Narcotic Effects Produced by Nitrous Oxide and Hyperbaric Nitrogen Narcosis in Rats Performing a Fixed-Ratio Test

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TURLE–LORENZO, N., B. ZOUANI AND J.-J. RISSO. Narcotic effects produced by nitrous oxide and hyperbaric nitrogen narcosis in rats performing a fixed-ratio test. PHYSIOL BEHAV 67(3) 321–325, 1999.—Narcosis is a neurological syndrome that reduces capacities of divers. Although this phenomenon appeared at the end of 19th century, the mechanisms are not yet elucidated. The greatest technical problem is that these studies are carried out under hyperbaric conditions. Nitrous oxide is known to be an inducer of narcosis, at atmospheric pressure. The aim of this study is to compare two narcotic environments; a normobaric narcosis under several percentages of nitrous oxide, and an hyperbaric narcosis under 0.9 MPa of Nitrox (N₂O mixture). This comparison is realized on rats submitted to a fixed-ratio 15 test, in which they have to press a lever to get rewarded. The results show significant performances decreases: the number of pressed lever are reduced by 50% under Nitrox and by 70% under N₂O. Nitrous oxide could be considered as a normobaric model of hyperbaric narcosis. © 1999 Elsevier Science Inc.

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INERT gases at raised pressure exert narcotic effects in both human divers and experimental animals. Symptoms of inert gas narcosis include euphoria, psychomotor disturbances, impaired judgement, spatial disorientation, hallucinations, and, ultimately, loss of consciousness (2). For instance, in humans, breathing air at pressure of more than 0.5 Mpa induces the first signs of nitrogen narcosis; at pressures higher than 3.5 Mpa, nitrogen produces anaesthetic effects in mice. Whatever the inert gas used, previous studies clearly reported a homogeneity in the manifestation of the behavioral disturbances (1,7,9). It is assumed that the mechanisms of action of inert gases are fundamentally similar to those of general anesthetics. However, do general anesthetics, including inert gases, bind directly to proteins or influence activity by indirectly perturbing membrane lipids remains a major question. Although this is still under discussion, it is well established since the discovery by Meyer and Overton of a correlation between narcotic potency and lipid solubility, that the onset pressure of inert gas required to produce narcosis varies inversely with lipid solubility (14).

In a recent neurochemical study, it has been demonstrated that exposure to 50% nitrous oxide or to 0.9 Mpa nitrogen resulted in a similar decrease of 70% in extracellular dopamine (DA) concentrations compared to concentrations at normal atmospheric pressure (16). In contrast, exposure to 50% nitrous oxide decreased Dihydroxyphenylacetic acid (DOPAC) concentration, the main metabolite of DA, whereas exposure to 0.9 MPa increased it. This was proposed to be the consequence of either some pressure effect under 0.9 Mpa nitrogen, a condition that is absent under 50% nitrous oxide, or some different mechanisms of action of nitrogen and nitrous oxide at the cellular level.

Behavioural experiments, performed in our laboratory, have put on evidence that several N₂O percentages could imply a decrease of locomotor activity. (a). A spontaneous locomotor activity, estimated by a video-tracking system, have shown that, up to 40% of N₂O, the animals produce high movements, and the authors observed that these movements decreased at a high percentage of nitrous oxide (15). (b). A vigilance task has been performed by Courtiere and Har-
douin (4). In this test, rats had to detect a brief intensity variation of the ambient light in the Skinner box. These experiments showed that, for N\textsubscript{2}O percentages lower than 30%, animals did not present any degradation of their performances. The first significant decrease of the behavioural capacities of the animals appeared around 50% N\textsubscript{2}O, and continuously increased until 80% of N\textsubscript{2}O, where the animals seemed to be unable to perform any behavioural test.

For this reason, a percentage of 50% N\textsubscript{2}O was chosen to give a normobaric normoxic N\textsubscript{2}O–Nitrogen breathing mixture.

Concerning experiments under hyperbaric condition, we referred to Jennings (13), for whom pressures lower than 1.2 MPa were narcotic but not lethargic, the animals being able to perform behavioural tests. Therefore, the pressure chosen, in our experiment, was set at 0.9 MPa.

Thus, to determine whether such differential effects may occur at the behavioural level between nitrogen narcosis and nitrous oxide narcosis, we examine the effects of 50% nitrous oxide and 0.9 MPa nitrogen on locomotor activity, using a fixed-ratio operant test, during which the animals have to press a lever to get rewarded. All experiments were performed in a hyperbaric chamber under normoxic conditions.

