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Environmental Specificity of Tolerance to Morphine-Induced Analgesia in a Terrestrial Snail: Generalization of the Behavioral Model of Tolerance

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KAVALIERS, M. AND M. HIRST. *Environmental specificity of tolerance to morphine-induced analgesia in a terrestrial snail: Generalization of the behavioral model of tolerance.* PHARMACOL BIOCHEM BEHAV **25**(6) 1201–1206, 1986.—Terrestrial snails, *Cepaea nemoralis*, develop tolerance to morphine-induced analgesia, such that after 7–9 days of treatment with morphine (10 mg/kg) their response latencies to an aversive thermal stimuli (38.5°C) are not significantly different from those of untreated control animals. In Experiment A snails were rendered tolerant to morphine using either of two pre-injection cues (light and dark background brightness or color) and then assessed for morphine-induced alterations in thermal nociceptive responses in both environments. In Experiment B snails were made tolerant to morphine in the presence of one of two different thermal cues (a stressful temperature of 35°C that is normally avoided or an ambient temperature of 22°C) and then tested for morphine-induced alterations in nociceptive responses in both environments. In the two experiments tolerance to morphine-induced analgesia was displayed when snails were exposed to the pre-injection environmental cue normally associated with the administration of morphine, but not when exposed to the alternative pre-injection cue. These results demonstrate that various environmental factors (background colors or brightness as well as temperature cues and potentially thermal stress), can function as environmental specific cues for the development of tolerance to morphine-induced analgesia in molluscs, in a manner consistent with a behavioral mechanism of tolerance. Thus, these results suggest that environmental specificity of tolerance involving either classical (Pavlovian) conditioning or habituation may be a general phenomenon having an early evolutionary development and broad phylogenetic continuity.

Analgesia Environmental cues Environmental tolerance Conditioning Opiate Morphine Snails Stress Classical conditioning Evolution Habituation Learning Pavlovian

A characteristic effect of repeated administrations of opiates is the development of tolerance. The behavioral and physiological effects that are initially produced by substances such as morphine show a progressive decline in intensity until they are indistinguishable from responses of control animals [3, 13, 40]. A variety of mechanisms have been proposed to explain the development of tolerance; the majority of these have dealt with the physiological and pharmacological consequences of repeated opiate administrations. It has been suggested that systemic changes arising within the organism may modify opioid receptor sensitivity, induce functional neurochemical and/or neurotransmitter changes and alter the disposition and metabolism of opiates [6, 13, 28, 40].

Relatively recently, it has been recognized that the display of tolerance is also dependent on behavioral factors and environmental cues in a manner consistent with either Pavlovian conditioning and/or habituation [10, 11, 35, 37]. According to both the compensatory classical conditioning and habituation proposals, tolerance depends not only on repeated administrations of the drug, but also on experience with specific environmental cues present at the time of drug administration [2, 10, 37]. For example, rats tested in the context of the 'usual' pre-drug cues are more tolerant to the analgesic, thermic, locomotor and behaviorally sedating effects of repeated doses of morphine than are equally drug-experienced rats tested in the context of 'alternative' cues

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[12, 33, 34, 37]. According to the habituation account this may involve learned and unlearned responses while the alternative conditioning explanation involves learning experience with the cues. Behavioral involvement in tolerance development has now been demonstrated with a variety of opiate and non-opiate drugs [11, 21, 26, 27, 37], and this environmentally influenced response is hypothesized to be a general characteristic of mammalian drug tolerance [37].

In invertebrates, and, in particular, molluscs, there is behavioral, biochemical, electrophysiological and pharmacological data supporting the existence of regulatory opioid systems that resemble those present in mammals [15–20, 23, 24]. Endogenous opioid systems are involved in the mediation of the thermoregulatory, nociceptive and analgesic responses of the terrestrial gastropod snail, *Cepaea nemoralis*. Morphine, as well as opioid peptides, enhance, in a dose-dependent manner, the aversive thermal response latencies of *Cepaea* to a 'hot-plate' in a manner analogous to that associated with the production of analgesia in mammals [15, 16, 20]. These 'analgesic' responses display a stereospecific requirement similar to that reported in mammals [20]. In addition, the opiate antagonist, naloxone, can suppress and reverse the analgesic effects of morphine in *Cepaea*, as well as reducing response times of control snails [15], further supporting a direct role for endogenous opioids in the mediation of thermal nociception. As in mammals, after 5–7 days of daily administration of morphine, *Cepaea* develop tolerance to the morphine-induced prolongation of response time [16]. Moreover, pre-treatment with the protein synthesis inhibitor, cycloheximide, disrupts this development of tolerance.

