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CHANGES OF SLEEP CYCLES AND EEG IN RATS CHRONICALLY EXPOSED TO TOLUENE VAPOR

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ABSTRACT

Workers exposed to toluene often develop central nervous symptoms. In the present experiment, changes of the sleep cycle and EEG were studied in order to make clear the effects of toluene on the central nervous system. Male albino rats of the Wistar strain were daily exposed to 2000 ppm toluene vapor for 4 hours (from 4:00 to 8:00 pm) during 24 weeks. Electrodes were permanently implanted in the rats in order to ensure continuous recording of biopotentials. The biopotentials and behavior of the rats were observed from 0:00 to 4:00 p.m. just before the first exposure, and from 0:00 to 8:00 p.m. after 4, 12 and 25 weeks of exposure.

The sleep cycle was divided into five phases according to the cortical and hippocampal EEG, the cervical EMG and behaviour. The wakeful phase increased and the slow-wave decreased but the paradoxical phase did not change in the exposure group during sleeping time (from 0:00 to 4:00 p.m.). The reaction to toluene exposure changed in the exposure group during the exposure time (from 4:00 to 8:00 p.m.) while the paradoxical phase increased slightly more during exposure than before exposure time. The sleep cycle in the exposure group was often interrupted while the duration time of the slow-wave decreased after 4, 12 and 24 weeks.

The EEGs of the cortex and hippocampus were continuously monitored by an EEG frequency analyser. The EEGs were recorded on magnetic tape and afterwards analysed in power spectrum by means of a signal processor. The fast components in cortical EEG increased more in the exposure group than in the control group during the exposure time after 4 and 12 weeks, while the component increased before the exposure time after 24 weeks. The θ component decreased in the exposure group during the exposure time after 4 and 12 weeks, but increased after 24 weeks.

These results suggest that toluene may play an important part in the cause of the sleep disturbance in workers exposed to organic solvents and that toluene exposure may lead to changes in the components of EEG. These results are believed to coincide in some degree with clinical observations.

Workers chronically exposed to organic solvents were reported to have many complaints regarding their central nervous system and autonomic nervous system, such as headache, headheaviness, giddiness, easy fatigability, sleep disturbance, etc. However, it is very difficult to detect the effects of organic solvents by means of the usual clinical examinations of blood or urine, even when the workers have apparent subjective symptoms of the central nervous system. Thus, some appropriate methods which could objectively detect the effects of organic solvents on the central nervous system seem to be necessary

from the point of view of occupational hygiene. There are some reports regarding the EEG of workers exposed to organic solvents, and EEG is regarded as useful means for detecting the effects of organic solvents on the central nervous system[2–9]. However, the relationship between changes in EEG and the exposure to organic solvents has not yet been sufficiently clarified. The present experiment was carried out to make clear the effects of organic solvents on the central nervous system by exposing rats to toluene, one of the most widely used organic solvent which produces comparatively strong effects on the central nervous system.

METHODS

The experiment was made on 14 male albino rats of the Wistar strain. Electrodes were permanently implanted in the rats to ensure continual recordings of EEG, EMG and the pulse rate. The locations of the recording electrodes in the rat skull and hippocampus were the same as shown in a previous report[10]. The EEGs of the cortex and hippocampus were continuously monitored by an EEG frequency analyser (MF-5; Nihon-Koden). The EEGs were recorded on magnetic tape and subsequently analysed by a signal processor (7TO7; Sanei--Sokki). The behavior of the rats during the experiment was observed on a monitor screen. Seven rats in the exposure group were daily exposed to 2000 ppm toluene vapor in the exposure chamber for 4 hours (from 4:00 to 8:00 p.m.) during 24 weeks, while another 7 rats were used as control. The rats in the control group, although confined to the same type of chamber as was that of the exposure group, breathed fresh air only, except on observation days. Some miniature sockets on the rat skulls were removed in the course of the experiment. After 12 weeks 6 rats of the exposure group and 5 rats of the control group, and after 24 weeks only 5 rats of the exposure group and 3 rats of the control group could be examined. The biopotentials, sleep cycle and behavior were observed from 0:00 to 4:00 p.m. just before the first exposure, and from 0:00 to 8:00 p.m. after 4, 12 and 24 weeks' exposure. The rats of the control group were exposed to 2000 ppm toluene in the same chamber as the exposure group, from 4:00 to 8:00 p.m. only on observation days in order to compare the reactions of the two groups.

