

Strain Differences in the Antinociceptive Effect of Nitrous Oxide on the Tail Flick Test in Rats

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To study strain differences in antinociceptive effects of nitrous oxide (N₂O), we examined various outbred and inbred strains of rats by using tail flick latency response. All outbred strains, i.e., Sprague-Dawley from two different breeders, Wistar, and Long-Evans, showed a similar antinociceptive response. Namely, the peak response occurred after 30 min of exposure, and tolerance to N₂O developed within 60 to 90 min. Each of the four inbred strains examined, i.e., Wistar-Kyoto, Brown-Norway, Fischer, and Lewis, displayed a unique pattern of antinociceptive response to N₂O. Wistar-Kyoto and Brown-Norway strains showed somewhat similar patterns as those observed in outbred strains, apart

from the fact that the Wistar-Kyoto displayed a more distinct development of tolerance, whereas, the Brown-Norway strain had a lower peak effect. The Fischer strain displayed the greatest antinociceptive response to N₂O, and did not develop tolerance. The Lewis strain showed no antinociceptive response to N₂O. These results indicate differences in the durability and the magnitude of the antinociceptive response to N₂O among various strains of rats. **Implications:** Because of the variability that already exists, we recommend that animal studies examining the antinociceptive effects of nitrous oxide should be performed on inbred rat strains. (Anesth Analg 2000;90:195-9)

The antinociceptive effect of nitrous oxide (N₂O) diminishes over time during continuous administration in humans (1,2). This biologic phenomenon, referred to as "tolerance," has also been demonstrated in rats and mice by using either temperature (3-7) or pressure (8) as the stimulus for nociception. Tolerance to other neurobehavioral effects of N₂O has been reported in cats involving electroencephalogram activity (9) and anticonvulsant action (10). However, Shingu et al. (11) found that tolerance does not develop to analgesic effects of N₂O determined in rats by using the tail flick test. Quock et al. (12,13) also found quantitative differences in the antinociceptive response of various mouse strains to N₂O using the abdominal constriction test (12,13). This differential sensitivity to N₂O among different strains of mice prompted us to study strain differences in antinociceptive effects of N₂O on the tail flick test in rats.

Methods

The study protocol was approved by the institutional animal investigation committee (Stanford University and Veterans Administration Palo Alto Health Care System). Animals were provided *ad libitum* food and water and artificial lighting between 6 AM and 6 PM. Adult male rats of four outbred strains, Sprague-Dawley (B & K Universal, Fremont, CA), Sprague-Dawley (Charles-River, Wilmington, MA), Wistar (Charles-River), and Long-Evans (Charles-River), were used along with adult male rats of four inbred strains (all from Charles-River), namely, Wistar-Kyoto, Brown-Norway, Fischer, and Lewis (Table 1). All experiments were performed between 11 AM and 4 PM, and each animal was used for only one set of studies to eliminate the effects of learning and chronic tolerance on tail flick latency (TFL).

The Tail Flick Test

A high-intensity light was focused on the dorsal side of the middle third of the rat's tail and the time for the rat to move its tail out of the light beam was recorded (Tail-Flick Analgesia Meter; Columbus Instruments, Columbus, OH) and referred to as TFL. The intensity of heat was set so that basal TFL occurred between 3 and 4 s. A cut off latency of 10 s was arbitrarily

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Table 1. Summary of Data from Experimental Groups in Different Strains

Strain (breeder, age) group	No. of animals examined	Body weight (g, mean \pm SD)
Outbred strains		
Sprague-Dawley (B & K Universal, 12-14 wk old)		
Control	6	490 \pm 17
N ₂ O	8	452 \pm 25
Sprague-Dawley (Charles-River, 11-13 wk old)		
Control	6	460 \pm 35
N ₂ O	7	410 \pm 19
Wistar (Charles-River, 7-10 wk old)		
Control	4	238 \pm 3
N ₂ O	6	304 \pm 16
Long-Evans (Charles-River, 9-10 wk old)		
Control	5	302 \pm 10
N ₂ O	7	304 \pm 16
Inbred strains		
Wistar-Kyoto (Charles-River, 8-10 wk old)		
Control	4	233 \pm 6
N ₂ O	8	194 \pm 20
Brown-Norway (Charles-River, 9-10 wk old)		
Control	5	219 \pm 7
N ₂ O	7	225 \pm 8
Fischer (Charles-River, 9-11 wk old)		
Control	5	273 \pm 15
N ₂ O	6	250 \pm 54
Lewis (Charles-River, 12-15 wk old)		
Control	5	344 \pm 26
N ₂ O	9	316 \pm 33

