male patients with Sipple's syndrome could prove a potent tool for understanding several aspects of the pathogenesis of this disease. First, the time point for development of the defect in neural crest tissue might be further elucidated. There is evidence that both the thyroid and adrenal tumors are preceded by a phase of hyperplasia bilaterally in the thyroid (*18*) and the adrenal medulla (*19*); this hyperplasia may appear or persist quite late into development since C cell hyperplasia in the thyroid has been recognized in patients up to 23 years of age (*18*) and bilateral adrenal medullary hyperplasia has been found in a 12-year-old patient (*19*). It seems unlikely that the somatic mutations suggested from our data would have occurred simultaneously at each of the separate sites of hyperplasia, but rather that the susceptible cells in these regions may have derived from stem cells that were already defective. If a population of black heterozygote females with Sipple's syndrome could be examined, and the thyroid and adrenal tumors proved to be not only monoclonal but also to contain the same G6PD isoenzyme in each tumor from the same patient, the evidence that the same mutated parent cells contribute to both lesions would be strong. Thus the defect could be pinpointed to a time prior to migration of neural crest elements to the thyroid and adrenal medulla. Obviously, since chance alone could account for the same G6PD form in the thyroid and adrenal tumors 50 percent of the time, the population of patients examined would have to be quite large.

It is intriguing that in our patient both the medullary carcinoma, a malignant lesion, and the pheochromocytoma, a benign lesion in Sipple's syndrome (*16, 20*), appear to be of monoclonal derivation. This finding indicates that the factors controlling malignancy and benignity for the tumors in Sipple's syndrome may be separate from the basic inherited defect; possibly factors in the thyroid and adrenal gland influence the behavior of the neoplastic cells, or differences evolve as the stem cells giving rise to the lesions mature and differentiate.

Finally, the mechanism for the third component of the syndrome, parathyroid hyperplasia or adenoma formation (*20*), might be clarified by performing studies such as those undertaken in our patient. The parathyroid lesions have been postulated by some to be part of the primary defect in this disease and by others to arise as a compensatory response to calcitonin excess (*21*). The former situation might be expected to show monoclonal origin in view of our findings, and the latter might show a multiclonal pattern. Unfortunately, although two hyperplastic parathyroid glands were seen in pathologic sections from our patient, fresh tissue was unavailable for study.

STEPHEN B. BAYLIN
DONALD S. GANN
SUSAN H. HSU

Clayton Research Laboratories and *Division of Medical Genetics, Department of Medicine, Oncology Center,* and *Department of Surgery, Johns Hopkins School of Medicine, Baltimore, Maryland 21205*

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Morphine Analgesic Tolerance: Its Situation Specificity Supports a Pavlovian Conditioning Model

Abstract. *Rats were made tolerant to morphine in either of two environments and then assessed for morphine-induced alteration of pain sensitivity in both environments. Analgesic tolerance was displayed when rats were tested in that environment in which they previously received morphine, but not in the alternative environment. The results indicate than an association between environmental cues and the systemic effects of morphine is crucial to tolerance development.*

Many interpretations of opiate analgesic tolerance have been proposed, most postulating some systemic change which either decreases the population or sensitivity of effective opiate receptors within the organism as a result of the initial drug administrations (*1*) or prevents the drug from gaining access to these central receptors (*2*). In marked contrast with such theories is an interpretation of tolerance which emphasizes the principles of Pavlovian conditioning (*3*). As suggested by Pavlov (*4*), the administration of a drug can be viewed as a conditioning trial, with environmental cues uniquely present at the time of drug administration constituting the conditional stimulus, and the actual pharmacological stimulation constituting the unconditional stimulus. According to the conditioning interpretation of tolerance, tolerance is a manifestation of the acquisition of an association between the systemic effects of the drug and those environmental cues which reliably precede these systemic effects. Such an association may be revealed if the subject, after a history of administration of the drug, is presented with the drug administration procedure not followed by the systemic effects of the drug—that is, if a placebo is administered.

