

HIPPOCAMPAL EEG AND BEHAVIOUR IN DOG. II. HIPPOCAMPAL EEG CORRELATES WITH ELEMENTARY MOTOR ACTS¹

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In previous studies (Lopes da Silva and Kamp 1969; Kamp et al. 1971; Arnolds et al. 1979), we have shown that there is a significant rhythmicity in the theta band of the dog's hippocampal EEG in the majority of behavioural states investigated (e.g. lying down, standing, eating, walking) and that transitions in motor behaviour are correlated with shifts in amplitude, frequency and rhythmicity of the theta component in the hippocampal EEG. These results in combination with data from the literature concerning rats, rabbits and cats (Vanderwolf et al. 1973; Kemp and Kaada 1975) suggest that the spectral properties of the dog's hippocampal EEG reflect motor activity and sensory inputs in a non-specific but predictable way, as was argued in our previous work (Arnolds 1978; Arnolds et al. 1979). In order to substantiate this notion further we designed an experiment to determine whether the *intensity* of motor behaviour is reflected in the hippocampal EEG (part I of this study).

The motor behavioural correlates of the hippocampal EEG are described in many cases in the literature at the level of goal-directed behaviour (walking towards or away from a goal) or in the context of particular moti-

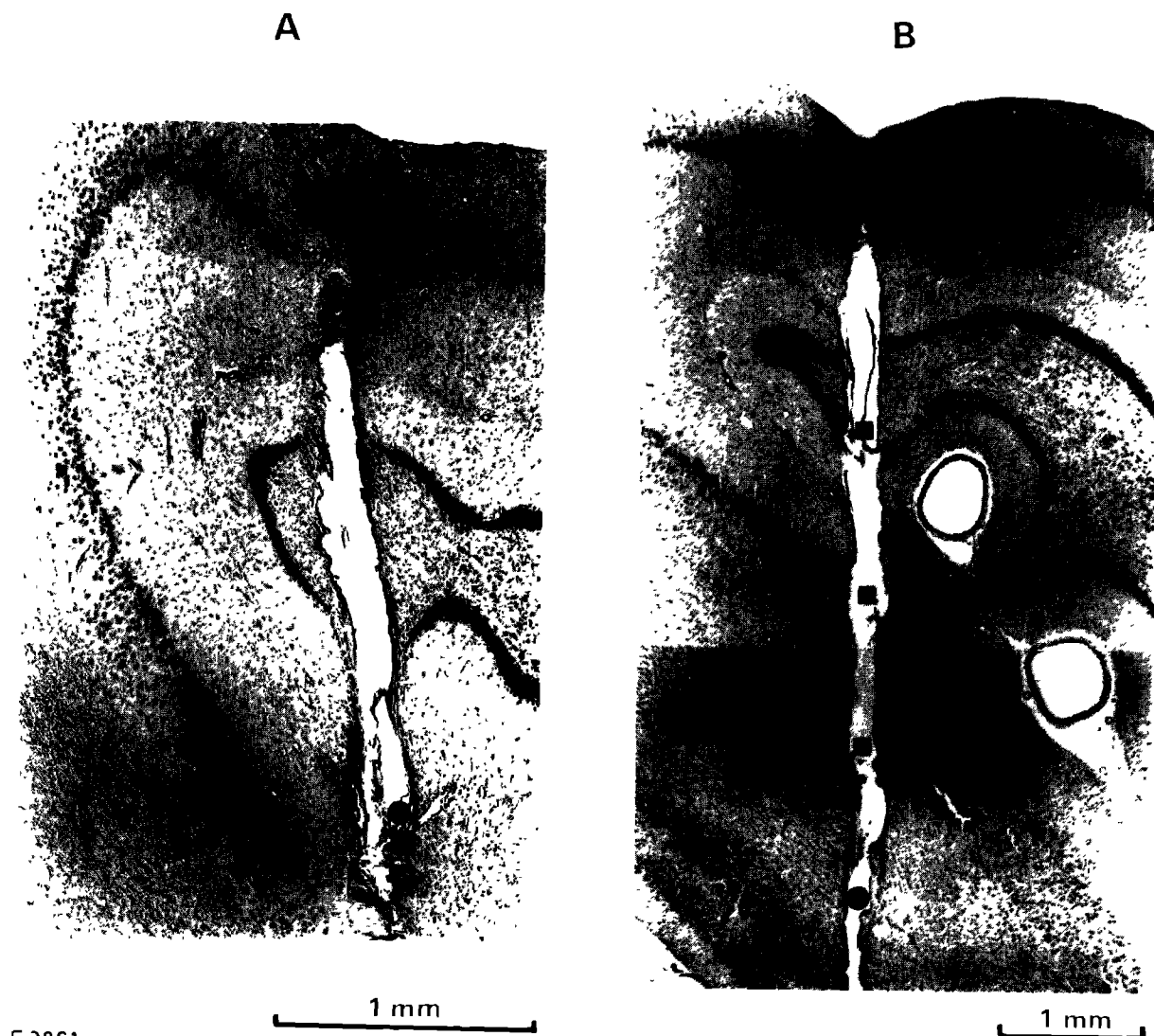
vational states (appetitive, aversive). Some investigators prefer to describe behaviour in a more direct way in terms of walking, eating, swimming etc. These behaviours can again be described in terms of sequences of more elementary behavioural acts such as steps, jaw movements etc.

