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Effects of variations in time pattern of nitrogen addition on development of HPNS in mice

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Brauer RW, Hinson WM. Effects of variations in time pattern of nitrogen addition on development of HPNS in mice. Undersea Biomed Res 1983; 10(4):281–298.—The effect of change in injection pattern in nitrogen on threshold pressures of three symptoms associated with compression in helium/nitrogen atmospheres was explored. Excitement threshold pressures decrease with increasing concentrations of N_2 and are not affected by changing from continuous to equivalent bolus N_2 injection. Coarse tremor onset is delayed in direct proportion to the amount of N_2 present with the same relative potency in compression at 60 atm/h as at 1000 atm/h. Bolus injection of N_2 is less than half as effective as continuous injection in this respect. Threshold pressures of convulsion from high pressure neurological syndrome (HPNS) increase with increasing amounts of N_2, the relative anticonvulsant potency of the gas being independent of compression rate when this is introduced by continuous injection. A bolus effect similar to, though smaller than with coarse tremors, is encountered at a compression rate of 60 atm/h but is absent at 1000 atm/h. Late injection of the bolus likewise abolishes this bolus effect. Possible mechanisms to give rise to these effects are discussed, and the bearing of the data on time sequence of HPNS development is explored.

HPNS
mice
nitrogen
helium
high pressure
tremors
convulsions
injection
nitrogen compression

Admixture of nitrogen to helium compression atmospheres has been used for some time to elevate the threshold pressures at which various symptoms of high pressure neurological syndrome (HPNS) manifest themselves (1–4). The original observations upon which such use was based involved modification of the compression gas by admixture to helium of some fixed proportions of nitrogen or other volatile constituent that is anesthetically effective and metabolically inert, a procedure adopted originally with the object of artificially creating gas mixtures possessing the pharmacological qualities of a single diving gas, hydrogen (2). In recent years Rostain and collaborators (4,5) have explored the possibility that the effectiveness of nitrogen in this context might be improved by modifying the pattern of its injection during compression so that chamber inert gas composition would vary along a predetermined profile. These authors were led to conclude that from the point of view of HPNS symptom development, repeated

small injections of nitrogen, more or less analogous to the earlier use of premixed He-N_2 atmospheres, tended to yield more favorable results than single and relatively large injections. The data on which this conclusion was based were largely clinical in nature and were based on multicelled designs in relatively small series of primate and human compression subjects.

The present report presents a more quantitative treatment of these phenomena using a small animal model in which the results of bolus injections of nitrogen could be compared with dose-response curves for series of compressions with several constant levels of nitrogen in the compression gas. In addition to their potential practical significance, the resultant data have bearing upon some aspects of the as yet controversial nature of the interaction between hydrostatic pressure and volatile anesthetic agents in intact animals (6).

METHODS

All experiments were carried out on adult female CD-1 mice. Details of animal maintenance and selection are contained in previous papers (e.g., Ref. 7). As in earlier work, too, compressions were carried out at chamber temperatures between 33°C and 34°C, P_{O_2} between 0.4 and 0.6 ATA, P_{CO_2} below 0.005 ATA. Chamber pressure was increased at average rates of either 60 or 1000 atm/h (cf. Ref. 7). At the lower compression rate, gases were added in such fashion that pressure was increased in 3-atm steps. At the higher compression rate compression was carried out continuously, and initial chamber temperature was held at 30°C to compensate for adiabatic heating; in all cases P_{O_2} was brought to 0.5 ATA initially and maintained at that level under analytical control. Gases utilized for compression included high-purity helium, high-purity nitrogen, and helium-nitrogen mixtures prepared to an accuracy of ±5% of the nominal N_2 concentration. Bolus injections of N_2, required in some of the procedures, were carried out in such a fashion as to maintain the predetermined compression profile; they were guided manometrically and verified at the time of symptom onset by gas chromatography. Actual bolus sizes given in this paper are based on the analytical results and are accurate to ±6%.

Four types of behavioral responses occurring successively during these compressions were noted, although only three of these were deemed suitable for scoring and quantitative comparisons: 1) Fine tremors, the onset of which is hard to diagnose with assurance in the mouse and which, therefore, will not be further discussed. 2) The appearance of what in human subjects would be qualified as *euphoria,* a stage of clearly recognizable excitement manifested in exaggerated motor activity, including grooming, exploring, and seemingly aimless locomotor activity (when fully developed referred to by our observers as the "student beer party stage"). This phenomenon is not recognizable in the absence of N_2, but it appears whenever sufficient amounts of N_2 are present at the appropriate total pressure. Scoring, somewhat subjective but reproducible to within ±3 or 4 atm at the lower compression rate, involves assessment of the behavior of the mice as a group. The corresponding results, therefore, are included without a numerical estimate of individual variability. 3) *Coarse tremors* in heliox compressions represent a rather dramatic transition from the minor locomotor disturbances associated with the fine tremor stage to coarse high-frequency shaking interrupted by occasional myoclonic jerks in the later stages, often persisting even in resting animals. The addition of N_2, especially at higher pressures, elicited a further distinctive stage of coarse shaking ("wet dog shakes") at what appeared to be rather lower frequencies. In animals in which N_2 administration was postponed until pressures were higher than 60 ATA, the intervention of this stage made the determination of subsequent coarse tremor development uncertain. Finally, 4) *convulsions* in the present context refers to Type I HPNS seizures (cf. Ref. 8). The convulsion threshold

pressure scored for each animal was the pressure at which the first seizure of this type was observed. Transition from coarse tremors to convulsions is usually sharp, although in about 25% of the animals one may observe minor myoclonic seizure events prior to development of the full seizure sequence. These last only a fraction of a second and do not cause the animals to lose their upright posture. No attempt was made in any of these experiments to observe Type II HPNS seizures, since threshold pressures in many of the cells of the design of Table 1 would have exceeded the safe operating pressure of our systems.

The design of the experiments is shown in Table 1. Two compression rates were used: 60 atm/h and 1000 atm/h. For both compression rates, control experiments were performed using for compression premixed He-N_2 atmospheres that varied in composition from 0 to 100% N_2 as shown in Table 1.

