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Reversal of pressure-induced tremors in rats by step decompression and by inert gases

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Bell PY, Macdonald AG. Reversal of pressure-induced tremors in rats by step decompression and by inert gases. Undersea Biomed Res 1984; 11(1):25–36.—A method for continuously recording the motor activity of pressurized rats was used to monitor their condition after 1) holding pressure constant after compression, 2) decompression, and 3) adding nitrogen prior to holding pressure constant. After procedures 1 and 2 the logarithm of activity plotted linearly against time in two distinct phases. A short initial decline was followed by a slower, longer decline that was incomplete after 5 h. The rate of the second, slower decline was inversely related to the holding pressure and unaffected by prior convulsions. The addition of 4.8 atm N_2 was very effective at decreasing the activity at constant pressure compared to a large (43%) decompression step. The slopes obtained from plots of activity against pressure, which provide a measure of the sensitivity of the animal to compression, were unaltered by the early inclusion of 4.8 atm N_2 or 0.19 atm N_2O but were displaced to higher pressures. Activity appears to be the net result of activity-generating and accomodation processes, and there was no evidence that it was in equilibrium with pressure.

motor activity decompression

inert gases

Reproducible changes in the motor activity of small mammals occur when they are subjected to a steady increase in pressure. Fine tremors are seen first, which gradually become coarse and then are interspersed with convulsions, both clonic and tonic (1, 2). This paper reports the reduction in the tremors and associated activity that is brought about 1) by holding pressure constant, 2) by a step decompression, and 3) by the addition of nitrogen or nitrous oxide. The purpose of the experiments was to reveal the closeness of the coupling between the primary kinetic disturbance that pressure causes, presumably in central neurons, and the complex motor symptoms seen in the integrated animal.

The experiments consisted of compressing rats to sub- and supraconvulsion threshold pressures and then either holding the pressure constant or decreasing it rapidly. In either case the decay in activity (defined below) was measured and the half-life of decay for different conditions compared. The effect of nitrogen added at the end of the compression profile was also studied in this way.

In a second group of experiments the influence of nitrogen and nitrous oxide injected at the start of the compression profile was studied. The object of these experiments was to examine the effect of the inert gases on the slope of the activity/pressure curves, and in particular to see if a change in slope or a displacement of the curve occurred along the pressure axis. The former would imply a pressure-dependent change in sensitivity of the animal to compression; the latter, a change in sensitivity by a fixed amount.

Both types of experiment used a method for continuously recording the motor activity of the animals and differed from many previous studies that have used end points such as the onset pressure for tremors or convulsions.

MATERIALS AND METHODS

Hooded Lister rats of both sexes and between 12 and 14 days old were used. They were reared in a normal light/dark cycle and experimented on between 1000 and 1500 h. The pressure chamber was used that was previously described in Ref. 3. The temperature of the gas in the chamber was measured with a fast-response thermistor (#427, Yellow Springs Instruments, Yellow Springs, OH) and was normally controlled at 29.5°C ± 1°C before compression and then increased to 34°C ± 0.5°C during the first 10 atm of compression. The chamber P_{O_2} was adjusted to 0.5–0.8 atm before each compression experiment and the P_{CO_2} was kept at a negligible level by absorption.

An individual rat was confined within a wire mesh cage within the chamber, and its tremorlike motor activity was recorded by monitoring the vibrations it transmitted to the cage. A phonograph cartridge served as a vibration transducer, as previously described (3). Simultaneously, the animal was observed through a pressure window.

The output from the phonograph cartridge was recorded by a preamplifier and pen recorder at a chart speed of 15 mm/min. The piezoelectric transducer detects very small movements of the cage at frequencies far in excess of those used in this study. The threshold sensitivity of the transducer was shown to be unaffected by pressures up to 60 atm by tests involving a calibrated solenoid device placed inside the animal cage. Thus a threshold (i.e., just detectable) movement of the cage recorded at atmospheric pressure would be similarly recorded at high pressure. Activity was scored by counting the pen deflections (peaks) in excess of a standardized threshold amplitude in a 5-min period. Base-line activity was obtained from the recording before the start of compression. Activity was expressed as deflections per 5-min period or as an amount in excess of the base-line activity level, expressed as a percentage of the peak level in the particular experiment. Thus 0% corresponds to a score indistinguishable from the base line, and 100% corresponds to the maximum activity within the experiment.

An arbitrary reference score of activity was obtained to enable various activity levels in different experiments to be compared. Twenty standard compressions to 47.6 atm yielded a mean maximum activity score of 220.7 ± 20.5 peaks per 5-min period. This value is used to compute the "activity, percent reference value" mentioned in the results. It should be remembered that this method is confined to a specific apparatus and has been used with animals of similar weight whose motor activity takes the form of various whole-body tremors and myoclonic jerks, which, judged by direct observation, mainly undergo quantitative changes in frequency and amplitude. Activity is thus an index of the frequency of movements that are greater in amplitude than the threshold level.