This led us to investigate whether such elementary motor acts would also be correlated with the properties of the hippocampal EEG. Thus in parts II and III of this study we describe the hippocampal EEG correlates of steps, sighs, and of a motor reflex.

This paper is based on an intensive longitudinal study of 2 dogs. This approach allows a precise comparison of the EEG and behaviour recorded in different but exactly reproducible situations in the same animal. A preliminary report on these investigations has been published elsewhere (Arnolds et al. 1977). In both dogs the electrodes were placed in the caudal 'knee' of the hippocampus near the CA1-subiculum border. The positions from which we recorded are indicated in Fig. 1 by round black dots. In Fig. 1B the squares indicate the positions of some other electrode tips in the same bundle. The uppermost yielded large amplitude theta rhythm with important high frequency components. The tip located in the pyramidal cell layer yielded a rather irregular EEG of smaller amplitude while the electrodes below the pyramidal cells all gave a highly isomorphic clear rhythmic slow activity (RSA). Hence one of these was used for analysis.

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Fig 1 The black dot in part A of this figure shows the position of the electrode in the dorsal hippocampus, signals from which are analysed in Fig 7 The dot in part B indicates the electrode position used for Figs 2-6 A and B refer to 2 different dogs

Material and methods

These were the same as described previously (Arnolds and Lopes da Silva 1977; Arnolds et al. 1979). Some special aspects are as follows:

(1) Experimental design

Recording took place under 3 experimental

circumstances. (a) With the dog strapped into a classical treadmill in which it could walk around over ground in circles of 2.60 m diameter. This allowed the displacement and hence the speed of walking to be measured. The resolving power of the displacement transducer on the axis of the treadmill was 3.4° , equivalent to a displacement of 8 cm. The dog

was trained to walk any number of rounds (up to 10) at a practically constant speed (approx. 1.5 m/sec), for a reward which was given by means of a noisy food dispenser. The dog's behaviour while in this treadmill was monitored by a closed circuit T.V. system. (b) With the dog placed on a conveyor belt which could be run at various speeds. At each stepping speed investigated, the animal was made to walk for at least 2 min in a stable fashion. After each such period, which was normally followed by a change in belt speed, the dog obtained a food reward which it ate while stepping. (c) To investigate the effect of whole-body linear acceleration on the hippocampal EEG and behaviour the dog was placed in a hammock suspended under a cart so that the paws of the animal could not touch the floor. In this way the dog was driven by the experimenter over a straight runway of approx 14 m from a standstill position, with a quick acceleration to a constant speed. This procedure was repeated a number of times. Care was taken that the animal hung quietly in the hammock at the start of each trial. The actual rate of displacement was measured by means of a photocell that scanned a black and white stripe pattern on the floor over which the cart was pushed. The interstripe distance was 10 cm. After each run the dog obtained some food.

(2) Data acquisition and computer processing

The procedures were the same as described previously (Arnolds and Lopes da Silva 1977; Arnolds et al. 1979). In addition, in order to determine linear relationships between different signals the Z transformed coherence function was used, as described by Lopes da Silva et al. (1973).

In all analyses 'EMG' refers to the rectified and smoothed EMG of a forepaw muscle (triceps), ACC to the integrated output of the movement transducer on the dog's back. I_{PF} is the index of the peak frequency in the theta band, I_{AM} indicates the amplitude in the theta band, I_R is an indicator of the apparent rhythmicity (i.e. bandwidth).

In the graphical presentation of the analysis of spectral or behavioural parameters, the following conventions apply: arrow indicates the trigger event; bar through each data point indicates the standard error of the mean; \bar{N} indicates the number of averaged events; \bar{D} the average duration of a number of periods which have been averaged. Where a curve is thickened there exists a statistically significant difference between that part of the curve and the value indicated by an asterisk, chosen as a reference (sign test). The thicker the line the higher the significance of the statistics ($P < 0.05$ in any case). The greatest thickness indicates $P < 0.001$. The series of histograms at the bottom of such an analysis represents the running spectral analysis of the EEG of a number of 200 msec time bins around the trigger event. The placement of the spectra corresponds to the time scale of the curves. \blacktriangle indicates arbitrary units.