**MATERIALS AND METHODS**

**Animals**

The animals were male Long–Evans rats obtained from Janvier Breeding (France) weighing 330–350 g. They were housed in individual cages located in a room at 22 ± 1°C, and maintained on a 12:12-h L/D cycle (lights on 0700–1900 h). Water was available ad lib, and a moderate food deprivation maintained the animals at 85% predeprivation weight.

**Skinner Box Training Sessions**

The experiments were conducted in a 180 l hyperbaric chamber because of its ability to receive the Skinner box. In this hyperbaric chamber, the ambient light is given by a specific COMEX hyperbaric lamp. The ventilation needed for the homogenisation of the breathing mixtures was performed by a remote control fan. Rats were trained in a Skinner box (IMETRONIC) placed in the hyperbaric chamber with ventilation. The operant cage was equipped with one retractable lever and a food pellet dispenser that delivered 45 mg pellets (Phymep food pellet precision) in a food tray. Two series of infrared beams situated 2 cm above the floor were displayed—one parallel to the task panel, and the other parallel to the back panel for forward and backward activity and rearings recordings. Each beam interruption was recorded as an index of locomotor activity. The luminance of the house light was set at 3 lx. The test cage was connected to and controlled by a computer, using operant conditioning software. The size of the cage has been reduced and all these components have been adapted to hyperbaric pressure. Some connections have been realised through the hyperbaric chamber to connect the Skinner box with the computer. The walls of the Skinner box have been designed in Plexiglas so as to observe the animal during the realisation of the test in the confined narcotic ambience.

A fixed-ratio test (FR 15) was chosen in this study. This test permitted us to access to information on the locomotor activity linked to a behavioural learning, so, this test gives us more behavioural details than a spontaneous locomotor activity. For this test, the animals were trained 30 min for 2 months to press on a pedal to get rewarded. They were initially trained to press the food lever for 45 mg food pellets on a continuous schedule. Progressively, animals were shifted to a FR3, FR5, FR10, and finally to a FR15 schedule. An ambient light was present along the test except when the animals pressed 15 times on the food lever. At this time, the ambient light was turned off and the light in the food pellet dispenser was turned on. The animal could begin the test by eating the pellet presented in the food dispenser, or the test began itself when 300 s had passed. The rats were trained until performances were stable.

**Exposure to Either 0.9 MPa Nitrogen (Relative Pressure) and 50% Nitrous Oxide**

On the day of the experiment, rats were placed in the Skinner box, in the hyperbaric chamber with oxygen calibration adjusted to normoxic value by the use of a microvalve. The hyperbaric chamber was either compressed with nitrogen up to 0.9 MPa at a rate of 0.15 MPa/min or filled with a breathing mixture containing 50% of nitrous oxide. The concentration of N\textsubscript{2}O was obtained by passing N\textsubscript{2}O through the hyperbaric chamber containing atmospheric air and finally adjusting oxygen concentrations to normoxic value (210 mbar O\textsubscript{2}). N\textsubscript{2}O and O\textsubscript{2} concentrations were continuously controlled by a gas analyser (Ohmeda RGM 5250). A final percentage of 50% N\textsubscript{2}O was setted. The test began when the animals were under narcotic conditions. Simultaneously to the fixed-ratio test, each beam interruption was recorded as an index of locomotor activity.

**Statistical Analysis**

Data were expressed as a percentage of the reference (100%) values, which were calculated by averaging all the results concerning the same animal.

The effects of 0.9 Mpa nitrogen and 50% nitrous oxide were analyzed using the nonparametric Wilcoxon \textit{t}-test to compare data under narcotic ambiances versus data concerning the reference series, and the Freeman test to compare all narcotic series together when they have been performed with the same conditions.

**RESULTS**

Under normobaric narcosis, rats presented a diminution in the number of pressed levers by 70% (\( p \leq 0.01 \)). Under 0.9 Mpa Nitrox, a diminution in response rate of 50% (\( p \leq 0.05 \)) was noticed (Fig. 1).

Concerning the reaction time, the first lever pression delay (time spent by the rat to press on the lever for the first time) increased by a factor of 3 under N\textsubscript{2}O (\( p \leq 0.01 \)) versus a factor 2 under 0.9 Mpa of Nitrox (this increase is nonsignificative). The last lever pression delay (time spent by the rat to press 15 times successfully) also increases by 2.5 under N\textsubscript{2}O (\( p \leq 0.01 \)) and by 2 under 0.9 Mpa of Nitrox (\( p \leq 0.05 \)) (Fig. 2).

The number of rearings were decreased under N\textsubscript{2}O and under Nitrox. They were both significative under N\textsubscript{2}O (\( p \leq 0.01 \)) and under Nitrox (\( p \leq 0.05 \)). Concerning the locomotor activity, the number of movements were decreased under N\textsubscript{2}O (\( p \leq 0.01 \)). Under Nitrox, the movements were similar to those of the reference (Fig. 3).

