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Development of the age-related spontaneous spike-wave discharges in rat neocortex and exposure to a model neurotoxin

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Abstract. In laboratory rats an epileptic-like spontaneous neocortical activity in the form of bursts of spike and wave discharges (SWD) develops gradually with age. High incidence of the SWD episodes is accompanied by other indices characteristic of advanced age: memory disturbances and atrophic changes within basal forebrain structures (Buzsaki et al. 1988b , Gage et al. 1988). Accordingly, it has been proposed that the number and duration of the SWD episodes be regarded as a diagnostic marker to distinguish between young and old brains (Buzsaki et al. 1988a). It is suspected that exposure to neurotoxins may accelerate the progress of age-related neurodegeneration by predisposing neurons to premature death and thus hasten the appearance of the age-related functional deficits (Weiss 1990). Analysing the development of SWD activity in exposed rats may be helpful for an assessment of the potency of the neurotoxin under study to exert such an effect. In the present work the influence of a three-month exposure to a model neurotoxin, ethanol (ETOH), on the development of the SWD activity in imp-DAK rats was investigated. It has been found that in rats given 10% ETOH solution as the only drink for three months, the incidence of the SWD episodes increased markedly. The increase was most clearly seen after ETOH withdrawal and on the 90th day after exposure no tendency to decline could be observed. The obtained data indicate that exposure to exogenous substances may exert a distinguishable long-lasting influence on the development of the SWD activity.

Short communication

Key words: spike-wave discharges (SWD), ethanol, ageing, rats

In the nervous system, natural ageing is marked by progressive atrophic changes and cell loss (Flood and Coleman 1988). It is assumed (Weiss and Simon 1975, Weiss 1990, Reuhl 1991) that exposure of neurones to toxicants may cause changes (e.g. metabolic alterations, changes in DNA or cytoarchitecture) which render the cells susceptible to premature senescence and death. Functional consequences may be absent at the time of exposure and for some time after its discontinuation. In the exposed subjects, however, symptoms and functional deficits characteristic of senility, may emerge faster than it can be expected on the basis of the subject's chronological age.

No direct experimental support of the above assumption was presented (see Weiss 1990, Reuhl 1991). One of the possible ways of checking its validity is to compare the rate of development of age-related CNS symptoms in untreated animals and animals exposed to a given neurotoxin. In laboratory rats of some strains, one of the symptoms which may be considered an index of the animal age is the spontaneous epileptic-like ECoG activity (Vanderwolf 1975). The seizures are characterized by bilateral and synchronous rhythmic 7-11 Hz spike-and-wave discharges (SWD). The number of SWD episodes and their duration increases with age (Aporti et al. 1986, Vergnes et al. 1986, Coenen and Van Luijtelaar 1987) and, according to Buzsaki et al. (1988a) these measures may serve "... as a diagnostic marker to distinguish between young and old brains". A high incidence of the SWD bursts accompanies other indices of advanced age: impaired memory and atrophic changes in the cholinergic structures of the basal forebrain (Aporti et al. 1986, Gage et al. 1988). On the other hand, the progressive increase in the number of SWD episodes may be blocked by i.p. administration of phosphatidylserine (Aporti et al. 1986), an endogenous phospholipid of plasma membrane, assumed to participate in cell communication and reactions promoting natural defense mechanism and tissue repair. Administration of phosphatidylserine counteracts also biochemical, morphological and behavioural changes in the aging nervous system and is recommended for clinical treatment of memory dysfunction in old people (see Nunzi et al. 1991)). The above data suggest that the increase in the SWD activity with age follows the progression of age-related neurodegenerative changes within brain systems involved in higher-order nervous functions. Measuring SWD may, therefore, serve as a useful tool in studying the effects of neurotoxins on the development of these changes. The main purpose of the present work was to check the validity of this assumption. Namely, we wanted to find out whether a prolonged exposure to a model neurotoxin, known to produce among other effects, biochemical, morphological and behavioural CNS alterations, resembling the ones which develop in the course of natrural ageing, would lead to a permanent increase in the SWD activity. Ethanol (ETOH) was selected as the model neurotoxin. The neurotoxic properties of ETOH are well known. ETOH consumption may exacerbate the effects of ageing in humans (Holden et al. 1988) and the similarities in the manifestations of alcoholic dementia and senile dementia have been pointed out (King 1986). In rats, prolonged intake of ETOH is known to induce neuron losses in the same basal forebrain areas of the rat brain in which age-related cell deficits and atrophic changes are most profound (Arendt et al. 1988, Gage et al. 1988). The above data justify the selection of ETOH as a model neurotoxin in the present studies.

