

cells regardless of source would ultimately acquire identical and multiple chromosome markers, lose specific chromosomes, and exhibit a uniform isoenzyme mobility pattern.

Type A isoenzyme mobility for G6PD is a sex-linked characteristic associated almost exclusively with a fraction of the world's Negro population (34). Cell lines with this feature must be considered rare. Therefore, when this characteristic and absence of the Y chromosome (both features of HeLa cells) appears in any cell line, further analysis by chromosome banding techniques should be undertaken in order to rule out the possibility of HeLa cell cross-contamination.

Note added in proof: Most recently, cells of a culture of line RT-4, presumably derived from a bladder carcinoma of a human male (35), were examined and found to have approximately 90 chromosomes including the Giemsa marker, the large isochromosome marker, and the four markers of Miller *et al.* (8). They exhibited G6PD type A mobility and lacked a Y chromosome. They, therefore, resemble the HEK cells and are of HeLa origin.

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16 April 1974

Acupunctural Analgesia? Evaluation by Signal Detection Theory

Abstract. *Pain responses to noxious thermal stimulation decreased in the acupunctured arm of subjects as compared to the arm not treated with acupuncture; this result suggested that effective analgesia had been induced. However, sensory decision theory analysis of the data revealed no difference in discriminability. This failure to find a sensory (physiological) change strongly suggests that analgesia had not been induced. The sole effect of acupuncture was to cause the subjects to raise their pain criterion in response to the expectation that acupuncture works.*

Is the reduction in the report of pain following acupunctural analgesia proof that amelioration of the pain experience has occurred, or is this decrease caused by suggestion that induces the subject to raise his criterion for reporting pain? This is not a new problem. A lengthy dispute surrounds the influence of hypnosis, placebos, anxiety, and "cognitive control" on perceived pain (1). Unequivocal proof of the effectiveness of these "psychic analgesics" has been difficult to obtain because the pain threshold is an unanalyzable amalgam of sensory (physiological) and attitudinal (psychological) variables. To answer the question one must resort to signal detection (or, more descriptively, sensory decision) theory (2). Sensory decision theory yields two measures of the subject's performance. The sensory or physiological parameter, d' , provides the measure of sensory sensitivity or discriminability. The attitudinal or psychological index, L_x , indicates the subject's response criterion, that is, his willingness or reluctance to report the presence of pain. We report here a portion of a larger, continuing study on the effect of acupunctural analgesia on d' and L_x .

Two types of pain experiments ana-

lyzed by sensory decision theory, one on the effect of suggestion on L_x (3) and others on the effects of analgesics on d' (4-6), underlie the present study of acupunctural analgesia. The magnitude of the likelihood ratio criterion, L_x , reflects the subject's response bias or attitude toward uttering a pain response. Clark (3) found that administration of a placebo described to the subject as a potent analgesic sharply decreased the proportion of pain responses to noxious radiant heat stimulation, a result suggesting that the threshold for pain had been raised. However, analysis of the data according to sensory decision theory demonstrated that d' remained unaltered and that the sole effect of the placebo was to raise the subjects' pain criterion, L_x . Since d' did not decrease, he concluded that the placebo had not diminished the subjects' sensory experience; that is, analgesia had not been produced.

The other sensory decision theory measure, d' , provides a relatively pure index of sensory sensitivity or discriminability that remains unaltered when nonsensory variables such as attitude, expectation, and motivation are changed. A low d' means that the subject tends to confuse stimuli of lower

and higher intensity; it is obtained when the physical intensities of the stimuli are very weak or similar, or when the subject's sensory system is insensitive. Thus, a decrease in d' after administration of an analgesic would suggest that the drug had attenuated neural activity in the sensory system. Such a relation between d' and analgesia was shown by Chapman *et al.* (4), who found that nitrous oxide, when given in amounts sufficient to produce an analgesic effect, decreased d' to noxious thermal stimulation, and by Dillon (5), who found a decrease in d' after administration of aspirin.

Radiant heat stimuli were presented by a modified Hardy-Wolf-Goodell dolorimeter (Williamson Development Co.). Unless the subject withdrew his arm, the stimulus lasted 3.00 seconds. The heat stimuli (2.0 cm in diameter) were presented to six patches on the volar surface of each forearm where India ink had been applied. The 12 subjects were instructed to rate each presentation along a 12-category intensity scale with categories ranging from "nothing" through various degrees of heat and pain to "withdrawal." Approximately 24 stimuli were presented to each subject in each of the cells of the two-by-three design (Table 1). Skin temperature was monitored throughout the experiment; no changes were found.

