Pharmacology Biochemistry & Behavior, Vol. 16, pp. 869–874, 1982. Printed in the U.S.A.

Selective Action of Morphine on Reflex Expression to Nociceptive Stimulation in the Rat: A Contribution to the Assessment of Analgesia

PHILIP H. WARREN[1] AND JAMES R. ISON

Reflex Modulation Laboratory, Department of Psychology, University of Rochester Rochester, NY 14627

Received 7 April 1981

WARREN, P. H. AND J. R. ISON. *Selective action of morphine on reflex expression to nociceptive stimulation in the rat: A contribution to the assessment of analgesia.* PHARMAC. BIOCHEM. BEHAV. **16**(6) 869–874, 1982.—In two experiments startle reflexes to both loud noises and electric shocks were elicited in rats. The two stimuli were paired so that the inhibitory effect of each stimulus on the response to the other could be assessed. In the first study it was shown that morphine (0–16 mg/kg) had a selective and dose related depressive effect on the response to shock. The response to a leading tone and inhibiton produced by the shock on the response to a following tone were minimally affected. In the second study, morphine (10 mg/kg) again depressed the reaction to shock but not to tone, and its effect was antagonized by naloxone (10 mg/kg). The selective effect on responses to shock, leaving responses to tone relatively unaffected, reveals that reflex depression should not be attributed to a loss in motor functions. Further, that morphine had little effect on reflex inhibition produced by the shock suggests that the nociceptive properties of shock were affected rather than simple sensory processes. It is proposed that the method described here is useful for assessing changes in nociception in laboratory animals, and for discriminating between nociceptive, afferent, and efferent processes.

Morphine Analgesia Nociceptive stimuli Startle reflex

THE two experiments reported here describe the effects of morphine on the short-latency reflex behaviors elicited by a tail shock and by an intense tone burst in the rat. Further, by pairing the two stimuli with a brief lag time between them we could assess the effect of morphine on the inhibitory consequence that shock normally has on a following acoustic startle reflex [11,12]. These experiments are intended as a preliminary contribution to the experimental analysis of morphine analgesia and, especially, to the development of a new procedure for measuring nociception in laboratory animals.

Although there do exist precise psychophysical tests for studying pain and analgesia in animals, which use operant conditioning methods [14], the "working methods", in general use because of their relative ease of administration, typically employ some sort of elicited reflex response. These methods include the "flinch-jump test" [1,5]; the "tail-flick test" [3]; the "hot plate test" [15]; and the "writhing test" [6,13]. While eminently servicable, these tests are not unflawed in either administration or interpretation. The apparatus is seldom instrumented and the occurrence of the response often depends on the quickness of an observer's eye and considered judgment. Further, the deficit in response amplitude or latency which is taken as the sign of analgesia may not be specific to the manipulated nociceptive stimulus but instead it may result from a nonspecific dysfunction in motor reactivity.

In the procedures we describe the reflexes are measured indirectly as the intensity of the force applied to the floor of a holding cage by the animal's reflexive jump response. (Prior work has shown that these force measures are highly correlated with EMG indices of startle behavior [7]). The stimuli are delivered automatically and the size of the reflex is measured on a CRT or digital voltmeter, these two mechanical procedures thus minimizing two sources of potential experimenter bias and observer variability. Our presenting two classes of reflexogenic stimuli allows us to discriminate between analgesia (a response deficit specific to an electric shock) and hyporeactivity (a deficit seen equally to shock

[1]Requests for reprints should be submitted to Dr. Philip H. Warren III, Laboratory of Psychobiology, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115.

and to acoustic stimuli). In this latter regard, it may be argued that because intense noises are commonly reported by humans as being painful, there is no *a priori* reason why startle inducing loud noises should be thought of as any less nociceptive to the rat than are electric shocks. This may be so: however, in counter argument we note that while the tones are intense (110 dB) they are very brief (20 msec in duration). In other experiments we routinely find that rats show little reaction to tones of even greater intensity, save for the momentary elicited startle reaction. And in the experiments we describe here we found that morphine does have a predominantly selective effect on the reflex jump to shock. We believe then that our procedure is advantageous from this standpoint.

