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Spontaneous seizure electrocortical activity in rats: relation to arousal level

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Abstract. The relationship between neocortical high voltage spindle activity (HVS) and the level of arousal (state of vigilance) was investigated in imp-DAK rats. Two groups of single records (6 h and 24 h) and one group of repeated ones (6 h daily for three consecutive days) were analysed. Five levels of arousal (states) were distinguished on the basis of hippocampal and neocortical EEG: high arousal (HA), moderate arousal (MA), low arousal (LA), slow wave sleep (SWS) and paradoxical sleep (PS). The number of HVS episodes and the cumulative amount (i.e. summed duration) of each state was calculated for each successive hour of recording. It was found that the number of HVS episodes correlated positively with the amount of the MA state, but not with the amount of the remaining states. The rate of HVS occurrence during the MA state, however, was not stable. It decreased along with the decrease in amount of MA in successive recording hours of the same recording session. However, at the beginning of each successive session, the HVS episodes became more numerous, whereas the MA amount decreased. The accompanying changes in the cumulative amount of the remaining states confirm indirectly the supposition that HVS occurrence is related to transitions from one state to another rather than to a given state itself.

Key words: rat, high voltage spindle activity (HVS), arousal

INTRODUCTION

Increasing attention is being paid to a peculiar form of seizure activity which occurs in some laboratory rats of different strains (see Coenen et al. 1992). This activity has the form of high voltage spindles (HVS) composed of 7-10 Hz spike and wave complexes (Vergnes et al. 1982, Buzsaki et al. 1988) and is considered to be reminiscent of human absence or "petit mal" epilepsy (Vergnes et al. 1982, (Micheletti et al. 1985, Peeters et al. 1988). Three factors determine the HVS occurrence. One of them is the genotype; it has been established that the propensity for HVS generation is transmitted through an autosomal gene (or genes) and that the mode of transmission is dominant (see Peeters et al. 1990, Marescaux et al. 1992). The age of the afflicted animal is the second factor; the HVS seizures appear at 40-120 days of age and last throughout the lifetime with an increase in number and duration with age (Aldinio et al. 1985, Aporti et al. 1986, Vergnes et al. 1986, Coenen and Van Luijtelaar 1987, Buzsaki et al. 1988). Of importance is the fact that the increase in HVS occurrence goes along with other age-related effects: deficient short-term memory (Aporti et al. 1986) and shrinkage of neurones of the nucleus basalis complex (Buzsaki et al. 1988). The third factor is the level of arousal; the bursts occur preferentially only during some behavioural states and are absent during others (Vergnes et al. 1982, Buzsaki et al. 1988, Coenen et al. 1991).

The above relationships could make rats with HVS not only a good animal model of absence epilepsy (Coenen et al. 1992), but also a preferable experimental material for assessment of the effects of exposure to drugs or industrial neurotoxins on CNS functional state and the rate of brain aging. There are, however, some problems which need elucidation before the use of rats with HVS for the latter purpose.

Theoretically, the factors under study (drug, toxic stimuli, age) may influence the rate of HVS occurrence through changes in the behavioral state of the animal, direct action on the neural substrate responsible for HVS generation or both. Therefore, not only the number and duration of HVS episodes but also the level of arousal require assessment. The literature on the relations between the HVS occurrence and the behavioural state of the rat, however, are not clear. According to some authors (Vergnes et al. 1982, Buzsaki et al. 1988), HVS episodes occur mainly during awake immobility, but are absent during slow-wave sleep. On the other hand, Coenen et al. (1991) found that almost 60% of HVS episodes occur during SWS.

In our previous studies on Wistar rats of the imp-DAK stock HVS episodes were found in almost 60% of 6-month old subjects (Gralewicz et al. 1989, Piasecka et al. 1989). The first purpose of the experiments presented below was to check whether the relationship of HVS activity and behavioural state (arousal level) in our animals was the same as that found by other authors. Taking advantage of the relationship of hippocampal activity to motoric behaviour (Vanderwolf 1975), the arousal level of the animal was determined on the basis of cortical as well as hippocampal EEG. Second, we attempted to find out whether the rate of occurrence of HVS episodes during a given state of arousal remained stable during successive recording periods, providing the external and internal conditions were unchanged. If it was stable, i.e if a good, reliable reference level could be found, it would strenghten the aplicability of this measure in studies aimed at detection of the effect of drugs or neurotoxins on the CNS functional state.

METHODS

The records

The records subjected to analysis in the present work were obtained from 22 rats selected from three rat populations used in three different experiments. Studying HVS activity was not the basic objective of these experiments. The records selected were control records, i.e. they were not preceded by any medication. In these three experiments, EEG was recorded continuously for 6 h, 24 h or 6 h daily during three consecutive days, respectively. The

presence of HVS activity in the cortical EEG of a given animal served as the criterion for selecting the record. Six records were from the six-hour (6 h) group, seven from the 24-hour (24 h) group and nine from the three day (3 x 6 h) group.