Bolus injection experiments involved injections of N_2 as already described. At 1000 atm/h bolus size alone was varied, all N_2 injections being started at a chamber pressure of 17.4 ATA. At 60 atm/h bolus injections of 17.1 atm were begun at 17.4, 70.4, or 76.6 ATA. In addition an experiment was carried out in which an attempt was made to postpone N_2 injection as much as possible without allowing premature HPNS seizures. This stipulation necessitated splitting the 17.1-atm N_2 bolus to be administered and injecting 6.5 atm N_2 when a pressure of 76.6 ATA had been reached and all animals were showing severe manifestations of the coarse tremor stage. After this partial bolus there was marked relief of HPNS symptoms, and compression could be continued to 102 ATA before recrudescence of symptoms required injection of the remainder of the 17.1-atm bolus, followed by resumption of injection of He, which was then continued until all animals in the chamber had undergone HPNS Type I seizures. The P_{N_2} profiles corresponding to these several regimes are illustrated schematically at the bottom of Fig. 5.

TABLE 1

EXPERIMENTAL DESIGN AND NUMBER OF MICE PER CELL

Inert Gas Composition Injected	Number of Mice at Compression Rate	
	60 atm/h	1000 atm/h
He	8	8
N_2 in He premix		
5–7%	8	2 × 8
10–12%	8	2 × 8
15–20%	8	3 × 8
30–35%	—	2 × 8
100%	8	—

Bolus N_2 injection	Number of Mice Beginning at				
	17.4 ATA	70.4 ATA	76.6 ATA	76.6 and 102 ATA	17.4 ATA
5–9 atm	8	—	—		3 × 8
10–12 atm	—	—	—		2 × 8
13–15 atm	—	—	—		2 × 8
17–18 atm	8	8	8	8	—
22–35 atm	8	—	—		2 × 8

All experiments involved 8 animals, i.e., two exposure sessions using 4 animals each. Table 1 shows the distribution of experiments among possible cells of the design; in the case of the compressions at 1000 atm/h, compression experiments lasting less than 5 min each were multiplied to take into account increased variance in terms of both determination of end point and replication of bolus size. Mean values, threshold pressures for each symptom, and standard deviations and standard errors for coarse tremor and convulsion data were computed for each combination of compression conditions, standard errors for mean threshold values being shown in the graphs. Student's t test was used to determine levels of significance of differences throughout, with a cutoff value of $P = 0.05$. Least-square linear regression equations were computed for each of the relations of Table 2; computations yielded, in addition to the parameters of the regression, correlation coefficients and standard deviations of the regression coefficients.

To assure intercomparability, all figures except those for the excitement stage are plotted, using as abscissa the partial pressure of N_2 attained at the mean onset pressure for the particular symptom and compression conditions being represented.

RESULTS

Clinical observations

Compression of mice in heliox atmospheres elicits a sequence of manifestations jointly described as the high pressure neurological syndrome (HPNS). The succession of fine and coarse tremors, as well as Type I and Type II HPNS convulsion stages, has been described extensively in the past (3, 8) and was reproduced in the present series. Substitution of nitrogen-containing helium for pure helium as the primary compression gas altered the absolute pressures at which the various symptoms were noted without changing their sequence, except for the development of an excitement stage (cf. Table 2). This latter was particularly noticeable at the higher concentrations of N_2: with both 12% and 15% N_2 indications of excitement were expressed as increases in general locomotor activity in all animals, beginning, in general, before or at about the same time as the development of fine tremors and persisting throughout most of the succeeding compression sequence.

Bolus injection of N_2 modified this picture: at 60 atm/h with bolus injections beginning at 17.4 ATA total pressure, even 7.1 atm N_2 elicited evidence of excitement by the end of the injection or shortly afterward. In the case of larger doses this excitement became very marked. As compression was continued the intensity of excitement seemed to subside by the time coarse tremors were developing. Bolus injections begun at 70.4 ATA caused only mild excitement. Bolus injections of N_2 begun at 76.6 ATA failed to elicit a recognizable excitement stage.

In general, animals in that series were near, or had actually entered, the coarse tremor stage at the time the bolus injection was begun. Progress of the N_2 injection at moderate total pressures resulted in reduction of the intensity of the tremors and of the associated occasional myoclonic jerks. Shortly after completion of the N_2 injection, however, when compression on He was resumed, coarse tremors reappeared, and the rest of the sequence progressed as in the previously described several series.

Shortly after the beginning of these N_2 bolus injections, all animals began to groom themselves furiously and this went on until several minutes after the end of the N_2 injection. Bolus N_2 injections begun at 60 or more ATA in 80% of the animals resulted in a striking modification of the coarse tremor activity, which appeared to be lower in frequency, but higher in amplitude,

TABLE 2

REGRESSION EQUATIONS AND PARAMETERS FOR REGRESSION OF MEAN THRESHOLD PRESSURES FOR 3 SYMPTOMS ON N_2 PARTIAL PRESSURE*

Regression Equation	$\dot{P}$	Parameters of Continuous N_2 Injection			Parmeters of Bolus N_2 Injection Beginning at 17.4 ATA			$b_{constant}/b_{bolus}$
		A	b	b/A, %	A	b	b/A, %	
$_{He}P_{ex} = A + b\sqrt{_{N_2}P_{ex}}$	60	54.4 ± 1.8	−10.1	—	(Same as continuous)			1
	1000	—	—			—	—	
$P_{CT} = A + b\ _{N_2}P_{CT}$	60	64.8	2.47 ± 0.01	3.81	67.6	1.13 ± 0.01	1.67	2.19
	1000	39.9	1.40	3.51	40.9	0.53	1.30	2.64
$P_c = A + b\ _{N_2}P_c$	60	97.8	1.68 ± 0.07	1.74	98.2	1.24 ± 0.03	1.26	1.35
	1000	74.5	1.30 ± 0.03	1.74	71.5	1.45 ± 0.01	2.03	0.86

Symptoms shown as subscripts to partial pressure: P_{ex}, excitement; P_{CT}, coarse tremors; P_c, convulsions. *Value of $r > 0.85$ throughout.

than before the beginning of N_2 administration. This "shaking" stage subsided shortly after termination of the N_2 injection, giving way again to coarse tremors of a more familiar pattern. These motor changes are rather striking and invite more detailed investigation in the future by means of appropriate accelerometer techniques. In the split N_2 bolus injection experiments of this series, injection of the first 6.8 atm, beginning at 76.6 ATA, substantially reduced the severity of coarse tremors; the second N_2 injection (10.8 atm N_2 begun at 102 ATA) elicited a shaking stage similar to that already described for the single bolus injections for 76.6 ATA.

There was no difference in the clinical manifestations of Type I HPNS seizures between bolus and continuous N_2 injection experiments. As mentioned above, Type II seizure thresholds in N_2-treated CD-1 mice are beyond the working level of our chambers and were not explored.