Results

(1) Forced walking at different speeds

Procedure. The dog first walked in the classical treadmill for a few minutes. Then it was made to step on the conveyor belt, which was run at a speed of 1.8 m/sec. After a few minutes of undisturbed stepping the speed was decreased in steps of 0.3 m/sec till a speed of 0.6 m/sec was reached.

Results and discussion. From the recorded signals at each stepping speed a number of independent 2 sec periods were selected (we assumed independency if the periods were separated from each other by at least 2 sec). The average spectra and average values of spectral and movement parameters for each of the stepping speeds were calculated. Results of this analysis are summarized in Fig. 2. There appears to be a clear and statistically significant increase in the amplitude (I_{AM}) of the hippocampal EEG in the theta band with an increase of stepping speed. I_{PF} tended to increase with stepping speed as well, but this trend was less pronounced. No clear relation-

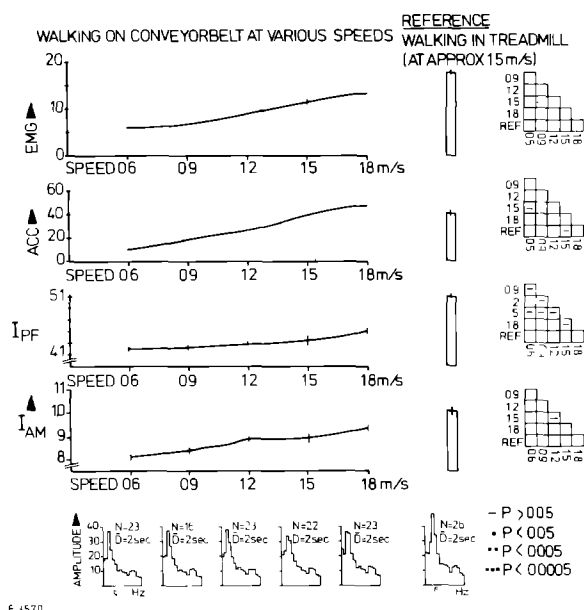


Fig. 2 Average values of EMG, ACC, I_P and I_{AM} as functions of conveyor belt speed. The bars to the right of the curves indicate the values of these same parameters while the dog was walking in the treadmill. For each parameter the values found under the different stepping conditions have been statistically compared. The results are shown in the matrix to the extreme right of each curve (Wilcoxon). Note the positive relationship between conveyor belt speed and the value of both behavioural and spectral parameters. Note also the disproportionately high values found for I_P and I_{AM} during active walking in the treadmill.

ship between I_R (not shown in Fig. 2) and stepping speed emerged. If anything I_R decreased slightly with increasing conveyor belt speed. It is striking that the amplitude of the hippocampal EEG was larger and the frequency higher when the dog walked around over ground in the treadmill than during forced stepping at the same speed, on the conveyor belt.

The results of this experiment support the hypothesis that the amplitude and frequency of the RSA component of the hippocampal EEG increase as the animal exerts itself more. They are in agreement with those of McFarland et al. (1975) who, in the rat, found that

the frequency of occurrence and the amplitude of hippocampal theta waves induced by forced locomotion was proportional to the speed of movement on a motor driven treadmill. Whishaw and Vanderwolf (1973), on the other hand, report no correlation between the speed of forced running in rats and the frequency of hippocampal EEG. Our findings indicate that especially the amplitude in the theta band increases with speed while the upward trend in frequency is weak. It could only be detected thanks to the frequency analysis and averaging which were employed here.

The differences in EEG correlates between active and forced stepping at the same speed is in accordance with the fact that the EMG output of the forepaw was larger while the dog was stepping actively than while walking on the conveyor belt. The difference in values of the spectral parameters correlated with the two stepping modes appears to be excessive, however, compared to the difference between the behavioural measurements (see also Fig. 3). The discrepancy may be due to differences in the motor aspects of the two stepping modes which exist but have not been covered by our limited measurements (Wetzel et al. 1975). There might also be differences in the sensory aspects which characterise the two modes of stepping, influencing the hippocampal EEG.

(2) Linear acceleration

Procedures. At the start of the experiment the dog walked for a few minutes in the treadmill and 2 min periods on the conveyor belt (one at 0.6 m/sec, the other at 1.8 m/sec). The data gathered during these introductory parts of the experiment served as reference values for the measurements made in the last part of the experiment: 20 trials of cart riding.