There is another advantage to the present procedure, which is that it allows for some analysis of the locus of an analgesic effect. In the rat, as in humans and other laboratory animals, the acoustic startle reflex is inhibited by irrelevant stimuli which just briefly precede the time of reflex elicitation. Inhibition of acoustic startle reflexes by preliminary stimuli depends largely on the intensity of the leading stimulus [9] and is unaffected by changes in response strength associated with the earlier event [10,12]. In general, an electric shock given just before reflex elicitation inhibits the acoustic startle reflex [11,12] to a degree determined by shock intensity. A specific analgesia, seen as a reduction in the size of the shock elicited startle reflex, might be accompanied by a reduction in the inhibitory effect of that shock on an immediately subsequent acoustic startle reflex. If this were obtained then we would suspect that the sensory impact of that shock had been impaired by the analgesic agent, because a dysfunction on the sensory side would result in decrements in both response elicitation and in the inhibitory consequence of the shock. But conversely, if the elicited reaction to shock was reduced and there was no accompanying decrement in its inhibitory effect, then the locus of analgesia would be presumed to lie more on the side of nociception of reflex expression, rather than simple sensation.

GENERAL METHOD

Subjects

The subjects were 30 male Long Evans rats weighing approximately 300 g each (mean = 312 g). They were housed in pairs in a temperature controlled colony room (68±2°F) on a 12/12 light/dark cycle. Animals were maintained on ad lib food and water and used in the experiment during the light part of the daily cycle.

Apparatus

During testing each animal was restrained in a small cage made of Plexiglas and brass bars (17 cm long × 9 cm high × 7 cm wide). The cage rested on a flexible Plexiglas platform with a Statham accelerometer attached on its undersurface. The animal's reactions to the eliciting stimuli were detected by this transducer (accelerometer), amplified, and either read as peak-to-peak maximum voltage on a Tektronix storage CRT within 100 msec of the stimulus (Experiment 2) or rectified, integrated over the same 100 msec and read in volts on a digital voltmeter (Experiment 1). The cage was placed in a small IAC sound attenuating chamber (approximately a 1-m cube) contained in an IAC sound attentuating room (approximately 2 × 2.5 × 2.5 m). All control apparatus was in an adjacent room. The acoustic stimuli were presented over a JBL tweeter placed about 50 cm from the cage. The tone was generated by a Hewlett-Packard oscillator, and gated through an electronic switch before amplification. It had a 10 kHz frequency and 110 dB (SPL) intensity, with 5 msec rise and decay times, and a 15 msec peak intensity. The animal's tail protruded out of the door of the restraining cage and was taped to a channel on the outer wall of the cage. Care was taken to assure a firm and secure contact without compressing the skin and possibly producing ischemia. Tail shock was presented over two Beckman miniature cup electrodes taped to the animal's tail. The electrodes were speced 3 cm apart with a proximal cathode. The animal's tail was carefully washed and slightly abraded so that electrode resistance was less than 25 kohms. The shock was 20 msec in duration and presented through a constant current DC stimulator constructed in the department. Stimulus durations, interstimulus intervals, and intertrial intervals were controlled by a bank of solid state timers. The animal was monitored over closed-circuit TV.