QUANTITATIVE RESULTS

The numerical results for the experiments of this series, in terms of mean threshold pressures for the three end points that could be scored with confidence—i.e., for the excitement stage, the coarse tremor stage, and Type I convulsions—showed substantial differences in their response to variation in the pattern of nitrogen administration. Their description, consequently, is conveniently subdivided into sections corresponding to each of these end points. The first section of this presentation deals with a comparison of the effects of N_2 administered by continuous injection and of equivalent bolus injections of N_2 administered beginning at 17.4 ATA, at compression rates of 60 atm/h and of 1000 atm/h.

Excitement stage

Excitement in the sense here defined was not observed unless nitrogen was added as a part of the compression mixture. Development of excitement appeared to require a measurable amount of time, of the order of a minute or more. Observations of threshold pressures for this stage in the rapid compression experiments, therefore, are so uncertain that they are not reported in detail other than by pointing out that in all such experiments excitement was recorded at pressures short of 40 ATA. At a compression rate of 60 atm/h, excitement developed in all experiments in which N_2 was added, regardless of whether this was done by bolus or by continuous injection. The threshold pressures at which excitement was noted decreased with increasing N_2 dose, and the points corresponding to bolus injection experiments seem to fall substantially on the same curve as those for continuous administration. By trial and error it was found that the results can be linearized by plotting He pressure at the point of onset of excitement as a function of the square root of N_2 partial pressure at the same time. The experimental results are represented graphically in this form in Fig. 1. Regression analysis showed that this result corresponds to the equation:

$$ {}_{He}P_{ex} = 64.8 - 10.3 \cdot \sqrt{{}_{N_2}P_{ex}} \qquad (1) $$

with a correlation coefficient of -0.96.

From the point of view of the present discussion the salient findings, thus, are that an excitement stage is elicited whenever N_2 partial pressures and He partial pressures reach

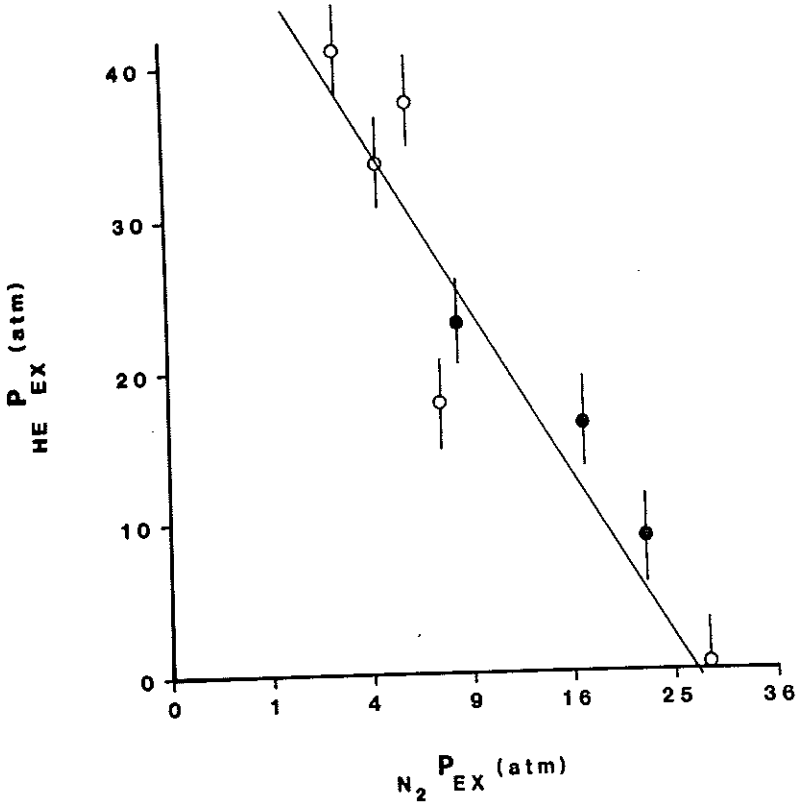

Fig. 1. Relation between mean threshold pressure of helium for excitement stage as a function of square root of nitrogen partial pressure in CD-1 mice. ⊢——⊣ Regression line: $_{He}P_{ex} = 54.4 - 10.3\sqrt{_{N_2}P_{ex}}$. ○, Continuous N_2 injection; ●, bolus N_2 injection beginning at 17.1 ATA. Compression rate 60 atm/h.

values given by Eq. *1*, i.e., whenever total pressure is related to N_2 partial pressure by the equation:

$$P_{ex} = {}_{N_2}P_{ex} - 10.3 \cdot \sqrt{_{N_2}P_{ex}} + 64.8 \qquad (2)$$

Thus threshold pressure for excitement decreases with increasing N_2 concentration, reaching a flat minimum of 38.3 ATA when $_{N_2}P_{ex}$ equals about 25 atm. The pattern of N_2 administration has little effect on the onset of this particular symptom.

Coarse tremors

At both compression rates tested, the data relating coarse tremor threshold pressures to nitrogen partial pressure at the onset of the coarse tremor stage can readily be fitted to linear regression equations. Four conditions were thus represented, as illustrated in Fig. 2, i.e., continuous N_2 injection and bolus N_2 injection, each at compression rates of 60 and 1000 atm/h. In each case correlation coefficients were higher than 0.85, and indeed were higher than 0.95 for all but the experiments involving bolus injections at the fast compression rate. The parameters *A* and *b* of the corresponding regression equations for continuous and for bolus N_2 injection are shown in Table 2, rows 3 and 4. Inevitably, the intercepts of the regression lines with the P_{CT} axis (i.e., $_0P_{CT}$ at $P_{N_2} = 0$) for continuous infusion do not differ significantly from