Twenty male Wistar imp: DAK rats, about 6 months old, and weighing approximately 350 g at the onset of the experiments, were used. They were housed individually in single rat cages under standard laboratory conditions (22-23 C, 12-h light/12-h dark photocycle (lights on at 6.OO a.m.). All rats had an unlimited access to food (standard pellets) in their home cages. For drinking, before the exposure and for three months after the exposure the rats were given plain tap water *ad libitum.*

Before the onset of exposure, chronic recording electrodes were implanted stereotaxically to all rats, under barbiturate anaesthesia (Nembutal, 50 mg/kg i.p.). Two bipolar electrodes, made of twisted stainles steel teflon-insulated wire (200 μm in diam) were implanted into frontoparietal cortex, bilat-

erally, (2.0 mm anteriorly to Bregma and 2.0 mm laterally to midline) in such a way that the uninsulated 0.5 mm tip of one member of the electrode pair penetrated the cortex to 1.0 mm depth and the second rested on the cortical surface. Two similar electrodes were implanted into the dorsal hippocampus, bilaterally (4.0 mm posteriorly to Bregma, 3.0 mm laterally to midline and 3.0 mm below the surface of the dura mater). The ground screw electrode was implanted over the cerebellum. The detailed description of the construction of electrodes and the surgery had been published in earlier papers (Gralewicz et al. 1989). After the surgery, the animals were allowed two weeks for convalescence.

After the period of convalescence but before the main experiment started, two 1h recording sessions were performed at two-day interval on each rat. SWD episodes (see Fig. 1) appeared in ECoGs of twelve animals (out of twenty). The rats were divided randomly into two groups, each consisting of six rats: ethanol group (ETOH) and sucrose group (SUC).

The exposure duration was three months. During that time the ETOH group had an unlimited access to 10% solution of ETOH as the only fluid in their home cages. The SUC group obtained sucrose solution at the concentrations adjusted so as to balance in both groups the daily caloric intake from fluid ingestion. The amount of fluid drunk by each animal was measured on each day of exposure. Cortical and hippocampal EEG was recorded for three hours daily before the exposure and then on day 28th, 56th and 84th of exposure and on day 2, 7, 14, 28, 42 and 90 after the exposure. The recordings were performed between 8.00 a.m. and 2.00 p.m. The rats were transferred from home cages to 25 x 25 x 30 cm open-top plastic containers and placed in a recording enclosure. (The level of the acoustic noise in the recording enclosure was similar as in the animal room and was not controlled). The cables connecting the recording electrodes with the electroencephalograph were attached and after a 15 min adaptation the recording started. The recordings were made with the use of an 8-channel electroencephalograph (Beckman - Acutrace). The behaviour of rats during the recording was observed on a TV screen.

The number and duration of the SWD episodes were calculated and the arousal level (state of vigilance) was assessed on the basis of visual inspection of the records. The SWD episodes were identified as regular 7-11 Hz spike and wave complexes of at least 2s duration and amplitude of at least twice the background amplitude. Using the criteria described in an earlier work (Gralewicz et al. 1994), five states were distinguished basing on the morphology of the cortical and hippocampal EEG: HA (high arousal - theta rhythm in hippocampus, fast desynchronized activity in neocortex), MA (moderate arousal - large irregular activity (LIA) in hippocampus, fast desynchronized activity in neocortex), LA (low arousal - periods with LIA in neocortex and hippocampus no longer than 10 s, SWS (slow wave sleep - periods with LIA in neocortex and hippocampus longer than 10 s), and PS (paradoxical sleep - theta rhythm in hippocampus and fast desynchronized activity in neocortex, sleeping posture). The

