Quantitative Study of Behavioral Disturbances in Rats Exposed to High Pressure

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When human divers or experimental animals are exposed to high pressure, they develop the High Pressure Neurological Syndrome (HPNS). Male Sprague-Dawley rats were exposed to high pressure in a conventional helium-oxygen breathing mixture to 80 bars. Pressure-induced behavioral motor disturbances including hyperlocomotor activity (HLA), tremor and myoclonia were monitored with a noninvasive piezoelectrical sensor device enabling a without discontinuity long-term analysis. New data were obtained on the development of the HPNS behavioral motor disturbances. Indeed, the present results suggest myoclonia would be more sensitive to constant high pressure exposure, while HLA and tremor would be more sensitive to increasing pressure. Moreover, myoclonia were found to occur significantly later in rats which developed epileptic seizures than in other. The present results constitute the quantitative basis of HPNS motor disturbances for future pharmacological pressure experiments.


Key words: High pressure neurological syndrome, Behavioral motor disturbances, Quantitative analysis

When human divers and experimental animals are exposed to high pressure, they develop the high pressure neurological syndrome (HPNS). The principal symptoms of HPNS include electroencephalo- graphic changes, sleep disturbances, tremor and myoclonia (Brauer et al, 1969; Fructus et al. 1969; Bennett and Towe 1971). Animals develop also hyperlocomotor activity (HLA) and for higher pressure epileptic seizures (For review, Halsey, 1982). In man, HPNS include other problems of motor coordination, disorientation, nausea, and loss of attention (For review, Lemaire and Rostain, 1988).

In most cases, behavioral motor disturbances of HPNS in free moving animals and also in restrained animals were generally visually estimated only by their onset pressure. Unfortunately, this method requires that one individual makes all the observations; also it is difficult to quantify motor disturbances by observation alone. However, several authors using noninvasive techniques have successfully performed quantitative analysis of one or more behavioral symptoms of HPNS. Indeed, interesting approaches using a mechanical transducer, a magnetic induction device, and small strain gauges were developed by Walker et al. (1977), Ackerman and Gruenau (1978), and Baker et al. (1981) respectively. Moreover, in a recent study we developed a new computerized device which enables, in free-moving rats exposed to high pressure, a without discontinuity long-term analysis of the behavioral motor disturbances of HPNS including hyperlocomotor activity (HLA), tremor, and myoclonia (Tomei et al. 1991).

We report here pressure experiments which were performed to be used as basic quantitative measures on HPNS behavioral motor disturbances for future
pharmacological studies.

METHODS

Male Sprague-Dawley rats weighing 350-450 g at time of the experiments were used (n=16). Rats were maintained on a 12-12 hour light-dark regime with lights on from 7:00 a.m. to 7:00 p.m.

The free moving animals (2 per pressure exposure run/test/trial) were placed in separate altuglass cylinders in a 50-liter pressure chamber (maximum pressure 200 bars). Rats were compressed with helium at a rate of 1 bar/min. Oxygen was maintained at a constant partial pressure of 0.4 bar which is the partial pressure generally used in human dives. Humidity was controlled and temperature progressively increased throughout the experiment from 25 to 34 °C, to prevent hypothermia because of the specific heat of helium as compared to air. Decompression was at a rate of 0.06 bar/min from 80 bars to 12 bars and 0.04 bar/min from 12 bars to surface. During decompression, partial pressure of oxygen was 0.5 bar. Animals were divided into 2 groups which were exposed to pressure as follows: (a) to 80 bars with a 4-h stay at this pressure (n=8); (b) until one of the two animals exposed to high pressure during each pressure experiments run/test/trial developed an epileptic seizure: stay at the maximal pressure: 20 min (n=8).

Behavioral analysis was performed as described previously by Tomei et al. (1991). Principles of analysis can be summarized as follows: behavioral motor disturbances of HPNS were obtained from piezoelectrical sensors which were fixed under the floor of each altuglass cylinder; signals were analysed, after amplification, on a PC-AT compatible computer and decomposed on line in HLA, tremor and myoclonia. HLA was calculated as the whole signal minus tremor and myoclonia; tremor was obtained from the 10-16 Hz signals since this symptom has been found to be characterized on this band frequency by several authors (Walker et al. 1977; Ackerman and Gruenau 1978; Baker et al. 1981). Myoclonia were detected as signals of unusual high amplitude with a threshold of detection adjustable for each rat. The values of HLA, tremor, and myoclonia were expressed in arbitrary units (U).