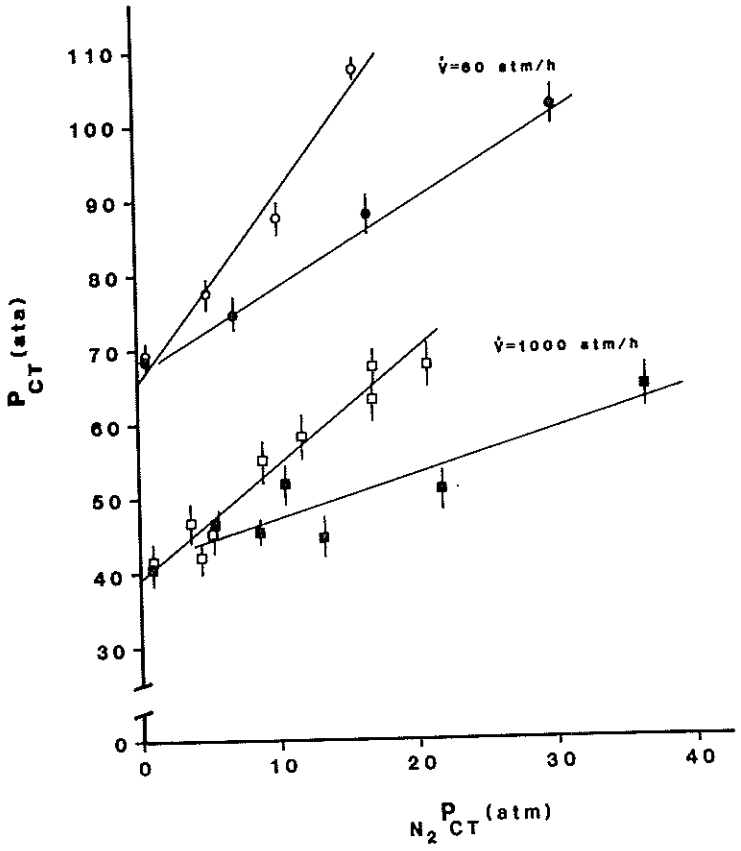

Fig. 2. Mean threshold pressures for coarse tremor stage as function of nitrogen partial pressure at tremor onset in CD-1 mice. ⊢——⊣, Regression lines (cf. Table 2 for equations); ○, ●, continuous or bolus N_2 injections at compression rate of 60 atm/h; □, ■, continuous or bolus injections at compression rate of 1000 atm/h.

the corresponding intercepts for bolus injection; this intercept for the slow compression was 60% larger than that for rapid compression, in both cases reflecting the effect of the compression rate in the absence of N_2. At both compression rates, the regression coefficients describing the continuous injection series were more than twice as large as the corresponding values for bolus injections of N_2. Under both injection conditions, furthermore, the regression coefficients for slow compression were about twice as large as the corresponding regression coefficients for rapid compression. The equations can be rewritten in the form $P_{CT} = {}_0P_{CT} \cdot (1 + a \cdot P_{N_2})$, where $a = b/{}_0P_{CT}$. The resultant values for a, then, are 4.1% and 2.0% for continuous and bolus N_2 injections at 60 atm/h, and 3.5% and 1.6% for the corresponding values at 1000 atm/h.

These results indicate, therefore, that the relative effectiveness of N_2 in antagonizing development of coarse tremors is about the same at slow as at fast compression rates, and that at either compression rate the antagonistic effect of N_2 is about twice as marked when the N_2 is administered by continuous injection as when it is administered by bolus injection.

HPNS convulsions Type I

The threshold pressures for HPNS convulsions are plotted as functions of nitrogen partial pressure at convulsion onset for the two patterns of nitrogen administration in Fig. 3. The data

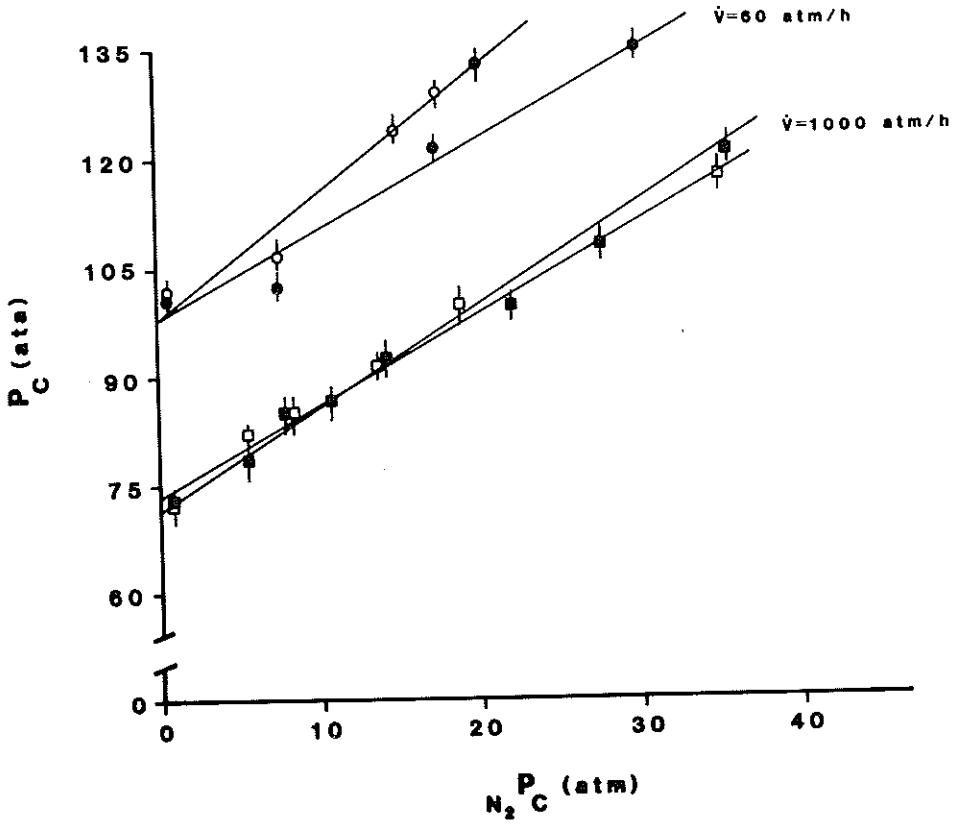

Fig. 3. Mean threshold pressures for Type I HPNS seizures as functions of nitrogen partial pressure at seizure onset in CD-1 mice. ⊢——⊣, Regression lines (See Table 2 for equations); ○, ●, continuous or bolus injections of N_2 at compression rate of 60 atm/h; □, ■, continuous or bolus injections of N_2 at a compression rate of 1000 atm/h; ⊙, delayed split bolus injection beginning at 76.6 and 102 ATA at compression rate of 60 atm/h.

obtained at rapid compression fit closely to a linear regression equation. The corresponding regression parameters are shown in Row 6 of Table 2. The corresponding data for a compression rate of 60 atm/h, however, show what appears to be a consistent and significant departure from linearity in the region of low N_2 pressures. This matter is to be discussed and analyzed in another communication. For present purposes, however, deviations from linearity are not so large as to preclude fitting the data to linear regression equations for ease of comparison. The regression parameters for this case are shown in Row 5 of Table 2 for the two modes of N_2 injection. As in the case of coarse tremors the intercept of the regression with the zero N_2 coordinate was not affected by varying the manner of N_2 administration, and as in that case, too, this intercept was substantially lower at the higher compression rate than at the lower one. At 1000 atm/h, the regression coefficients corresponding to continuous N_2 injection are slightly smaller than those corresponding to bolus injection of N_2, the ratio between the two being as 0.9 to 1. At the slower compression rate the regression coefficient for continuous injection of N_2 is significantly larger than that for bolus injections of N_2 ($P < 0.01$). The difference, however, is considerably smaller than in the case of the coarse tremor thresholds (a ratio of 1.35:1 vs. 2.19:1).