After the before-acupuncture test period, sterile 2- and 4-cm stainless steel acupuncture needles were inserted into the alcohol-cleaned skin in three electrically paired sites: anteriorly and posteriorly at the axial crease, radially and medially at the elbow crease, and in the hand at the metacarpal joint between the small and ring finger and between the thumb and first finger. Half of the subjects were treated in the left arm and the other half in the right arm. These acupuncture sites are standard for surgery of the arm (7). The positioning of the needles was based on the concept of Ten Ch'i, and involved the subject's report of soreness, heaviness, and numbness when the needle was twirled manually (8). The needles were then stimulated electrically by a battery-powered acupuncture apparatus (model 6.26, manufactured in Canton, People's Republic of China) at the maximum intensity acceptable to the subject. The characteristics of the biphasic, 88-hertz stimulus were measured on an oscilloscope with the subject in the circuit. The current

Table 1. Mean sensory discriminability (d') and the mean likelihood ratio criterion ($\log L_x$ and L_x) for pain to stimuli at 370 and 425 mcal sec⁻¹ cm⁻². Means and standard errors are given; acu., acupuncture.

Period	Acupunctured arm			Control arm		
	d'	$\log L_x$	L_x	d'	$\log L_x$	L_x
Before acu.	1.37 ± 0.22	0.029 ± 0.09	1.07	1.33 ± 0.18	- 0.083 ± 0.09	0.83
During acu.	1.42 ± 0.17	0.123 ± 0.10	1.33	1.49 ± 0.26	- 0.045 ± 0.08	0.90
After acu.	1.76 ± 0.24	0.189 ± 0.07	1.55	1.73 ± 0.24	0.008 ± 0.10	1.02

ranged from 4.0 to 1.8 ma, the mean peak-to-peak voltage from 600 to 360 mv, and the impedance (at peak current and voltage) from 200 to 150 ohms. After 15 to 20 minutes of stimulation, during which additional sensory measurements were obtained, the needles were removed and the final thermal sensitivity was determined.

Sensory decision theory was used to determine measures of discriminability, d' , and criterion, L_x (2-6). The mean values for d' and for the pain criterion for withdrawal to stimuli at 370 and 425 mcal sec⁻¹ cm⁻² appear in Table 1. Analysis of variance for period versus treatment revealed no significant differences for d' between the acupunctured and control arms ($F=0.12$; d.f. = 1,10; $P>.50$) or between the periods before and after acupuncture ($F=3.90$; d.f. = 2,10; $P>.10$). A separate analysis of variance for the pain criterion revealed that a much higher L_x was set for the acupunctured arm than for the control arm ($F=6.97$; d.f. = 1,10; $P<.025$). This difference was significant only during acupuncture stimulation ($t=2.58$, d.f. = 11, $P<.05$). The criterion did not shift significantly between the periods before and after acupuncture ($F=1.85$, d.f. = 2,10; $P>.10$). During the session both d' and L_x increased, but not significantly. Such changes had been observed previously (3). The higher d' reflects the improved discrimination that occurs with practice. The increase in L_x results because, as the experimental session proceeds without injury, the subject loses his fear that he will receive a second-degree burn, and thus ceases to use the response "pain" as a warning to the experimenter to avoid extreme stimulus intensities.

The results show that the acupuncture procedure used in this study decreased the proportion of withdrawals and reports of pain, which suggests that analgesia had been induced. However, sensory decision theory analysis of the response data revealed that this

decrease had its sole origin in a raised criterion for reporting pain. Acupuncture failed to decrease discriminability, d' . Since a low d' occurs when the subject's sensory system is insensitive—as, for example, after administration of an analgesic (4-6)—the failure to find a decrement in d' strongly suggests that the stimulus parameters used in this study did not produce acupunctural analgesia.

The dramatic role of expectation in studies of acupunctural analgesia is revealed in the much higher pain criterion that subjects set for the acupunctured arm. The interpretation of the data according to sensory decision theory is that subjects experienced equal amounts of "physiological" pain in the two arms, but were less likely to admit that a given sensory experience was painful when it occurred in the arm which had received the acupuncture treatment.

Although acupunctural analgesia was not obtained with the stimulus conditions used, a wide variety of stimulus parameters, including voltage, current, frequency, waveform, and duration of treatment, must be systematically investigated before it can be concluded that acupunctural analgesia is a myth. Many methods of acupuncture and types of pain remain to be investigated. However, the present study does prove that the pain threshold determined by traditional psychophysical techniques cannot be used to resolve the question of acupunctural analgesia. Until the role of suggestion on the readiness of the subject to report pain after acupuncture has been determined, speculations concerning the physiological mechanisms involved (9) are premature.

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17 September 1973; revised 16 November 1973 ■

Energy Cost of Animal Locomotion

Gold (1) has presented an interesting hypothesis regarding energy expenditure in animal locomotion, that the cost of a step, wingbeat, or swim stroke (in calories per gram per "step") is independent of body size and stepping (flapping, stroking) frequency. This hypothesis merits investigation; unfortunately Gold did not compare it with available information.

In order to check the specific energy cost (C) values derived by Gold, I calculated C for three animals, taking information from Tucker (2, 3), Brett (4), Gray (5), and Muybridge (6). The calculations for Table 1 may need some refinement, but of the three only the horse conforms to Gold's prediction. The hypothesis may fit for walking animals, but there are some fundamental differences in flying and swimming which indicate that Gold's hypothesis is oversimplified.