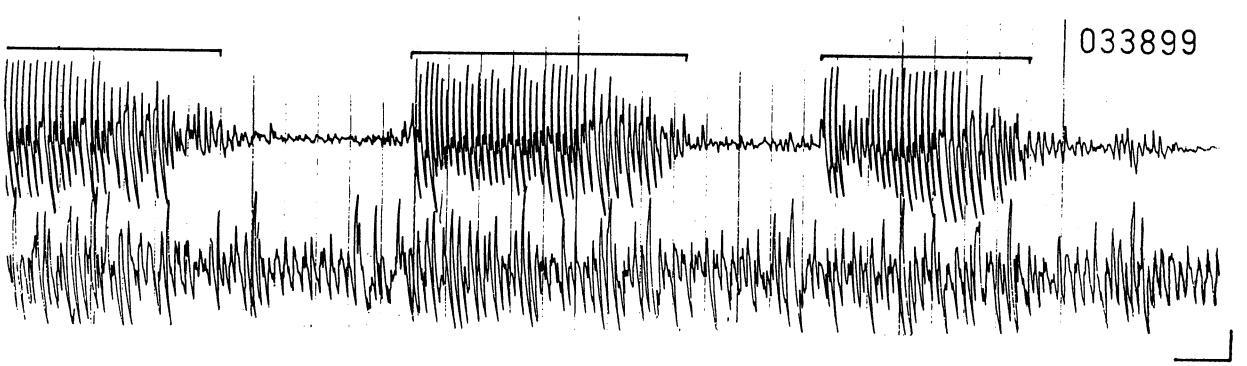

Fig. 1. A fragment of rat electroencephalogram. Upper trace: frontoparietal ECoG with SWD episodes (overlined). Lower trace: hippocampal EEG. Callibrations: vertical 200 μV, horizontal 1.0 s.

amount (cumulative duration) of each state, the number and duration of the SWD episodes as well as the percentage distribution of episodes within the distinguished states, were calculated for the whole record and for successive hours of recording.

Statistical evaluation of the differences between groups and between successive recording sessions was performed with the use of parametric or non-parametric ANOVA. Tukey test or Mann-Whitney U test were used for detailed comparisons (Siegel 1956, Winer 1962).

The average mean daily ETOH consumption in the ETOH group was 5.1±0.56 g/kg. The average mean daily SUC consumption in the SUC group was 9.1±1.0 g/kg. Figure 2 presents the time course of changes in body weight in both groups during the experiment. The Group x Successive measurements ANOVA, performed on relative data (value of the first measurement assumed to be 100%) revealed no significant effect of the Group factor. The Group x Successive measurements interaction was also insignificant.

The analysis of the EEGs revealed that in the first control session (before the exposure onset) the percentage distribution of the consecutive states was as follows: HA - 25.9±12.4; MA - 40.2±12.0; LA - 4.9±1.5; SWS - 27.5±8.9; PS - 1.5%±1.2. The comparisons of the contribution of each state in successive sessions (Groups x Sessions ANOVA) revealed no significant effect of the Groups as well as the Sessions factor on the amount of any state. The Groups x Sessions interaction was also insignificant. Comparisons within consecutive sessions (Groups x Hours) confirmed the significant effect of Hours in the case of HA and MA (a gradual decrease) and SWS (a gradual increase) in each session (see Gralewicz et al. 1994), but the effect of Groups as well as the Groups x Hours interaction, were not significant in any of the sessions.

No between-session differences in the distribution of the distinguished states were found. The effect of time (Hours) was similar in all sessions and the Groups x Hours interaction was not significant in any of the sessions. In view of the above, analysing the SWD occurrence in relation to particular state was omitted and only global numbers of the SWD episodes and their total duration in successive sessions and in successive hours of each session were subjected to the analysis.

In the first session (before the exposure) the mean number of the SWD episodes was 62.7±38.7 in the ETOH group and 84.5±57.6 in the SUC group. The mean total duration of the SWD activity in this session was 476.2±216.4 s in the ETOH group and 693.7±528.4 s in the SUC group. In the last recording session (three months after the end of