Thus the bolus effect on HPNS Type I convulsion thresholds is substantially smaller but significant at the slow compression rate, but it disappears or is even reversed at a compression rate of 1000 atm/h.

Delayed injection of bolus

These several sets of results can be summarized conveniently by noting that the linear regression equations for threshold pressures on nitrogen partial pressures can be subtracted to yield corresponding regression equations for the net effect of manipulation of injection mode or of compression rate on nitrogen partial pressure. The relations for the effect of difference in injection mode on coarse tremor and on convulsion threshold pressures plotted thus (Fig. 4 *top* and *bottom*) show, for coarse tremors at both compression rates and for convulsions at the slower compression rate, that the bolus effect (the difference in mean threshold pressures between continuous injection and bolus injection experiments) increases linearly with increasing N_2 concentration. In contrast thereto, in the case of convulsion onset at the rapid compression rate, the bolus effect at all N_2 partial pressures explored is negligible.

To further explore the significance of this last observation, i.e., the absence of a bolus effect at rapid compression rates, a series of experiments was conducted in which 17.1 atm of oxygen

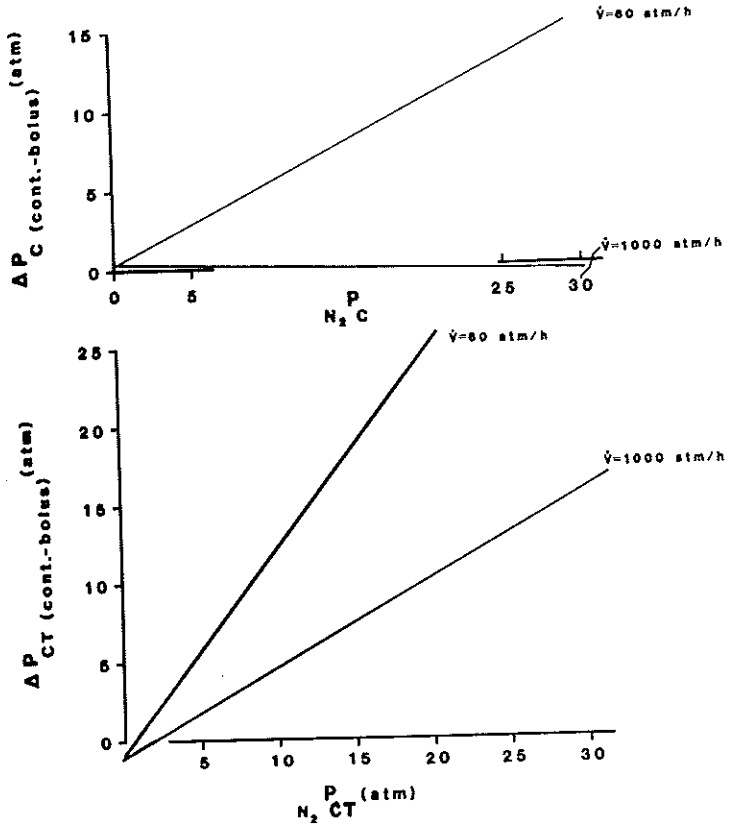

Fig. 4. Regression equations for difference between onset pressures on continuous or bolus injection of N_2 for coarse tremors (ΔP_{CT}) and for Type I convulsions (ΔP_c), at compression rates of 60 and 1000 atm/h, upon N_2 partial pressure at symptom onset in CD-1 mice.

were injected at various stages of compression. The pattern of this experiment is illustrated in Fig. 5. Bolus injections begun at 17.4 ATA, 70.4 ATA, and 76.6 ATA resulted in mean convulsion threshold pressures that were indistinguishable from one another (*top panel* of Fig. 5). A final experiment of this series sought to further delay the N_2 injection. To do so without incurring premature HPNS seizures required the use of a split-dose technique: the first 6.8 atm of N_2 were injected as before, beginning at 76.6 ATA; compression was then continued using He to 102 ATA, when the remaining 11.1 atm of N_2 were injected, and the compression was finally completed by further injection of He. This pattern of injection resulted in significant elevation of the mean convulsion threshold pressure to a point indistinguishable from the threshold pressure expected if the same amount of N_2 had been injected continuously (Figs. 3 and 5). The difference between the convulsion threshold pressures corresponding to the single bolus injection at 76.6 ATA and the split-bolus injection is well secured statistically ($P < 0.02$). Thus, it would appear that whatever change in response to compression under N_2 is attributable

