produced by these cultures, we suggest that the parenchymal hepatocyte is the major in vivo source of plasminogen.

Thus we have utilized a sensitive and specific immunological assay to demonstrate that primary cultures of rat parenchymal hepatocytes actively accumulate plasminogen in their medium. These hepatocyte cultures were further shown to synthesize and secrete both circulating forms of the plasminogen molecule. To our knowledge, this is the first unequivocal demonstration of plasminogen synthesis by any cell type. An abstract of these studies has been published (*19*).

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11. Rat plasminogen was purified by affinity chromatography on lysine-Sepharose (*1, 17*) from fresh rat plasma treated with heparin (100 U/ml), *p*-nitrophenyl-*p*′-guanidinobenzoate ($10^{-5}M$), and Aprotinin (Sigma; 50 U/ml). The plasma (10 to 60 ml) was applied to a column (2 by 5 cm) of lysine-Sepharose equilibrated at 4°C in 0.1*M* sodium phosphate, *p*H 8.0 (buffer 1), and the bulk of the plasma was washed through with this buffer. A variable amount of loosely bound, nonplasminogen protein was washed from the resin with a solution of 2.0*M* NaCl and 0.1 percent Tween-20. After reequilibrating the resin in buffer 1, the bound plasminogen was eluted with 0.05*M* 6-aminohexanoic acid in buffer 1. An antiserum to this protein was produced in a rabbit, and the antibodies against plasminogen were isolated from the antiserum by affinity chromatography on rat plasminogen-Sepharose [T. G. Cooper, *The Tools of Biochemistry* (Wiley, New York, 1977)]. Both the antiserum and the antibodies (purified by affinity chromatography) were judged to be specific for rat plasminogen by immunoelectrophoresis.
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Pain Responses in Nepalese Porters

Abstract. *When tested by the method of limits, Nepalese had much higher pain thresholds to electrical stimulation than Occidentals did. Discriminability was the same for both groups, however, indicating that there were no neurosensory differences. Nepalese had higher (stoical) criteria for reporting pain but were not less sensitive to noxious stimulation. The battery of sensory measurement procedures described may be applied to any modality and are particulary applicable to difficult field conditions.*

Members of non-Western ethnocultural groups are said to be less sensitive to pain (*1*). We determined that this difference was due to a culturally imposed stoicism (that is, pain was felt as intensely but was not reported) and was not due to a true hypalgesia caused by genetic or climatic differences or perhaps local botanicals. Sensory decision theory was used since this method distinguishes between sensory (d') and attitudinal (L_x) components of the report of pain (*2, 3*). Although sensory decision theory has proved invaluable in the laboratory, it had not, to our knowledge, been tested in the field, where there is little time to gather data and where language and literacy barriers often exist. We now describe a combination of methods—the method of limits followed by forced-choice and one-interval binary decision —applicable to field conditions.

Responses to noxious transcutaneous electrical stimulation were obtained from Nepalese, East Indians, and Occidental visitors during a trek in the Himalayas (*4*). The five trekkers were English-speaking, college-educated, and of Christian or Jewish background. Their mean age was 45 years (range, 30 to 68). Although familiar with the minor discomforts encountered in hiking and camping at moderate altitudes (3000 m), they were not regularly exposed to the hardships that were routine for the Nepalese. The six Nepalese did not speak English, had little schooling, were devout Buddhists, and some were illiterate. Their mean age was 32 years (range, 23 to 42). The four porters were accustomed to carrying 77-pound packs at high altitudes wearing only light clothing, even at freezing temperatures. The cook and his assistant were more literate and accustomed to slightly better living conditions. Two additional Indian subjects from Darjeeling, whose life-style was comfortably Westernized (for example, their homes were well heated) were included in a correlational study between pain threshold and living conditions, but not in the comparisons between the Western trekkers and Nepalese.

The method of limits was followed by forced-choice and one-interval binary decision procedures of sensory decision theory to obtain measures of pain threshold and discriminability (d'). With the method of limits, the stimulus is increased stepwise until the subject rates it as "painful." In a forced-choice task the subject judges which of two stimuli has the higher intensity. In a one-interval binary decision task, the subject rates individual stimuli as "high" or "low." Sensory decision theory analysis uses the proportion of incorrect judgments to estimate the subject's sensory sensitivity.