Results and discussion. In reacting to the acceleration from 0 to approximately 3 m/sec in approximately 1.5 sec at the start of the cart ride, the dog showed a reflex comprising extension of legs and neck. In the hip-

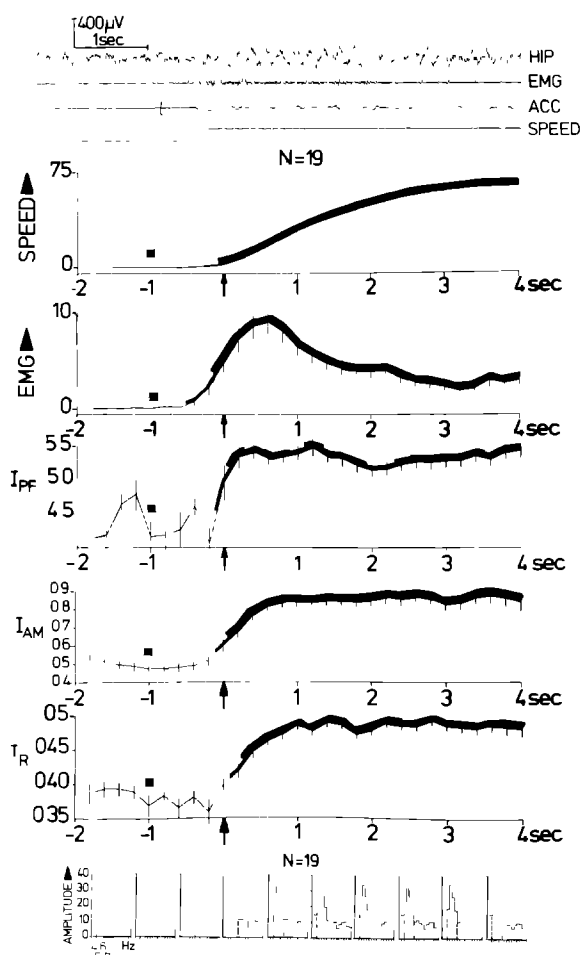


Fig 3 Registration of a typical run in the cart. Speed is proportional to pulse density, displacement between two pulses: 10 cm. The next 5 curves show the integrated output of the displacement transducer (speed), the integrated EMG of a forepaw muscle (EMG) and I_{PF} , I_{AM} and I_R of the hippocampal EEG as a function of time in relation to the start of cart movement

pocampus the displacement and concomitant motor activity were correlated with a significant increase in amplitude and frequency of the RSA component of the hippocampal EEG.

These findings are illustrated in Fig. 3. In another experiment in which the cart was accelerated to a speed of approximately 1.5 m/sec in approximately 2 sec so that no

overt stretch reflex occurred, no significant increases in frequency and amplitude of the RSA component could be found. Passive displacement as such appears therefore not to be sufficient to cause a change in hippocampal EEG activity; a motor response, however slight, may be a necessary prerequisite for the occurrence of such a change. Fig. 4 shows the amount of motor activity involved in this reflex response to be much lower than that during stepping. The frequency and amplitude of the RSA accompanying the linear acceleration, however, are high compared to the stepping case. These results indicate a clear discrepancy between the degree of motor activity, as measured by our behavioural parameters, and the characteristics of the hippocampal EEG expected on the assumption that there would exist a linear relationship between these and the amount of motor activ-

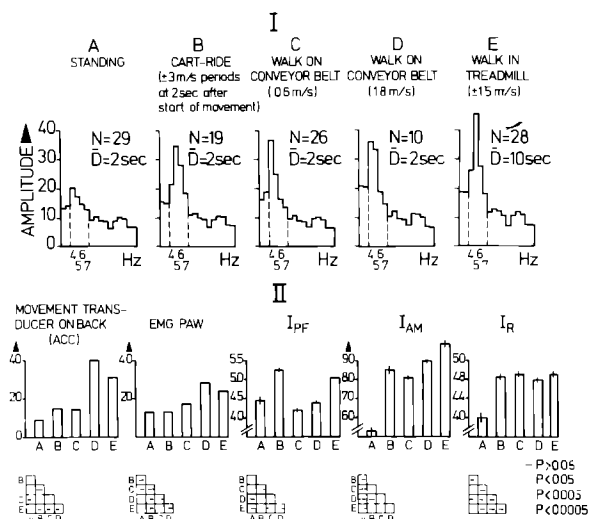


Fig 4 Each histogram (A through E in part I) represents the amplitude spectrum of the hippocampal EEG while the dog is in a certain stationary behavioural state, as indicated. Each bar diagram in part II shows the average value of a behavioural or a spectral parameter during these same independent periods of behaviour A through E. The statistical comparison of the 5 values comprising each bar diagram is presented in the matrix below in a way similar to Fig. 3. Note the apparent discrepancy between the values of movement and spectral parameters under B, D and E

ity. In this case sensory factors, probably from the vestibular system, may play a role in the generation of this hippocampal response.