EXPERIMENT 1

In this experiment we were concerned with two problems. First, we wanted to assure ourselves that the planned procedures would yield sensible reactions to the two eliciting stimuli as well as inhibition of the acoustic startle reactions by a leading shock. Second, we questioned whether the behaviors would be sensitive to low and graded applications of an analgesic agent, morphine. There is a classic distinction, going back to Head in 1920 [8], between two kinds of pain. The first is epicritic pain, which is both spatially and temporally localized, and is characterized most readily by the flexor withdrawal reflex. The second is protopathic pain, which is diffuse in bodily location and prolonged in duration, and is characterized best in deep tissue injury and resulting spasms. It has been proposed that these two sorts of pain are functionally and pragmatically different. On the pragmatic side, it has been proposed sometimes that only protopathic pain is of importance to human distress because only protopathic pain persists beyond the finish of the precipitating conditions. A functional characteristic which has been suggested as distinguishing between the two sorts is their responsiveness to analgesic agents: thus Bowsher [2] asserted that epicritic pain does not respond to subanesthetic doses of opiate analgesics. For this reason, he severely questioned the utility of the several reflexive measures of pain sensitivity in laboratory animals as being at all applicable to understanding problems of human distress. As we too use brief cutaneous stimuli as the precipitating conditions, and abrupt and momentary reactions as the indicator variables, we though it important to present a dose response curve for these behaviors as affected by morphine.

Procedure

Ten Long-Evans rats were used, each animal run under each drug condition. In this experiment the shock was 1.2 mA, 20 msec in duration, and the tone was fixed at 110 dB, 20 msec duration as described above. A 60 dB background noise was present at all times. The shock and tone were given in pairs, alternately shock or tone leading, with a 1-sec interval within the pair and a 30 sec interval between pairs. Just 8 pairs of each sort were given, this including a "warm-

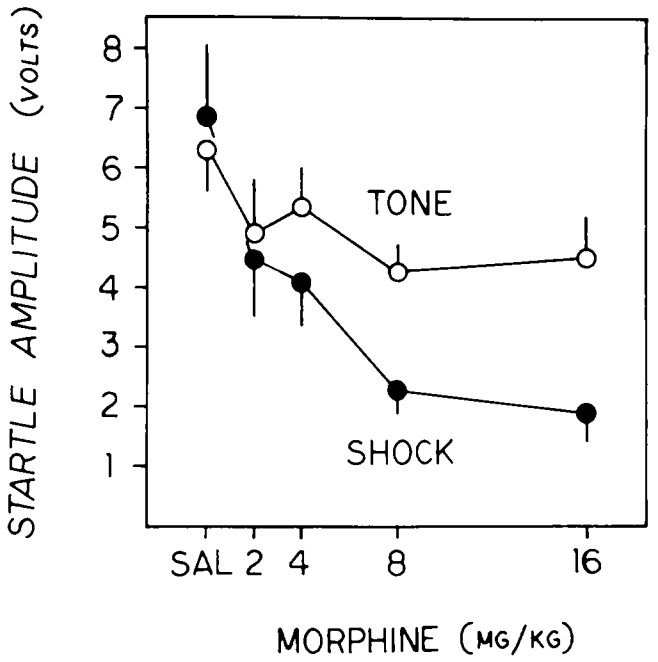

FIG. 1. Effect of saline and four doses of morphine on the amplitude of the startle response to shock and tone when each stimulus was the first of the pair. Plotted are means and standard errors.