First, with the method of limits, two sets of ascending trials were run with the stimulus intensities increasing in steps from zero until the subject's pain tolerance was reached. After each stimulus, the subject selected a response from a list of seven categories in his native language. The categories ranged from "nothing" through various degrees of "discomfort" and "pain" to "withdrawal." If the subject was illiterate, the list was rehearsed aloud. Next, each subject received four to eight trials in the forced-choice task, with stimulus intensities lying within the range of his threshold determined by the method of limits for "slightly painful" (high) and "uncomfortable" (low). The subject judged which interval contained the higher intensity stimulus and was told whether he was right or wrong. This procedure established the intensities for the subsequent binary decision task, familiarized the subject with the decision tasks, and ensured that he understood the instructions. In the final test, the stimulus intensities determined by the forced-choice procedure were used in the one-interval binary decision procedure of sensory decision theory. Ten high- and ten low-intensity stimuli were presented in random order. The subject identified each stimulus as being high or low, but knowledge of results was withheld. The "hit" and "false affirmative" rates were used to compute values of d' and L_x (a hit is the high response to the stimulus of higher intensity; a false affirmative is a high response to the stimulus of lower intensity). Since slightly different intensity separations between the high and low stimuli were used in individual subjects, discriminability was expressed as volts and milliamperes per d'.

Electrical stimuli were generated by the EPC personal stimulator (Stimtech) (*5*). Electrode paste (Spectra) was applied to flexible carbon electrodes and to stimulus sites on the left wrist over the median nerve (cathode) and on the left forearm (anode). The width of the unipolar pulses was 0.32 msec, the frequency, 11 Hz. For the method of limits, the stimulus intensities were increased from zero in steps averaging 7 V and 8 mA. The stimulus duration was 2.0 seconds unless the subject withdrew. Informed consent was obtained. The Nepalese subjects were paid 2 rupees, equivalent to the pay for 2 hours of work, for a test session lasting about 12 minutes.

Table 1. Threshold values obtained by the method of limits from Nepalese porters and Occidentals. Abbreviations: $\bar{X}$, mean; S.E., standard error.

Group	N	Statistics	Detection		Faint pain		Very painful	
			V	mA	V	mA	V	mA
Nepalese	6	$\bar{X}$	13.0	7.0	54.2	64.5	71.3	82.8
		S.E.	2.4	2.0	3.9	5.1	4.2	5.2
Occidentals	5	$\bar{X}$	13.8	7.4	37.0	35.0	51.4	58.8
		S.E.	2.8	2.4	3.4	5.3	3.4	5.4
		t*	0.20	0.12	2.96	3.62	2.65	2.27
		P	> .8	> .9	< .05	< .01	< .05	< .05

*Two-tailed t-test, d.f. = 9.

Table 2. Measures of discriminability (d') and report criterion (log L_x) obtained by sensory decision theory from Nepalese porters and Occidentals.

Group	N	Statistics	Volt/d'	Milliampere/d'	Log L_x
Nepalese	6	$\bar{X}$	3.8	5.6	0.008
		S.E.	1.2	1.2	0.13
Occidentals	5	$\bar{X}$	3.3	4.7	−0.040
		S.E.	0.51	0.67	0.05
		t*	0.32	0.60	0.28
		P	> .7	> .5	> .7

*Two-tailed t-test, d.f. = 9.

The Nepalese had a considerably higher threshold (by the method of limits) and fewer reports of pain (Table 1). However, their ability to detect nonpainful electrical stimulation did not differ from that of the trekkers. Sensory decision theory analysis (Table 2) revealed no differences between the groups with respect to discriminability and report criterion (log L_x) for high or low (*6*).