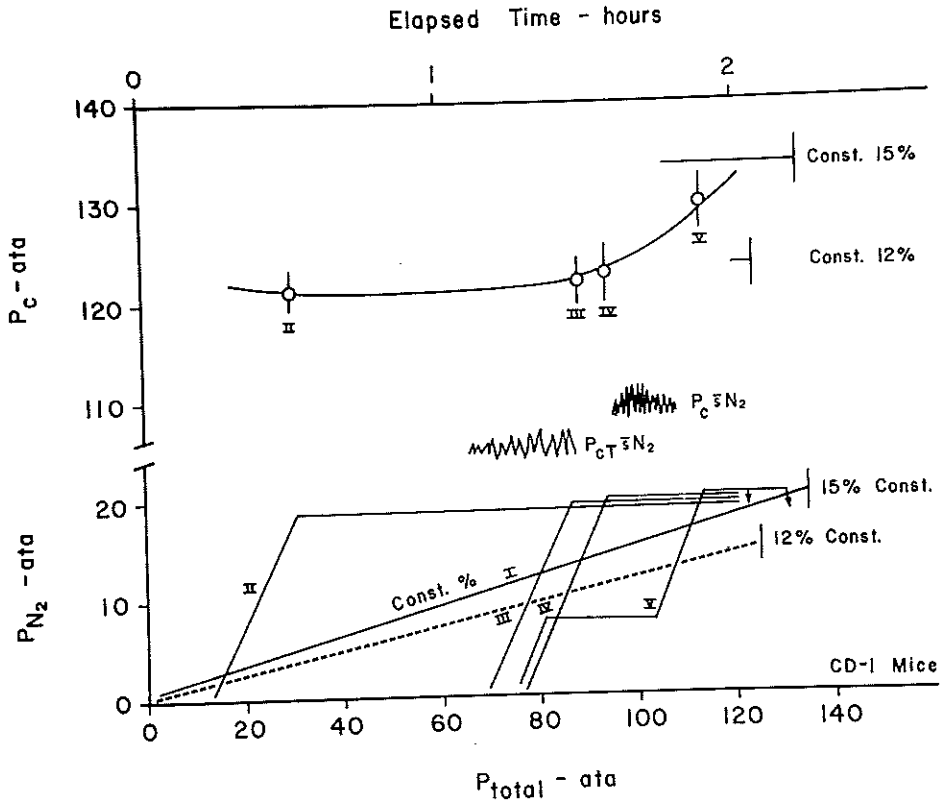

Fig. 5 Convulsion threshold pressures *(upper curve)* and time course of nitrogen partial pressure in chamber *(lower diagram)* plotted as function of elapsed time (abscissa marking at *bottom*) for different patterns of N_2 administration. The patterns of N_2 administration designated by Roman numerals are as follows: I: Compression gas He-15% N_2 (*dashed line* showing corresponding data for He-12% N_2 is also included for reference). II–V: All involve bolus administration of 17.1 atm N_2. This was begun at 17.4 ATA for II, at 70.4 ATA for III, and at 76.6 ATA for IV and V. V: Split bolus 6.8 atm being injected beginning at 76.6 ATA, and 10.3 atm beginning at 102 ATA. *Insert* between upper and lower graphs indicates ranges of onset pressures for coarse tremors (P_{CT}) and Type I seizures (P_c) observed under same compression conditions with N_2 free He as compression gas.

to the difference between continuous and bolus injection must concern an event that supervenes during the very last stages preceding seizure onset.

DISCUSSION

The investigation has dealt with changes in development of neurological manifestations associated with compressions in helium-nitrogen mixtures resulting from manipulation of the pattern of nitrogen administration. The results suggest striking differences in the effects on the three symptoms singled out for observation.

Euphoria is a well-known manifestation accompanying compression of human subjects as well as of animals in helium-nitrogen mixtures (9). So far as we know, the quantitative aspects of the interaction of hydrostatic pressure with the effects of nitrogen in this regard has not been previously explored. The present data indicate that the threshold pressure for the form of excitement scored decreases as the amount of nitrogen is increased. It is useful to turn this statement around and conclude that as the pressure is increased, the amount of nitrogen required to induce a particular level of excitement is decreased. This finding then becomes reminiscent of the interaction of hydrostatic pressure with hyperoxia in the production of hyperoxic seizures: there, too, as pressures were increased within certain limits, oxygen partial pressures required to elicit the first hyperoxic seizure decreased (10). In both of these situations, it would appear that the action of the pharmacologically active gas on the central nervous system was potentiated at the higher pressures. In the case of the hyperoxic seizures it was suggested that this effect might be related to changes in metabolic rate, and perhaps to associated changes in cerebral circulation. Whether the latter play a role in the present situation remains to be established by future investigations. Because of the relation between the amount of nitrogen required to produce measurable effects and the critical pressure at which the recognizable excitement stage supervenes, manipulation of the timing of bolus injections in this case was impractical. The present data are, therefore, merely suggestive, but they are not conclusive in indicating that onset of the excitement stage does not appear to be modified significantly by changing the pattern of nitrogen administration from continuous injection to bolus injection. The available data do, however, seem to indicate that even if there is in this case a bolus effect, its magnitude will have to be quite small.

The tremor stage is the one stage for which data are available for both human and animal subjects (11, 12). Both clinical and neurological evidence attest that the mechanisms giving rise to the two stages of the HPNS tremors (fine and coarse tremors in our terminology) are distinct. In the case of the mouse, while fine tremors are not readily diagnosed, onset of the coarse tremor stage is well defined. Nitrogen markedly affects this stage and, in the CD-1 mouse at least, strongly delays its onset. The effect is directly proportional to the concentration of nitrogen present at the onset of this symptom, each atmosphere of nitrogen increasing the coarse tremor onset by 3.5%–4%, regardless of compression rate (cf. Table 2). Substituting bolus administration of nitrogen for continuous nitrogen injection reduces this effect of nitrogen to less than half (i.e., 1.3%–1.7% per atm N_2). As has been described previously, onset of tremors of this type is markedly accelerated by increasing the compression rate (13). It is not clear yet whether this effect should be interpreted as the result of more intense stimulation during the more rapid compression or whether, as in the case of convulsions, this effect is due in whole or in part to the action of compensatory mechanisms triggered during the compression and requiring a certain amount of time to develop (cf. Ref. 14). If the former were the case, the bolus effect might be interpreted as contributing further to intensification of the stresses

to which the animal is subjected during compression. The only possible way in which this could come about would be by the continuous change in composition of the compression atmosphere to which the animal is subjected in the case of bolus injections. This is particularly true during the actual time when the bolus is injected: At that point, in the case of the early bolus injections, the compression medium is changed from a helium atmosphere containing small admixtures of nitrogen and oxygen to an atmosphere that, immediately after completion of the bolus injection, is made up of more than 50% nitrogen. A clinical manifestation underscoring this consideration is the development of intense grooming behavior during the bolus injection and for some minutes thereafter. It seems plausible to associate this with counterdiffusion effects (15). In the present case such effects possibly should be termed *isobaric decompression*. In the mice this effect is quite transient, the symptoms disappearing within 4–5 min after termination of the bolus injection. In the case of the rapid compression experiments, however, these bolus effects extend almost to the point of onset of coarse tremors and could possibly account for the more marked effect of bolus injection on coarse tremor onset at rapid compression rates (cf. Table 2, last column).

The possibility that these effects may not be merely peripheral but may include perceptible changes at the central nervous system level is suggested by the curious alteration in tremor behavior, the shaking stage, observed regularly when bolus injections were made at a time when the animals were near or already in the coarse tremor stage. The clinical event observed here is reminiscent of the phenomenon described as wet dog shakes by a number of investigators (16), and associated with epileptiform activity in various structures of the limbic system (17). Clearly, these interpretations must remain tentative at the present time; they do, however, invite analogous experiments substituting a more potent agent, such as nitrous oxide, for nitrogen so that the partial pressures required to elicit measurable effects will be small enough to substantially eliminate counterdiffusion effects.