All recorded animals were male Wistar rats of imp-DAK stock about seven months old. The conditions of housing (single cages, 12/12 h light/dark cycle with light on at 6h AM) and the neurosurgery (implantation of intrabrain electrodes) were the same in all cases. The animals had been experimentally naive before the analysed records were taken. All recordings were made in the same recording room and with the same recording equipment.

Electrode placements

All rats had bipolar electrodes implanted bilaterally into parietal cortex, and into dorsal hippocampus. The construction of electrodes and details of the surgery have been described previously (Gralewicz et al. 1989).

Apparatus and recording

For recording, the animals were transferred from their home cages to the recording room and placed in opaque plastic containers (30 x 30 x 40 cm), with food and water available. Records were made with an 8-channel electroencephalograph. The low and high filters were set at 1 Hz and 35 Hz, respectively. Two days before the records were made the animals were adapted to the recording conditions for about 1 h. In all cases the recording started about 8 AM, 15 min after placing the animal in the recording cage.

Data analysis

Each record was separated into one-hour successive segments. The analysis consisted in counting the number and duration of HVS episodes and the duration of the following states: high arousal (HA), moderate arousal (MA), low arousal (LA), slow wave sleep (SWS) and paradoxical sleep (PS). The states were distinguished on the basis of the hippocampal and cortical EEG morphology. Notes which had been made below the traces during the recording, concerned the rat posture and locomotor behavior. They helped to make distinction between HA and PS. The states were classified according to the following criteria. Sections characterized by low voltage fast activity (LVFA) in the cortex and rhythmic slow (5.0-9.0 Hz) activity (RSA) in the hippocampus, not shorter than 1.0 s, were classified as HA (Fig 1A). Sections characterized by LVFA in the cortex and no RSA in the hippocampus, not shorter than 1.0 s, were classified as MA (Fig. 1B). Sections longer than 1.0 s but shorter than 10.0 s, characterized by dominance of high voltage slow activity (HVSA) in the cortex and large irregular activity (LIA) in the hippocampus were classified as LA (Fig. 1C). Sections longer than 10 s with LIA in the hippocampus and HVSA in the cortex were classified as SWS (Fig.1D). Sections with continuous RSA in the hippocampus and LVFA in the cortex were classified as PS if they started after a SWS episode and the animal was lying immobile (Fig.1E). Intermediate stages with RSA in the hippocampus and HVSA in the cortex were classified as PS.

Changes in EEG morphology, lasting less than 1.0 s, were neglected. State present during a 2 s period preceding directly a given HVS episode was regarded as the state on which this episode occurred no matter what state was present immediately after the end of the episode.

The following numerical data were collected during the analysis: the number of HVS episodes, the average episode duration and the total duration of the HVS activity in each hour of recording and in the entire record, the percentage amount of each state in each hour of recording and in the entire record, the proportion of HVS episodes occurring during each state in the entire record and in each one-hour segment. Statistical evaluation of differences was performed with the use of nonparametric and, where possible, parametric tests (Siegel 1956, Winer 1962).