RESULTS

The sleep cycles were divided into five phases according to the EEG, the EMG of cervical muscles and the behavior of the rats, i.e. wakeful phase (W), spindle phase (S), slow-wave phase (SW), preparadoxical phase (PP) and paradoxical phase (P). Details of the classification of sleep cycles were shown in an earlier report[10]. The percentage of each phase was found to change as shown in Figure 1. Before the first exposure, the average percentages of the phases were 16.6% of the wakeful, 11.1% of the spindle, 53.4% of the slow-wave, 6.7% of the preparadoxical and 12.3% of the paradoxical phase in the exposure group. There was no statistically significant difference between the exposure group and the control group. After the exposure started, during deep-sleep time (from 0:00 to

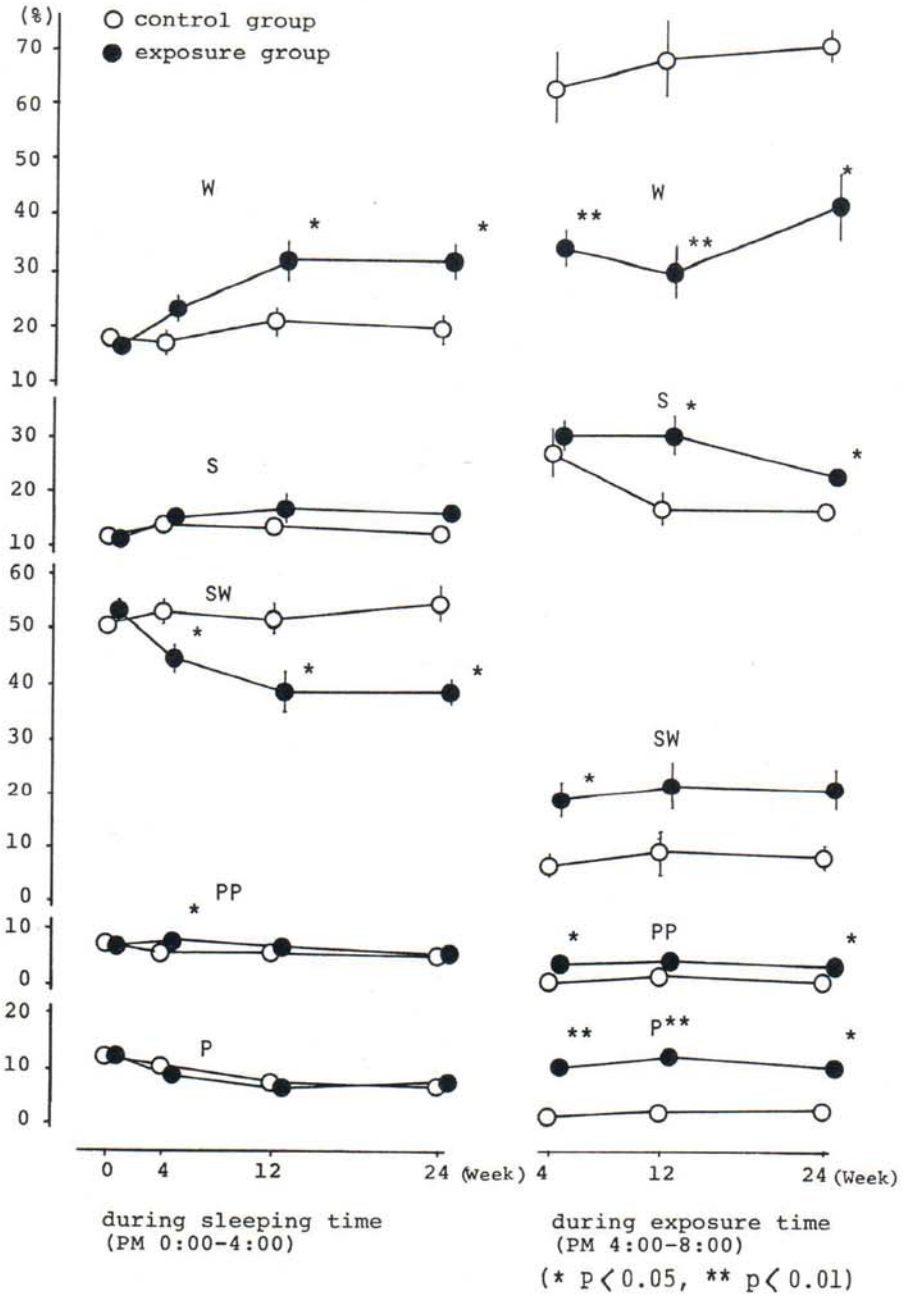

FIG. 1 – Changes in the percentages of the sleep cycle.