It has frequently been reported that conditional drug responses are opposite in direction to the unconditional effects of the drug (*3, 5*). Thus, in the case of a subject with a history of drug administration, the administration ritual may elicit responses antagonistic to those elicited by the drug, and these anticipatory drug responses should serve to attenuate the effects of the drug. As is generally the case with conditional responses, the compensatory conditional drug responses are expected to become more pronounced as conditional and unconditional stimuli are paired more and more often (that is, the drug administration

Table 1. Mean responsivity during analgesiometer and hot-plate test sessions.

Tolerance acquisition condition	Analgesiometer test: mean paw-withdrawal threshold (grams of pressure)		Hot-plate test: mean paw-lick latency (seconds)	
	Morphine	Saline	Morphine	Saline
Functional hot plate	245*	81	6.0	10.7
Functional analgesiometer	106	57	22.5†	14.2
Nonfunctional hot plate	316*	81	13.6	10.7
Nonfunctional analgesiometer	120	70	23.1†	14.2

*The difference in paw-withdrawal thresholds between these two groups was not significant, but both displayed significantly higher withdrawal thresholds than any other group. †The difference in paw-lick latencies between these two groups was not significant, but both displayed significantly longer lick latencies than any other group.

procedure is associated with increasing frequency with the unconditional systemic effects of the drug); therefore, the net effect of the drug should decrease over the course of successive administrations. Such a decreased response to a drug, as a function of successive experiences with the drug, defines tolerance (*6*).

Of special relevance to the role of conditional drug responses in morphine analgesic tolerance is the finding that rats with a history of morphine administration, in which each administration of the same dose has less and less of an analgesic effect, display hyperalgesia when confronted with the usual drug administration ritual but actually injected with a placebo (*3*). Thus, in anticipation of the systemic effects of morphine (and its analgesic consequences), rats show hyperalgesia. According to the conditioning theory of tolerance, it is this conditional hyperalgesic response together with the unconditional analgesic effect of morphine that is responsible for the net decrease in the analgesic effect of the opiate over the course of successive administrations.

According to the conditioning theory of morphine tolerance, cues that reliably predict the systemic effects of the drug are crucial to the development of tolerance because they enable the subject to make timely compensatory responses in anticipation of the central responses elicited by the drug. For tolerance to the analgesic effects of morphine to be obtained, subjects must have a consistent set of cues correlated with the morphine administration (*3, 7*). My investigation extends these findings by demonstrating that the display of tolerance is specific to the environment in which the drug has been administered, and that "morphine tolerant" rats, when assessed for the effects of the narcotic in an environment other than that in which they became tolerant, evidence a relatively nontolerant response.

The experiment consisted of ten sessions, one session every other day. The first eight sessions constituted the tolerance acquisition phase of the experiment, and the remaining two sessions constituted the tolerance test phase. The experimental groups, each consisting of 12 experimentally naive rats (male, Wistar-derived, 90 to 110 days old), differed in their treatment during the tolerance acquisition phase of the experiment. Half the groups experienced this phase of the experiment in one environment, while the remaining groups experienced this phase in a distinctly different environment. During the final test sessions the effects of the drug were assessed for all groups in both environments.

One environment in which morphine was administered was the colony room, where the rats were housed in individual cages. For each of the eight tolerance acquisition sessions, rats in one group were removed from their cages, subcutaneously injected with morphine sulfate (5 mg per kilogram of body weight, via a solution containing 5 mg/ml), and returned to their cages. One-half hour later each rat in this group was again removed from its cage and its analgesia level was assessed with the Randall-Selitto paw pressure analgesiometer (*8*). The rat was positioned in such a way that it was free to withdraw its paw from a source of gradually and constantly increasing pressure. The amount of pressure applied before the paw-withdrawal response occurred provided a measure of the subject's pain sensitivity. Thus, relatively high paw-withdrawal thresholds are indicative of analgesia. This group provided a measure of the initial analgesic effect of morphine and the development of tolerance over eight successive drug administrations when the drug was administered in conjunction with one set of environmental cues (the colony room and paw-pressure analgesiometer assessment).