Gold regards as similar the slopes relating $\log C$ and $\log$ body mass for runners and flyers, -0.40 (7) and -0.227 (2), respectively. In allometric analysis these values would not be regarded as similar.

There is also a fundamental difference between flying and swimming. The specific energy cost can be obtained as:

$$\begin{aligned} C &= \text{energy cost} \times \text{stride length} \\ &= \text{cal g}^{-1} \text{ cm}^{-1} \times \text{cm step}^{-1} \\ &= \text{cal g}^{-1} \text{ "step"}^{-1} \end{aligned} \quad (1)$$

or

$$\begin{aligned} C &= \text{metabolic rate/step frequency} \\ &= \text{cal g}^{-1} \text{ hour}^{-1} / \text{step hour}^{-1} \\ &= \text{cal g}^{-1} \text{ "step"}^{-1} \end{aligned} \quad (2)$$

If Eq. 2 is used, note that the speed and tailbeat frequency ("stepping frequency") of a fish are linearly proportional (5), while wingbeat frequency is independent of airspeed (3, 8).

Finally, Gold is qualitatively correct in stating that the proportion of body mass devoted to propulsion increases from mammals to birds to fish. According to Gray (5), however, only 45 percent of the mass of a fish is muscle, which does not support Gold's remark that most of the body structure of the fish seems to be devoted to propulsion.

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Table 1. Values of the specific energy cost (C) in calories per gram per "step" calculated from the information available for the horse, budgerigar, and salmon are compared with the values derived by Gold (1).

Animal	C (cal g ⁻¹ "step" ⁻¹)		Calculated/Gold's
	Calculated	Gold's derivation	
Horse (walking)	$2.87 \times 10^{-4*}$	3×10^{-4}	0.96
Budgerigar (flying)	$2.02 \times 10^{-3†}$	1×10^{-4}	20.2
Salmon (swimming)	$1.26 \times 10^{-4‡}$	4×10^{-5}	3.15

* From (2), $(2.61 \text{ cal g}^{-1} \text{ hour}^{-1}) / (5 \text{ km/hour}^{-1}) = 0.522 \text{ cal g}^{-1} \text{ km}^{-1}$. From (6), for the horse "Eagle," $(2.2 \text{ m/stride}) / 4 = 5.5 \times 10^{-4} \text{ km/step}$; $0.522 \text{ cal g}^{-1} \text{ km}^{-1} \times 5.5 \times 10^{-4} \text{ km/step} = 2.87 \times 10^{-4} \text{ cal g}^{-1} \text{ step}^{-1}$. † From (3), $102 \text{ cal g}^{-1} \text{ hour}^{-1} = 1.70 \text{ cal g}^{-1} \text{ min}^{-1}$; dividing this by 840 wingbeats per minute gives $2.02 \times 10^{-3} \text{ cal g}^{-1} \text{ wingbeat}^{-1}$. ‡ From (4, p. 82, figure 2), 880 mg of O₂ per kilogram per hour = $8.21 \times 10^{-4} \text{ cal g}^{-1} \text{ sec}^{-1}$; dividing this by 78 cm/sec gives $1.053 \times 10^{-5} \text{ cal g}^{-1} \text{ cm}^{-1}$ for 20-cm salmon. From (5, p. 48), the distance traveled between tailbeats is 0.6 times the length; $0.6 \times 20 \text{ cm} = 12 \text{ cm}$. Then $1.053 \times 10^{-5} \text{ cal g}^{-1} \text{ cm}^{-1} \times 12 \text{ cm/tailbeat} = 1.26 \times 10^{-4} \text{ cal g}^{-1} \text{ tailbeat}^{-1}$.

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14 September 1973

Calder has demonstrated that for swimming and flying the "step rule" cannot be interpreted as implying the constancy of energy expenditure for each contraction of the animal's propulsive muscles, but must be more narrowly construed to imply only the constancy of energy expenditure per characteristic length.

Herschman (1) has noted that the reported interspecific constancy of the strength of muscle (approximately 4 kg/cm²), in fact, implies a constancy in the quantity of energy available per contraction per unit of muscle mass (approximately 1 cal/kg). It is instructive to combine this with Calder's observation and some rough anatomical assumptions.

If c is the derived energy cost per step (per characteristic length), then we may write

$$c \propto (m/M)n$$

where m is the mass of propulsive muscle, M is the animal's body mass, and n is the number of muscle contractions required for the animal to travel one characteristic length. The ratio of the c values for running, flying, and swimming, respectively, is 15 : 5 : 2 (2). For runners, a reasonable value of m/M is 0.1; for flyers and swimmers, respectively, 0.2 and 0.45 are rough values.

Taking Calder's observation that $n = 1$ is an excellent approximation for running, we find that

$$\begin{aligned} n &= 1/6 = 0.167 \quad (\text{flying}) \\ n &= 2.96 \quad (\text{swimming}) \end{aligned}$$

These are at least qualitatively consistent with Calder's demonstration that a salmon requires about two strokes to swim its own body length, whereas a budgerigar traverses about a meter, some four or five times its wingspan, per wingbeat.

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21 February 1974