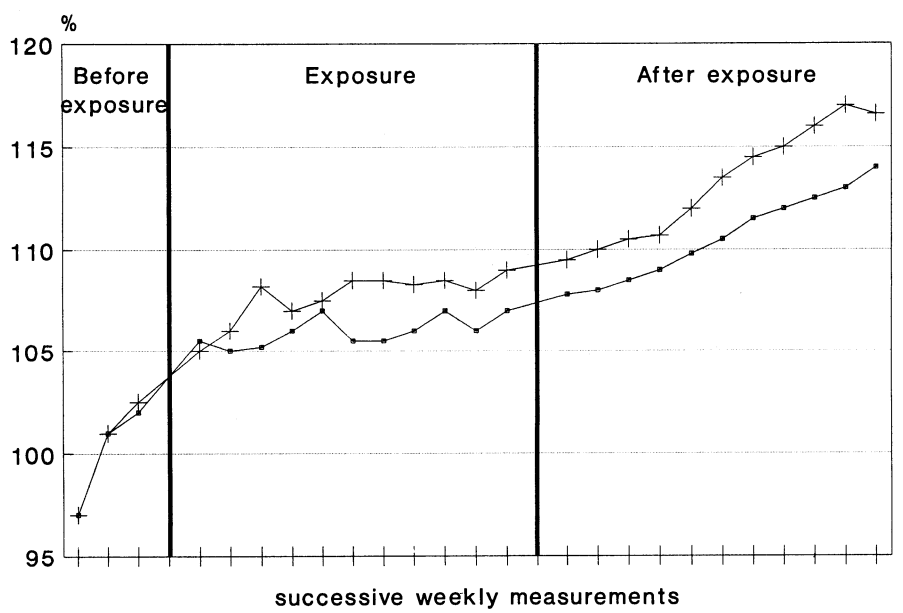

Fig. 2. Effects of prolonged (three months') intake of 10% ETOH solution (ETOH group) or sucrose solution (SUC group) on body weight of rats. The measurements were performed once each week. The mean from the three measurements performed before the exposure was assumed to be 100%.

exposure) the mean number of the SWD episodes was 151.7±46.9 in the ETOH group and 97.5±75.3) in the SUC group. The mean total duration of the SWD activity in the last session was 1075.8±306.23 s in the ETOH group and 821.3±668.9 s in the SUC group. The Friedman nonparametric ANOVA was used for within-group comparisons. The analysis of the data on the ETOH group revealed significant differences between successive recording sessions in the total number of the SWD episodes (X^2_8=23.5, P<0.01) and in their total duration (X^2_9=26.9, P<0.01). In the sessions performed on day 28, 42 and 90 after the exposure, the values of both these parameters were significantly increased as compared with those from the control session and sessions performed on 28th and 56th day of exposure (Wilcoxon test, P<0.05 in each case). In the SUC group, the analysis revealed no differences in the number of SWD episodes and in the total duration of SWD activity between successive recording sessions. Between-group comparisons of relative values (percents), performed separately for each recording session (Mann-Whitney, two-tailed test) revealed that in sessions performed 4 and 12 weeks after the exposure the increase in the number of SWD episodes in the ETOH group was significantly higher than in the SUC group, and that on 4th, 6th and 12th week after the exposure the total duration of the SWD activity in the ETOH group was significantly higher than in the SUC group (Fig. 3).

The above results suggest that the three-month intake of ETOH, as opposed to the three-month intake of SUC, results in a gradual increase in the SWD activity in the rat neocortex. Considering the duration of the experiment (six months), the lack of any overt changes in the SUC group may give rise to some doubts as to the relation of the SWD activity to age, or it may suggest that drinking sucrose solution had an inhibitory influence on the development of this form of activity. A closer examination of the relevant literature reveals, however, that a simple relationship between the SWD activity and age, i.e. almost linear increase in the number and duration of the SWD episodes, is evident only while comparing separate groups of rats of different age (Aporti et al. 1986, Coenen et al. 1987, Buzsaki et al. 1988b). When the same rats are tested repetitively, a biphasic course of the increase becomes evident, with a plateau between the sixth and fourteenth month of age (Vergnes et al. 1986). Considering the fact that in our rats the first record was made when they were about seven months old, the above data may account for the lack of overt changes in the SWD activity in the SUC group over the course of