4:00 p.m.), the wakeful phase gradually increased in the exposure group while the percentages of the wakeful phase were significantly higher than those of the control group after 12 and 24 weeks. The slow-wave phase gradually decreased in the exposure group, and after 4, 12 and 24 weeks the percentages of the slow--wave phase were significantly lower than those of the control group. However, in the control group the percentages of all the phases showed only a slight change throughout the 24 weeks. During exposure time (from 4:00 to 8:00 p.m.) the percentages of the wakeful phase were much lower in the exposure group than in the control group. However, the percentages of the other phases were higher in the exposure group than in the control group, especially the percentages of the paradoxical phase which were much higher in the exposure group than in the control group.

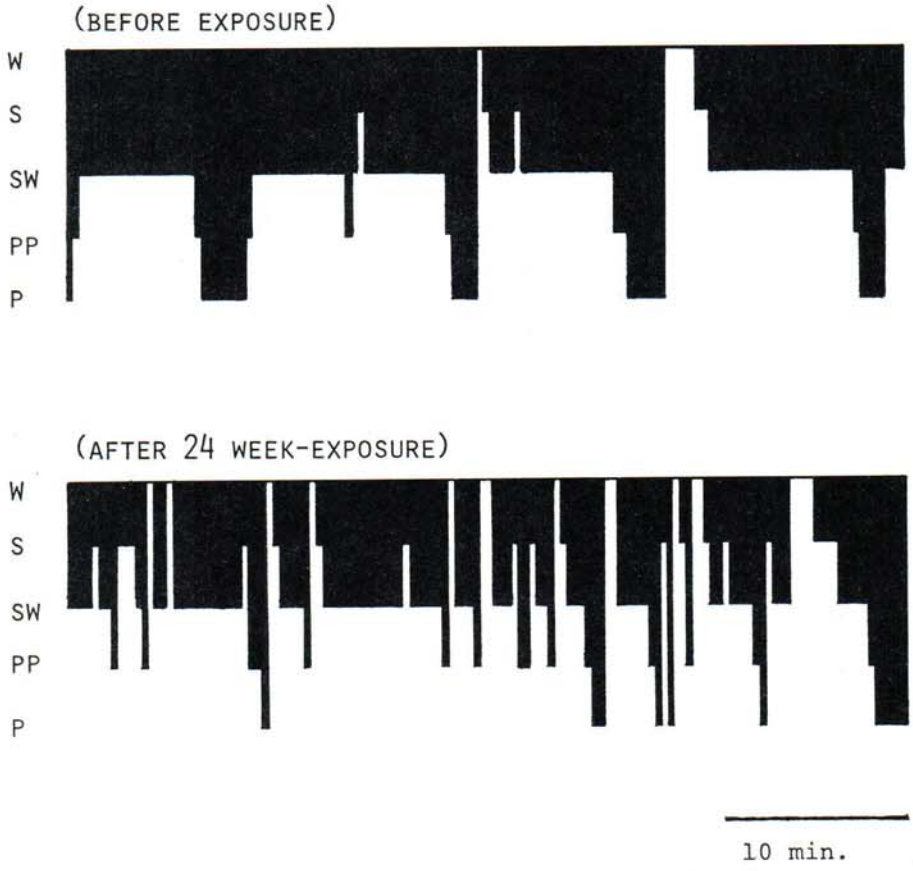

FIG. 2 – Interruption of the sleep cycle in the rat exposed to 2000 ppm toluene for 24 weeks. (w: wakeful, s: spindle, sw: slow-wave, pp: preparadoxical, p: paradoxical)