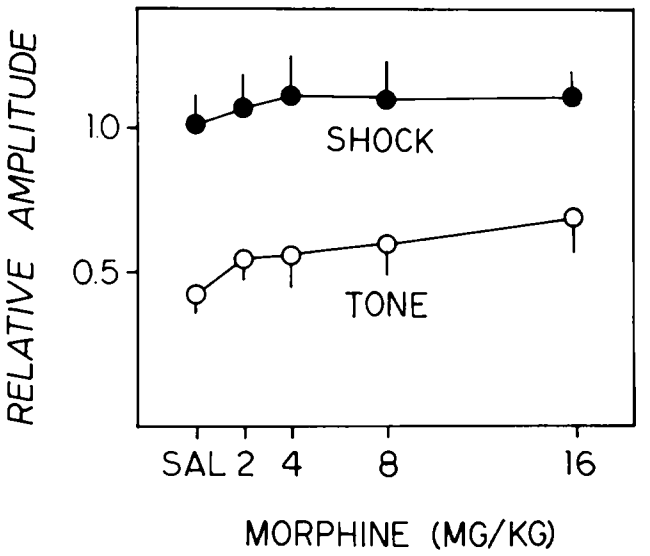

FIG. 2. Effect of saline and four doses of morphine on the inhibitory effect of shock on the response to tone and of tone on the response to shock when each stimulus was the second of the pair. Plotted are means and standard errors.

up" pair which was not included in the analyzed data. The rats were injected (IP) 45 min before behavioral testing, with saline (1 ml/kg) or one of four doses of morphine (2, 4, 8, and 16 mg/kg), the order for each animal varying according to its assignment to the row of a Latin-Square design. Tests were at least 7 days apart to minimize tolerance to the drug and habituation to the stimuli. Analyses were performed on the mean response voltage in each stimulus condition and on mean inhibition. Inhibition was calculated separately for each animal as the difference in the mean response to a stimulus when it was first in the pair (the "conditioning stimulus") compared to the mean response to the second in the pair (the "test stimulus"), expressed as a proportion of the control response to the first stimulus.

Results and Discussion

The data are presented in two figures. Figure 1 shows the mean response to shock and to tone at each dose of drug when each stimulus was the first member of the pair. Figure 2 shows the mean inhibition of the response to tone and to shock at each dose of the drug when each stimulus was the second member of the pair. In Fig. 1 it is clear that in the saline condition the responses to shock and to tone were about equal, the standard error bars overlapping considerably. The curves then diverged with the response to shock being considerably more affected by morphine than was the response to the tone. The linear functions which describe the two curves were significantly different, $F(1,9)=6.11$, $p<0.05$. Analysis of the shock function revealed a reliable effect of morphine dose level, $F(5,36)=7.14, p<0.01$. A t-test of the control value against the 2 mg/kg value yielded significance, $t(19)=2.28, p<0.05$. Analysis of the response to tone did not yield a significant overall effect of the morphine treatment ($p>0.1$), though an analysis of extreme values, 0 mg/kg vs either 8 or 16 mg/kg, did yield significance ($p<0.05$).

It is evident, firstly, that morphine affects responses elicited by shock more so than responses elicited by tone. This finding indicates that the effectiveness of morphine is largely selective, and that this drug is having only a minimal effect on general reactivity. The same conclusion was reached by Davis [4] in his study of the effect of morphine on potentiated startle reflexes in the rat. Secondly, we note that the lowest level of morphine used did have a substantial effect on the reaction to shock, indicating that the functional distinction between epicritic and protopathic pain which has been proposed [2] does not hold, in the present circumstances at least.

Figure 2 shows that in general the shock inhibited the response to tone whereas the tone did not inhibit the response to shock. There seemed to be a small but systematic dampening effect of morphine on the inhibitory effectiveness of the shock. Morphine did not reliably reduce reflex inhibition in an overall test, but a post-hoc analysis of changes in inhibition of the acoustic startle reflex produced by the shock did reveal a significant linear trend in response to the increased level of morphine, $F(1,9)=7.66, p<0.05$. This finding suggests that morphine may have had a modest but dose related effect on the sensory process produced by the tail shock. This small effect is out of proportion to the large effect of the drug on reflex amplitudes, and that the change in inhibition was minimal compared to the change in reflex strength argues that morphine had its effect on reflex strength by some additional decremental process. We conclude that a major share of morphine's effect in this situation is to reduce shock aversion rather than diminish shock detection.