(3) Breathing and stepping

Procedures. We further explored whether hippocampal EEG correlates of simple motor acts could be shown to exist by analysing the hippocampal EEG in relation to respiratory movements. We analysed this relationship in a dog which was lying down quietly as well as in a dog stepping at a speed of 1.8 m/sec on the conveyor belt.

Moreover, we tried to determine whether a correlation exists between the hippocampal EEG spectral parameters and the fluctuations occurring with every step in the movement transducers and the paw EMG. The dog was investigated while stepping at 0.6 m/sec on the conveyor belt, and while walking around actively at a relatively low speed of 1 m/sec. In the former case we used not only our event-related averaging technique but we also performed coherence analysis between the signal represented by the integrated paw EMG and the time signal formed by I_{PF} , I_{AM} and I_R .

Results and discussion. (a) *Respiratory movements:* the end of each deep expiration (sigh) is clearly indicated in the EEG signal derived from the olfactory bulb (Storm van Leeuwen et al. 1967). An analysis of the spectral properties of the hippocampal EEG in relation to these trigger points showed a significant increase in amplitude and rhythmicity to be correlated with each sigh (Fig. 5). The I_{PF} did not show a significant modulation. Equivalent results were found on analysis of breathing during conveyor belt stepping at 1.8 m/sec, although the modulations in I_{AM} and I_R were superimposed upon higher baseline levels for these parameters. I_{PF} showed no significant modulation in this case either. The fact that respiratory movements are reflected in the hippocampal EEG may contribute to an explanation of the finding that rats swimming under water (and thus not breathing) have an RSA peak frequency which is lower

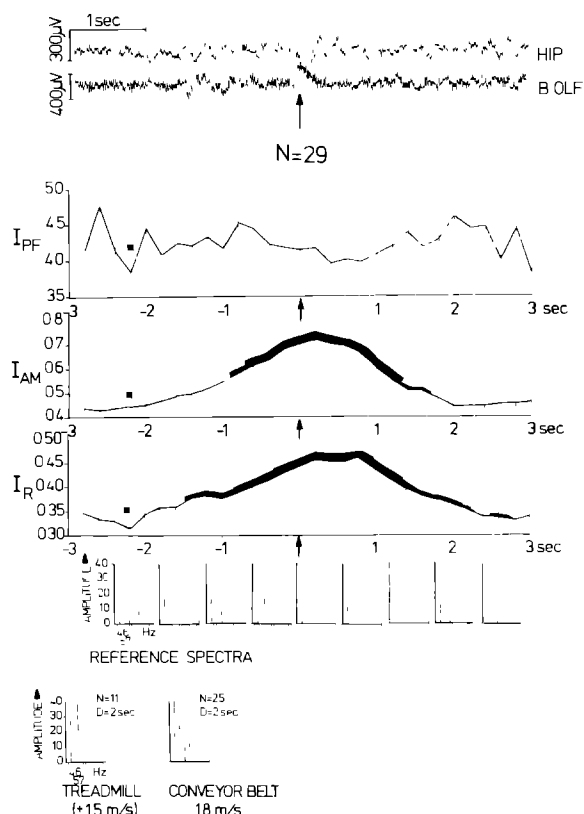


Fig. 5 Upper part: hippocampal EEG and the EEG of the olfactory bulb during a single sigh heaved by a dog which was lying down quietly. Next 3 curves: average values of the I_{PF} , I_{AM} and I_R in relation to the end of the inhalation phase of the sigh (arrow). Spectral analyses of the hippocampal EEG during 2 well-investigated stationary behavioural situations (during the same experimental session) have been provided for comparison.

than that of rats swimming with their nostrils above the water (Whishaw and Schallert 1977). (b) *Stepping:* stepping on the conveyor belt at 0.6 m/sec was correlated with a significant modulation in I_{AM} , I_{PF} and I_R related to each step (Fig. 6). The point was even more rigidly established by the use of a cross-correlation technique. Upon spectral analysis of the rectified and smoothed EMG signal, sampled at 200 msec intervals, there appeared to be a peak at about 0.8 Hz corresponding to a stepping frequency of one step