The sleep cycle of the rats before the exposure started, smoothly changed from the wakeful to the spindle, the slow-wave, the preparadoxical and the paradoxical phase. However, it appeared disturbed after exposure to 2000 ppm toluene. Figure 2 shows a sample of the sleep cycle in the exposed rats. The sleep cycle before exposure was smooth and rhythmical but after 24 weeks of exposure it often appeared interrupted. After 4 and 12 weeks of exposure the sleep cycles were found interrupted in a similar way. Figure 3 shows changes of the duration time of the slow-wave phase as an index of the disturbance of the sleep cycle. The duration time of the slow-wave phase in the exposure group decreased after the exposure started, while that in the control group showed only a slight change. The differences in the duration time of the slow-wave phase between the exposure group and the control group were statistically significant after 4, 12 and 24 weeks.

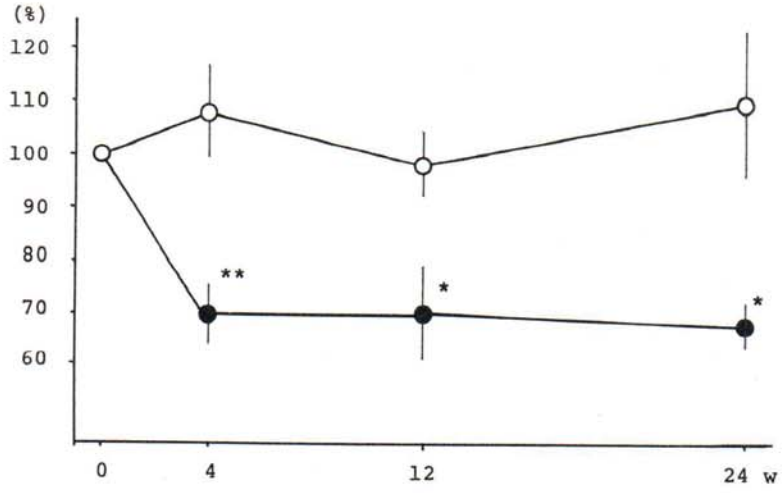

FIG. 3 – Changes of duration time of slow-wave phase. ○ control group, ● exposure group; *$p < 0.05$, **$p < 0.01$.

EEGs were analysed at 10-second intervals by means of an EEG frequency analyser. Figure 4 shows the changes in energy percentages of each frequency band of EEG in the wakeful phase. There was no statistically significant difference between the two groups before the exposure started. After 4 weeks' exposure, the θ component significantly decreased while the β_1 and β_2 components significantly increased in the cortex during exposure time (from 4:00 to 8:00 p.m.) in comparison with those of the control group. After 12 weeks of exposure the δ_1 component in the exposure group was significantly greater than that in the control group. The θ component significantly decreased while