Results were analysed using median value ± 25th-75th percentiles, and nonparametric statistical tests (Mann-Whitney U test, and Wilcoxon paired T test) (Siegel and Castellan 1988).

RESULTS

Compression at 80 bars

Rats compressed to 80 bars showed all the behavioral motor disturbances of HPNS, except epileptic seizure. HLA occurred at 38 bars (25th-75th percentiles: 29-47 bars) and the maximum value recorded for HLA was 112 U (p<0.005, W paired T test) (Fig. 1). The onset pressure and maximum value of tremor were respectively 51 bars (25th-75th percentiles: 47-54 bars) and 208 U (p<0.005, W paired T test) (Fig. 2). Myoclonia were found to occur at 73 bars (25th-75th percentiles: 65-78 bars)

![Fig. 1](image)

Quantitative analysis of hyperlocomotor activity in free-moving rats exposed to high pressure (80 bars). Left: compression up to 80 bars; duration 1 h 20 min. Middle: 4-h stay at 80 bars. Right: decompression from 80 bars to surface; duration 24 h. Y-axis: hyperlocomotor activity expressed in arbitrary units, median value obtained from n = 8 rats. X-axis: pressure expressed in bars. Hyperlocomotor activity during compression can be seen <p <0.005, W paired T test).
with a maximal value of 40 U (p<0.005, W paired T test) (Fig. 3). Myoclonia appeared later than HLA and tremor (p<0.01, W paired T test).

When compression was stopped, HLA, tremor and myoclonia decreased (p<0.01, W paired T test) but continued during the entire 4-h stay. However myoclonia were found to decrease only 1-2 h after

HLA and tremor (p<0.01, W paired T test).

Behavioral symptoms of HPNS disappeared during decompression (HLA and tremor: 76-75 bars; myoclonia: 65-64 bars).

Compression until epileptic seizure

In animals which presented no epileptic seizure

Fig. 2 Quantitative analysis of tremor in free-moving rats exposed to high pressure (80 bars). Left: compression up to 80 bars: duration 1 h 20 min. Middle: 4-h stay at 80 bars. Right: decompression from 80 bars to surface; duration 24 h. Y-axis: tremor expressed in arbitrary units, median value obtained from n = 8 rats. X-axis: pressure expressed in bars. Tremor during compressin can be seen (p<0.005, W paired T test).

Fig. 3 Quantitative analysis of pressure-induced myoclonia in free-moving rats exposed to 80 bars. Left: compression up to 80 bars; duration 1 h 20 min. Middle: 4-h stay at 80 bars. Right: decompression from 80 bars to surface; duration 24 h. Y-axis: myoclonia were expressed in arbitrary units, median value obtained from n = 8 rats. X-axis: pressure expressed in bars. Pressure-induced myoclonia can be seen (p<0.005, W paired T test).

Fig. 4 Quantitative analysis of behavioral symptoms of HPNS in free-moving rats from 30 min before epileptic seizure to 30 min after seizure. Left: in rats with epileptic seizure (n = 4). Right: in rats without epileptic seizure (n = 4). Behavioral symptoms were expressed in arbitrary units using median value and the 25th -75th percentiles. The arrow indicates the time of the epileptic seizure (pressure of occurrence: 95 ± 4 bars). Intensity of behavioral symptoms was compared between rats which developed epileptic seizure and rats which did not ( *p<0.05, U test).
(n=4), onset pressure of HLA was found to be 50 bars (25th-75th percentiles: 37-53 bars). Maximal value of HLA was 174 U (25th-75th percentiles: 85-254 U). Tremor occurred at 51 bars with the 25th-75th percentiles of 46-62 bars. The maximum value recorded for tremor was 216 U (25th-75th percentiles: 0-398 U). Myoclonia occurred at 68 bars (25th-75th percentiles: 61-71 bars). The maximum value recorded for myoclonia was 130 U (25th-75th percentiles: 0-150 U).

In rats which developed epileptic seizure, onset pressure of seizures was 95 bars with the 25th-75th percentiles of 90-99 bars. Onset pressure and maximum value of HLA were respectively 66 bars (25th-75th percentiles: 58-69 bars) and 158 U (25th-75th percentiles: 86-188 U). Tremor occurred at 48 bars (25th-75th percentiles of 45-53 bars). At time of seizure, the maximum value of tremor was 269 U (25th-75th percentiles: 142-284 U). Myoclonia appeared at 77 bars (25th-75th percentiles: 75-79 bars). The maximum value recorded for myoclonia was 55 U (25th-75th percentiles: 40-150 U). Myoclonia occurred at significantly higher pressures in rats which developed an epileptic seizure (n=4) that in rats which did not (p<0.02, U test).