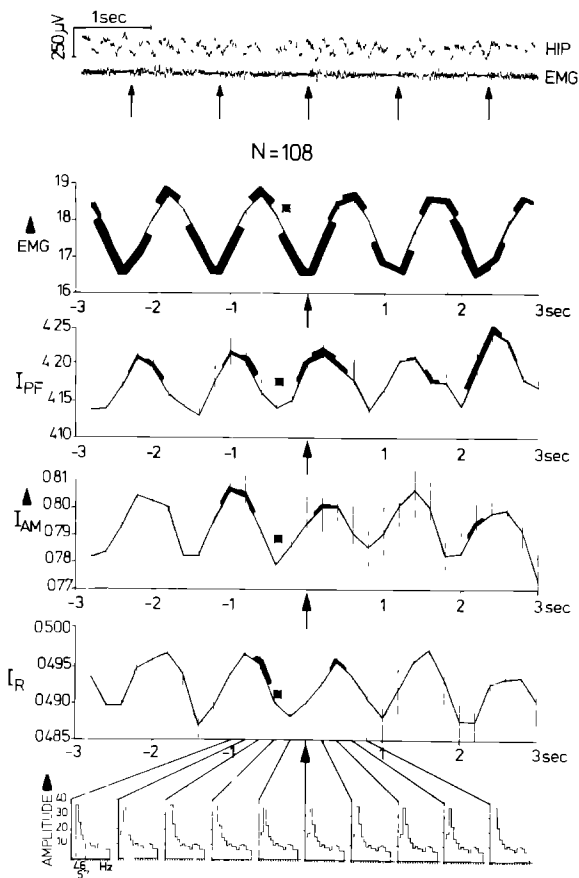


Fig 6. Upper part: hippocampal EEG and EMG of the forepaw triceps recorded during stepping on the conveyor belt at a speed of 0.6 m/sec. Next 4 curves: average values of EMG, I_{PF} , I_{AM} and I_R in relation to the phase in the step cycle indicated by the initiation of the burst in the recorded EMG signal.

in 1.2 sec. In the frequency band of 0.8 Hz a significant coherence between the integrated EMG signal and a rhythmical component at 0.8 Hz present in the time signal formed by I_{PF} , I_{AM} and I_R was established (coherence

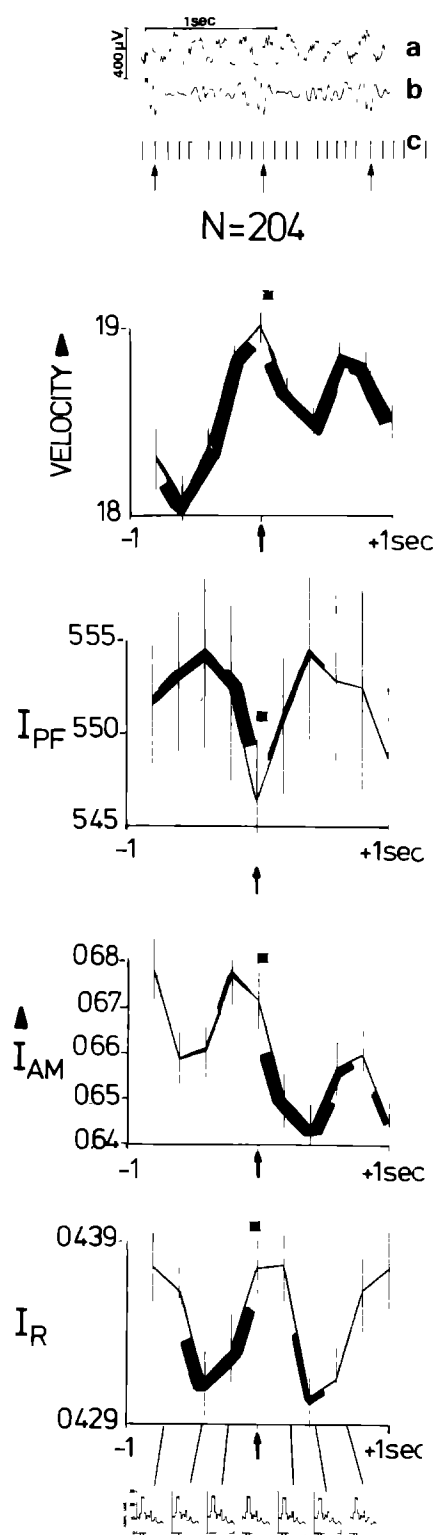


Fig 7. Upper part: for a few typical steps, hippocampal EEG (a), output of the movement transducer on the dog's back (b), and output of the speed transducer on the treadmill (pulse density is proportional to speed). Next 4 curves: average values of the integrated speed transducer output, I_{PF} , I_{AM} and I_R in relation to the peaks in the output of the movement transducer on the dog's back.