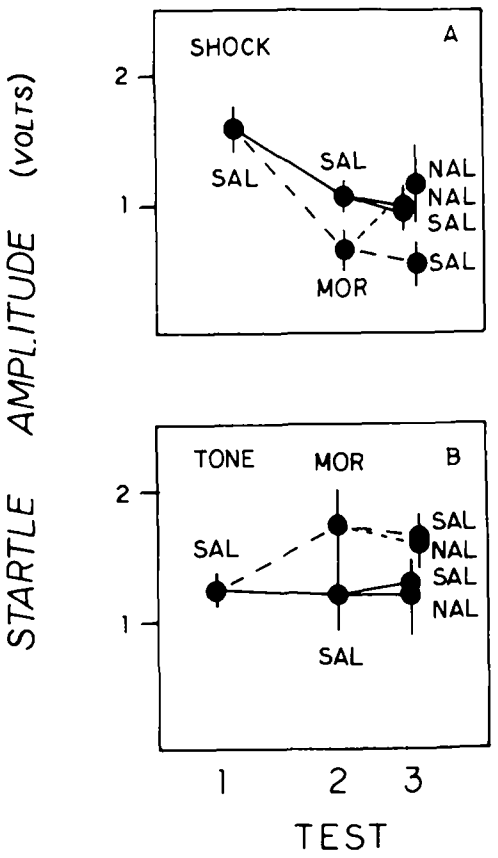

FIG. 3. Effects of saline, morphine, and naloxone on the amplitude of the startle response to shock and to tone. Panel A shows means and standard errors for startle responses elicited by shocks. Panel B shows means and standard errors for responses elicited by tones. For both graphs the ordinate units are arbitrary (volts read from an oscilloscope) but are equal for panels A and B.

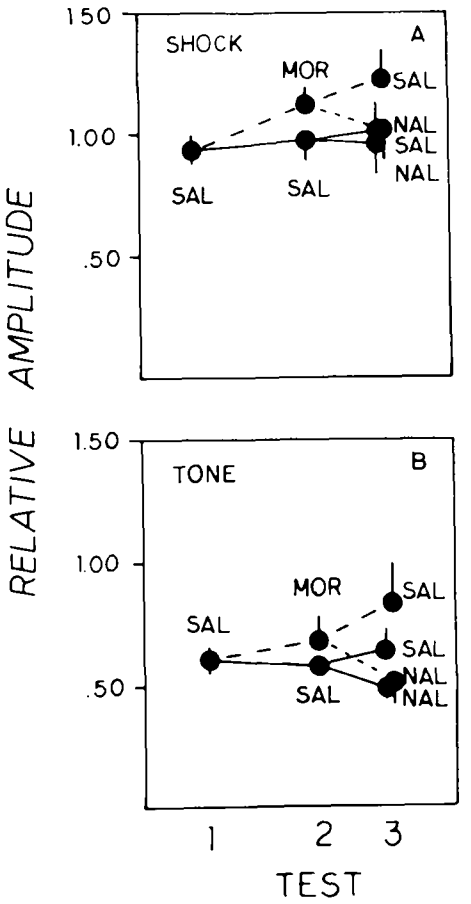

FIG. 4. Effects of saline, morphine, and naloxone on the amplitude of the startle response to shocks and to tones which were preceded one second earlier by a tone or shock respectively. Panel A shows that tone has a negligible inhibitory effect on the response to shock. Panel B shows that shock has a large inhibitory effect on the response to tone.

EXPERIMENT 2

One criterion for concluding that analgesia is mediated by the opiate receptor is that the process can be reversed by treatment with naloxone. In this second experiment, therefore, we used conditions in which this reversal could appear. An experimental session was divided into three components, namely, (1) baseline, then (2) morphine treatment, then (3) naloxone, along with appropriate control substances. The primary interest was in whether the decrement in the reflexive response to shock would be reversed by naloxone in the third part of the experiment.

