S_3 are escape latencies measured as the number of shocks (40 per minute) administered during trials 1 to 10, 11 to 20, and 21 to 30. One-half of the fish receiving 25 μg of camptothecin and all of the fish receiving 10 μg of camptothecin were from a group of fish in which the control animals had low responding rates. For these fish, $P = 4.518 + .233\ A_1 - .409\ A_2 + .573\ A_3 - .0198\ S_1 - .0013\ S_2 + .0118\ S_3$. Fish that failed to show an avoidance response during acquisition training or had more than five avoidances or failures to escape during the first ten trials (about 20 percent of the animals) were excluded from further study.

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18. Eighteen of 20 fish injected intracranially with 2 μg of actinomycin died between 11 and 20 days of the injection, while two fish survived. In 20 fish injected intracranially with 50 μg of camptothecin, there were no deaths.
19. J.H.N. was supported by NIMH training grant 07417. Supported by NIMH grant MH-12506 and NSF grant GB-5125X.

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Analgesic Strength of 33 Percent Nitrous Oxide: A Signal Detection Theory Evaluation

Abstract. *Radiant heat stimulation was applied to volunteers and rating scale responses were obtained to assess the analgesic properties of 33 percent nitrous oxide. The methodology of signal detection theory was applied to the data to demonstrate that nitrous oxide reduces both sensitivity to pain and willingness to report pain. This method is superior to threshold estimation for the evaluation of analgesics.*

The efficacy of analgesic agents for pain in humans has been studied extensively in the laboratory by using the sensory threshold as an index of sensitivity to pain, but, as in other areas of behavioral research, this index has proved to be unreliable. As Goldiamond (*1*) noted, the threshold reflects both sensory sensitivity to stimulation and response bias (willingness to report the experience of the stimulation). Thus, an elevated pain threshold following drug administration may reflect changes in either or both of these factors. Recently, an alternative methodology based on signal detection theory (SDT) has been applied to the problems of quantifying human pain in the laboratory in terms of sensory and response factors (*2*). Our purpose in this report is to demonstrate the usefulness of SDT methodology in assessing the analgesic effects of agents commonly used to relieve pain. We chose nitrous oxide because in a concentration of 33 percent, its analgesic effects are well documented (*3*) and it still permits subject control and cooperation.

The Hardy-Wolff-Goodell dolorimeter was used to deliver potentially painful intensities of radiant heat ranging from 0 to 500 mcal sec^{-1} cm^{-2} to ink-blackened spots on the skin for 3-second periods. We defined four intensities for each subject: zero (stimulus 1), pain threshold minus 60 mcal sec^{-1} cm^{-2} (stimulus 2), pain threshold (stimulus 3), and pain threshold plus 60 mcal sec^{-1} cm^{-2} (stimulus 4).

Fourteen male subjects participated individually in two 1-hour sessions on different days (*4*). Each subject was randomly assigned to one of two groups to determine whether he would receive 33 percent nitrous oxide in the first or the second session. Before the first session, we introduced the dolorimeter and explained its function as we blacked five areas (each about 3 cm in diameter) on the volar surface of each of the subject's forearms. An estimate of the subject's pain threshold was obtained by using the method of limits, and the heat and pain stimuli were calculated on the basis of this estimate.

Before the first experimental session, subjects were instructed to assign each stimulus experienced to one of six categories: nothing, warm, hot, faint pain, moderate pain, and strong pain. We did not restrict how often each category could be used, nor did we say how many heat intensities would be employed or that one intensity was zero.

During each session, 200 stimuli were delivered sequentially: 50 trials were given at each intensity level with the levels interspersed according to a random schedule. We introduced a brief rest and measured the subject's skin temperature after every 50 trials.

The supine subject wore an airtight anesthesia mask in each testing session, breathing a mixture of 33 percent nitrous oxide with oxygen in one session and room air in the other (*5*). He indicated responses on the rating scale by finger signals. Before the session involving 33 percent nitrous oxide, the gas was administered for a 15-minute period to ensure that the subject was at a stable level of intoxication (*6*).