Decompression proceeded at a rate of approximately 60 atm/min. The chamber gas cooled to around 30°C but returned to within 0.5°C of the normal level within 5 min. The animals,

which were also subjected to significant noise during decompression, showed no sign of decompression sickness or other deleterious effects.

RESULTS

A typical experiment is described to illustrate the details of the method and the nature of the measured activity. First the chamber was flushed with oxygen and the confined animal left for 20 min to settle down. Compression with helium (99.995%) at a rate of 80 atm/h was achieved by smoothly bleeding gas into the chamber; the transient temperature increase of less than 2°C lasted less than 15 s. The animals were normally disturbed by the start of compression and scratched their ears. This caused an initial high level of activity (Fig. 1). At 15 atm mild tremors appeared in the forelimbs and head, subsequently spreading to the hindlimbs. Activity plots above a pressure of 15 atm provide an index of pressure-induced movements (Fig. 1). Their severity increased, particularly on the rare occasion when the animal turned or walked. The pressure-induced movements became grouped into bouts of coarse tremor. These occurred more frequently and lasted longer as pressure increased. Type I convulsions (4), which caused the animal to be thrown about inside the cage, were seen at a mean pressure of 46.4 ± 1.0 atm. A typical animal would appear to try to control its movements by gripping the cage, during which time it arched its back, retched, and then relaxed several times. Type I convulsions were not recorded distinctively on the chart recorder because of their similarity to coarse tremors in activity level, but their occurrence was separately noted. They continued intermittently against a background of tremor and, as compression proceeded,

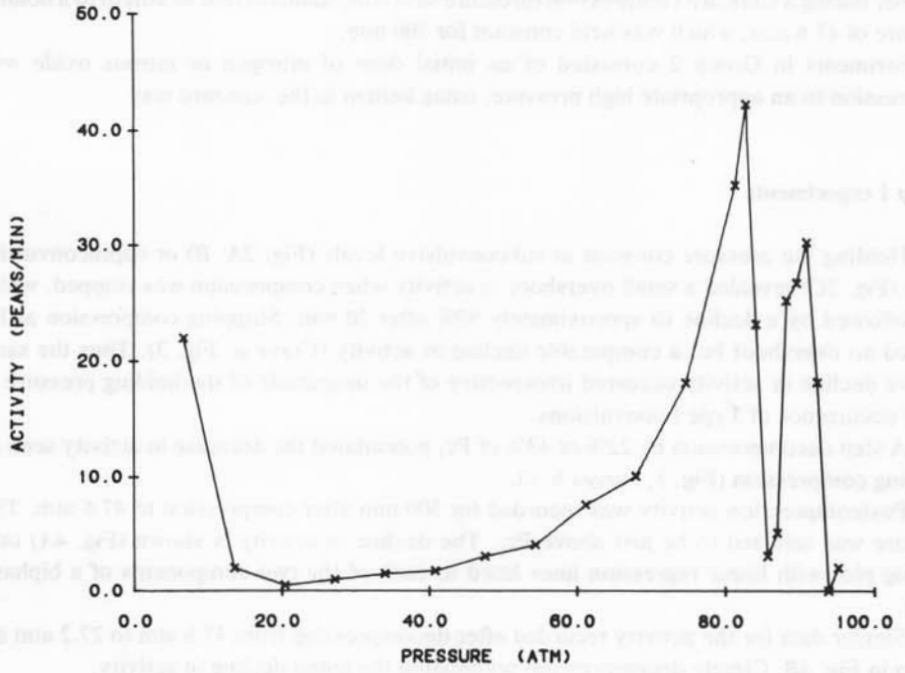

Fig. 1. Change in activity (peaks/min, see text) with increase in pressure. Result of an individual experiment is shown in which the preamplifier gain was reduced to record the full range of activity that was likely to occur during compression at 80 atm/h to convulsion pressures.

the first of the Type II convulsions were seen at a mean pressure of 83.6 ± 1.75 atm (4). In these the limbs were rigidly extended from the fallen animal, followed by a weak cycling motion. Tremors were then resumed giving a second peak (Fig. 1), interrupted by a second Type II convulsion at a mean pressure of 87 ± 1.78 atm. In the experiment illustrated in Fig. 1, compression was halted at 95 atm with the animal exhibiting a low level of tremor activity and some respiratory difficulty after its second Type II convulsions.

Thus defined, activity traced in Fig. 1 is an arbitrary reproducible score of the frequency of movements induced by high pressure.