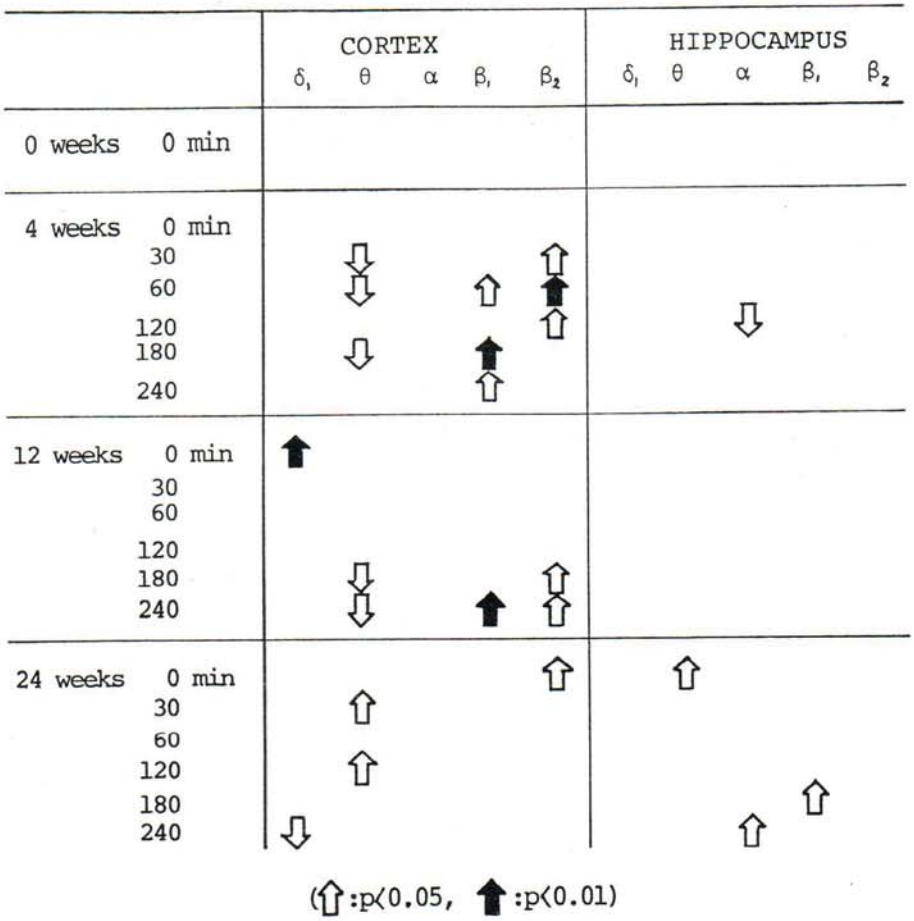

FIG. 4 – Changes in energy percentages of each frequency band of EEG in comparison with the control. (δ_1: 2–4Hz, θ : 4–8Hz, α: 8–13Hz, β_1: 13–20Hz, β_2: 20–30Hz).

the β_1 and β_2 components significantly increased in the cortex during the exposure time (from 4:00 to 8:00 p.m.) in comparison with those of the control group. After 24 weeks of exposure, the β_2 component in the cortex and the θ component in the hippocampus in the exposure group significantly exceeded those in the control group before exposure. The θ component significantly increased, while the β_1 component significantly decreased in the cortex, and the α_1 and β_1 components significantly increased in the hippocampus during exposure. Figure 5 shows changes in the power spectra of the EEG analysed by means of a signal processor during exposure time (from 4:00 to 8:00 p.m.) after 12 weeks' exposure. In both groups the fast components remarkably increased