According to the d' measure of the one-interval binary decision procedure, the ability of the Nepalese to discriminate between the higher and lower mildly noxious stimuli equaled that of the Occidentals. This result suggests that the neurosensory information arriving centrally, and hence the amount of pain experienced, was the same for the two groups. Nevertheless, the method of limits demonstrated that the Nepalese had much higher pain thresholds. The threshold by the method of limits is a mixture of criterion locus (L_x) and discriminability (d'). Thus, the high threshold must have been due to their high criterion for reporting pain, that is, their stoicism. The finding that d''s were equal eliminates the possibility of genetic or other physiological differences between the two groups. Since analgesics have been shown to decrease d' (*2*), the finding also means that the high pain threshold was not caused by the chewing of betel nuts, potentially a mild analgesic, by some of the Nepalese. Nor can peripheral variables such as differences in skin impedance account for the results, since detection thresholds for the presence of a stimulus did not differ.

In order to investigate the effect of continued exposure to a harsh environment, a correlational study, which included the two well-off Indians, was undertaken. The entire group (N = 13) was ordered by rank from most to least comfortable life-style. Comfortable life-style was correlated with the thresholds for "faint pain" (for voltage, $r = -.67$; for current, $r = -.69$) and for "very painful" (for voltage, $r = -.59$; for current, $r = -.60$) [R (1,11) = .55, $P < .05$]. There were no significant correlations with d' or L_x. The correlation between uncomfortable living conditions and pain threshold suggests that continual exposure to a harsh environment, rather than ethnocultural factors, caused the high report criterion of the Nepalese.

Subsequently, a similar result was obtained from two Touareg camel drivers during a trip through the Hoggar Mountains of southern Algeria. The Touaregs, who lead a rigorous life in the desert, also had a very high pain threshold, yet d' equaled that of the Westerners.

The response to pain is an important component of behavior and deserves study by anthropologists and others. In their extensive review of this field, Wolff and Langley (*1*) concluded that ethnocultural differences in pain threshold are poorly documented. Our study confirms that at least one group, the Nepalese, are much more tolerant of pain than are Occidental trekkers. However, because they discriminated equally well, the two groups experienced the same amount of pain. We conclude that the high pain threshold of the Nepalese is due to their stoicism, probably induced by their harsh living conditions, and perhaps in part by other ethnocultural factors such as religion.

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3. The index of discriminability (d') describes the functioning of the neurosensory system independently of the report, "pain." For example, analgesics that interfere with neural transmission decrease d'. The other measure of the subject's performance is the criterion for reporting pain (L_x) which depends upon psychological factors such as stoicism. For example, placebos and suggestion alter L_x without affecting d'. In the threshold of traditional psychophysics, these separate quantitative measures of discriminability and report bias are confounded (*2*).
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Pradhan served as interpreter and solicited volunteers.

5. The EPC mini-stimulators were obtained through the generosity of A. F. Battista, New York University Medical Center, and M. L. Mashburn, president, Stimulation Technology, Inc.
6. This binary decision criterion, which was used in place of a pain rating scale to simplify the decision task, bears no relation to the criterion for reporting pain. In designing this study, we were aware that the one-interval pain rating scale procedure with six response categories would measure the pain report criterion directly. However, we were concerned that such judgments would prove too complex for poorly educated subjects. Experience suggests that we probably were mistaken; we recommend that the multicriteria pain rating scale (*2*) be tried for a more direct assessment of the pain criterion locus.

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Associative Behavioral Modification in *Hermissenda*: Cellular Correlates

Abstract. *Three days of training consisting of trials of light paired with rotation produces a long-term modification of photopositive behavior in* Hermissenda crassicornis. *The behavioral modification depends on the temporal association of light and rotation. For animals that received light paired with rotation, significant increases in the spontaneous activity of type B photoreceptors were correlated with changes in photopositive behavior after training. A persistent tonic depolarization of type B photoreceptors can explain the cellular changes correlated with the long-term behavioral modification produced by the temporal association of light and rotation.*