B & K Universal, Fremont, CA. Charles-River, Wilmington, MA.

selected. TFL was measured to the nearest 0.1 s. Each TFL measurement consisted of a mean of three separate trials over 5 min. This was repeated every 30 min.

Before ascertaining baseline TFL, each rat was placed in a cylindrical plastic restrainer (Broome Rodent Restrainer; Harvard Apparatus, South Natick, MA), which prevented free movement while allowing easy access to the length of the tail. Rats were allowed to accommodate to their restrainers for 2 h before baseline tail flick measurements were taken. All rats were confined to their individual restrainers throughout the duration of the experiment.

N₂O Exposure

All gas exposure was performed in a Plexiglas chamber (20 in. long, 35 in. wide, and 15 in. high) with a sliding door on one side (for insertion of animals). This airtight chamber was large enough to enclose the Tail-Flick Analgesia Meter and up to five rats, each in its individual restrainer. Fresh test gases (10 L/min) were delivered from an anesthetic machine into the chamber via an inflow port, circulated throughout the chamber by two small fans, and purged by a vacuum set to aspirate at the same rate as the fresh gas inflow. Gas concentrations, including those for N₂O, oxygen (O₂), and carbon dioxide (CO₂), were monitored continuously by infrared gas spectrometry (Datex 254

airway monitor; Datex Medical Instrument, Tewksbury, MA), and recorded on a strip chart recorder. N₂O concentration was maintained between 70% and 75%, while O₂ concentration was maintained between 20% and 25%. Male rats of different strains were exposed to either 75% N₂O/25% O₂ (N₂O group) or room air (control group).

The data were expressed as percent of maximal possible effect (%MPE) as follows:

%MPE =

$$\frac{\text{TFL with treatment} - \text{baseline latency}}{\text{cut off time (10 s)} - \text{baseline latency}} \times 100$$

This approach normalizes the distribution of data while retaining the graded analysis (14). Data between control and N₂O groups were analyzed by one-way analysis of variance. A *P* value <0.05 was considered significant.

Results

All outbred strains we examined, i.e., Sprague-Dawley, Sprague-Dawley, Wistar, and Long-Evans, showed a similar antinociceptive response to 75% N₂O

(Fig. 1). In all strains, the peak response occurred after 30 min of exposure, and their %MPE ranged between 60% and 80%. All strains also developed tolerance to N₂O within 60 to 90 min.

Each of the four inbred strains we examined, i.e., the Wistar-Kyoto, the Brown-Norway, the Fischer, and the Lewis, displayed a unique pattern of antinociceptive response to N₂O (Fig. 2). The Wistar-Kyoto strain showed a similar pattern as those observed in outbred strains; namely, the peak response occurred after 30 min of exposure, and tolerance developed within 60 min. Although the peak %MPE was similar to that seen in the outbred strains, development of tolerance was more distinct, i.e., %MPE decreased to almost 0% after 60 min, and the variance at each time point was much smaller than that seen in outbred strains. The Brown-Norway strain also showed a similar pattern of antinociceptive response to N₂O. However, the peak %MPE at 30 min was much smaller, i.e., approximately 25%.

The Fischer strain displayed the greatest antinociceptive response to N₂O; %MPE of all tested animals reached 100% after 30 min. Additionally, tolerance did not develop in Fischer rats. Although the variation was relatively large among tested animals, the mean %MPE never decreased to <50% during the 180-min period. However, the Lewis strain showed no antinociceptive response to N₂O at any time. In fact, the TFL actually decreased after N₂O exposure, suggesting the development of hyperalgesia.