Group 1 experiments, which involved a standard compression at 80 atm/h, were developed from an investigation into the immediate effects of holding pressure constant or decompressing rapidly by a set amount. The initial experiments consisted of the following: 1) Pressure was held constant at Pc_1 (the pressure that elicits the first Type I convulsion in an individual—close to 46 atm) for 30 min. 2) Pressure was decreased from Pc_1 by 22% and by 43%, and activity was recorded for 30 min. To remove the effects of the decompression process per se the following experiments were carried out: 3) Pressure was held constant at 27.2 atm, 34 atm, and $Pc_1 \times 1.2$ (20% higher than Pc_1, the mean convulsion pressure). These experiments demonstrate that there was considerable effect on the activity recorded in the first 30 min after the procedure; therefore, certain experiments were repeated using 47.6 atm as a compression end point. 4) Pressure was increased to 47.6 atm and then held constant for 300 min. 5) Pressure was increased to 47.6 atm, then rapidly reduced to 27.2 atm, which was held constant for 300 min.

To compare the effect of the addition of a narcotic agent with the observed effect of a decompression step the following experiment was performed: Nitrogen was injected into the chamber during a standard compression (pressure 42.8 atm, standard rate 80 atm/h) to a holding pressure of 47.6 atm, which was held constant for 300 min.

Experiments in Group 2 consisted of an initial dose of nitrogen or nitrous oxide with compression to an appropriate high pressure, using helium in the standard way.

Group 1 experiments

1. Holding the pressure constant at subconvulsive levels (Fig. 2*A*, *B*) or supraconvulsive levels (Fig. 2*C*) revealed a small overshoot in activity when compression was stopped, which was followed by a decline to approximately 50% after 30 min. Stopping compression at Pc_1 showed no overshoot but a comparable decline in activity (*Curve a*, Fig. 3). Thus the same relative decline in activity occurred irrespective of the magnitude of the holding pressure or of the occurrence of Type I convulsions.

2. A step decompression by 22% or 43% of Pc_1 potentiated the decrease in activity seen on stopping compression (Fig. 3, *curves b, c*).

3. Postcompression activity was recorded for 300 min after compression to 47.6 atm. This pressure was selected to be just above Pc_1. The decline in activity is shown (Fig. 4*A*) on a semilog plot with linear regression lines fitted to each of the two components of a biphasic curve.

4. Similar data for the activity recorded after decompressing from 47.6 atm to 27.2 atm are shown in Fig. 4*B*. Clearly decompression potentiated the initial decline in activity.

5. The standard compression profile was modified by injecting 4.8 atm N_2 at 42.8 atm, at a rate of 80 atm/h, to a total holding pressure of 47.6 atm. Figure 5 shows the ensuing biphasic decay in activity, which is to be compared with the data in Fig. 4*A*, *B*. (See DISCUSSION.)

