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BEHAVIOURAL RESEARCH AND NONCONVULSIVE SPONTANEOUS ELECTROCORTICAL SEIZURES IN LABORATORY RATS. A NOTE OF CONCERN

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Abstract. A proportion of subjects of rat strains and lines commonly used for behavioural research in neurotoxicology, neuropharmacology and related fields show spontaneous generalized nonconvulsive EEG seizures. The seizures have the form of bursts of spike and wave discharges (SWD) reminiscent of human petit-mal epilepsy. Behavioural manifestations of SWD seizures are poor and, therefore, their occurrence is not taken into account either during selection or during grouping of rats for the experiment. This paper comprises a short discussion of literature data which allow one to suspect that the rats with SWD seizures differ from normal nonepileptic ones with respect to behavioural performance in some test situations and behavioural effects of exposure to drugs or other chemicals. Thus, considering the fact that the proportion of subjects with SWD seizures may vary considerably in rat samples, it seems likely that the broad use of rat populations heterogenous with respect to this EEG pathology may be responsible, at least in part, for inconsistent results of similar experiments performed at different laboratories. Therefore, attempts should be made in order to reduce the use of such rat population in behavioural research, particularly those intended to supply data which may consist the basis of hygiene standards of exposure to xenobiotics.

INTRODUCTION

In neurotoxicology and neuropharmacology, the primary goal of experimental studies is to supply information on whether and how the nervous system functions are being affected by exposure to a variety of chemical substances. For obvious reasons, such studies are performed on laboratory animals. Among the experimental tools, the neurobehavioural methods are regarded as particularly valuable not only for the detection but also for the characterization of neurotoxicity resulting from

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an exposure. It is generally believed that behaviour, the final product of coordinated action of many neural subsystems, constitutes a very sensitive index of the brain functional state. The NOAEL (no observable adverse effect level) and LOAEL (the lowest observable adverse effect level) values established in behavioural animal studies serve as the basis for setting occupational exposure limits. Therefore, it is essential to ensure that the data from such studies are reliable. However, quite frequently similar experiments performed at different laboratories (or even at the same laboratory) give conflicting results. When attempting to find causes of those discrepancies, one is usually concerned with the differences in the experimental procedure, technical conditions, data collection and analysis, strain, sex and age of the experimental animals. Less attention is paid to the possible differences in the quality of the experimental material. This may constitute an important, frequently overlooked, source of inconsistencies in experimental results.

The rat, *Rattus norvegicus*, is the main laboratory animal species. Today, there are nearly 150 inbred rat strains and numerous lines from outbred stocks. In majority of toxicological studies the use of outbred rats is preferred. Due to its genetic heterogeneity, such material is regarded to be a better model of the human general population than rats of inbred strains. High between-subject variability which may cause problems during statistical comparisons is an obvious disadvantage. The problem is solved to some extent by rejecting animals showing some functional abnormalities or excessively differing from the rest of the sample in some physical, physiological or psychological features. Some traits or pathologies, however, can be revealed only by specific, work-intensive and traumatic testing which are usually omitted and, therefore, neither the supplier nor the experimenter are aware that rats in a given sample are encumbered with such traits. The heritable tendency for spontaneous generalized nonconvulsive electrocortical seizures is one of such pathologies. According to the existing data, in rat samples from the commonly used rat populations: Wistar (38), Long-Evans (31), Sprague-Dawley (5), the proportion of animals with this pathology may vary from several to several tens per cent. There are good reasons to suspect that it may be an important cause of inconsistencies in results of experiments with behavioural endpoints.

FACTORS DETERMINING THE INCIDENCE OF SWD SEIZURES

The literature concerning factors determining the occurrence of spontaneous nonconvulsive electrocortical seizures in rats have been reviewed by Coenen et al. (7) and Marescaux et al. (19). Therefore, only the most important facts are discussed shortly below.

In the electrocorticogram of the afflicted rat, the seizure has a form of a burst of 7–10 Hz spike-wave discharges (SWD). The burst duration may vary from seconds to minutes. Behavioural manifestations of SWD are poor (immobile posture, fixed staring, vibrissal twitching) and usually pass unnoticed during a casual observation. The spontaneous occurrence of SWD seizures in laboratory rats, as well as in other rodent species, has been mentioned in more than 100 papers. However, many authors experimenting in behavioural neurotoxicology and related fields are unaware of this pathology in a proportion of subjects used for their experiments. Even if the SWD bursts are noticed in an experiment performed with the EEG control, they are regarded as an EEG artefact resulting, for example, from scratching.