**DISCUSSION**

The aim of this study was to valid normobaric N\textsubscript{2}O as a model of pure narcosis through a behavioural experiment in which rats were submitted to a fixed-ratio test.
Two narcotic conditions were compared: 50% of N₂O normobaric normoxic ambience, and 0.9 Mpa of Nitrox hyperbaric normoxic ambience. In both cases, a decrease of response rate have been noticed.

In our laboratory, the same experiment has been performed under several percentages of N₂O: 30, 40, and 60% (Hardouin, data not published). Rats submitted to the fixed-ratio test (15) have shown a dose-dependent decrease of their
locomotor activity. Time spent responding for the first hit, in all narcotic ambience, was always increased comparing to the reference. These results were in agreement with previous studies concerning N$_2$O effects in humans (11).

Another important result consists of the fact that response latencies of hits were all increased by N$_2$O such as by Nitrox. The first and the last hit were given late by the animal compared to their performances on their own reference. These results were significative, regarding the N$_2$O experiments. They were not significative for the Nitrox experiment, according to the hypothesis that a 0.9 Mpa Nitrox mixture was less narcotic than a 50% N$_2$O environment. This hypothesis was confirmed by global performances that are more affected under 50% N$_2$O than under 0.9 Mpa of Nitrox: diminution of the number of levers pressed by 70% under 50% N$_2$O versus a diminution by 50% under 0.9 Mpa of Nitrox. These results confirmed previous studies performed in humans (8,10), where exposure of subjects to 20–35% of N$_2$O was accompanied by an increased reaction time.

Concerning the rearings, experiments performed under the two narcotic ambiences put in evidence a decrease versus the reference day with a higher significativity under N$_2$O than under hyperbaric nitrox. Under N$_2$O, the forward and the backward activity were significantly decreased as well as the forward/backward activity. Under Nitrox, the activity was similar to the data obtained under 0.9 Mpa Nitrox. These results confirmed previous studies performed in humans (8,10), where exposure of subjects to 20–35% of N$_2$O was accompanied by an increased reaction time.

It has been established that inert gas narcosis depends upon the solubility of gases in the phospholipidic bilayer. Consequently, Nitrogen narcosis depends upon the partial pressure of N$_2$. Several theories exist on the mechanism of the narcosis. Several experiments have been performed to study the action of N$_2$O. This gas possesses different actions, depending of the environmental test and the receptors affected. N$_2$O induces analgesia, anxiolysis, and hyperphagia. All these symptoms proceed from direct or indirect action on dopaminergic receptors, opiate receptors, benzodiazepine receptors, or proceed from nitrogen monoxide production (NO).

This gas would bind on opiate receptors and, in this case, would induce analgesia hyperlocomotion and hyperphagia, according to the opiate receptor activity. Hynes and Berkowitz (12) have shown that the stimulation of mouse locomotor activity, produced by nitrous oxide, appears to be mediated by both endogenous opioids and catecholamines, especially dopamine. Czech (6) showed that the feeding behaviour of nondeprived rats is induced by an opiate mechanism.

N$_2$O also produces an anxiolytic action, inducing similar than those obtained by benzodiazepines. Some studies on this interaction have shown several behavioural consequences: inhibition of the burying paradigm (5) and inhibition of fright (15).

This N$_2$O anxiolytic effect could proceed from the production of nitrogen monoxide (3). By all its properties, N$_2$O may lead to different consequences, according to the test chosen. As a matter of fact, in an open-field test, the locomotor activity will increase because of the anxiolytic properwtie, whereas in an operant test, like a fixed ratio, the number of hits will decrease. So, N$_2$O could affect more than a basal locomotor activity, and acts on memory processes.

As all these mechanisms are well established, it seems that N$_2$O could act on several receptors, and so, any opioid or benzodiazepine antagonist would be sufficiently specific or adequate if applied alone. Pharmacological studies remain to be performed to elucidate the mechanism of action of N$_2$O.
BEHAVIOURAL STUDIES OF NARCOSIS

In conclusion, in the present study, the results show that N₂O narcosis in normobaric conditions could be used as a narcotic model for inert gas narcosis studies, even if the narcosis mechanisms of action have not as yet been totally elucidated. Moreover, it seems interesting to establish a correlation between N₂O percentages and the Nitrox hyperbaric pressure, concerning the narcotic manifestations. This correlation could be performed in behavioural but also in neurochemical studies to establish the neuropsychomotor symptoms in both narcosis.

REFERENCES