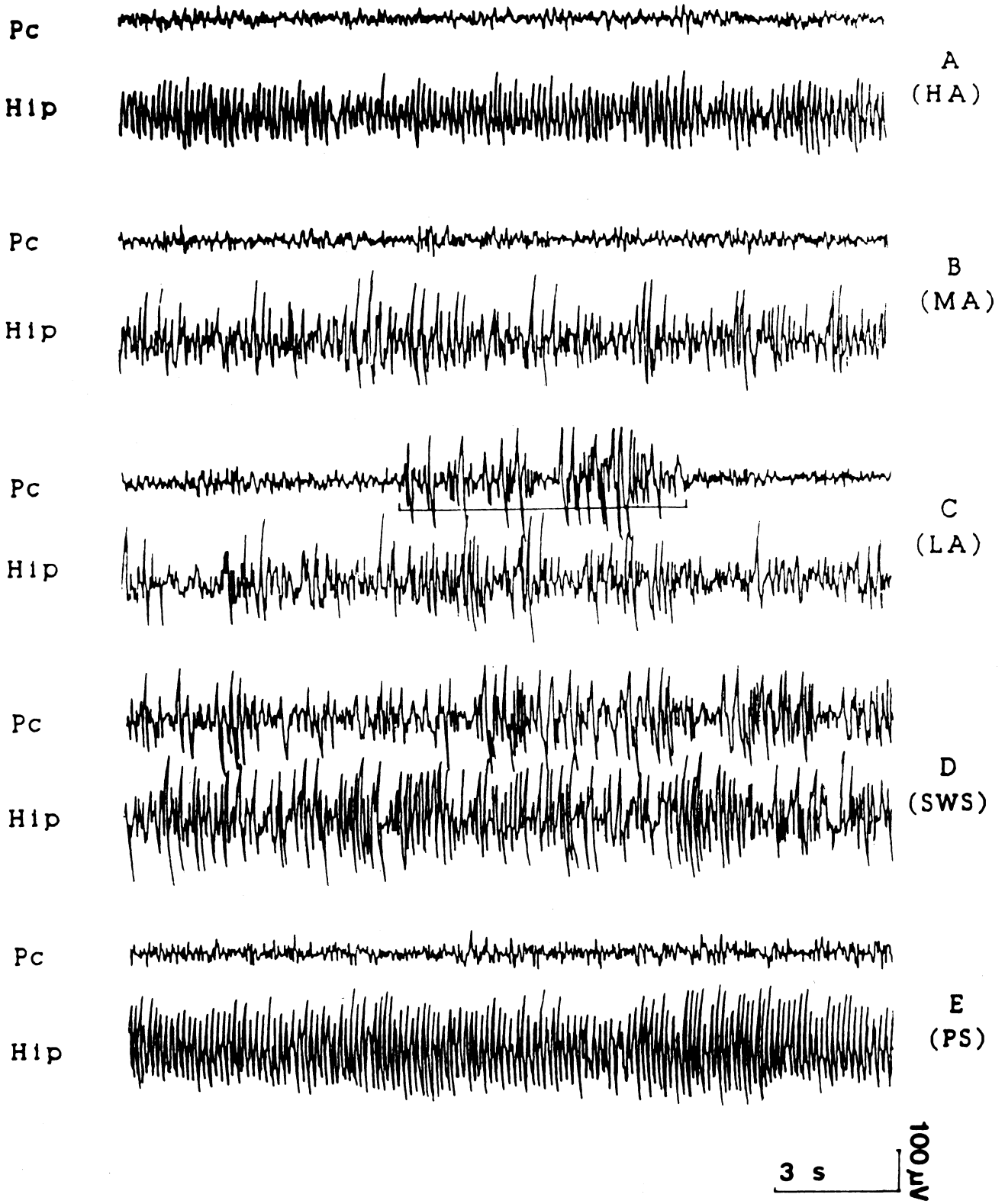

Fig. 1. Fragments of records illustrating the neocortical and hippocampal EEG of the rat during the distinguished states (levels of arousal). Abbreviations to the right: A, high arousal (HA); B, moderate arousal (MA); C, low arousal (LA); D, slow wave sleep (SWS); E, paradoxical sleep (PS). Denotations to the left: Pc, parietal cortex, Hip, hippocampus.

RESULTS

Single records (6 h group and 24 h group)

An example of a HVS episode is presented in Fig 2. The animals differed markedly with respect to the rate of HVS occurrence. The average number of episodes per hour varied from 4.4 to 52.0 (global mean=18.3, SD=14.3). Mean episode duration varied from 2.7 to 9.3 s in particular subjects (global mean= 6.5, SD=2.9), but in a given animal the value of this parameter was relatively stable during successive hours of recording; in ten cases (out of thirteen), the SD value did not exceed 15% of the value of the mean episode duration. Therefore, only the number of episodes was taken into account during further analyses.

In the 6 h group about 40% of the entire record was classified as MA. HA, LA and SWS constituted each about 20% of the record. The amount of PS was negligible. In the 24 h group, two states dominated: MA and SWS (Table I, rows A)

In both groups, HVS episodes occurred most frequently during MA and LA, and only occasionally during the remaining states (Table I, rows B). In order to visualize better the HVS "affinity" to particular states, a "preference index" was calculated by dividing the percentage of the total HVS number during a given state by the percentage amount of this state in the whole record. Value higher than 1 means that the state was "HVS-preferred". Friedman nonparametric ANOVA, performed for the combined data from both groups, showed a significant effect of state on the value of the preference index (X^2=49.3, *df*=4, P<0.001). In case of MA and LA the values of the preference index markedly exceeded 1 (Table I, rows C) and were significantly higher than those calculated for HA, SWS and PS (Wilcoxon signed rank test, P<0.01 in all comparisons). The differences between MA and LA as well as between HA, SWS and PS were not significant. The above suggested that the most convenient conditions for HVS occurrence existed during LA and MA.

Friedman ANOVA revealed significant differences in the number of HVS episodes between successive hours of recording in the 6 h group (X^2=17.33, *df*=5, P<0.01) as well as in the 24 h group (X^2=74.08, *df*=23, P<0.001). In the 6 h group, in the first and in the second hour of recording the episodes were significantly more numerous than in the remaining hours, but in the second hour they were significantly less frequent than in the first one (Wilcoxon test, P<0.05 in all comparisons). The remaining hours did not differ from each other with respect to the HVS number.

In the 24 h group the HVS episodes were significantly (Wilcoxon test, P<0.05) more frequent in the first hour of recording than in the remaining hours except hour 10, 11, 12 and hour 17. In hour 10, 11, 12 and 17, however, the HVS were significantly more numerous only in comparison with hour 4, 5, 6, 7, and the last four hours of recording, i.e. hour 21-24 (Wilcoxon test, P<0.05 in all cases).