In the case of HPNS Type I convulsions, the effect of nitrogen admixture to the compression gas was found to deviate systematically from direct proportionality to the amount of nitrogen present, but to do so to a relatively small extent, still compatible with linear regression analysis. Such analysis showed that when the nitrogen was administered continuously, the relative magnitude of the effect on convulsion onset was less than half as large as the corresponding effect on coarse tremor threshold pressures (Table 2, Column 4). As in that case, the relative magnitude of the nitrogen effect under those conditions was independent of compression rate. With bolus injections, on the other hand, a quite different pattern is observed (Table 2, Column 7): At the slower compression rate, as in the tremor case, nitrogen injected by bolus was less effective than the same amount introduced continuously, though the ratio of the effects was only as 1:1.35, rather than as 1:2.2 or 2.6 for the tremors. At rapid compression, however, the opposite was observed, bolus injection being more effective in postponing HPNS convulsions than continuous injection of an equal amount, the effectiveness ratio being 1:0.86. Time relations in this case are such that it is unlikely that the transient effect of the nitrogen bolus could be reflected in the ultimate development of seizures, since the seizures develop no less than 1.5 h after the end of the bolus injection. This conclusion is strengthened by the observation that changing the point at which the nitrogen was injected from 17.1 to 76.6 ATA did not affect the threshold pressure of the final convulsion (Fig. 5).

The two most striking observations in this series, then, are on the one hand the lack—or really inversion—of the bolus effect on rapid compression, and the disappearance of the bolus effect when bolus injection was delayed so that the bulk of the nitrogen was injected beginning at 102 ATA only.

Analysis of these data (Fig. 6) shows that there is no way of correlating these results with differences in the nitrogen profile, regardless of whether this is attempted by comparing the time integrals of total nitrogen concentration to which the animals were subjected during the course of the different compressions, or by focusing on the period during which nitrogen concentrations in the bolus-exposed animals were higher than in the continuous-injection experiments. It is only when one focuses on events at high total pressures that a semblance of conformity between the magnitude of the bolus effect and the time course of nitrogen partial pressures can be attained (Fig. 6 *C, D*). Some years ago we proposed (14) that the compression rate effect on HPNS convulsion threshold pressures could best be described by hypothesizing a dual mechanism in which tendencies for convulsion development increased with total pres-

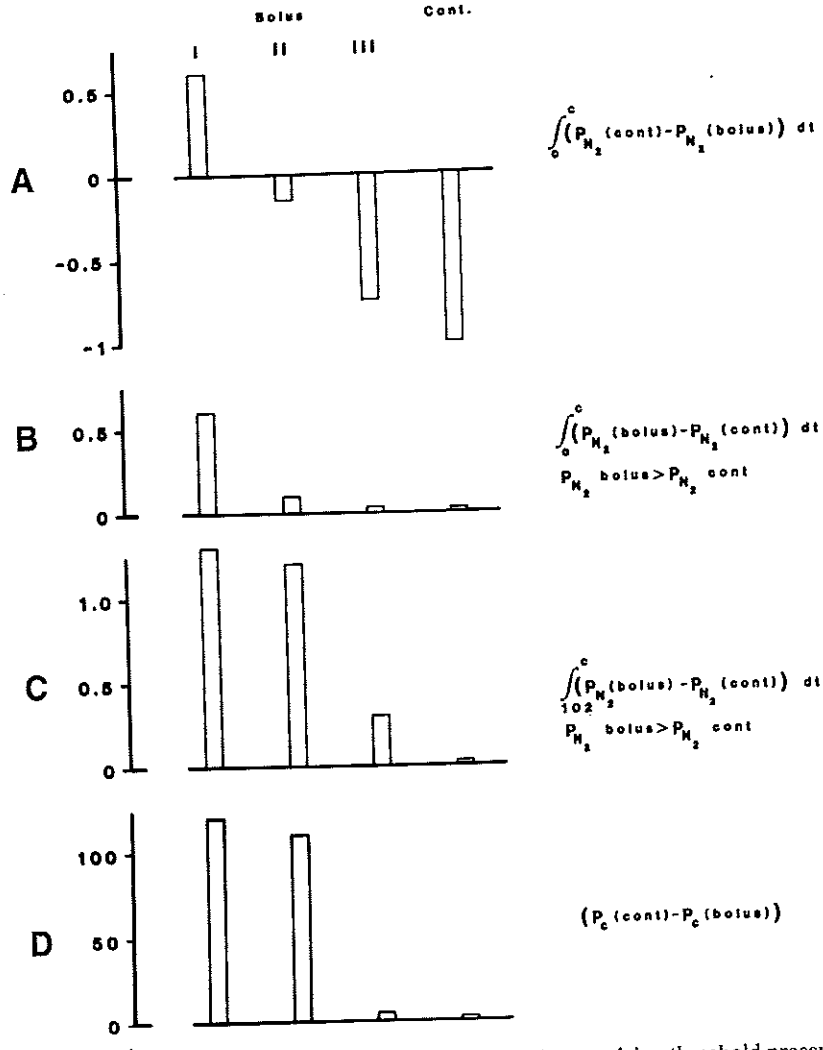

Fig. 6. Comparison of N_2 partial pressure/time integrals with convulsion threshold pressures resulting from four modes of N_2 administration as indicated at *top*. *A:* Net difference in $\int P_{N_2}\,dt$ between continuous and bolus N_2 injections from beginning of experiment to convulsion onset. *B:* $\int P_{N_2}\,dt$ integral for periods where P_{N_2} was greater in bolus than in continuous injection experiments. *C:* Same as *B*, but covering only portions where total pressure was 102 ATA or greater. *D:* Mean difference in convulsion threshold pressures between continuous injection and bolus injections of N_2 for same experiments.