Learning behavior analogous to vertebrate classical conditioning and habituation has been demonstrated and is under intensive investigation in a number of species of molluscs [1, 4, 5, 8, 9, 29, 30]. It has been proposed that at least some of the cellular and neuronal mechanisms underlying associative learning may be consistent from molluscs through to mammals [1, 8, 9]. It was considered of interest, therefore, to determine whether or not the development of tolerance to morphine in *Cepaea* is also influenced by environmental cues in a manner consistent with the proposed behavioral models of tolerance. In the present study we show that environmental features (background color or brightness cues) as well as an elevated temperature, and potentially thermal stress, can function as specific environmental cues for the development and expression of tolerance to morphine-induced analgesia in *Cepaea*.

METHOD

Animals

Cepaea were collected locally in London, Ontario, and maintained as separate color banding morphs [14] under a 12 hr light-12 hr dark cycle (LD 12:12, L=25 μw/cm^2) at 22°C. The snails were fed daily with lettuce supplemented with vitamins, with water being freely available.

Experimental Procedures

Aversive thermal responses of hydrated snails were determined using a modified hot-plate technique [39]. Since the activity of snails can be modified by their state of hydration [38], all snails were allowed to fully hydrate (15 min) before being tested. Snails were placed in sealed containers with a saturated atmosphere and water in the bottom. After hydration, snails were injected with either morphine sulfate (BHD, Toronto) in a physiological saline solution (1.0 μl containing 10 μg) or the saline vehicle (1.0 μl) [25]. No corrections in dose were made for variations in the mass of the individual snails (the mass of the snail's body without shell ranged from 0.7–1.3 g). The solutions were injected with a microsyringe (5.0 μl Hamilton, NV) into the side of the foot in either the vicinity of, or directly in, the mantle cavity into the haemocoel. Snails were returned to the hydration chambers for an additional 15 min. Individual snails were then placed on a hot-plate (Technilab, NJ) and the latency of their foot lifting response to a 38.5±0.5°C thermal stimulus was recorded. When placed on a thermally aversive surface, fully hydrated *Cepaea* display a characteristic elevation of the anterior portion of their foot (Fig. 1 in [20]). This response, indicative of discomfort or aversion, has not been observed in snails exposed to temperatures normally present in their natural habitats [20], but becomes evident as the experimental temperature is raised to 40°C [16]. Previous studies had established that maximal responses to morphine by *Cepaea* occurred 15 min after injection [15,16]. All determinations were carried out in the early light period (1000–1200 hr).

Tolerance Acquisition

Tolerance to morphine-induced analgesia was established in snails that were exposed to one of two different specific environmental cues. Administration of morphine was associated with exposure to either (A) light or dark background colors (high and low brightness, respectively), or (B) ambient or elevated environmental temperatures (22°C and 35°C, respectively). The latter investigation also encompasses consideration of the possible roles of stress [35°C being aversive to some morphological varieties of *Cepaea* ([20] and Kavaliers, in preparation)] as a cue for tolerance development. Details followed in experiments A and B are given below.

A. Background color cues. Snails were treated as described previously with saline or morphine every day for 10 days and the latencies of their thermal responses were determined. Hydration of the snails for 15 min both before and after injection occurred in either a bright light colored (white opaque chamber with a transparent top) or dark (black, closed chamber) environment. Chamber sizes, temperature and humidity levels (saturated) were similar. Twenty different snails were used for each of the four groups.

B. Temperature cues. Snails were treated with saline or morphine as above every day for 10 days and the latencies of their thermal responses were determined. One group of these snails was exposed to a 35±0.5°C hot-plate for 1 min before being injected with morphine or saline, while another group was placed on the hot-plate at an ambient temperature of 22±1°C. Although the snails do not show any evidence of an aversive response to 35°C after 1 min, the majority of morphs of *Cepaea* avoid this temperature and in a number of cases show aversive responses after 1.5–2.0 min. This latter avoidance is taken as evidence to support the contention 35°C is a stressful environment to this particular morphological type of *Cepaea*. All groups were hydrated in similar light environments. Twenty different snails were used in each of the four groups.