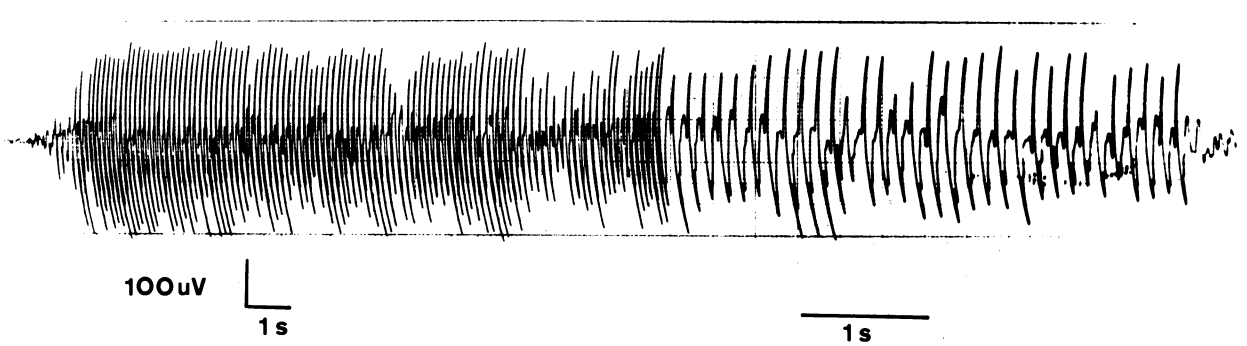

Fig 2. An example of the high voltage spindle (HVS) activity in the neocortical EEG of a rat recorded at 1 cm/s (left part) and 3 cm/s (right part) paper speed.

TABLE I

Relations between the occurrence of HVS episodes and the level of arousal (state) estimated on the basis of 6 h and 24 h EEG records

	Rate of HVS occurrence per hour (group mean and SD)	Episode duration (group mean and SD)		Percentage amount of states within the whole record (a), percentage distribution of HVS episodes (b) and the "preference index" (c). (mean and SD)				
				HA	MA	LA	SWS	PS
6 h group (n=6)	26.55 ±17.04	8.9 ±2.52	a	17.65 ±7.80	42.76 ±10.20	17.60 ±5.60	18.50 ±6.60	3.40 ±2.20
			b	0.60 ±0.90	77.70 ±12.70	21.40 ±12.30	0.10 ±0.20	0.00
			c	0.00	1.80 ±0.30	1.40 ±0.90	0.00	0.00
24 h group (n=7)	11.21 ±4.75	4.3 ±1.05	a	15.00 ±8.30	38.20 ±6.60	9.90 ±2.90	31.30 ±2.80	5.60 ±1.40
			b	0.50 ±0.70	73.00 ±10.90	18.00 ±5.50	7.30 ±8.70	0.60 ±0.50
			c	0.00	1.90 ±0.20	2.00 ±0.80	0.20 ±0.30	0.10 ±0.10

These differences reflected the existence of two additional peaks of HVS activity in the 24 h group, one between 5 h PM and 7 h PM and the other about midnight.

In both groups of records, changes in the amount of the dominant states (HA, MA) assumed the expected course which reflected changes in arousal due to habituation (i.e. a progressive decrease at the initial phase of recording) and to the circadian variability. Therefore, the reliability of these changes was not tested statistically. The changes in the amount of MA, and in some cases of HA, paralleled those in HVS number. Changes in the SWS amount went in the opposite direction, but this relationship was less pronounced (Fig. 3A and B).

In order to determine the mutual relations between HVS activity and arousal level, correlation between HVS number and amount of state was computed for each state and for each animal separately. In all animals positive correlation between HVS number and MA amount was found. The lowest value of the correlation coefficient (r) in rats of the 6 h group and 24 h group, was 0.87 and 0.32, respectively. It reached significance (P<0.05) in all animals of the 6 h group and five of the 24 h group. Only in two rats (both from the 24 group) HVS number was negatively correlated with SWS amount (r=-0.48 and -0.52, P<0.05 in both cases). No significant correlation was found in the case of the remaining states in any animal.

In the subsequent analysis, the rate of HVS occurrence during a given state was calculated according to the following formula:

$$X = \frac{3600}{t} N$$

where t denotes the cumulative state duration (in seconds) in a given one-hour EEG section and N denotes the number of HVS episodes occurring during this state. Because HVS episodes were so rare during HA, SWS and PS, and the amount of LA was small, the calculations were performed for MA