Cellular analysis of long-term behavioral modifications in invertebrates may elucidate neural mechanisms of learning (*1-3*). Conditioning procedures have produced long-term behavioral modifications in gastropod mollusks in which some progress has been made toward defining the neural circuitry and the neural activities involved in behavior (*2*). The eyes, optic ganglia, and statocysts of *Hermissenda* consist of relatively few cells whose synaptic relations and cellular organization have been examined in detail (*4*). Stimulation of the animals' eyes and statocysts with light during rotation resulted in a short-term nonassociative change in photopositive behavior (*5*). Recently, Crow and Alkon (*3*) reported long-term modification of a photopositive response—approaching and moving into an illuminated area (*3, 5*)—in the nudibranch mollusk *Hermissenda crassicornis*. Response latencies of individual *Hermissenda* to enter an illuminated area were significantly longer after training than before in animals receiving training with diffuse light paired with rotation on a modified turntable. Trained animals were significantly different from groups that received random control procedures (*3*). The behavioral modification persisted for several days after training, depended on the temporal association of the training stimuli, increased with practice, reversed to levels before training with repeated testing, and may have exhibited some savings (*3*). The response was specific to the light test and not a change in general activity: When the trained animals were tested in both light and dark, only latencies to enter the illuminated area increased (*6*). The increase can be accounted for by a significant increase in the latency to initiate movement in response to light (*6*). Changes in hair cell responses to light stimuli have been observed after short-term training with light paired with rotation, and stimulation of the isolated nervous system with trials of light paired with rotation resulted in a change in type A photoreceptor responses to light (*7*). We have now found that nervous system modification of the photopositive response in *Hermissenda* is correlated with cellular changes in the type B photoreceptors.

Animal maintenance and automated training (*8*) and testing procedures were used (*3*). The animals were placed on a cycle of $6^{1}/_{2}$ hours of light in 24 hours for 3 days before the start of behavioral training. Training, behavioral testing, and intracellular recording from the isolated nervous systems took place during the animals' light cycle. For three consecutive days trained animals ($N = 25$) received 50 trials per day of light (30 seconds) paired with rotation (30 seconds) (average intertrial interval, 2.0 minutes). Control groups (total $N = 25$) received identical trials of random light and random rotation or random rotation alone. Immediately after the last training trial on the third training day, response latency to enter an illuminated area was measured. The circumesophageal nervous systems were then removed and pinned to the Sylgard (Dow Corning) bottom of a recording chamber filled with seawater maintained at 15°C during the recording session. The nervous system was incubated in a solution of digestive enzyme prior to microelectrode penetration of the photoreceptors (protease, type VII, Sigma). Photoreceptors were impaled with single microelectrodes filled with $4M$ potassium acetate for conventional intracellular recording and current injection. A bridge circuit was used to pass current through the recording electrode for input resistance measurements. Current was monitored by a virtual ground current-to-voltage converter or by the voltage drop across a resistor in the ground path. Measurements of spontaneous activity of the photoreceptor, responses to brief light flashes, and input resistance were taken after 15 minutes of dark adaptation. Frequency of B activity was determined for all groups by counting the number of spikes in a continuous 20-second period immediately after dark adaptation. Illumination was provided by a quartz-iodide incandescent lamp. The light was attenuated by neutral density filters. The measurements were taken from one type B cell in an eye, and data from only one eye per preparation were used in the statistical analysis. We found the spontaneous activity in the dark of type B photoreceptors from the group receiving paired light and rotation was significantly higher than that of the two random control groups (Fig. 1). A Cochran test for homogeneity of variance (*9*) showed that the sample variances were not significantly different. The spontaneous activity of the B photoreceptors between the groups differed significantly [$F_{(2,47)} = 7.32$; $P < .01$]. The a posteriori tests showed that the spontaneous activity of the B photoreceptors from the paired light and rotation group was significantly higher than that of the two random control groups (Newman-Keuls test, $P < .01$). The two random control groups were not significantly different from each other. A replication of the experiment was conducted with a blind procedure. The spontaneous activity of B photoreceptors in trained animals ($N = 6$) was compared with random controls ($N = 5$) under identical maintenance conditions. Trained animals [mean ($\bar{X}$) = 2.84 spikes per second] were significantly different from random controls ($\bar{X} = 2.09$ spikes per second) ($t = 2.47$; $P < .025$).

As before (*3*), the group that received light paired with rotation took significantly longer to enter the illuminated area after training than the random light and rotation control group ($z = 1.79$, $P < .03$) and the random rotation control group ($z = 2.12$, $P < .01$) (Fig. 1B). The latencies to enter the light were not significantly different for the two random control groups. The relationship be-