To test for the possible effects of experience with the 35°C hot-plate on the latency of thermal responses, additional

groups of 10 snails were injected with morphine or saline for 10 days and tested on a non-functional 38.5°C hot-plate after receiving morphine or saline. Previous studies had shown that daily injections and handlings by themselves have no significant effects on nociception and response latency of snails [16].

Tolerance Test Conditions

A. Background color cues. After ten days of treatment with morphine the specificity of the light and dark cues for morphine tolerance induction was examined. Ten randomly chosen snails that had been exposed to light pre- and post-injection cues were now hydrated in the dark, while 10 individuals that had received the dark cues were hydrated in the light. The other ten individuals in each group were maintained and tested with their original background color conditions. Similar tests of the specificity of the background hydration cues were also carried out with 10 each of the saline injected individuals. As before, the other 10 individuals remained under their original conditions fifteen min after injection with morphine or saline and hydration. The thermal response latencies of the snails were determined as described previously. On the next day, the nociceptive response of all the morphine and saline-treated snails were determined under their original light or dark tolerance acquisition conditions.

B. Temperature cues. After ten days of treatment with morphine and pre-exposure to either 35°C or 22°C the specificity of these temperatures as cues for morphine tolerance was examined. Ten randomly chosen snails that had been exposed to 35°C were now exposed to the hot-plate at 22°C, while ten individuals that had been placed on the non-functional 22°C hot-plate were exposed to 35°C. The other 10 individuals in each group were kept under their original cues. Similar tests of the specificity of the temperature cues occurred with 10 each of the saline-treated individuals. Fifteen min after injection with morphine and hydration, thermal response latencies of the snails were determined. On the next day the nociceptive responses of morphine and saline injected snails were determined under their original thermal tolerance acquisition conditions.

All data were analysed by analysis of variance for repeated measures. Student-Newman-Keuls multiple range test was used for post-hoc comparisons of means. The significance level for hypothesis testing was set at 0.05.

RESULTS

A stereotyped elevation of the anterior portion of the extended foot was observed in fully hydrated snails that were exposed to 38.5°C for a short period of time. This foot-lifting behavior was never observed at ambient temperatures of 22°C conditions and as indicated previously occurred only after 1.5–2.0 min exposure to 35°C in individuals of this particular morphological type.

A. Light and Dark Cues and Tolerance Development

Administration of morphine resulted in an initial (day 1) significant ($p<0.001$, for the light or dark environment) increase in the latency of the foot-lifting response as compared to saline-treated individuals (Fig. 1). There was a significant ($p<0.01$) decrease in the analgesic effect of morphine between days 1 and 3. By days 7–10, the response latencies were not significantly different from those observed in saline treated animals and the *Cepaea* were considered to have become fully tolerant to the analgesic effects of morphine. There were no significant changes in the response latencies of saline-treated animals. Similar response latencies are present for days 1–10, (Fig. 1). For clarity of presentation only days 1–7 of the saline treated animals are presented in Fig. 1.

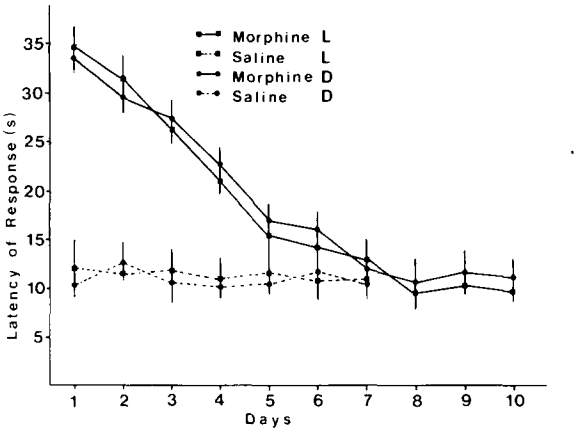

FIG. 1. Thermal response latencies (38.5°C) of snails (n=20) exposed to either light (L) or dark (D) backgrounds and receiving daily injections of morphine (10 μg) or saline (1.0 μl) for 10 days. Saline responses over days 7–10 (not shown) are the same as for the first 7 days. Vertical lines denote two standard errors of the mean.