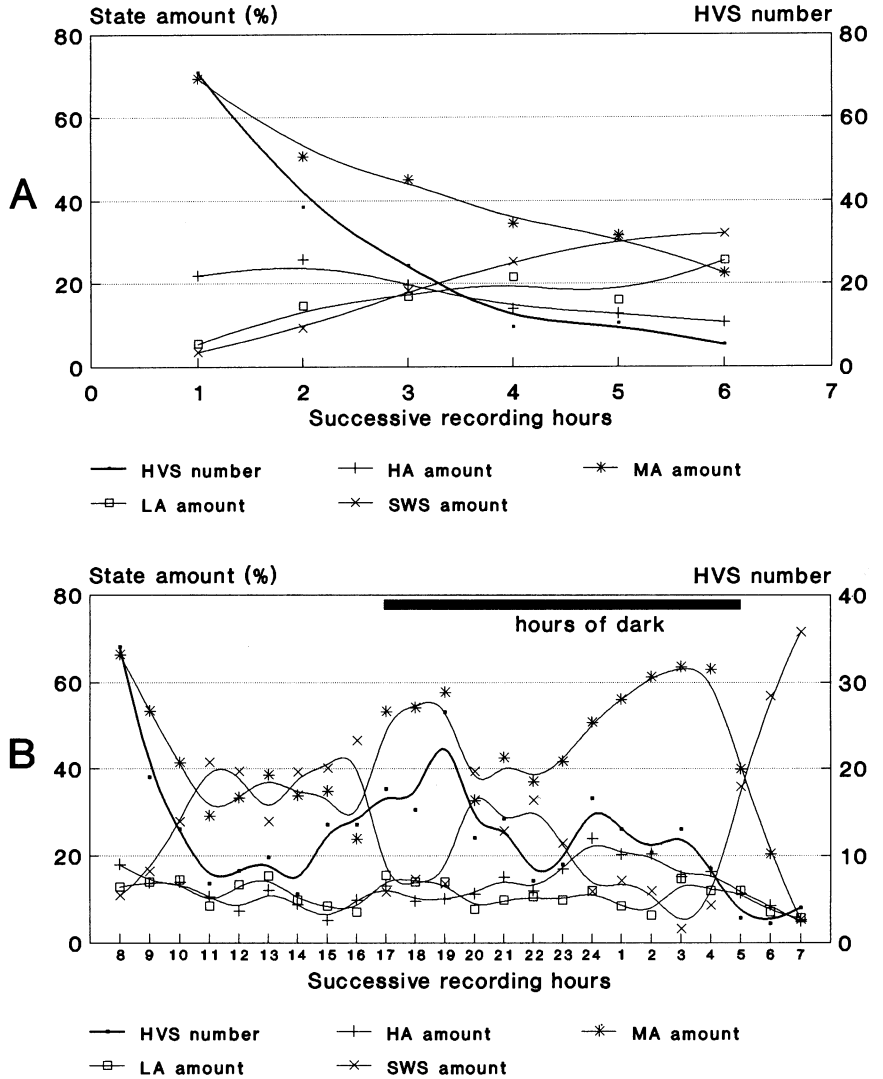

Fig 3. Changes in the number of the HVS episodes and the cumulative amount of the distinguished states during consecutive hours of recording. For clarity, the curve illustrating changes in the PS amount has been erased. A, 6 h Group; B, 24 h Group.

only. The rate of HVS occurrence during successive hours was expressed as the percentage of the value calculated for the first hour of recording.

Figure 4 illustrates changes in the cumulative MA amount and changes in the rate of HVS occurrence during that state in the first 6 hours of recording (data from the 6 h and 24 h group were combined). In order to limit the influence of the individual variability in HVS number, (which was particularly high in rats with few HVS episodes), only the data from five (out of thirteen) animals in which more than 50 HVS episodes occurred during the first hour of recording were taken into account. It has been found, contrary to our expectations, that the rate of HVS occurrence during MA was not stable; it decreased along with the decrease in MA amount. The correlation analysis confirmed the positive correlation between the MA amount and HVS occurrence during that state in all five rats ($r=0.89$ or higher, $P<0.05$ in all cases). It suggested that in sections with similar amount of MA, the rate of HVS occurrence during that state might be rela-

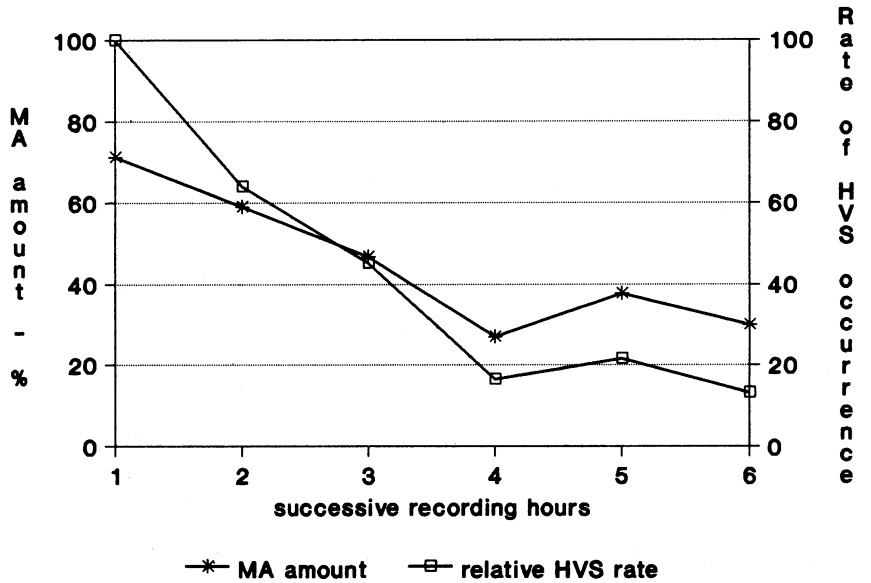

Fig. 4. Relationship between the rate of HVS occurrence in MA state and the cumulative MA amount during successive hours of recording. Note: The curves were drawn on the basis of data from five rats in which the rate of HVS occurrence during the first recording hour was no less than 50/h. The rate of HVS occurence is expressed as percent of that noted in the first hour of recording.

tively stable. Records from the 24 h group were the only ones which contained sufficient number of such sections to attempt tentative comparisons. The results are presented in Table II. In spite of the fact that in each animal in the selected one-hour sections the MA amount was similar (in no case did the SD value exceed 15% of the mean), the frequency of HVS occurrence during this state varied greatly; the SD values varied from 45% (rat no 18) to 108% (rat no 2) of the mean.