>0 , $P < 0.05\%$, established by means of the Z transformed coherence function). At 0.8 Hz the phase lag was 80° ($\pm 2^\circ$, $P < 0.05$) between I_{PF} and 'EMG', 90° ($\pm 20^\circ$, $P < 0.05$) between I_{AM} and 'EMG' and 45° ($\pm 14^\circ$, $P < 0.05$) between I_R and 'EMG'. In all cases the EMG was leading.

The same type of step-correlated modulations in I_{PF} , I_{AM} and I_R was shown to occur while the dog walked around actively over ground. During a few sessions the animal walked less fast than usual, possibly due to the heat in the experimenting room. In this case the output of the accelerometer on the dog's back clearly indicated each separate step the animal made, while the treadmill output showed slight modulations in speed related to each step. In the analysis of the EEG correlates of this stepping, we used the maximal output of the movement transducer on the back as a trigger. The results, as shown in Fig. 7, indicate that step-related modulations in the spectral parameters of the hippocampal EEG can be found in a dog which walks around actively.

General discussion

We found significant modulations in the spectral properties of the hippocampal EEG in relation to 3 elementary motor acts: steps, respiratory movements and a reflex movement induced by linear acceleration. Furthermore, a positive correlation was established between the speed of forced running on a conveyor belt and the spectral properties of the hippocampal EEG. We made it plausible that a motor act, however small, will be related to a change in the spectral properties of the RSA component of the hippocampal RSA, which, in various species including the dog, is confined to the theta band but in other species such as the rat can reach up to 12 c/sec. These results fit the hypothesis that the spectral properties of the hippocampal EEG reflect motor behaviour in a non-specific but predictable way, as stated more elabo-

ately in our previous work (Arnolds 1978; Arnolds et al. 1979). A simple relationship between hippocampal EEG characteristics and the intensity of motor behaviour could only be demonstrated, however, if EEG records were compared which had been obtained under the same experimental circumstances during behaviours of comparable morphology, the only difference being the amount of motor behaviour involved. The relationship does not necessarily hold across experimental situations. This phenomenon may eventually be found to be related to essential differences in the motor components exhibited under these different circumstances, which escaped our crude method of measuring behaviour. It may also reflect the differences in 'commands' required at a neural level for the generation of different behaviours. Possibly this phenomenon may also be due to differences in sensory input during the various experimental situations. Further experiments, specifically designed with these options in mind, may help to decide between these possibilities. Such experiments could at the same time bring us closer to an understanding of the functional importance of the hippocampus.

The notion that elementary sensorimotor events such as reflex movements, stepping and breathing are reflected in the hippocampal EEG is supported by several observations from the literature.

Storm van Leeuwen et al. (1967) reported that in the dog lambda waves recorded from the visual cortex, which are indicative of eye movements, occur phase-locked to the hippocampal RSA. Recently Semba and Komisaruk (1978) reported phase relationships between hippocampal EEG and various limb movements in the rat. Clearly our results show that, at least in the dog, the behaviours which occur in relation with RSA are not covered by the term *voluntary* movements, as proposed for the rat by Vanderwolf et al. (1973). Grastyán et al. (1959), working with cats, has proposed a correlation between RSA and the occurrence of the *orientation* reflex. In the dog at any rate our results have shown that

other reflex behaviour involving a motor response finds its representation in the hippocampal EEG as well.

The fact that both sensory input and motor output during the reflex movements contingent upon linear acceleration are easy to quantify and to manipulate lends extra interest to the EEG correlates observed in relation to this behaviour. This experimental design opens the possibility of a systematic investigation into the effects of the manipulation of various accelerations and of related variations in the motor response on the spectral properties of the hippocampal EEG. This may yield material fit to construct a quantitative descriptive model of the relationship between hippocampal spectral parameters, sensory input and motor output. It seems also important to us that such an elementary approach opens the possibility for comparative research on the hippocampal EEG correlates in different species and thus may contribute to solving current controversies concerning hippocampal-behaviour correlates (Lopes da Silva and Arnolds 1978).

Summary

A positive correlation has been shown between the speed of forced stepping on a conveyor belt and the amplitude and frequency of the concomitant hippocampal EEG.

Significant modulation in the spectral properties of the dog's hippocampal EEG has been found in relation to 3 elementary motor acts: stepping, respiratory movements and a reflex movement in reaction to linear acceleration of the animal.

The findings support the idea that within a certain experimental situation the hippocampal EEG reflects the intensity of motor behaviour in a non-specific but predictable way.

However, some experimental results indicate that sensory inputs may modulate the hippocampal EEG as well.

Résumé

EEG hippocampique et comportement chez le chien. II. Corrélation avec des activités motrices élémentaires

Chez le chien une corrélation positive a été trouvée entre la vitesse de 'marche' sur un tapis roulant et l'amplitude et la fréquence de l'EEG de l'hippocampe.