Discussion

To date, several investigators have reported that tolerance develops to the antinociceptive effect of N₂O in rats and mice as measured by the TFL response (Table 2). However, one group of investigators has reported that tolerance does not develop to antinociceptive effects of N₂O, determined in rats using the same variable (11). Another group has also reported on quantitative differences in the antinociceptive response of various mouse strains to N₂O using the abdominal constriction test (12,13). This differential sensitivity to N₂O among different mouse strains prompted us to study strain differences in antinociceptive effects of N₂O on the tail flick test in rats. We found, in the present study, that while four outbred strains we examined showed similar responses to N₂O, four inbred strains showed variable responses to N₂O.

In all outbred strains, the peak response occurred after 30 min of N₂O exposure, with the %MPE ranging approximately between 60% and 80% (Fig. 1). All outbred strains also developed tolerance to N₂O within 60 to 90 minutes. These results are consistent with reports of most other investigators (3-7). However, each of

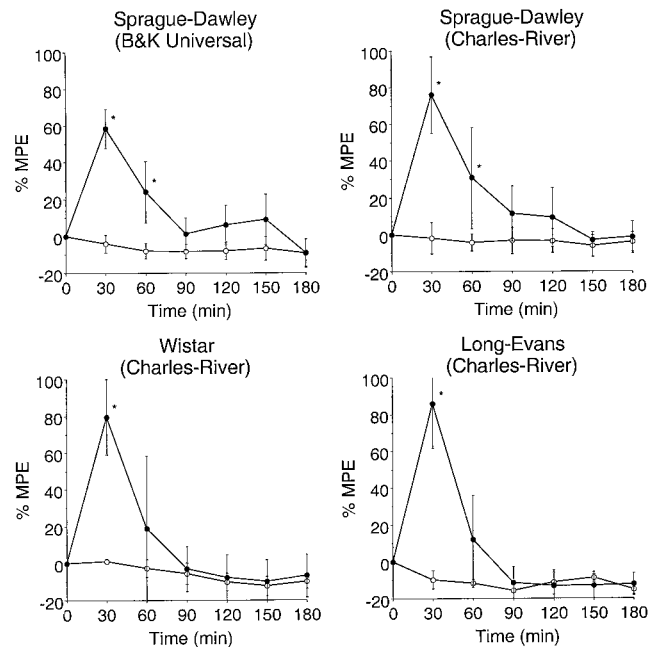


Figure 1. Time course of the effect of 75% nitrous oxide on the tail flick test in four different outbred strains of rats. Open circle indicates control groups, and closed circle indicates nitrous oxide groups. %MPE (mean \pm sd). **P* < 0.05 versus control.

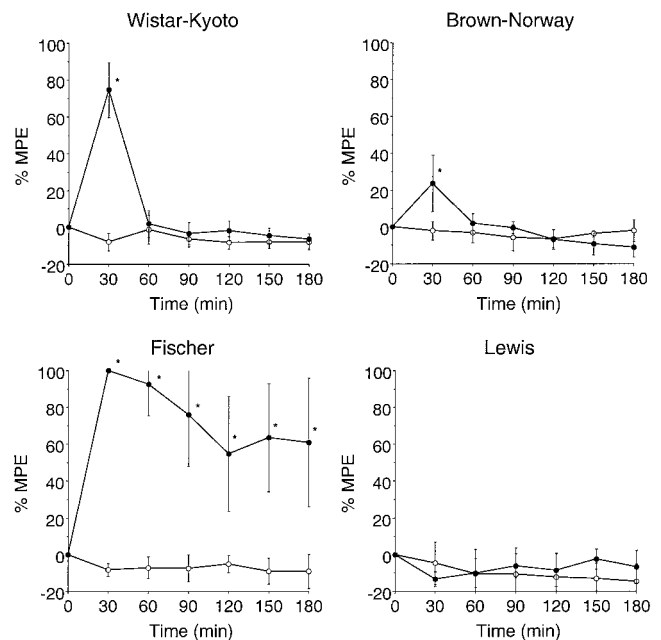


Figure 2. Time course of the effect of 75% nitrous oxide on the tail flick test in four different inbred strains of rats. Open circle indicates control groups, and closed circle indicates nitrous oxide groups. %MPE (mean \pm sd). **P* < 0.05 versus control.