During the tolerance acquisition period there were no significant differences between the thermal response latencies displayed by the morphine-treated snails exposed to either the light or dark cues. However, when the responses of the light or dark hydrated groups were assessed after exposure to dark or light environmental cues, respectively, there was a significant ($p<0.001$) elevation of thermal response latencies (Table 1). Neither group of animals held in the reversed cue condition displayed tolerance to morphine-induced analgesia; their response latencies were not significantly different from one another, or from that obtained on the first day of treatment with morphine (Table 1). The thermal response latencies of the saline-treated snails subjected to equivalent treatments were not different. Although not shown, the increase in morphine response latencies disappeared upon subsequent re-testing under the original light and dark environmental cues. These thermal response latencies did not differ significantly from either those found on day nine, before imposition of the novel cues, or from the response latencies displayed on days 10 and 11 by snails maintained under their original acquisition cues.

B. Temperature Cues and Tolerance Development

During the tolerance acquisition period there were no significant differences between the thermal response latencies displayed by the morphine-treated snails exposed to either 35°C, or the non-functional 22°C hot-plate, prior to injection (Table 2). The initial levels of morphine-induced analgesia and patterns of tolerance development were equivalent to those obtained for the snails exposed to the light and dark cues in Experiment 1 (Fig. 1, Table 1). Addi-

tionally, there were no evident differences between the basal nociceptive responses of all the groups of saline-treated snails.

Snails that had been treated with morphine and tested with a non-functional hot-plate did not significantly differ in their response to the hot-plate from individuals that were tested daily with the hot-plate (Table 3). A similar pattern of response was also observed for the saline-treated individuals. This indicates that the increased responsiveness to the hot-plate observed during the development of tolerance cannot be attributed to an acquired proficiency in responding to aversive stimulation. Whether or not just exposure to a non-functional hot-plate can serve as environmental cue for the development of tolerance was not, however, examined. When the responses of the 35°C and 22°C exposed groups were assessed after pre-injection exposure to either a non-functional 22°C hot-plate or a 35°C temperature, respectively, there was a marked elevation ($p<0.001$) in thermal response latencies (Table 2). Both groups showed no evidence of tolerance to morphine-induced analgesia, their thermal response latencies being similar to those obtained during the initial treatments with morphine. There were no significant differences in response latencies between the stressed and non-stressed groups. No significant differences were evident in saline-treated snails exposed to novel pre-injection cues (Table 3). Although not shown, in the subsequent re-test with the original pre-injection temperature cues, there was no evidence of tolerance to morphine-induced analgesia; the thermal response latencies being similar to those of the saline-treated individuals as well as to that of the morphine-injected animals that were continuously exposed to the same pre-injection cues.

TABLE 1

SPECIFICITY OF THE LIGHT AND DARK BACKGROUNDS AS CUES FOR THE DISPLAY OF TOLERANCE TO MORPHINE-INDUCED ANALGESIA BY SNAILS (n=10, FOR EACH CATEGORY)

Tolerance Acquisition Condition	Latency of Response (sec)			
	Light		Dark	
	Morphine	Saline	Morphine	Saline
Light	11.8±1.7	11.4±1.8	29.8±2.7	12.6±1.9
Dark	32.7±2.4	14.6±1.6	12.5±1.8	15.4±1.7

Saline responses are given for comparison. All thermal response latencies are ± two standard errors.

TABLE 2

SPECIFICITY OF THE THERMAL STIMULUS (35°, STRESS) AND AMBIENT CONDITIONS (22°, NON-STRESS) AS CUES FOR THE DISPLAY OF TOLERANCE TO MORPHINE-INDUCED ANALGESIA BY SNAILS (n=10, FOR EACH CATEGORY)

Tolerance Acquisition Condition	Latency of Response (sec)			
	Stress		Non-Stress	
	Morphine	Saline	Morphine	Saline
Stress	9.8±1.6	12.7±6.9	23.7±2.4	14.4±1.6
Non-Stress	29.6±2.4	13.9±2.1	10.6±1.8	13.2±1.7

Saline responses are given for comparison. All thermal response latencies are ± two standard errors.