The methodology of SDT (*7*) may be used as an alternative to the unreliable pain threshold employed in early work with the dolorimeter. While the threshold provides a single index of sensitivity to stimulation, SDT methods yield two dependent variables: (i) a relatively pure index of perceptual sensitivity, *d'*, and (ii) an index of response bias which reflects the willingness of the observer to categorize the stimulation in a certain way, for example, as painful. It is the uncontrolled interaction of these two factors which led to the unreliability of the older dolorimeter research. We used the threshold only to adjust the demand characteristics of the experimental task to each subject.

A discrete stimulus experience is represented in SDT as a sensory event, *x*, along a hypothetical subjective continuum such that the intensity of the stimulus determines the location of *x*

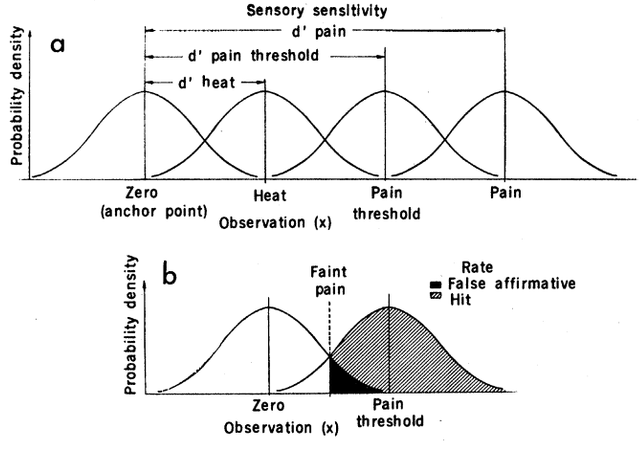

Fig. 1. (a) Representation of sensory events that occur in response to stimuli along the hypothetical subjective continuum. (b) Location of a simple response criterion corresponding to a rating scale category along the hypothetical continuum.

on the continuum. While a single x may fall at any point along the continuum, repeated sensory events tend to cluster about a mean value with a hypothetical infinite repetition of events yielding a Gaussian distribution. Thus, when a subject experiences four levels of stimulation, detection theory locates four mean points along a continuum. Responses to a stimulus intensity of zero are assumed to be like those to other stimuli (x falls about a mean location), except that zero serves as an anchor point and the distances of the other means from the zero mean serve as indices of sensitivity to stimulus intensity, as in Fig. 1a.

The rating scale categories may also be located along the continuum for every hypothetical comparison of two stimulus intensities; that is, every rating scale category is represented by a response criterion location. Each criterion is a decision cutoff point, as in Fig. 1b, such that, when x from an unknown stimulus source falls to the right of the criterion, the stimulus is labeled according to the criterion category (for example, faint pain). When x falls to the left of the criterion, the stimulus is labeled as the next lower category (for example, hot). It is the conservative or liberal location of the criterion for each category that is assessed as response bias.

We have represented our results visually by plotting the hit rate (the rate at which a stimulus is identified correctly) and the false affirmative rate (the rate at which a stimulus is identified incorrectly as the next stronger stimulus) associated with each rating scale category on normal-normal (z-score) coordinates. There is a function, termed a receiver operating characteristic (ROC), for each nonzero stimulus, and the distance of each ROC from the principal diagonal in Fig. 2 is d', that is, the distance of the mean of each curve in Fig. 1 from the mean of the zero curve. As the ROC indicates, d' was reduced for all three nonzero stimuli with 33 percent nitrous oxide (*8*), and the application of a G-test (*9*) to the d' values for the control and nitrous oxide at stimulus 4 ($z = 2.83$, $P < .01$), stimulus 3 ($z = 5.80$, $P < .001$), and stimulus 2 ($z = 3.67$, $P < .001$) yielded statistical significance. These results indicate that 33 percent nitrous oxide does reduce sensitivity to heat and painful stimulation.