The tendency to spontaneous SWD seizures is a heritable feature (SWD trait), determined by one autosomal gene with dominant pattern of inheritance (23). Inbred rat strains homogenous with respect to this trait have been obtained in some laboratories (7,19). The strains with spontaneous SWD seizures (SWD rats) are regarded as animal models of the human petit-mal epilepsy (7,38) and used in studies on epileptogenesis as well as for testing the pro- and antiepileptic potential of drugs. To date, rat strains free of the SWD trait (nonSWD rats) have not aroused a broad interest among behavioural scientists.

In the SWD rats, spontaneous SWD seizures start to appear at 40–120 days of age and increase in the number and duration as the animal gets older (2,4,6,39). Thus, the older the rats selected for the experiment, the greater the chance of a higher proportion of the animals with seizures in particular groups and the more intense the SWD activity. The incidence of SWD seizures is strongly related to the level of arousal; the seizures occur preferably during a state of relaxed immobility and are absent during active movements, slow wave sleep and paradoxical sleep (9,13,37). It means that in a behavioural experiment, the task requirements will determine the frequency of SWD seizures.

SWD SEIZURES AND BEHAVIOUR

Considering the widespread use, in behavioural studies, of rat populations heterogenous with respect to the SWD trait, it is essential to explain the relationship between this trait and the rat behavioural performance. As stated earlier, without an EEG control, the experimenter is not able to distinguish between the SWD and nonSWD rats. Thus, the groups may differ in the proportion of the SWD rats. In the extreme case the SWD rats may dominate in one group whereas the nonSWD ones may prevail in another. In case the proneness to SWD seizures and the seizures themselves were not related to behaviour, unequal distribution would not matter. If that is not the case, however, the groups are incomparable and the results confusing. Even when the proportion of the SWD rats in each group is similar, the within-group variability of some behavioural parameters may overshadow the effect of the studied factor in statistical comparisons.

A relationship between the SWD trait and behavioural capabilities is indirectly suggested by some neurochemical data. According to the published reports, animals genetically predisposed to SWD show an impaired $GABA_A$ system in superficial layers of the frontoparietal cortex and in the anterior thalamic areas (18,35) and possibly a hyperactive $GABA_B$ system (16). There is an evidence that GABAergic mechanisms are involved in a variety of behaviours, including ingestive, sexual and agonistic behaviour, anxiety response, motor functions, learning and memory, some physiological functions, and perhaps in several mental disorders (1,22). The SWD rats show also some alterations in synaptosomal neurotransmitter amino acid content (33), and increased sensitivity of the cortical glutamatergic system (25), i.e. the system involved in motor control (the corticostriatal glutamatergic pathway), synaptic plasticity, learning and memory, and neuronal death (8,24,32). The rats are also characterized by altered metabolism in some brain regions (20,21), an increased level of some neuropeptides in several subcortical and cortical areas (29), a lowered density of adenosine receptors in the thalamus (11), and changed sleep parameters (12). Adenosine is an important regulatory substance, which mediates several

biological functions, such as neurotransmitter release, spontaneous neuronal firing and synaptic transmission (27,28). It also has a regulatory effect on the sleep-wake cycle (26), and sleep was reported to be related to cognitive functions (36).

Data from behavioural studies on SWD behaviour relationship are not univocal. In two cases, the SWD and nonSWD rats were compared for the behavioural performance in selected test situations. Individual differences in the level of the SWD activity and the presence or absence of the seizures during testing were not controlled in either of the two studies. In one of these studies, the SWD and nonSWD rats came from the same heterogenous population (2). The results showed that, compared to nonSWD rats, the SWD rats were impaired in passive avoidance and spatial alternation. In the other study (40), the SWD and nonSWD rats came from inbred strains homozygous with respect to the SWD trait. In that study, the rats of a strain with inherited tendency towards SWD generation (Genetic Absence Epilepsy Rats from Strasbourg – GAERS) appeared behaviourally unimpaired or even better in some test situations than rats of a nonepileptic control strain. The authors have concluded that the SWD rats are not impaired in usual behaviours, such as spontaneous activity, exploration, feeding, social interaction or learning of positively or negatively reinforced tasks. It is worth noting, however, that improved performance in tests in which the GAERS appeared better – the two-way active avoidance in a shuttle box and a bar pressing for food in a fixed ratio (FR) schedule – characterizes rats with lesions of some limbic structures (14).