TABLE 3

MEAN RESPONSITIVITY DURING HOT-PLATE TEST SESSIONS

Tolerance Acquisition Condition	Latency of Response (sec)	
	Morphine	Saline
Functional hot-plate	11.7±1.3	11.4±1.7
Non-Functional hot-plate	13.9±2.4	13.2±1.9

All thermal response latencies are ± two standard errors.

DISCUSSION

The present results demonstrate that environmental specificity of tolerance development to morphine-induced analgesia occurs in invertebrates as well as in vertebrates. Snails were significantly more tolerant to the analgesic effects of morphine when the drug was administered in the context of cues previously associated with the drug, than in the context of alternative cues. These responses are analogous to the environmental specificity of tolerance to morphine-induced analgesia that has been observed in rodents [10, 11, 33, 34]. Furthermore, and as in mammals, different stimuli were shown to be effective environmental cues for tolerance development [10,37]. The present results showed that background color or brightness and temperatures, and potentially the presence or absence of an elevated and potentially aversive thermal stimulus or stress can function as specific environmental cues for the expression of tolerance to morphine-induced analgesia in *Cepaea*. These similarities to mammalian systems provide further support for the generality and fundamental nature of environmental specificity of tolerance.

Previous investigations had revealed that the pharmacological aspects of opiate tolerance and withdrawal in *Cepaea* were analogous to those reported in mammals [16]. This study provides further evidence of this similarity, demonstrating that, as in mammals, morphine tolerance in snails is dependent on the environmental context in which the drug affect is assessed. This environmental specificity of tolerance in mammals has been explained by a classical conditioning model [12, 33, 34, 37, 40], though a modified homeostatic-conditioning model [27], as well as habituation have also been proposed [2, 7, 10, 22]. According to the Pavlovian associative conditioning model, environmental stimuli accompanying drug administration serve as the conditional stimulus, with the drug effect constituting the unconditional stimulus. In the habituation proposal both learned and unlearned processes are postulated to be involved in the determination of the environmental specific responses [2]. The conditioning and habituation models both agree with the growing number of reports which show that conditioning procedures, as well as habituation related learning, can significantly affect the behavior of molluscs [4, 5, 8, 9, 29, 30]. Simple associative learning in molluscs has been

shown to be in most respects similar to that observed in mammals [9], and could account for the observed environmental specificity of tolerance to morphine-induced analgesia. Whether or not molluscs display environmental specific tolerance to other opiate or non-opiate mediated behaviors remains to be determined. However, the present results do suggest that the investigations of the neuronal mechanisms of associative learning, which are being carried out with several species of molluscs [1, 8, 9], may provide insights into the basic mechanisms underlying the impact of environmental cues on opiate-induced tolerance.

Environmental specificity of opiate-based tolerance necessitates a thorough account of and understanding of the effects of the various environmental stimuli present at the time of drug administration. Investigations with mammals have revealed that a variety of factors may serve as distinct cues [21, 37, 38, 40]. The present results indicate that this is also true for *Cepaea*, with distinctive background and thermal environment serving as effective cues. Whether or not it is the differences in temperatures and/or the stress associated with exposure to 35°C that serves as the effective environmental cue remains to be determined. It has been suggested, however, that tolerance may be enhanced when the drug is experienced in conjunction with stress induced by exposure to distinctive cues associated with drug administration and assessment [12]. In the present study the results of the control procedures indicated that the decreasing analgesic effectiveness of morphine was not due to experience with the aversive test surface. This is similar to the results obtained from control studies by Siegel [33] using the 'hot-plate' test with rats. Exposure to stress has been shown to enhance morphine-induced analgesia in rats [31,32]. However, the intensity of stress imposed in those studies activated endogenous opioid systems and was, by itself, capable of inducing analgesia. Although molluscs can also display stress-induced analgesia [18], the duration and/or intensity of the thermal stress or in the present study was not sufficient to induce analgesia or potentiate the effects of morphine. In this regard, it would be of interest to determine whether a pre-exposure to a low level thermal or other type of apparently non-analgesic stress could serve as an effective environmental cue for tolerance to morphine-induced analgesia in mammals.