Procedure

Twenty Long-Evans rats were used, five in each of four conditions. Shock intensity was varied across animals on the basis of five initial trials in which an attempt was made to roughly equate response levels to the shock and the tone: the average was 1.0 mA. The three stages of the experiment each followed an injection (IP) with a lead time of 45 minutes for saline and morphine, 30 minutes for naloxone. In the first injection animals were injected with saline; for the second injection one-half of the animals were selected randomly and received morphine (10 mg/kg), the others saline; and for the final injection half within each earlier subdivision received naloxone (10 mg/kg), the others saline. The injections were given immediately after the preceding behavioral test. Each test consisted of 30 pairs of trials, 15 with shock leading tone, 15 with tone leading shock. The data were analyzed in the same fashion as before. (Note, however, that because of the changes in response measurement direct comparisons of response magnitudes between the two experiments are not meaningful.)

Results and Discussion

Figure 3 shows the mean response amplitude to shock and to tone in each component of the experiment when shock or tone was the first member of the pair. Figure 4 shows the mean inhibitory effect when tone or shock was the second member of the pair. There are three features in Figure 3 which stand out. The first is that the shock (but not the tone) diminished in effectiveness across the three test periods. This effect, which appears to be habituation, was not seen in the earlier experiment which employed briefer tests (eight

trial pairs only) with a minimum of 7 days, rather than 30–45 min, between tests. Second, note that the injection of morphine prior to the second test greatly diminished the subsequent reactions to shock, but had a minimal (and perhaps contrary) effect on the reactions to the tone. The effect of morphine on the reactions to shock is exactly that found in the earlier experiment. Its effect on the reaction to tone is not the same in the two experiments, but in both cases the effect was of marginal significance relative to its standard error. Third, it can be seen that the injection of naloxone reversed the depressant effect of morphine on the reaction to tail shock, but had no effect on reactions to tone nor on reactions to shock in those animals who had not previously had morphine. Analyses of these data substantiated the reliability of the depressing effect of morphine on the reaction to shock, $F(1,7)=7.57$, $p<0.05$, as well as the overall decrement in the reaction to tail shock with repeated testing for the rats given saline on all three tests, $F(1,4)=8.13$, $p=0.01$. Effects of morphine, naloxone, or repeated testing were not significant on the reactions to the tone ($p>0.10$).

None of the several effects of morphine and naloxone on reflex inhibition suggested in Fig. 4 was reliable, using analysis of covariance. In agreement with the earlier data, presented in Fig. 2, tone had little effect on the response to tail shock, whereas the shock had a major and reliable ($p<0.01$) inhibitory effect on the response to the tone. As in Fig. 2, morphine had a slight effect on reducing the inhibitory impact of the shock, which was apparently reversed by naloxone. The consequences of these drugs, minimally evident in the graph, did not stand up to statistical analysis. Although these effects have systematic coherence, they resulted in part because the animals randomly chosen as the morphine subgroup to receive naloxone had a somewhat reduced preinjection response; analysis of covariance suggested that the graphic consequence of naloxone resulted in part because of this inadvertant bias in subject selection. It will be remembered that in the first experiment morphine had only marginal effects on the inhibitory consequence of shock, in contrast to its major effect on the response eliciting consequence of that stimulus; the pattern of substantial effects on reactions to shock, and marginal effects on shock produced inhibition conforms to the earlier results.

GENERAL DISCUSSION

The procedures used in these experiments, particularly the distributed trial sequences used in the first experiment, were successful in attaining our objectives: responses to shock and to tone were readily obtained as was the inhibitory effect that shock has for the response to the tone. The efficacy of these procedures then allowed the analysis of morphine effects in terms of their specificity and locus of action. Here our major findings can be simply summarized. First, morphine primarily affected the response to tail shock and had relatively little effect on the response to tone. This reveals that morphine is not very effective as a general motor depressant for all sorts of reflex activity, but rather, is relatively selective for nociceptive stimulation. Second, morphine had little effect on the inhibitory consequence of shock, in contrast to its major effect on the animals' reflex reaction to shock. This reveals that morphine is not attenuating the simple sensory aspects of the noxious stimulus as much as reducing its nociceptive properties, or aversiveness. Third, in the second experiment we found that the depressive effect of morphine on reflex amplitudes is reduced by naloxone. This finding suggests that cells containing opiate receptors modulate the reaction to shock.