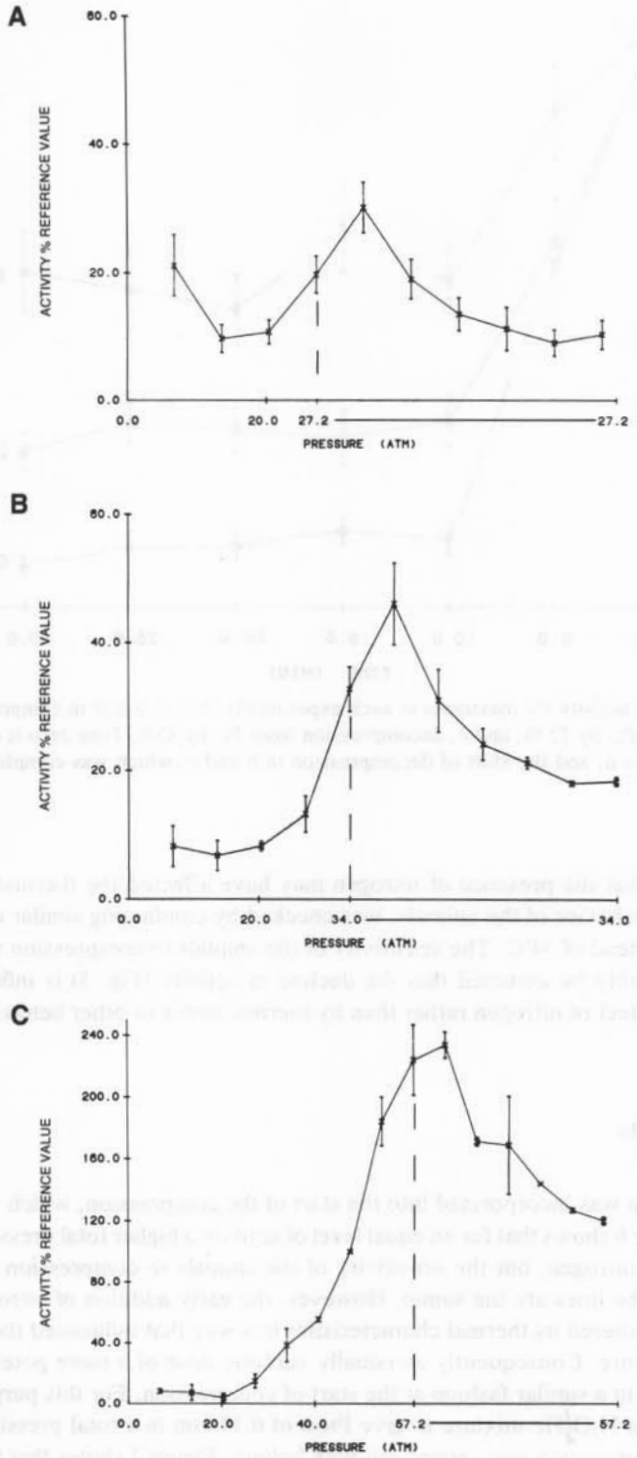

Fig. 2. Change in activity (% reference value, see text) during compression and then upon halting of compression at *A*, 27.2 atm; *B*, 34 atm; and *C* $Pc_i \times 1.2$. Points are means ± SE; $n = 4$ in *A* and *B*, and 2 in *C*. Dashed line indicates the cessation of compression, after which it was held constant for 30 min.

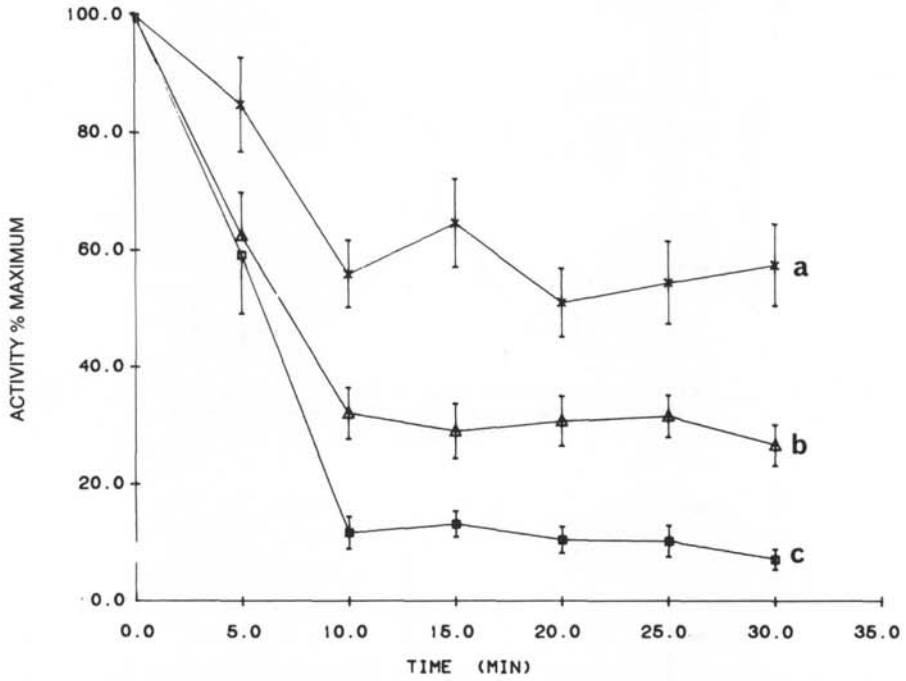

Fig. 3. Change in activity (% maximum in each experiment) after *a*, a halt in compression to Pc_1; *b*, decompression from Pc_1 by 22 %; and *c*, decompression from Pc_1 by 43%. Time zero is onset of Pc_1 and halt in compression in *a*, and the start of decompression in *b* and *c*, which was completed within 30 s. Mean ± SE; $n = 6$.

The possibility that the presence of nitrogen may have affected the thermal comfort temperature, and the behavior of the animals, was checked by conducting similar experiments at 30.5°C ± 0.5°C instead of 34°C. The sensitivity of the animals to compression was unaltered, and it may reasonably be assumed that the decline in activity (Fig. 5) is influenced by the pharmacological effect of nitrogen rather than by thermal stress or other behavioral factors.

Group 2 experiments

A P_{N_2} of 4.76 atm was incorporated into the start of the compression, which was continued with helium. Figure 6 shows that for an equal level of activity a higher total pressure is required in the presence of nitrogen, but the sensitivity of the animals to compression was unaltered (i.e., the slope of the lines are the same). However, the early addition of nitrogen to the gas mixture may have altered its thermal characteristics in a way that influenced the sensitivity of the animal to pressure. Consequently an equally narcotic dose of a more potent gas, nitrous oxide, was applied in a similar fashion at the start of compression. For this purpose the initial compression used a N_2O–He mixture to give P_{N_2O} of 0.19 atm in a total pressure of 6.8 atm; the subsequent compression was carried out with helium. Figure 7 shows that the increase in activity resembles that seen in the presence of nitrogen; the experimental curves are displaced to higher pressure and are parallel to the control slope at pressures higher than 30 atm.