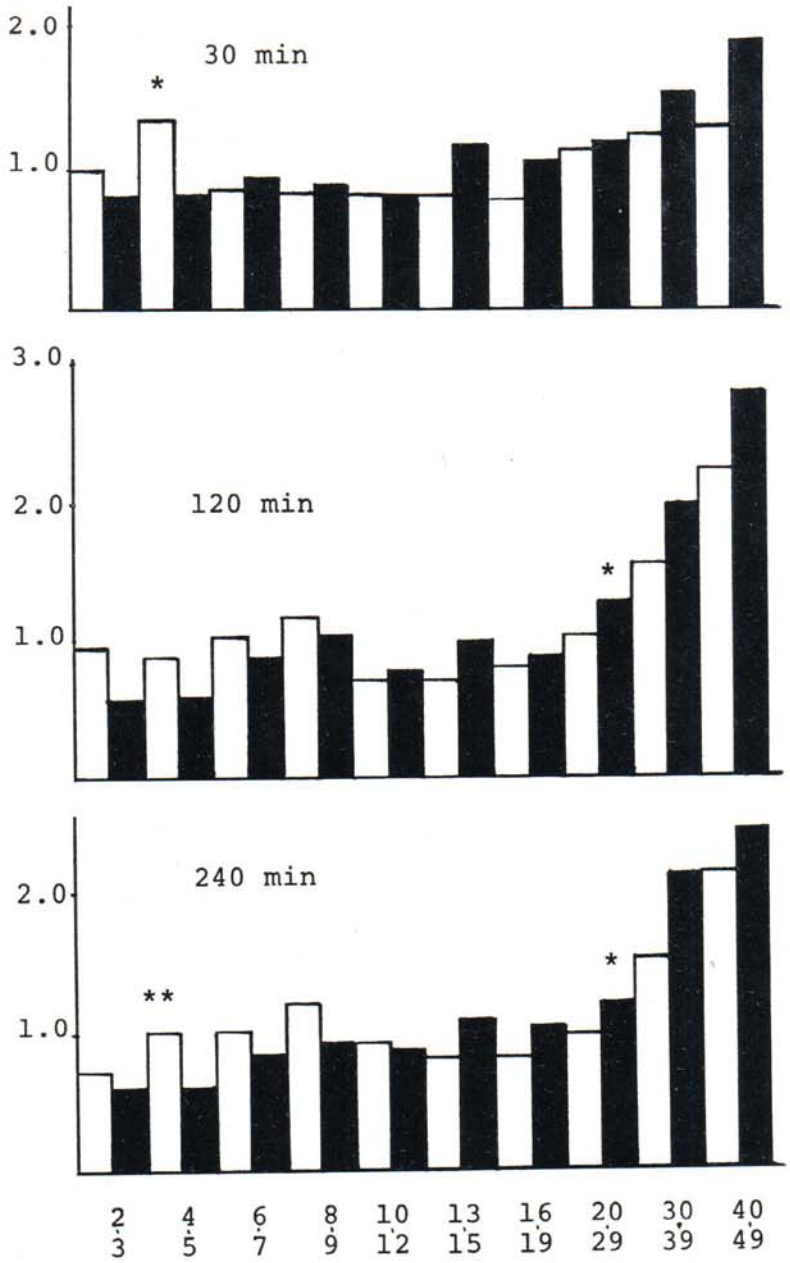

FIG 5. – Changes in energy percentages of each frequency band of EEG by exposure of toluene after 12 weeks (cortex). Open bars: control, solid bars: exposure group.

during exposure. The increase of the power spectra in the fast components (>20 Hz) was greater in the exposure group than in the control group and the differences between the two groups were statistically significant in 20–29 Hz after exposure for 120 min and 240 min. The slow components (5 Hz) gradually decreased in the exposure group and the differences between the two groups were found statistically significant in 4–5 Hz after 240 min of exposure. The changes of the components of EEG had the same tendency after 4 and 24 weeks as those after 12 weeks but there was no statistically significant difference between the two groups.

DISCUSSION

As regards changes in the sleep cycle, the wakeful phase increased while the slow-wave phase decreased but the paradoxical phase did not change in the exposure group during sleeping time (from 0:00 to 4:00 p.m.). The reaction to toluene exposure changed during exposure time (from 4:00 to 8:00 p.m.) in the exposure group while the paradoxical phase was found to increase a little more during exposure than before exposure time. The sleep cycle in the exposure group was often interrupted by the wakeful or spindle phase, while the duration time of the slow-wave phase decreased after 4, 12 and 24 weeks. These results suggest that toluene may play an important part in the cause of the complaints of sleep disturbance in workers exposed to organic solvents[1,5].

As regards changes in EEG, the fast components in cortical EEG increased more in the exposure group than in the control group during exposure time after 4 and 12 weeks, while the β_2 component increased before exposure time after 24 weeks. The θ component decreased in the exposure group during exposure time after 4 and 12 weeks but increased after 24 weeks. The β_1 component increased before exposure time after 12 weeks and decreased during exposure time after 24 weeks. These results suggest that toluene exposure may lead to changes in the components of EEG. There exist clinical reports on changes in EEG in workers exposed to organic solvents. Hirano[4] reported that in cases of chronic intoxication with organic solvents, the basic wave becomes fast in medium--degree poisoning while slow basic waves, slow wave burst, spike waves etc. appear in high-degree poisoning. Mabuchi and co-workers[7] reported that abnormal basic waves were found in 30 cases out of 42 patients intoxicated with organic solvents, and that the abnormal basic waves were as follows: 11 diffuse irregular α wave, 10 irregular α wave mixed with fast wave, 6 low voltage fast pattern, 2 high voltage α and 1 low voltage diffuse slow wave. The present experiment suggests that in the early stage (after 4–12 weeks) of intoxication the fast wave may increase and the slow wave may decrease, while in the later stage (after 12–24 weeks) the slow wave may increase in the cortex. These results are believed to coincide in some degree with the clinical observations mentioned above.

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