A second group received its morphine injections and analgesia assessment in the alternative environment during tolerance acquisition. For each session, rats in this group were transferred, in their home cages, from the colony room to a different room, in which a constant background of white noise at 60 db above 0.002 dyne/cm^2 was maintained, and injected with morphine. One-half hour later, tolerance was assessed with the "hot-plate" procedure (*9*); that is, the rat was placed on a 52.2°C (±0.2°C) copper plate for 30 seconds, and the number of seconds that elapsed until the rat licked a paw was recorded. Thus, relatively long paw-lick latencies are indicative of analgesia. This group provided a measure of the initial analgesic effect of morphine and the development of tolerance in a second administration and assessment situation.

Two additional groups were treated in the same manner as the above groups except that the substance injected was physiological saline.

Figure 1 shows the mean analgesiometer paw-withdrawal thresholds and hot-plate paw-lick latencies for groups injected with morphine and saline on each tolerance acquisition session. With both procedures, the analgesic effect of morphine was observed on the first session (that is, rats injected with morphine showed significantly lower pain sensitivity than rats injected with saline; both t's > 11, both P's $< .001$) and the analgesic effect of the opiate decreased over the course of successive administrations (both morphine groups had significantly higher pain sensitivity the eighth time they were injected with the drug than the first time; both t's > 20, both P's $< .001$).

It is possible that the apparent acquisition of analgesic tolerance shown in Fig. 1 may be due to increasing practice in making the pain-ameliorating paw-withdrawal or paw-licking response while drugged, rather than to any functional decrease in the narcotic's analgesic properties. Therefore, the design of the experiment included four additional groups (12 rats in each group) that were treated like the four groups in Fig. 1, but for whom the analgesia assessment apparatuses were nonfunctional during this first phase of the experiment. In the case of groups experiencing this phase of the experiment with a nonfunctional analgesiometer, half an hour after injection of either morphine or saline the rats were positioned in the analgesiometer but no pressure was applied. In the case of groups experiencing this phase of the experiment with a nonfunctional hot plate, the rats were placed on the plate while it was at room temperature (21.2° to 22.2°C). Animals in these groups, al-

though they received either morphine or saline in one of the two environments, never practiced the indicant response during the first phase of the experiment; therefore, when the analgesia assessment apparatuses were functional during subsequent test sessions, the responsivity of these groups could not be attributable to acquired proficiency in responding to stimulation.

After the eight tolerance acquisition sessions, all rats were tested with both the analgesiometer and hot-plate procedures in a counterbalanced order. These two tolerance test sessions were initiated with an injection of the same substance, morphine or saline, that the rat received during tolerance acquisition. The analgesiometer withdrawal thresholds and hot-plate lick latencies for these test sessions are summarized in Table 1 (*10*). Separate multidimensional analyses of variance of the analgesiometer and hot-plate test session data revealed similar patterns of results. There was no evidence that either hot-plate or analgesiometer test session performance was affected by whether or not the assessment apparatus was functional or nonfunctional during the acquisition of tolerance phase of the experiment. Thus, rats that never practiced either response prior to the test showed test responsivity to pain similar to that of groups with previous experience in responding to aversive stimulation, indicating that such practice is irrelevant to test session performance. With both test procedures, there was a significant interaction between the drug (morphine or saline) and pretest tolerance acquisition situation (hot plate or analgesiometer) [F (1/80) = 5.56, P = .02 for analgesiometer test; F (1/80) = 12.3, $P < .001$ for hot-plate test]. Pairwise comparisons (Tukey's test) to analyze the source of the interaction indicated that when rats injected with morphine were tested in the environment other than that in which they received their tolerance acquisition sessions, they evidenced significantly lower pain sensitivity (that is, longer paw-lick latencies and higher paw-withdrawal thresholds) than any other condition ($P < .05$ for analgesiometer test, $P < .01$ for hot-plate test). For both the analgesiometer and hot-plate tests, none of the other differences between pairs of groups were statistically significant.