TABLE II

Variability in the rate of HVS episodes in MA state in selected one-hour EEG sections with similar cumulated MA amount

No of rat and number of 1 h EEG sections analysed	Cumulative MA amount (mean and SD)	Rate of HVS occurance during MA (mean and SD)
1	82.1	26.1
n=6	±8.7	±13.3
2	55.6	26.3
n=8	±4.6	±28.5
3	64.8	11.1
n=5	±6.3	±5.3
4	62.8	10.5
n=4	±8.3	±7.0
9	56.2	23.3
n=8	±1.8	±21.1
18	76.3	8.2
n=7	±9.7	±3.7
25	56.0	28.6
n=6	±3.9	±15.7

Repeated records (3 x 6 h group)

Data from this part of the experiment are presented in Table III. The comparisons between records were made with a parametric two-way ANOVA (Days x Hours). Detailed comparisons were performed with Tukey test. In the case of HVS, relative number of HVS episodes and relative rate of HVS occurrence during MA were analysed separately. State amounts were expressed in percents of record duration. The analysis of the relative number of HVS episodes (the HVS number from the first hour of the first day of recording = 100%) revealed no significant global effect of Days but the effect of Hours and the Days x Hours interaction were significant (see Table III). Within-day com-

TABLE III

Hour-to-hour changes in the HVS occurrence and cumulated state amounts in three successive daily recording sessions

	day 1						day 2						day 3						ANPVA (Days x Hours)
	Successive hours of recording						Successive hours of recording						Successive hours of recording						D-effect of days H-effect of hours
	1	2	3	4	5	6	1	2	3	4	4	6	1	2	3	4	5	6	I-interaction
Relative HVS number	100	67.8	61.4	35.1	19.8	21.3	107.2	47.1	37.5	13.6	14.0	17.1	* 166.7	63.5	36.9	19.4	35.8	21.3	D-F(2,24)=0.67 NS H-F(1,24)=22.65 $P<0.0001$ I-F(2,24)=5.31 $P<0.02$
Relative HVS occurance during MA	100	60.7	77.2	66.9	42.3	31.1	123.8	76.3	90.4	33.6	42.9	39.6	* 190.7	99.7	79.6	62.4	66.1	39.7	D-F(2,24)=0.97 NS H-F(1,24)=12.1 $P<0.02$ I-F(2,24)=4.85 $P<0.02$
HA amount	12.7	10.9	11.6	5.3	3.5	7.3	17.3	14.3	8.8	4.4	3.9	4.5	* 23.9	17.5	4.3	7.9	7.5	5.6	D-F(2,24)=1.32 NS H-F(1,24)=20.08 $P<0.0002$ I-F(2,24)=5.99 $P<0.001$
MA amount	74.9	62.0	38.5	30.6	21.5	24.0	55.2	* 34.2	23.9	24.7	21.0	31.5	* 50.6	* 33.0	26.5	21.1	18.1	21.6	D-F(2,24)=3.52 NS H-F(1,24)=71.89 $P<0.0001$ I-F(2,24)=13.02 $P<0.0001$
LA amount	6.5	13.5	12.8	12.3	12.5	12.1	13.0	13.7	12.6	12.6	11.4	11.9	* 13.6	14.0	12.9	11.8	11.4	11.7	D-F(2,24)=0.16 NS H-F(1,24)=7.65 $P<0.02$ I-F(2,24)=8.61 $P<0.02$
SWS amount	5.8	13.2	33.0	41.4	49.2	43.6	14.5	* 35.9	49.6	49.4	50.3	42.2	11.8	* 32.2	* 45.1	47.9	47.5	47.9	D-F(2,24)=3.81 NS H-F(1,24)=68.57 $P<0.0001$ I-F(2,24)=5.85 $P<0.01$
PS amount	0.0	0.5	4.0	10.4	13.6	13.0	0.5	2.5	5.4	9.0	13.5	9.9	0.5	3.3	6.5	11.3	15.5	13.6	D-F(2,24)=1.61 NS H-F(1,24)=18.19 $P<0.001$ I-F(2,24)=2.50 NS

Notes: The HVS data are percents of the values noted in the first recording hour of day 1. State amounts are expressed as mean percentage distributions in cuccessive one-hour recording periods.Asterisks denote values differing significantly ($P<0.05$) from those calculated for corresponding hours of day 1.

parisons revealed significant differences in all cases (day 1: $F(5,120)=4.61$, $P<0.001$; day 2: $F(5,120)=6.07$, $P<0.001$; day 3: $F(5,120)=14.63$, $P<0.001$). On day 1 the HVS episodes were significantly more numerous in the first hour of recording than in the fourth, fifth and sixth hour and on day 2 and 3 they were significantly more numerous in the first hour than in the following hours ($P<0.05$ in all comparisons). Between-day comparisons revealed significant differences only in the case of the first hour of recording ($F(2,144)=4.46$, $P<0.02$). Detailed comparisons showed that on day 3 the HVS number in the first hour of recording was significantly higher than in their coresponding hours of day 1 and day 2 ($P<0.05$ in both cases).