In contrast, the gas did not change sensitivity to the differences between

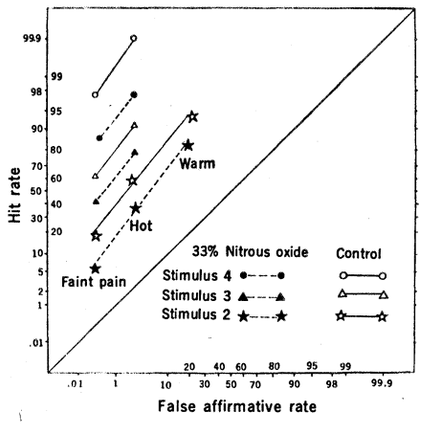

Fig. 2. Receiver operating characteristics for the detection of nonzero stimuli under control conditions and under 33 percent nitrous oxide.

adjacent nonzero stimulus pairs. Tests of the d' difference for control and gas sessions demonstrated nonsignificance for stimuli 4 and 3 ($z = 1.44$, $P > .05$) and stimuli 3 and 2 ($z = -0.11$, $P > .05$). Thus, 33 percent nitrous oxide did not interfere with the ability of the subjects to discriminate among adjacent nonzero stimuli, even though the gas did reduce absolute sensory sensitivity to each of the nonzero stimuli (*10*).

While sensitivity to pain determines the pain report, response bias is also a factor. We quantified response bias as percent bias (*11*) for each subject for the rating categories used. The most appropriate category was chosen for each pair comparison of adjacent stimulus intensities. Thus, percent bias for the moderate pain category was obtained from the data associated with the discrimination of stimulus 4 from stimulus 3 [$t = -3.03$ (13 d.f.); $P < .01$]. A bias measure for faint pain was obtained from the discrimination data for stimulus 3 compared to stimulus 2 [$t = -3.42$ (13 d.f.); $P < .01$], and percent bias was obtained for the hot category from the data associated with stimulus 2 and stimulus 1 [$t = 0.04$ (13 d.f.); not significant]. Thus, subjects were less willing to report pain under the influence of the gas than under control conditions, although willingness to label a stimulus as hot did not change. The observed bias shift indicates that a significant change in cognition occurred with the gas.

Melzack and Casey (*12*) have provided a model which specifies three determinants of pain behavior. The sensory-discriminative and motivational-emotional systems control, respectively, the spatiotemporal analysis of the incoming stimulation and the drive with unpleasant affect associated with that stimulation. The central control system consists of processes in the higher central nervous system and can influence either or both of the other systems. We observed that 33 percent nitrous oxide affected the sensory-discriminative determinant, and the reduction in response bias implies that the gas also affected one or both of the other determinants.

The complex model of human pain offered by Melzack and Casey (*12*) stands in contrast to the simple concept of pain implied in the many analgesia studies of pain threshold. Such work is based on the assumption that pain is a simple sensory experience that occurs with sufficient stimulation of the appropriate receptors, a concept analogous to the all-or-none principle in the firing of neurons. Analgesic agents are thought to alter the readiness of the pain sensory system to activate. Pain reports are taken at face value as though the subject under heat, mechanical, or electrical stimulation is able to identify the point at which the pain system begins to function.

In the alternative SDT framework, it is assumed that sensory information is sometimes processed incorrectly and that human perception involves sensory and cognitive factors. This methodology is applied to study pain without assuming that there is a boundary point in stimulus intensity above which the pain system is activated. Rather, the analysis is concerned with the discriminability of those stimuli normally defined as painful, and the willingness of subjects to classify stimuli as pain experiences. More simply, the SDT approach leads us to think of pain perception as a sensory experience, and a cognitive reaction to that experience. The information generated by these methods is consistent with a broadened conception of pain, and it demonstrates that pain need not be conceptually oversimplified in order to be measured.