In summary, the present results suggest that environmental specificity of tolerance, consistent with a classical conditioning or habituation model, may be a basic and general characteristic. Moreover, the results also suggest that both the associative (learning) and non-associative (pharmacological) mechanisms that contribute to the development of tolerance may have had an early evolutionary development and broad phylogenetic distribution.

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REFERENCES

1. Alkon, D. L. Calcium-mediated reduction of ionic currents: a biophysical memory trace. *Science* **226:** 1037–1044, 1984.
2. Baker, T. B. and S. T. Tiffunx. Morphine tolerance as habituation. *Psychol Rev* **92:** 78–108, 1985.
3. Brase, D. A., H. H. Loh and E. L. Way. Comparison of the effects of morphine on locomotor activity, analgesia and primary protracted physical dependence in six mouse strains. *J Pharmacol Exp Ther* **201:** 368–374, 1977.
4. Carew, T. J., E. T. Walters and E. R. Kandel. Classical conditioning in a simple withdrawal reflex in *Aplysia californica*. *J Neurosci* **1:** 1426–1427, 1981.
5. Carew, T. J., R. D. Hawkins and E. R. Kandel. Differential classical conditioning of a defensive withdrawal reflex in *Aplysia californica*. *Science* **219:** 397–400, 1983.
6. Chavkin, C. and A. Goldstein. Opioid receptor reserve in normal and morphine-tolerant guinea pig ileum myenteric plexus. *Proc Natl Acad Sci USA* **81:** 7233–7257, 1984.
7. Dafters, R. and L. Bach. Absence of environment specificity in morphine tolerance acquired in nondistinctive environments: habituation or stimulus over-shadowing? *Psychopharmacology (Berlin)* **87:** 101–106, 1985.
8. Furley, J. and D. L. Alkon. Cellular mechanisms of learning, memory, and information storage. *Annu Rev Psychol* **36:** 419–494, 1985.
9. Gelperin, A., J. J. Hopfield, and D. W. Tank. The logic of *Limax* learning. In: *Model Neural Networks and Behavior*, edited by A. Severston. New York: Plenum Press, 1985, pp. 237–261.
10. Goudie, A. J. and J. W. Griffiths. Behavioral factors in drug tolerance. *Trends Pharmacol Sci* **7:** 192–196, 1986.
11. Hinson, R. E. and S. Siegel. Nonpharmacological bases of drug tolerance and dependence. *J Psychosom Res* **26:** 495–503, 1982.
12. Hinson, R. E. and S. Siegel. Anticipatory hyperexcitability and tolerance to the narcotizing effect of morphine in the rat. *Behav Neurosci* **97:** 759–767, 1983.
13. Iversen, S. D. and L. L. Iversen. *Behavioral Pharmacology*. New York: Oxford University Press, 1981.
14. Jones, J. S., B. H. Leith and P. Rawlings. Polymorphism in *Cepaea:* A problem with too many solutions? *Annu Rev Ecol Syst* **8:** 109–143, 1977.
15. Kavaliers, M., M. Hirst and G. C. Teskey. A functional role for an opiate system in snail thermal behavior. *Science* **220:** 99–101, 1983.
16. Kavaliers, M. and M. Hirst. Tolerance to the morphine-influenced thermal response in the terrestrial snail, *Cepaea nemoralis*. *Neuropharmacology* **22:** 1321–1326, 1983.
17. Kavaliers, M., M. Hirst and G. C. Teskey. Opioid systems and feeding in the slug (*Limax maximus*): Similarities to and implications for mammalian feeding. *Brain Res Bull* **14:** 681–685, 1985.
18. Kavaliers, M. and M. Hirst. Naloxone-reversible stress-induced feeding and analgesia in the slug (*Limax maximus*). *Life Sci* **38:** 203–209, 1986.
19. Kavaliers, M. and M. Hirst. Slugs and snails and opiate tales: opioids and feeding behavior in invertebrates. *Fed Proc*, in press, 1986.
20. Kavaliers, M. and M. Hirst. Evolutionary aspects of opiate involvement in thermoregulation. In: *Handbook of Comaprative Aspects of Opioid and Related Mechanisms*, Vol I, edited by G. R. Stefano. Florida: CRC Press, 1986, pp. 139–152.
21. Kavaliers, M. and K.-P. Ossenkopp. Tolerance to morphine-induced analgesia in mice: magnetic fields function as environmental specific cues and reduce tolerance development. *Life Sci* **37:** 1125–1135, 1985.
22. Kesner, R. P. and D. G. Cook. Role of habituation and classical conditioning in the development of morphine tolerance. *Behav Neurosci* **97:** 4–12, 1983.