sure, while a monoamine-dependent convulsion-delaying mechanism was called into action during the compression and progressed at a rate with a half time of approximately 1 h. No attempt was made at that time to decide whether this latter mechanism progresses throughout the entire course of the compression, or whether it is triggered to begin its development at some critical pressure. The present data would be compatible with this latter hypothesis if it were enlarged by specifying a particular time course for this event, and by postulating that nitrogen interferes with its development. The argument can be phrased as follows: 1) If nitrogen partially blocked development of this convulsion-delaying effect, one would predict that bolus injections, which subject the animals to higher mean nitrogen partial pressures than the continuous-injection experiments, should result in lower convulsion thresholds—i.e., that bolus injection should result in lower anticonvulsant effects of nitrogen at the slower compression rates than continuous injection of nitrogen, in accordance with the data. 2) If, as postulated in the earlier analysis of the effect of the compression rate, this effect is minimal at rapid compression rates, one would predict either that at rapid compression the bolus effect ought to be also absent or even that it might be inverted as a result of the action of the higher mean nitrogen levels on the convulsant effects of hydrostatic pressure; again, this is in accordance with the observed facts. 3) Finally, if the beginning of development of this convulsion-delaying effect did not coincide with the beginning of the compression but were delayed until a specific stage of HPNS severity had been attained (to some such pressure as 85 ATA, for instance, when unprotected animals are beginning to develop HPNS seizures at the compression rate being used in Fig. 5), one would predict that postponing bolus injection until its completion coincided with this critical stage should exert no effect on the magnitude of the bolus effect; again, this is in accord with the data, although it is not clear whether the critical pressure in this case should be sought at the intercept of nitrogen partial pressures between bolus and continuous injection, or at the point of completion of the bolus injection. This leaves an uncertainty of approximately 6 or 7 atm or as many minutes of time. 4) Finally, if the convulsion-delaying effect could be assumed to have gone to completion, or at least to approach completion, by the time the animals had reached a pressure of just over 100 ATA, or a time 15–20 atm short of seizure onset, one would predict that the split bolus injections used here (the bulk of the nitrogen being injected beginning at 102 ATA) should exert no effect on the eventual convulsion threshold and should result in abolition of the bolus effect, again as observed in the present series.

Taken together, these data suggest substantial refinement in the time structure of development of the convulsion-delaying effect in these compression experiments. On the whole, the agreement of prediction with observed facts, utilizing only a minor extension of the hypothesis previously proposed on other grounds, renders this approach a most appealing working hypothesis.

It is interesting to compare the present data with observations presented some years ago concerning barbiturate effects on HPNS convulsions in the same strain of mice (18). Recalculating those old data, it can be shown that they fit, to an acceptable extent, regression equations similar to those used for the present series. For the case of phenobarbital, and expressed in μg/kg of this drug, the regression equation for a compression rate of 40 atm/h is

$$^{40}P_c = 107.7\ (1 + 2.14\ D) \qquad (3)$$

where D is drug dosage in mg/kg body wt, while for a compression rate of 1000 atm/h it is

$$^{1000}P_c = 71.1\ (1 + 5.20\ D) \qquad (4)$$

In this case, therefore, the relative potency of the drug at slow compression is substantially

smaller than in the case of rapid compression, the ratio between the two being as 1 to 0.4. Comparing this with the nitrogen series (Table 2, columns 4 and 7), we find that the corresponding potency ratio for continuous injection experiments is 1.0, while for bolus injections the ratio is 1:0.55. It would appear, therefore, that the phenobarbitol experiments show characteristics similar to those of the experiments with bolus injections of nitrogen, a conclusion well in keeping with the fact that, inevitably, the phenobarbital injections were carried out several hours before the beginning of the compression and thus have to an exaggerated extent the character of bolus injections. The fact that this similarity is observed between phenobarbital and nitrogen provides additional support for the view that transient effects due to changes in the makeup of the compression atmosphere are not involved in development of the bolus effect of nitrogen on HPNS convulsions.

From a practical point of view, the present data support the recommendation made by Rostain and collaborators (4, 5) to the effect that constant nitrogen administration during compression is more effective in suppressing HPNS symptoms than bolus injections. From the point of view of understanding of the neurophysiology of the HPNS, the present data provide new insights into the complex time patterns associated with the development of this entity. In addition, they once again underscore the wide differences in the behavior of the several components of the symptom complex designated as the high pressure neurological syndrome in the face of modifications of compression procedures. Finally, it may be in order to note that review of the material presented leads us to conclude that the phenomena described here do not contribute to the discussion of the two possible ways of viewing the nature of the effect of nitrogen on the development of HPNS—i.e., either as a manifestation of a direct reversal of pressure effects on central nervous system function, or alternatively as the result of masking by the anesthetic effects of nitrogen of manifestations of pressure-induced changes in central nervous system function (cf. Ref. 6).

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Brauer RW, Hinson WM. Effets des variations dans le temps et la façon d'ajouter l'azote sur le développement du syndrome nerveux des hautes pressions chez la souris. Undersea Biomed Res 1983; 10(4):281–298.—L'effet de changer la façon d'injecter l'azote (N_2) sur les pressions seuils des trois symptômes associés avec la compression dans des atmosphères d'azote-hélium a été investigué chez la souris. Les pressions seuils d'excitation diminuent avec l'augmentation des concentrations de N_2 et ne sont pas affectées par le changement de l'infusion continuelle de N_2 à l'injection d'un bolus équivalent de ce gaz. Le temps d'apparition du tremblement grossier est retardé en proportion directe avec la quantité de N_2 présente et avec la même puissance relative pour une compression à 60 atm/h ainsi que 1000 atm/h. A ce propos, l'injection de N_2 sous forme de bolus est plus de la moitié moins efficace que l'infusion continuelle. Les pressions seuils pour les convulsions reliées au syndrome neurologique des hautes pressions (SNHP) croissent avec l'augmentation des quantités de N_2; la puissance anti-convulsante du gaz étant indépendante de la vitesse de compression quand il est introduit par infusion continuelle. Un effet similaire de bolus, quoique plus petit que celui avec du tremblement grossier, survient à la vitesse de compression de 60 atm/h, mais est absent à 1000 atm/h. L'injection tardive du bolus de N_2 abolit également cet effet de bolus. Les mécanismes possiblement responsables de ces effets sont discutés et l'impact des résultats sur la séquence temporelle du développement du SHNP est analysé.

souris
azote
hélium
convulsion
tremblement
injection
SNHP
haute pression
compression à l'azote

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APPENDIX

SYMBOLS USED

Symbol	Meaning
$\dot{P}$	Compression rate, atm/h
P_{CT}, P_c	Threshold pressures for coarse tremors or convulsions
${}^{V}P_c$	P_c at compression rate V
${}_{N_2}P_{ex}$, ${}_{N_2}P_{CT}$, ${}_{N_2}P_c$	Nitrogen partial pressures at moment of onset of excitement, coarse tremors, or convulsions, respectively
${}_{He}P_{ex}$	Helium partial pressure at moment of onset of excitement stage
ΔA (. . .)	Difference in A-values under conditions described in parentheses
$\underline{r}$	Correlation coefficient