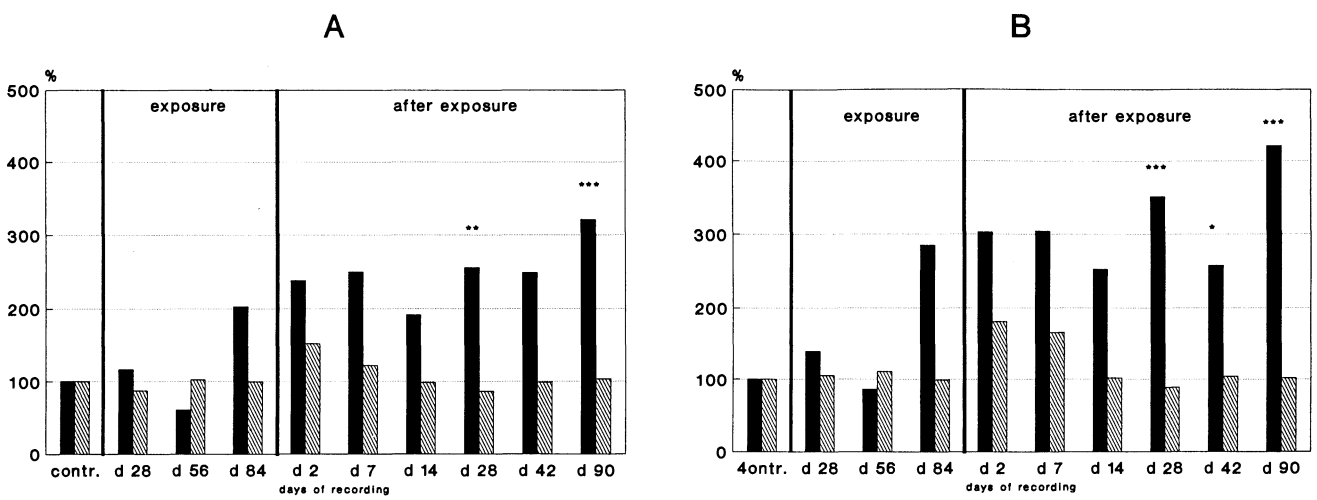

Fig. 3. The development of the spontaneous SWD activity in rats drinking 10% ETOH solution (ETOH group, black bars) or sucrose solution (SUC group, shadowed bars) for three months. A, percentage changes in the number of the SWD bursts; B, percentage changes in the total duration of the SWD activity. *P<0.05; **P<0.01; ***P<0.005.

the experiment. They also justify the conclusion that the differences in the number and duration of the SWD episodes between the ETOH and SUC groups in our experiments reflected a facilitation of the development of this form of activity by prolonged ETOH consumption rather than its suppression by SUC consumption.

It is worth emphasizing that in the ETOH group the increase in the SWD activity progressed after ethanol had been withdrawn from the diet; in fact the differences in the number of HVS episodes between the ETOH and SUC groups attained significance in the sessions performed during the third month after exposure. This may mean that the effect of ETOH develops slowly and that the changes which have been initiated or stimulated by ETOH, proceed after ETOH withdrawal.

If the incidence of the SWD episodes is a diagnostic marker that enables distinction between old and young brains (Buzsaki et al. 1988a), then after the exposure the brains of our rats from the ETOH group might be regarded as older than those of the rats of the SUC group. The existing evidence suggests that SWD's appear in response to rhythmic bursting discharges of the thalamic reticular nucleus (RTN) neurones, transmitted via thalamic relay nuclei to the cortex (see Gage et al. 1988, Avanzini et al. 1992). According to Gage et al. (1988) and Buzsaki et al. (1988b), the bursting activity of the RTN neurones is under control of the cholinergic neurones of the nucleus basalis magnocelullaris (NBM) complex. Consequently, the age-dependent deterioration (atrophic changes and cell loss) within the NBM complex is regarded by these authors as the main cause of the increase in the SWD activity in rats. The NBM complex neurones appear to be particularly vulnerable to ETOH. In rats given a 20% ETOH solution as the only drink for three months, Arendt et al. (1988) found out a marked reduction in the number of the cholinergic neurones and a decrease in choline acetyltransferase (ChAT) activity and acetylcholine (ACh) content in the NBM. These changes are similar to those found in aged rats (see Gage et al. 1988), which suggests that prolonged exposure to ETOH may accelerate the ongoing process of structural and functional deterioration within the NBM complex. This may explain why, judging by the number and duration of the SWD episodes, the brains of rats from the ETOH group appear older than those of the SUC group.

Summing up, the results of the present experiment show that prolonged exposure to a neurotoxin may influence the development of the age-related SWD activity after the exposure. In the light of the existing literature data, it may suggest an interaction of the neurotoxin with some age-related processes within the brain. Thus, analysing the SWD development during and after the exposure may be considered as a potential tool for detecting such interactions in the case of other neurotoxins.

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