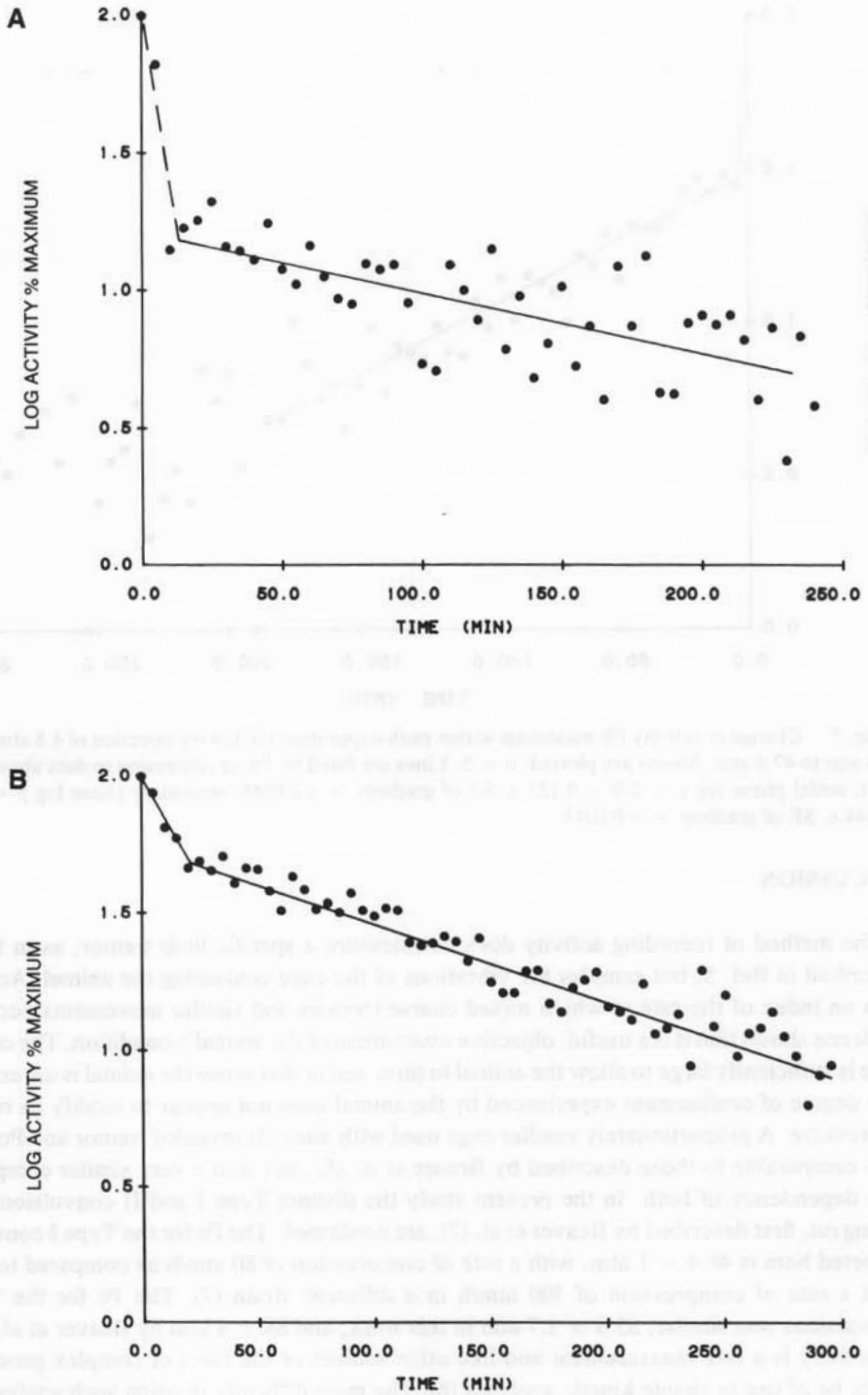

Fig. 4. Change in activity (% maximum within each experiment) after *A*, compression to 47.6 atm; *B*, decompression from 47.6 atm to 27.2 atm. Time zero is the onset of 47.6 atm and in *B* is also the start of decompression. Means are plotted; $n = 5$. Lines are fitted by linear regression: *A*, initial phase $\log y = 2.0 - 0.0148\,x$, SE of gradient $= \pm 0.0021$; secondary phase $\log y = 1.75 - 0.00316\,x$, SE of gradient $= \pm 0.00041$. *B*, secondary phase $\log y = 1.21 - 0.0022\,x$, SE of gradient $= \pm 0.00056$.