After compression was stopped, i.e., after epileptic seizure occurred, HLA and myoclonia but not tremor were found to be significantly depressed in rats which developed an epileptic seizure as compared to rats which did not (U test: HLA, p<0.05; tremor, p<0.1, n.s; myoclonia, p<0.05) (Fig. 4).

**DISCUSSION**

The present study was designed to quantitatively assess the behavioral motor disturbances which occur in rats exposed to high pressure. Indeed, in most cases the severity of HPNS has often been estimated, in free-moving animals, only by the onset pressure of the behavioral symptoms of HPNS. Recently, Wardley-Smith et al. (1990) have visually scored disturbances of HPNS in rats exposed to high pressure. Interest of such an approach is evident, particularly for pharmacological experiments. Nevertheless in these conditions of analysis the severity of HPNS is subjective, whereas the present device that we used enables a computerized and without discontinuity long-term analysis of the HPNS' behavioral motor disturbances (Tomei et al. 1991). During our hyperbaric experiments, all the behavioral motor disturbances of HPNS were found to occur. The onset pressures and the developments of the HPNS motor disturbances that we recorded in rats exposed to high pressure were found to be in total agreement with previous data (for review: Rostain 1981; Halsey 1982). Indeed, HLA, tremor, and myoclonia were found to occur during the compression phase of the pressure exposure and then progressively decreased during the stay and decompression phases. This confirms and extends that the behavioral motor disturbances which occur under high pressure are directly in relationship with the increase of pressure (Rostain et al. 1974; Brauer et al. 1975). As suggested by Brauer et al. (1974) the present results also confirm, that for a given species and for a given rate of compression, tremor is a useful variable to monitor HPNS because of its very reproducible onset pressure. However, although the present experiments essentially confirm previous other data, this is the first report to our knowledge to clearly demonstrate that myoclonia both occur and decrease at around the same range of pressure (65-75 bars), at significantly higher pressure than HLA and tremor. These results suggest that the development of myoclonia would be sensitive to constant high pressure, while HLA and tremor would be more sensitive to increasing pressure. Such an hypothesis seems to be confirmed since myoclonia have been found to occur in the same range of pressure despite the use of fast rate of compression of 3 bars/min (Rostain et al. 1986). Moreover, as described previously, HLA was found to progressively occur as an ambulatory activity (Tomei et al. 1991). Since striatal dopamine release been found to be increased in animals exposed to...
high pressure (Forni and Rostain 1989), and the nigro-striatal pathway is well known to be involved in the extrapyramidal locomotor activity, the use of HLA in hyperbaric experiments could be of great interest for further studies in the field of behavioral neurochemistry and pharmacology.

During experiments performed until an epileptic seizure occurred, no significant differences were found during compression, i.e. before epileptic seizure, in the occurrence and the development of the HPNS motor disturbances between rats which developed epileptic seizures and rats which did not, except for myoclonia. Indeed, myoclonia were found to occur at significantly higher pressures in rats which developed an epileptic seizure than in rats which did not. At the present time we have no physiological explanation for this fact despite the consistency of these behavioral observations (p < 0.02). Alternatively, after epileptic seizure occurred HLA and myoclonia, but not tremor, were found to be significantly depressed in rats which developed seizures as compared to rats which did not. This result suggest that the brain disturbances involved in tremor would be essentially different than those involved in HLA, myoclonia, and epileptic seizures.

In conclusion, the present study confirms that the piezoelectrical sensor device enables without discontinuity long-term behavioral analysis in rats exposed to high pressure. Our results confirm and extend previous data on HPNS. However, this is the first time to our knowledge to clearly demonstrated: (a) myoclonia appear and disappear in the same range of pressure at around 70 bars; (b) myoclonia occur at significantly higher pressures in rats which developed seizures than in rats which did not; and (c) HLA and myoclonia, but not tremor, are significantly depressed in rats which developed an epileptic seizure. However, further experiments are needed to determine the mechanisms of the behavioral and neurochemical disturbances of HPNS, and therefore the present results constitute the quantitative basis of these future phar-

macological pressure experiments in the field of behavioral neurochemistry.

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