Des modulations significatives des caractéristiques spectrales de cet EEG de l'hippocampe accompagnent 3 types d'activités motrices élémentaires: une marche pas à pas, des mouvements respiratoires, un mouvement réflexe déclenché par une accélération linéaire du chien. Ces résultats renforcent l'idée que l'EEG de l'hippocampe reflète l'intensité des comportements moteurs d'une façon non-spécifique mais prévisible.

Toutefois, certains de nos résultats indiquent que l'EEG de l'hippocampe peut être également modulé par des afférences sensorielles.

References

- Arnolds, D.E.A.T. Behavioural Correlates of Hippocampal EEG in Dog. Ph.D. Thesis University of Utrecht, Utrecht, 1978, 108 pp.
- Arnolds, D.E.A.T. and Lopes da Silva, F.H. Computer assisted determination of brain behaviour correlates. *Physiol Behav*, 1977, 19: 377-380.
- Arnolds, D.E.A.T., Lopes da Silva, F.H., Kamp, A. and Aitink, J.W. Hippocampal EEG correlates with movements in dog. *Electroenceph. clin. Neurophysiol*, 1977, 43: 5678.
- Arnolds, D.E.A.T., Lopes da Silva, F.H., Aitink, J.W. and Kamp, A. Hippocampal EEG and behaviour in dog I. Hippocampal EEG correlates of gross motor behaviour. *Electroenceph. clin. Neurophysiol.*, 1979, 46: 552-570.
- Grastyán, E., Lissák, K., Madarász, I. and Donhoffer, H. Hippocampal electrical activity during the development of conditioned reflexes. *Electroenceph. clin. Neurophysiol.*, 1959, 11: 409-430.
- Kamp, A., Lopes da Silva, F.H. and Storm van Leeuwen, W. Hippocampal frequency shifts and behaviour. *Brain Res*, 1971, 31: 287-294.
- Kemp, I.R. and Kaada, B.R. The relation of hippocampal theta activity to arousal, attentive behav-

- ious and somatomotor movements in unrestrained cats. *Brain Res.*, 1975, 95 323-342
- Lopes da Silva, F.H. and Arnolds, D.E.A.T. The physiology of the hippocampus and related structures. *Physiol Rev.*, 1978, 40 185-216.
- Lopes da Silva, F.H. and Kamp, A. Hippocampal theta frequency shifts and operant behaviour. *Electroenceph. clin. Neurophysiol.*, 1969, 26 133-143
- Lopes da Silva, F.H., Hoeks, A., Lierop, T.H.M.T. van, Schrijer, C.F. and Storm van Leeuwen, W. Confidence intervals of spectra and coherence functions their relevance for quantifying thalamo-cortical relationships. In: G.K. Schenk (Ed.), *Beiträge des Symposiums 'Die quantifizierung des Elektroencephalogramms'*. Publ. AEG Telefunken, Konstanz, 1973: 437-449
- McFarland, H. and Hedges, E.K. Relationship between hippocampal theta activity and running speed in the rat. *J. comp. physiol. Psychol.*, 1975, 88 324-328
- Semba, K. and Komisaruk, B.K. Phase of the theta wave in relation to different limb movements in awake rats. *Electroenceph. clin. Neurophysiol.*, 1978, 44: 61-71.
- Storm van Leeuwen, W., Kamp, A., Kok, M.L., de Quartel, F.W., Lopes da Silva, F.H. et Tielen, A.M. Relations entre les activités électriques cérébrales du chien, son comportement et sa direction d'attention. *Actualités neurophysiol.*, 1967, 7 167-186
- Vanderwolf, C.H., Bland, B.H. and Whishaw, I.Q. Diencephalic hippocampal and neocortical mechanisms in voluntary movement. In: J.D. Maser (Ed.), *Efferent Organisation and the Integration of Behaviour*. Academic Press, London, 1973, 368 pp.
- Wetzel, M.C., Atwater, A.E., Wait, J.V. and Stuart, D.G. Neural implications of different profiles between treadmill and overground locomotion timings in cat. *J. Neurophysiol.*, 1975, 38 492-501.
- Whishaw, I.Q. and Schallert, T. Hippocampal RSA (theta), apnea, bradycardia and effects of atropine during underwater swimming in rat. *Electroenceph. clin. Neurophysiol.*, 1977, 42: 389-397
- Whishaw, I.Q. and Vanderwolf, C.H. Hippocampal EEG and behaviour. changes in amplitude and frequency of RSA (theta rhythm) associated with spontaneous and learned movement pattern in rats. *Behav. Biol.*, 1973, 8 461-484.