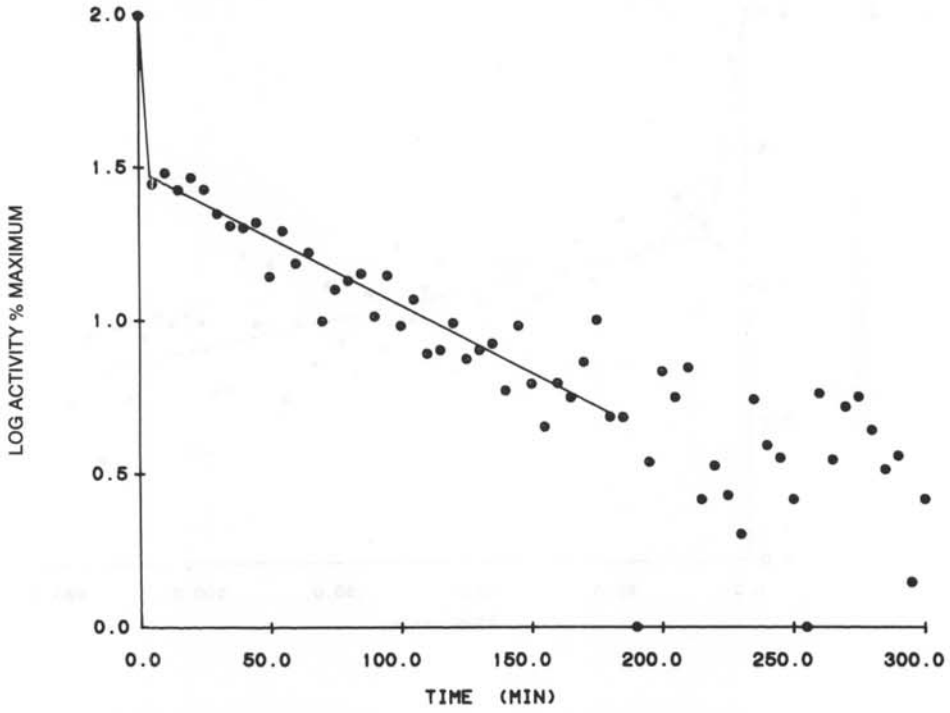

Fig. 5. Change in activity (% maximum within each experiment) following injection of 4.8 atm N_2 from 42.8 atm to 47.6 atm. Means are plotted; $n = 5$. Lines are fitted by linear regression to data above the 5% level; initial phase $\log y = 2.0 - 0.125\,x$, SE of gradient $= \pm 0.0265$; secondary phase $\log y = 1.49 - 0.0044\,x$, SE of gradient $= \pm 0.0013$.

DISCUSSION

The method of recording activity does not measure a specific limb tremor, as in the case described in Ref. 5, but samples the vibrations of the cage containing the animal. Activity is thus an index of the rate at which mixed coarse tremors and similar movements occur. The evidence shows that it is a useful, objective assessment of the animal's condition. The confining cage is sufficiently large to allow the animal to turn, and in that sense the animal is unrestrained. The degree of confinement experienced by the animal does not appear to modify its response to pressure. A proportionately smaller cage used with mice (3) revealed tremor and Pc thresholds comparable to those described by Brauer et al. (6), and also a very similar compression rate dependency of both. In the present study the distinct Type I and II convulsions in the young rat, first described by Beaver et al. (7), are confirmed. The Pc for the Type I convulsions reported here is 46.4 ± 1 atm, with a rate of compression of 80 atm/h as compared to 60 atm with a rate of compression of 500 atm/h in a different strain (7). The Pc for the Type II convulsions was similar, 83.6 ± 1.7 atm in this work, and 88 ± 4 atm by Beaver et al. (7).

Activity is a rate measurement and like other indices of the rates of complex processes it might be of use in simple kinetic analyses (8). The main difficulty in using such analyses here is the loose coupling between pressure and activity and the virtual absence of a steady-state level of activity. In Fig. 2*A* and *B* for example, activity clearly increases after compression is stopped at 27.2 atm and 34 atm. The subsequent decline in activity appears to be a 2-stage process. The initial rapid rate of decline seen when compression is stopped is not related to