An advantage of this methodology is that it can deal with the variability in one individual's reports of pain in response to constant levels of noxious stimulation in different situations. For example, SDT studies of the placebo effect (*2*) have shown that the analgesic effects of suggestion on pain are not sensory changes but rather response bias shifts. This research has emphasized the importance of cognitive fac-

tors in pain in humans, and it has indicated that the sensory aspects of pain have been overemphasized in the past.

Our study suggests that SDT is a very useful and versatile tool for the evaluation of analgesic agents. The improved precision in measurement, and the increased information yielded by these methods, will permit investigators to evaluate human pain in the laboratory with more confidence than threshold methodology has warranted.

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On the Ascent of Sap

Plumb and Bridgman (*1*) suggest various ways of producing zero hydrostatic gradient in a vertical tube containing movable water. One way is by means of a series of semipermeable membranes separating solutions of increasing concentrations, another is by means of a tubing with an increasing density of wiggling molecular hairs affixed to the wall. If it were possible in a state of equilibrium to interfere with the hydrostatic gradient of water itself by means of captive molecules, a mere submersion of the system would create perpetual flow. In reality all such systems arrive at a hydrostatic gradient of pure water. This is borne out by direct experiments starting with Perrin's celebrated work on Brownian motion and, more recently, by measurements on gels, suspensions, and magnetic solutions (*2*). However, as contrary claims are commonly inferred for gels, counterions, secretory crypts, and so forth, the issue merits attention.

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2 October 1972

Scholander implies that it is impossible to interfere with the hydrostatic gradient of pure water by the constrained chemical activity gradient we have proposed (*1*) and asserts that if it were possible one could create a perpetual motion machine by submersion of the column in pure water. We show these tenets to be incorrect by the following thermodynamic analysis of the osmotic pressure effects.

Consider a vertical column, with a constrained activity gradient, the base of which is at elevation $z = 0$, and a reservoir of pure water, the surface of which is at elevation $z = 0$. The chemical potential of water in the reservoir is

$$\mu(z,P) = \mu^0 + Mgz + \bar{V}[P(z) - 1]$$

where P is the pressure in atmospheres, M the molecular weight, g the gravitational constant, and $\bar{V}$ the molar volume. If the pressure at $z = 0$ is 1 atm, the functional dependence of P on z is

$$P(z) = 1 - \frac{Mgz}{\bar{V}}$$

The chemical potential of water in the column is

$$\mu(z,h,P,\pi) = \mu^0 + Mgz + \bar{V}[P(h) - \pi(h) - 1]$$

where h is the distance from the base and π is the osmotic pressure. The functional dependence of π on h will be chosen as

$$\pi(h) = \frac{Mgh}{\bar{V}}$$

and, if the column is upright ($z = h$), the hydrostatic pressure is 1.0 atm throughout. If the column were rotated to a horizontal position the solvent would redistribute to produce a new thermodynamic equilibrium in which

$$P(h) = \frac{Mgh}{\bar{V}} + 1$$

Now specifically assume a 10-m column in which $\pi = 1.0$ atm at $h = 10$ m, and $\pi = 0.0$ atm at $h = 0$ m, such that $P = 1$ atm throughout when the column is vertical. If the column were placed horizontally the pressure would be 1.0 atm at $h = 0$ m and 2.0 atm at $h = 10$ m. If the horizontal column were submerged in the reservoir it would be at thermodynamic equilibrium with the reservoir. At the base end there is no hydrostatic or osmotic pressure differential. At $h = 10$ m the hydrostatic pressure differential is just the correct value to produce osmotic equilibrium. Thus, no flow would occur. A similar argument applies if the column is lowered vertically into the reservoir.

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Plumb and Bridgman (*1*) have suggested that the ascent of sap in trees may be accomplished by two mechanisms which permit the hydrostatic pressure of the xylem sap to remain near ambient pressure at the highest part of the tree: (i) by a concentration gradient of filamentary monomolecular chains with one end attached to the xylem vessel wall and (ii) by solute injection.

If flagella-like molecules were projecting into the xylem sap and if they could lower the chemical potential of the sap, they would also lower the melting point of the sap. Recent experiments on winter-hardened conifer