The analysis of the rate of HVS occurrence during MA revealed no significant global effect of Days but the effect of Hours and the Days x Hours interaction were significant (see Table III). Generally, on all days the rate of HVS occurrence during MA was significantly higher in the first hour of recording than in the remaining hours ($P<0.05$ in all cases). Between-day comparisons revealed differences only in the case of the first hour [$F(2,144)=4.61$, $P<0.02$]; on day 3 the rate of HVS occurrence was significantly higher than on day 1 and 2 ($P<0.05$ in both cases).

The analysis of state amounts revealed no significant effect of Days in any case but the effect of Hours was significant in all cases and the Days x Hours interaction was insignificant only in the case of PS (see Table III). The amount of HA changed significantly in successive hours of recording on day 1 ($F(5,120)=3.27$, $P<0.01$), on day 2 ($F(5,120)=7.95$, $P<0.001$) and on day 3 ($F(5,120)=11.85$, $P<0.0001$). On all days the amount of HA decreased in successive recording hours; in the first hour of recording it was significantly ($P<0.05$) higher than in the last two hours and this difference was especially well pronounced on day 3. Comparisons between corresponding hours of successive days revealed differences only in the case of the first hour of recording ($F(2,144)=4.97$, $P<0.0.01$); on day 3 the amount of HA was significantly higher than on day 1 ($P<0.05$).

In each of the three recording sessions the amount of MA decreased gradually in successive hours (day 1: $F(5,120)=42.05$, $P<0.0001$, day 2: $F(5,120)=18.33$, $P<0.0001$, day 3: $F(5,120)=19.92$ $P<0.0001$). Each day the amount of MA in the first two hours of recording was significantly higher than in the last two hours. Comparisons between corresponding hours of successive days revealed differences in the case of the first ($F(2,44)=3.90$, $P<0.03$) and the second ($F(2,44)=10.48$, $P<0.001$) hour of recording. The amount of MA in the first hour of day 3 was significantly lower than during the corresponding hour of day 1 and the amount of MA in the second hour on day 2 and 3 was significantly lower than in corresponding hours of day 1.

The amount of LA changed significantly in successive recording hours on day 1 ($F(5,120)=4.64$, $P<0.02$) and on day 3 ($F(5,120)=6.49$, $P<0.001$). On day 1 the amount of LA in the first hour was lower than in the remaining ones but the difference assumed significance only in comparison with the second hour ($P<0.05$). On day 3 the amount of LA in the fourth, fifth and sixth hour of recording was significantly smaller than in the first and the second one ($P<0.05$ in all cases). Successive days differed significantly only with respect to the LA amount in the first hour of recording ($F(2,144)=4.64$ $P<0.02$); on day 1 the amount of LA in this hour was lower than on the remaining days but only in comparison with day 3 did the difference reach significance ($P<0.05$).

Changes in amount of SWS during successive recording hours were significant on day 1 ($F(5,120)=30.92$, $P<0.0001$), day 2 ($F(5,120)=18.56$, $P<0.0001$) and day 3 ($F(5,120)=22.01$, $P<0.0001$). On each day the SWS amount increased gradually reaching nearly 50% by the third or fourth hour and remained at that level till the end of recording. On day 1, 2 and 3 the SWS amount in the first hour was significanly lower than in the remaining hours and in the second hour lower than in the fourth, fifth and sixth one ($P<0.05$ in all cases). The days differed between themselves with respect to the SWS amount only in the second and third hour of recording (second hour: $F(2,144)=7.55$, $P<0.001$, third

hour: $F(2,144)=3.25$, $P<0.05$). On day 2 and 3 the SWS amount in the second hour was significantly higher than in the corresponding hour of day 1, and on day 3 the SWS amount in the third hour was significantly higher than in the corresponding hour of day 1 ($P<0.05$ in all cases).

Changes in PS amount proceeded similarly on each day (Days x Hours interaction was not significant). In the first hour of recording, PS episodes were very rare but became more frequent in the successive hours. In each of the last three hours the amount of PS was significantly higher than in the first and in the second one ($P<0.05$ in each case).

Summing up, the above comparisons have shown that in each of the following three six-hour recording sessions the global number of HVS episodes as well as the rate of HVS occurrence during MA decreased in successive recording hours. This decrease was accompanied by a decrease in the amount of MA and HA states and an increase in the amount of SWS and PS. Changes in LA amount were less pronounced. Comparisons between corresponding one-hour EEG sections of successive daily records suggest, however, an increase in HVS number as well as in the rate of HVS occurrence during MA at the beginning of successive recording sessions. Unexpectedly, this increase was accompanied by a decrease in the amount of MA state and an increase in the amount of HA and LA states.