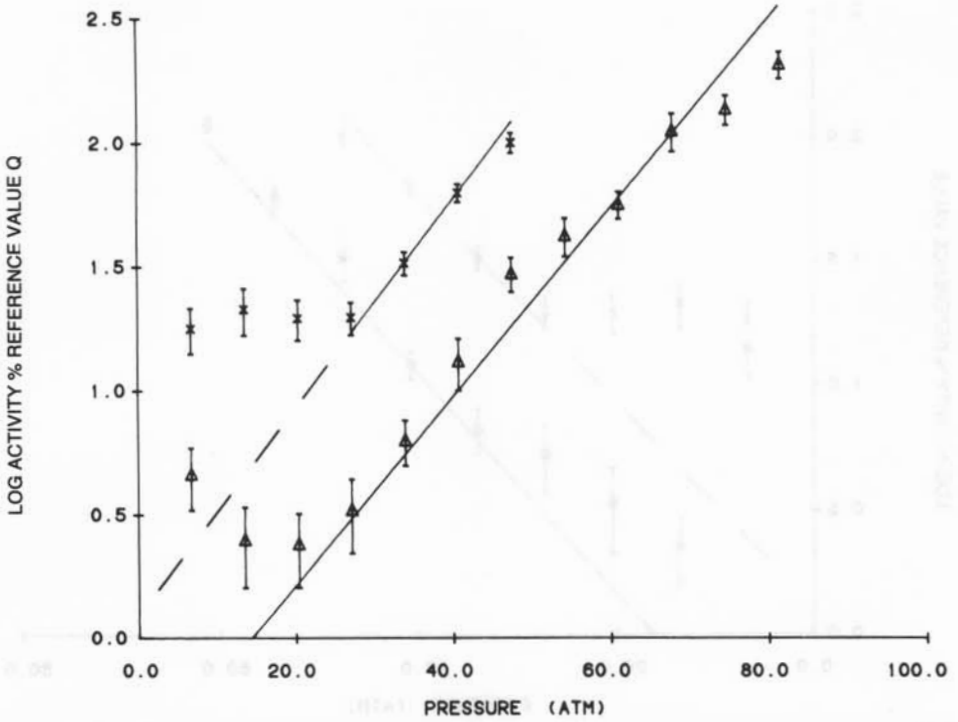

Fig. 6. Change in activity (ordinate: activity, % reference value, see text) during compression with △, a P_{N_2} of 4.76 atm injected at start; ×, control, no added nitrogen. Means ± SE are plotted (△, $n = 5$; ×, $n = 20$). Lines are fitted by linear regression: △, $\log y = 0.379 - 0.0026$, SE of gradient = ±0.0038; ×, $\log y = 0.0416 - 0.133$, SE of gradient = ±0.0029.

the holding pressure, and is unaffected by convulsions; activity is approximately halved at 30 min at 27.2 atm, 34 atm (Pc × 1.2), and 47.6 atm. The slower secondary phase in the decline in activity is inversely proportional to pressure and lasts for at least 5 h after compression is stopped or after decompression to a lower, constant pressure. There is clearly no evidence of an equilibrium being established between pressure and activity.

Decompression modifies both the initial and the secondary phases in the decline of activity seen when compression is halted. After decompression to 27.2 atm (Fig. 4*B*) or by 22% (Fig. 3) there occurs a decrease in activity of approximately twice that seen when compression is merely stopped.

Inspection of the curves in Fig. 4*A* and *B*, shows that decompression from 47.6 atm to 27.2 atm reduced activity to 50% of its peak level by 75 min, whereas at a constant 47.6 atm, 260 min were required for the activity to decline to 50%. A P_{N_2} of 4.8 atm injected at the end of the compression profile (Fig. 5) reduced the animal's activity to 50% of the peak level while still at the holding pressure of 47.6 atm, within 100 min. More quantitative comparisons can be obtained by computing the half times for the activity decay curves (Table 1). Nitrogen has a greater effect on the intial decay phase than on the secondary phase, and it accelerates the rate of decline in the secondary phase, while decompression slows it. Generally, the experiments show that in contrast to severe decompression, addition of an inert gas is very effective in reversing the motor symptoms caused by pressure (9, 10).

It is interesting to compare these results with the recordings made of tremor in the forelimb of guinea pigs restrained at the neck (5). Obvious points of similarity are the buildup in coarse