Prior to the test sessions, all animals injected with morphine, regardless of the environment in which they received the drug and regardless of whether or not they had the opportunity to practice either of the responses, were subjected to the same morphine-induced systemic effects equally as often and at the same intervals. These rats should all have been subjected to the same metabolic, cellular, or immunifacient modifications hypothesized to be responsible for tolerance; thus, on the basis of any theories of tolerance which emphasize these modifications (*1, 2*), it is expected that all morphine groups should be equally tolerant to the analgesic effects of the opiate in both test environments. However, only when rats were tested in the environment in which they previously experienced the narcotic did they show the high pain sensitivity indicative of analgesic tolerance. When the same rats were tested with morphine in the environment other than that in which they had previously experienced the drug, they evidenced relatively nontolerant (that is, analgesic) responses.

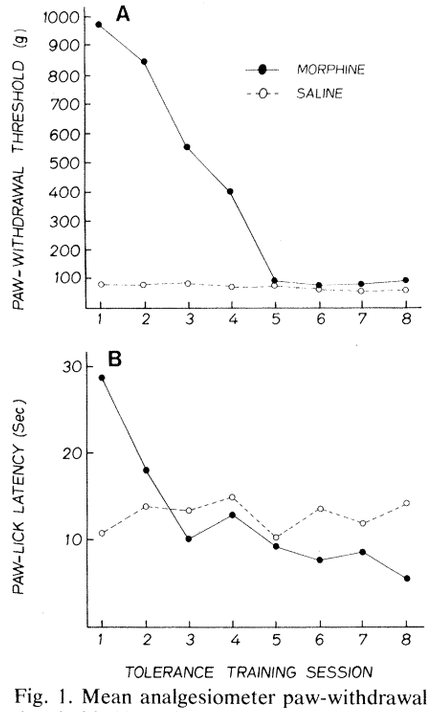

Fig. 1. Mean analgesiometer paw-withdrawal thresholds (A) and hot-plate paw-lick latencies (B) for rats injected with morphine or saline for each tolerance acquisition session. Each of the four independent groups consisted of 12 rats.

The importance of environmental cues in the display of tolerance cannot be attributed to practice in making the analgesia-indicant response while drugged (*7*), since groups that experienced the tolerance acquisition phase of the experiment with a nonfunctional assessment apparatus responded on the test sessions in the same way as groups that had the opportunity to practice the indicant response. The results of my experiment indicate that tolerance to the analgesic effects of small doses of morphine is highly dependent on the pairing of a drug administration ritual with the systemic effects of the drug, rather than merely the frequency of opiate stimulation. The findings, although contrary to most theories of tolerance (*1, 2*), are predicted by the conditioning theory of tolerance (*3*), which stresses the role of drug-associated environmental cues in the display of tolerance.

SHEPARD SIEGEL
Department of Psychology,
McMaster University,
Hamilton, Ontario L8S 4K1, Canada

References and Notes

1. Examples of such central theories of tolerance would be those postulating that the relevant effect of the early drug administrations is to (i) cause receptor sites to become occupied, thereby decreasing the population of sites that can be stimulated by that same drug on a later occasion [A. E. Livingston, *J. Pharmacol. Exp. Ther.* **47**, 433 (1933)]; (ii) induce the formation of "silent receptors," which functionally reduce the effects of later drug administrations by serving as "dead spot" receptors for drug molecules that would otherwise stimulate active "pharmacological receptors" [H. O. J. Collier, *Adv. Drug Res.* **3**, 171 (1966)]; or (iii) change the opiate receptor so that it is less likely to assume a conformation that will bind the drug [S. H. Snyder and S. Matthysse, *Opiate Receptor Mechanisms* (MIT Press, Cambridge, Mass., 1975)].
2. Examples of such peripheral theories of tolerance are those suggesting that early experiences with the opiate alter the organism's metabolism in such a way that the drug is subsequently more efficiently metabolized [S. J. Mulé and L. A. Woods, *J. Pharmacol. Exp. Ther.* **168**, 251 (1969)], or that narcotics act as antigens, with tolerance reflecting a process resembling the development of immunity [J. Cochin, in *Narcotic Drugs: Biochemical Pharmacology*, D. H. Clouet, Ed. (Plenum, New York, 1971)].
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