DISCUSSION

The analysis of the records has shown that HVS occurs most frequently during MA and LA and less frequently during HA, SWS and PS. In the course of a long-lasting recording, HVS incidence is positively correlated with the amount of MA state, and, less frequently, negatively correlated with the amount of SWS. The amount of other states shows no correlation with HVS.

The dependence of HVS on arousal level has been emphasized by all authors studying this form of rat neocortical activity. According to Vergeness et al. (1982) and Buzsaki et al. (1988), the episodes of HVS occur during awake immobility, but not during sleep. Our observations are consistent with this statement, since what we have defined as MA and LA, are in fact bioelectrical correlates of awake immobility (Vanderwolf 1975). These observations do not confirm, however, those of Coenen et al. (1991), who noted that almost 60% of HVS episodes occurred during SWS. On the other hand, the time course of changes in HVS number during 24h recording in our experiments and in those of Van Luijtelaar and Coenen (1988), were similar. This suggests that the cause of the discrepancies concerning the state during which HVS occur most frequently may lie in the criteria for state classification. It is very likely that what we defined as LA would be classified as SWS or light SWS by the automated system used by these authors (Van Luijtelaar and Coenen 1984). Experimental procedure might be another source of differences. For example, a high incidence of HVS episodes in the first hours of recording (morning hours) was observed in our experiments, but not in the experiment of Van Luijtelaar and Coenen (1988). This was most probably due to the fact that our rats had to be transferred from their home cages to the recording container and that they were allowed a short (15 min) adaptation period before the onset of recording. All this resulted in a high amount of the MA state, i.e. a state preferrable for HVS occurrence, at the beginning of each session.

On the basis of some previous reports (Vergnes et al. 1982, Buzsaki et al. 1988) we expected that the rate of HVS occurrence during a preferred state would be relatively stable. The obtained results revealed, however, that the rate of HVS occurrence during MA (the only state the amount of which was positively correlated with the HVS number) varied; it might decrease in the course of the same recording session along with the decrease in MA content, but in the first hour of successive recording days, the HVS episodes became more numerous, whereas the MA content decreased. One can therefore, suppose that apart from the MA content, other variables may affect the rate of HVS occurrence.

According to a recent report by Drinkenburg et al (1991), HVS occurs most frequently during awake immobility and light SWS. However, after

analysing the EEG directly preceding and following HVS episodes, those authors concluded that HVS occurs most preferably during the transitions from one state to another rather than during a given state. It follows from the above that what determines the rate of HVS occurrence is not the amount of a preferred state but rather the stability of arousal level. The more frequent the shifts from one state to another, the higher the probability of HVS occurrence. From this point of view, the results obtained in the present studies become more clear. In the course of each of the 6 h recording sessions the HA and MA amounts decreased gradually. This decrease went along with an increase in SWS amount. It is worth reminding here that, according to the adopted criteria, sections as short as 1 s contributed to the cumulative amounts of HA, MA and LA states, whereas in the case of SWS, they had to be longer than 10 s. Thus, the decrease in HA and MA and the increase in SWS amount in a given recording hour means a decrease in the number of EEG fluctuations and, consequently, a decreased probability of HVS occurrence. The differences between the first one hour sections of three successive records went in the opposite direction: the decrease in MA amount was accompanied by an increase in the amount of HA and, although less clearly, LA, and no significant changes in SWS amount. This change in the distribution of the state amounts suggests a decrease in EEG stability and, accordingly, a higher probability of HVS occurrence.

If one wishes to use HVS as an index in the studies on the effects of chemicals, aging, etc., on the functional CNS state, then, in the light of the Drinkenburg et al. (1991) and the present data, it is important to consider how to schedule the experiment, which variables are to be controlled and how? In the literature two procedures have been used in such experiments. One consists in the application, when necessary, of mild sensory stimuli during recording in order to maintain the required state of moderate arousal. The rate of occurrence of the HVS episodes is then calculated from the episode number and duration of the recording session (e.g. Vergnes et al. 1982). The other procedure consists in counting HVS episodes only in cumulative 10-30 min sections of EEG taken during "waking immobility" (e.g. Buzsaki et al. 1988, Riekkinen et al. 1991). In both cases, it is assumed that the rate of HVS occurrence during a forced or selected behavioural state is relatively stable and a change, if it occurs, is due to the effect of the factor under study (e.g. a drug, age). As Drinkenburg et al. (1991) and our data suggest this would be true only when the proportions of the remaining states as well as the frequency of transitions from one state to another were similar in successive recording periods. Moreover, neither procedure takes into account the possible influence of the factor under study on the arousal level as such.

If the propensity for the HVS occurrence is highest during the transitions from one state to another then, apart from the percentage distribution of states within the recording period, the number of such transitions and their direction should be carefully observed during the experiment. One can expect that the HVS number/number of transitions, ratio will appear more stable in consecutive hours of recording than the ratio: HVS number/cumulative duration of a given, "preferred" state. This will be examined in our further studies.

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