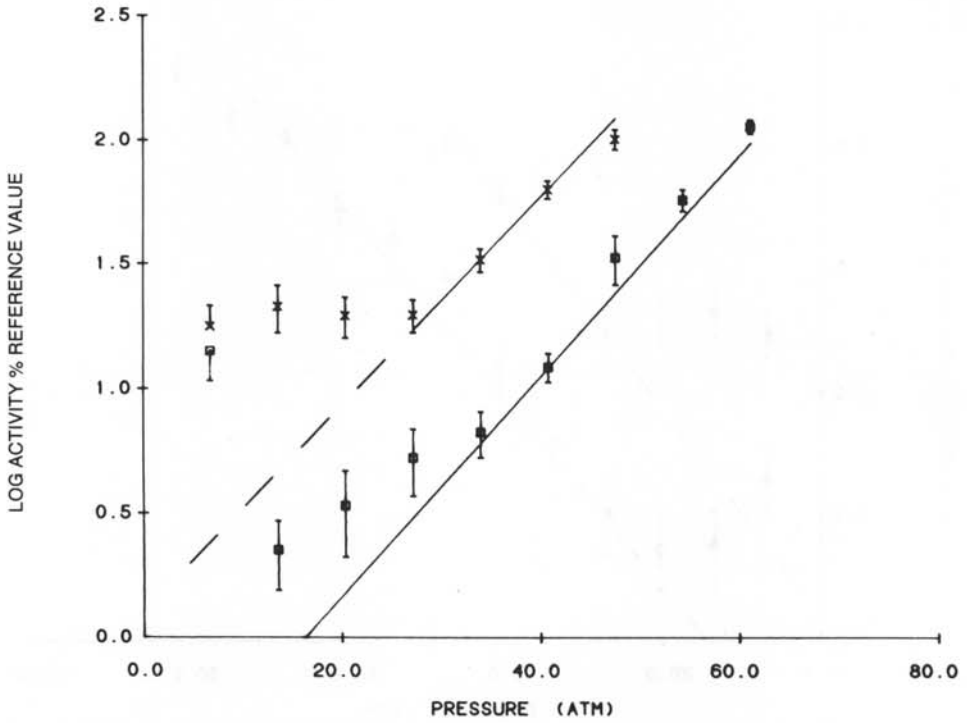

Fig. 7. Change in activity (ordinate: activity, % reference value, see text) during compression with □, a P_{N_2O} incorporated at the start; ×, control, no N_2O present. Means ± SE are plotted (□, $n = 6$; ×, $n = 20$). Lines are fitted by linear regression: □, $\log y = 0.044\,x - 0.0014$, SE of gradient = ± 0.0020; ×, as in Fig. 6.

TABLE 1

RATE OF REDUCTION IN ACTIVITY UNDER CONDITIONS OF A HALT IN COMPRESSION OF RATS AND DECOMPRESSION OF RATS

Condition	*Initial Phase* Half-life, min	*Secondary Phase* Half-life, min
Compression halted, pressure constant, 47.6 atm	20	103
P_{N_2} 4.8 atm injected from 42.8–47.6 atm. Compression halted; pressure held constant, 47.6 atm	2.4	68
Compression halted at 47.6 atm, decompression to 27.2 atm	—	136

Means of at least 4 determinations. Compression rate 80 atm/h. For details see text.

tremors during compression and their initial rapid decline when compression was halted. In the case of the guinea pig forelimb the tremors within the 11-to-20-Hz band (likely to arise centrally, from the rubro-olivocerebellar complex) declined rapidly to near base-line level within 10 min of halting of compression. This is a faster and more complete decrease than is

seen in the activity recordings in the present work. It is not clear whether this is due to a species difference or the lack of intentional or postural tremor in guinea pigs restrained at the neck.

The rationale behind the compression experiments (Figs. 6, 7) involving nitrogen and nitrous oxide is as follows. If it could be shown that activity is a measure of a pressure-dependent rate of reaction, then the inert gas data (Figs. 6, 7) would be open to kinetic interpretation. The results obtained from the present experiments show that the level of activity recorded during compression is probably not immediately determined by pressure, as would be the case in a simple chemical reaction, but is more likely the outcome of pressure-dependent processes generating activity, and accomodation processes (see above). Thus, plots of activity against pressure provide a measure of sensitivity (slope) but not a manageable kinetic relationship. It is, however, interesting that both nitrogen and nitrous oxide offset the curves in Figs. 6 and 7 without affecting their slope, suggesting a constant rather than a pressure-dependent interaction between pressure hyperactivity and inert gas molecules.

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Bell PY, Macdonald AG. Renversement du tremblement induit par la pression chez le rat par la décompression en étapes et les gaz inertes. Undersea Biomed Res 1984; 11(1):25–36.—Une technique pour enregistrer continuellement l'activité motrice des rats sous pression fut utilisée pour surveiller leur condition à la suite de: 1) pression de séjour constante après la compression, 2) la décompression, et 3) l'addition d'azote avant le maintien d'une pression constante. Après les procédures (1) et (2), une relation linéaire fut établie entre le logarithme de l'activité et le temps écoulé en deux phases distinctes. Une diminution initiale courte fut suivie d'une réduction lente et plus longue qui se prolongea au dela de 5 heures. Le taux du second déclin fut inversement lié à la pression de séjour et ne fut pas affecté par les convulsions préalables. L'addition de 4.8 atm de N_2 fut très efficace pour réduire l'activité à pression constante comparée au grand (43%) échelon de décompression. Les pentes obtenues des tracés de l'activité versus pression, lesquelles fournissent une mesure de la sensibilité de l'animal à la décompression, ne furent pas modifiées par l'introduction précoce de 4.8 atm de N_2 ou de 0.19 atm de N_2O, mais furent déplacées à des pressions plus élevées. L'activité apparaît être le résultat net de processus d'accomodations et générateurs d'activité, sans indiquation qu'elle fut en équilibre avec la pression.

activité motrice décompression
gaz inertes

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