four inbred strains showed different patterns of antinociceptive response to N₂O (Fig. 2). Among them, the Wistar-Kyoto and Brown-Norway strains showed somewhat similar patterns to that observed in outbred strains. In Wistar-Kyoto strain, the peak %MPE was in

Table 2. Summary of Previous Reports on Antinociceptive Effects of N₂O in Rats and Mice by Using the Tail Flick Test

	Strain	Development of tolerance	Side of stimulation on the tail
Rat			
Berkowitz et al., 1977 (3)	Sprague-Dawley	Yes	?
Berkowitz et al., 1979 (4)	Sprague-Dawley	Yes	?
	Long-Evans	Yes	?
Shingu et al., 1995 (11)	Wistar	No	Dorsal
Guo et al., 1996 (5)	Sprague-Dawley	Yes	Dorsal
Ohara et al., 1997 (6)	Sprague-Dawley	Yes	Ventral
Mouse			
Guo et al., 1999 (7)	129/svj	Yes	Dorsal

? = information not available.

the same range as those in outbred strains. However, development of tolerance was more distinct; %MPE decreased to almost 0% after 60 minutes, and the SD of %MPE at each time point was much smaller than those observed in outbred strains. The Brown-Norway strain also showed a similar pattern of antinociceptive response to N₂O as those in outbred strains. However, the peak %MPE at 30 minutes was much smaller, approximately 25%.

Of particular interest are the Fischer and Lewis strains that displayed the most widely divergent antinociceptive response to N₂O. The Fischer strain showed the greatest antinociceptive effect to N₂O; %MPE at 30 minutes of all tested animals was 100%. Furthermore, tolerance did not develop during three hours of N₂O exposure. The Lewis strain, however, showed no antinociceptive effect to N₂O at all. Interestingly, these two strains are known to differ in willingness to self-administer "dependence-producing" drugs, e.g., alcohol and cocaine (15-18). Thus far, differences in catecholamine biosynthesis in various regions of the brain (19) and differences in basal levels of opiate peptides and responsiveness to morphine administration (20) have been demonstrated. Notably, the Lewis strain of rats has lower basal levels of opioid peptides than the Fischer strain, and the administration of morphine increases opioid peptide levels in the Fischer strain but not in the Lewis strain.

The most recent studies have shown that N₂O produces a release of endogenous opioid peptides in the periaqueductal gray area of the midbrain, which activates noradrenergic descending inhibitory pathways, leading to modulation of nociceptive processing via α_2 adrenoceptors in the dorsal horn of the spinal cord (6,21). Although the underlying mechanism for development of tolerance to N₂O is not yet well understood, acute depletion of opioid peptides in the central nervous system has been hypothesized to be a possible cause (8). Differences between the Fischer and Lewis strains in antinociceptive response to N₂O and in development of tolerance could originate from a disparity in the biochemical and physiological consequences

of opioid peptides in the central nervous system in each strain. A possible explanation of our observation is that the lesser amount of endogenous opioid peptides in the periaqueductal gray area of the Lewis strain of rats is insufficient to activate the noradrenergic descending inhibitory pathway. However, the Fischer strain has abundant opioid peptides that produce a powerful antinociceptive effect of N₂O and confers resistance to the development of tolerance, because it is not easily depleted.

Our results and results from previous reports by Quock et al. (12,13) imply that investigators should use a specific inbred strain of animal when studying the antinociceptive effects of N₂O, because different strains of animals show variable responses to N₂O. Also, a less variable genetic stock of inbred strain animals will display fewer phenotypic variations than the more genetically diverse outbred strains, as is evident in the comparison between the Wistar (outbred) and the Wistar-Kyoto (inbred) rat strains. Furthermore, the genetic backgrounds of certain outbred strains might vary significantly among animals from different breeders. Thus, the results from different investigators may not be comparable, even when the results come from the same outbred strain of animals. Furthermore, any time a breeder changes an animal colony, e.g., to prevent spread of disease, it can result in a different phenotype in the outbred strain.

In summary, we have demonstrated that various strains of rats, both outbred and inbred, show different antinociceptive effects of N₂O. Our results suggest that investigators should use a specific inbred strain of animal when studying issues related to the antinociceptive effects of N₂O.

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