Neurobehavioural consequences of ongoing SWD activity were investigated in several studies. Electrophysiological observations showed that both primary and secondary components of visual evoked potentials were differentially disrupted during SWD (15). In rats pressing a lever for food according to a fixed (60 s) interval schedule, appearance of SWD prolonged the postreinforcement pause and the prolongation was usually longer than the SWD burst itself (37). In an instrumental discrimination task, when a discriminative auditory stimulus was presented during the ongoing SWD activity, the instrumental conditioned response did not appear before the end of the SWD burst, and the response accuracy was markedly reduced (10). The authors have concluded that SWD discharges might result in a dysfunction in more advanced stages of information processing that affect motor response programming and response accuracy after the SWD. It was also assumed that SWD might induce amnesia similar to, although milder than, the amnesia induced by electroconvulsive shock (37). If so, then in situations of learning, SWD bursts occurring during trials or during intertrial intervals would disrupt learning. However, the occurrence of SWD is strongly related to the level of arousal (see above). In many test situations used commonly in behavioural studies, the task demands require a level of arousal incompatible with the SWD. Thus, there may be no differences in performance between rats with and without SWD as, for example, in studies of Vergenes et al. (40). However, occasional occurrence of SWD bursts, and hence behavioural impairment, seem quite likely in the afflicted rat during performance of a nonstressful task in a well known experimental setting.

Usually the SWD rats differ considerably between themselves in the incidence of SWD bursts and their duration. Thus, it would be reasonable to try and find a correlation between the level of SWD activity characterizing a given animal and its behavioural performance. We have not found literature data concerning this

problem directly. In one of our studies (in preparation) rats from our own breeding colony were tested for their response to novelty, accuracy of finding water reward in a radial maze, passive avoidance and hot-plate behaviour before and after a footshock. Then, they were implanted with intrabrain electrodes and the level of SWD activity (the number of episodes and the total duration of SWD during a 3-h recording session) were assessed in each animal. A subsequent correlation analysis has revealed that the number of perseveration errors in a radial maze was positively, and the exploratory response to a new object negatively correlated with the level of the SWD activity. On the other hand, no correlation was found between SWD activity and passive avoidance performance, as well as the latency of the paw-lick response to heat before or after a footshock. The results suggest a relationship between the rat behaviour in some test situations and the level of the SWD activity.

The data presented above address possible differences in behaviour of SWD and nonSWD rats not subjected to any stressors other than the task demands. In neurotoxicology and neuropharmacology, the main objective of behavioural experiments is to find out whether and how exposure to a given substance influences the animal behaviour in a given test situation and what is the dose-effect--relationship. In case of many chemicals, the neurobehavioural effects of exposure result from a direct or indirect interaction of the substance with neurotransmitter systems. It seems obvious that a behavioural effect resulting from the activation of a neurotransmitter system would be less pronounced in rats with subsensitivity of this system, and vice versa. The variable interactions between neurotransmitter systems may make this relationship more complex. As mentioned earlier, the SWD rats differ from the non SWD ones in the activity of the GABAergic, glutamatergic, peptidergic, and adenosinergic systems. Thus, it seems reasonable to suspect that in the SWD and nonSWD rats, the behavioural effects produced by exposure to many xenobiotics may differ quantitatively if not qualitatively. According to our knowledge, published reports from studies dealing with this problem are not available. It does not mean, however, that such differences do not exist.

CONCLUSIONS

To sum up, the data presented above allow one to suspect that the SWD trait is somehow related to the rat behaviour and may quantitatively or qualitatively modify the behavioural consequences of exposure to chemical substances or other factors affecting the brain functions. Considering that the rat populations may differ in the proportions of animals with and without SWD seizures, and that the experimenter is usually unable to separate the afflicted rats from the rest of the sample during the selection of animals and group formation, it seems quite likely that the use of rat populations heterogenous with respect to the SWD trait may be responsible, at least in part, for the inconsistencies in the results obtained by different laboratories. Therefore, although there is no conclusive evidence in support of the relationship between the SWD trait and behaviour, attempts should be made to reduce the use of such rat populations in behavioural research, especially those intended to supply data which may be extrapolated to the general human population. In the general human population, the prevalence of epilepsy is estimated to be about 1.0%, while the corresponding number for the absence epilepsy is about 0.003% (29).

In the outbred or random bred rat stocks, which serve as the main source of experimental material for behavioural (and other) studies, the proportion of SWD rats may vary from several to more than eighty per cent. Is it reasonable to think that a sample taken from any such rat population represents a valid model of the general human population?

As stated above, SWD-homogeneous rat lines have been obtained in some laboratories by selective cross-breeding. Some authors regard SWD rats as animal models of human absence epilepsy and use them in assessment of anti- and proepileptic properties of drugs. In neurotoxicology, these rats might find a similar use. Studies performed in several laboratories supplied data concerning the mechanism of the spontaneous SWD seizures in the rat (3,17,34). Therefore, a change in the level of the SWD activity resulting from exposure to a chemical may serve as an important clue as to the presence or absence of an effect, as well as to the possible mechanism of action of that substance. However, the SWD rats do not seem to be appropriate material for studies in which the SWD activity is neither the matter of interest nor serves as an index of the effects produced by the factor under study. For such studies, the use of rats from lines free of the SWD trait seem to be most appropriate.

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