical bars in Fig. 3. The mean amplitudes of the H responses elicited at 0·5, 1·5, 1·8, and 1·9 seconds after the warning click (SI)—that is, during the development of the CNV—are consistently
FIG. 5. The upper figure shows the percentage change of the average amplitude of the H response at various times during the interstimulus and intertrial periods. The lower figures show the amplitude of the CNV during the interstimulus period for the same eight subjects. In all subjects the amplitude of the H response was greater during the interstimulus interval when the CNV developed. Note that this increase already occurred 500 msec after the first stimulus, even though the amplitude of the CNV was still rising. Time scale shown is non-linear.

larger than the amplitudes of the H responses elicited after the second stimulus and termination of the CNV. The two superimposed CNV traces in Fig. 3 are each averages of 25 trials and represent the CNVs when the H eliciting stimulus is interposed between the click and tone (thick line) and when the H eliciting stimulus follows the termination of the CNV (thin line). The similarity of these two traces shows that the occurrence of the electrical stimulus has little effect on the development of the average CNV.

In Fig. 4, H responses recorded from three subjects are shown when elicited during the interval between click and tone (Hd) and after
the tone (Ho). In these subjects (and all others) the mean amplitude of the H response elicited during the occurrence of the CNV was greater than that elicited after its termination and in most subjects both were larger than the H response elicited at the end of the experiment when the subjects were told that the click and tones were to be discontinued. The shape of the CNV varied slightly from subject to subject.

A two way analysis of variance for all subjects showed that the H response during the interval between click and tone was significantly greater than the H response after CNV (P < 0.001). The mean increase over all subjects was $12\%$.

All the five H responses recorded during the interval between stimuli were augmented in amplitude. The earliest H response (occurring 0.5 sec after the first stimulus) was as large as any of the others during the interstimulus interval; at this time the CNV was still rising from the pre-stimulus baseline. The relationship of the amplitudes of CNV and H response for eight subjects is shown in Fig. 5. The termination of the CNV by the motor action was correlated with a decrease of amplitude of H reflex which continued for up to 20 sec after the second stimulus.

Of the various other physiological variables monitored only the heart rate showed a consistent decrease during the interval between the click and tone. There was no discernible CNV or H response change related to the respiratory cycle. Changes of the palmar skin resistance occurred about 2 sec after the warning stimulus but there was considerable variation of amplitude during the experiment and from subject to subject. For all subjects there was little or no myographic activity recorded from electrodes on the triceps surae and tibialis anterior muscles during the experiment. The arm muscles showed some EMG activity during the foreperiod before pressing the switch but the amount was an individual characteristic and generally reduced during the course of the experiment.

**DISCUSSION**

All the subjects tested showed similar effects—that is, an increase of the amplitude of the H-reflex during the foreperiod when the CNV develops. Whether these two phenomena are causally related or whether they are both mediated through some common mechanism is not directly determined by our studies. However, the rate of change of the two measures during the inter-stimulus interval is not the same—the H response increasing to maximum more rapidly than the CNV—suggesting that the hypothesis of a common mediating mechanism is more likely.

The observed increases of the H-reflex could be due to a change of the excitability of the relevant motor neurone pool or in presynaptic action or a combination of both. An increase of the excitability of the motor neurones could be by direct action on the alpha motoneurones or by changes of the interneurones by activation of the $\gamma$ system (Granit et al., 1966). These two processes could also act together via the $\alpha-\gamma$ linkage. On the other hand, the presynaptic inhibition of the primary afferent neurones in the spinal cord (Eccles, 1964) could be reduced and the resulting facilitation give rise to the increase of the H reflex. Matthews (1969) has shown excitatory action upon extensor motoneurones by secondary afferent neurones and stimulation of such fibres by the H reflex eliciting stimulus cannot be excluded completely. Involvement of the brainstem in these processes is well established.

The changes of the electrical activity of the brain as shown by the development of the CNV can also be attributed to the influence of the brain stem. Arduini et al. (1957) showed that stimulation of the brain-stem reticular formation produced increases of negativity of the cortical steady potentials (like the CNV). In conditioning procedures in animals the learned association of stimuli is accompanied by the appearance of slow negative shifts of cortical potential (Rowland, 1967) and an increase of brain-stem cellular activity (Olds et al., 1969). Recently, potentials similar to the CNV were recorded in subcortical structures in monkey (Rebert, 1973) and man (McCallum et al., 1973). In the measurements in man the onset of the CNV was earlier in the midbrain structures than in the cortex, suggesting a subcortical origin. Our findings of the changes of heart rate during the interstimulus interval also indicate brain-stem involvement.

The changes of spinal cord activity as shown by the H reflex, the cortical activity as shown by the CNV, and the autonomic activity as shown by the changes of heart rate suggest that within the brain-stem is a common mechanism integra-
ting the whole body reaction to the stimulus and response situation. This, in turn, is dependent on the cortical activity of perception and interpretation of the stimuli according to the instructions given to the subject.

This view is supported by the work of Gazzaniga and Hillyard (1973) who studied the CNV of patients who had undergone surgical transection of the forebrain commissure. They showed that CNVs occurred simultaneously in both hemispheres, even though the visual warning stimulus (S1) was presented to one hemisphere only.

In this integrative process some systems will be excited, some inhibited, and others unchanged. It ought to be possible to use a cluster of the phenomena associated with such changes as a measure of the semantic and integrative processes of the various parts of the brain of intact man.

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