23. Kream, R. M., R. S. Zukin and G. B. Stefano. Demonstration of two classes of opiate binding sites in the nervous tissue of the marine mollusc *Mytilus edulis*. *J Biol Chem* **225:** 9218–9224, 1980.
24. Leung, M. K. and G. B. Stefano. Isolation and identification of enkephalins in pedal ganglia of *Mytilus edulis* (Mollusca). *Proc Natl Acad Sci USA* **81:** 955–958, 1984.
25. Meng, K. 5-Hydroxytryptamin und acetylcholin als Wirkungsantagonisten beim Helix-Herzen. *Naturwissenschaften* **19:** 480–481, 1958.
26. Poulos, C. X. and R. E. Hinson. Pavlovian conditional tolerance to haloperidol catalepsy: Evidence of dynamic adaptations in dopaminergic systems. *Science* **218:** 491–492, 1982.
27. Poulos, C. X. and R. E. Hinson. A homeostatic model of Pavlovian conditioning: tolerance to scopolamine-induced adipsia. *J Exp Psychol [Anim Behav]* **10:** 75–89, 1984.
28. Redmond, R. E, Jr. and J. H. Krystal. Multiple mechanisms of withdrawal from opioid drugs. *Annu Rev Neurosci* **7:** 443–478, 1984.
29. Sahley, C., A. Gelperin and J. W. Rudy. One-trial associative learning modifies food odor preferences of a terrestrial mollusc. *Proc Natl Acad Sci USA* **78:** 640–642, 1981.
30. Sahley, C., J. W. Rudy and A. Gelperin. An analysis of associative learning in a terrestrial mollusc. 1. high-order conditioning, blocking and a transient US pre-exposure effect. *J Comp Physiol* **144:** 1–8, 1981.
31. Sherman, J. E., C. Proctor and H. Strub. Prior hot-plate exposure enhances morphine analgesia in tolerant and drug-naive rats. *Pharmacol Biochem Behav* **17:** 229–232, 1982.
32. Sherman, J. E., H. Strub and J. E. Lewis. Morphine analgesia: enhancement by shock-associated cues. *Behav Neurosci* **98:** 203–209, 1984.
33. Siegel, S. Morphine analgesic tolerance: its situation specificity supports a Pavlovian conditioning model. *Science* **1983:** 323–325, 1970.
34. Siegel, S. Morphine tolerance acquisition as an associative process. *J Exp Psychol [Anim Behav]* **3:** 1–13, 1977.
35. Siegel, S., R. E. Hinson and M. D. Krank. The role of predrug signals in morphine analgesic tolerance: support for a Pavlovian conditioning model of tolerance. *J Exp Psychol [Anim Behav]* **4:** 188–196, 1978.
36. Siegel, S., R. E. Hinson, M. D. Krank and J. McCully. Heroin "overdose" death: contribution of drug-associated environmental cues. *Science* **216:** 436–437, 1982.
37. Siegel, S. and J. MacRae. Environmental specificity of tolerance. *Trends Neurosci* **7:** 140–143, 1984.
38. Smith, L. H., Jr. Quantified aspects of pallial fluid and its effect on the duration of locomotor activity in the terrestrial gastropod *Triodopsis alborabria*. *Physiol Zool* **54:** 407–414, 1981.
39. Woolfe, G. and A. D. MacDonald. The evaluation of the analgesia action of pethidine hydrochloride (demerol). *J Pharmacol Exp Ther* **80:** 300–307, 1944.
40. Wuster, M., R. Schulz and A. Herz. Opioid tolerance and dependence: re-evaluating the unitary hypothesis. *Trends Pharmacol Sci* **2:** 64–67, 1985.