Overall the data we present provide a modest addition to the accumulating knowledge of the behavioral effects of morphine and naloxone. We would like to emphasize here, however, the relevance of the general methodology to studies of nociception and analgesia in laboratory animals. This preparation, by virtue of its stimulus control and objective measurement of behaviors, is well suited to automation both of the experimental procedures and of data collection. This is a major advantage in behavior test development. Its fractionation of reactivity across stimulus modalities, and its separation of inhibitory potential from the excitatory potential of nociceptive stimuli, allows the experimenter to distinguish between general and specific effects of analgesic agents. Our finding that the method yields coherent and sensible effects for morphine, effects at least as sensitive as some other tests [5, 6, 16], should encourage its continued development and use with other agents of known and questioned analgesic properties.

ACKNOWLEDGEMENTS

We wish to thank J. M. Russo and J. Foss for their advice during the planning stages of this work, M. Davidson for his advice on statistical analysis, and J. Foss, C. Kellogg, B. Weiss, and R. Wood for their thoughtful comments on preliminary drafts of this report. We wish also to thank Endo Laboratories for their kind donation of naloxone hydrochloride. Philip H. Warren III is now at the Laboratory of Psychobiology, Harvard Medical School. The work reported here was supported in part by research grants from the Public Health Service, ES 01248, and from the Environmental Protection Agency, R-8068201. Although this research was supported by the United States Environmental Protection Agency it has not been subjected to the Agency's required peer and policy review. Therefore it does not reflect the views of the Agency and no official endorsement should be inferred.

REFERENCES

1. Bonnet, K. A. and K. E. Peterson. A modification of the flinch-jump test for measuring pain sensitivity in rats. *Pharmac. Biochem. Behav.* **3**: 47–55, 1975.
2. Bowsher, D. Pain pathways and mechanisms. *Anaesthesia* **33**: 935–944, 1978.
3. D'Amour, F. and D. Smith. A method for determining loss of pain sensation. *J. Pharmac. exp. Ther.* **72**: 74–79, 1941.
4. Davis, M. Morphine: Effects on conditioned fear as measured with the potentiated startle paradigm. *Eur. J. Pharmac.* **54**: 341–347, 1979.
5. Evans, W. O. A new technique for the investigation of some analgesic drugs on a reflexive behavior in the rat. *Psychopharmacologia* **2**: 318–325, 1961.
6. Fennessy, M. R. and J. R. Lees. The assessment of the problems involved in the experimental evaluation of narcotic analgesics. *Narcotics Research*. New York: Marcel Dekker, Inc., 1975, pp. 75–99.
7. Hammond, G. R., D. W. McAdam and J. R. Ison. Effects of prestimulation on the electromyographic response associated with acoustic startle reaction in rats. *Physiol. Behav.* **8**: 535–537, 1972.
8. Head, H. *Studies in Neurology*, Vol. 1. London: Oxford University Press, 1920, pp. 189–201.
9. Hoffman, H. S. and J. R. Ison. Reflex modification in the domain of the startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. *Psychol. Rev.* **87**: 175–180, 1980.

10. Ison, J. T. and E. E. Krauter. Reflex-inhibiting stimuli and the refractory period of the acoustic startle reflex in the rat. *J. comp. physiol. Psychol.* **86:** 420–425, 1974.
11. Pinckney, L. A. Inhibition of the startle reflex in the rat by prior tactile stimulation. *Anim. Learn. Behav.* **4:** 467–472, 1976.
12. Russo, J. M., L. A. Reiter and J. R. Ison. Repetitive exposure does not attenuate the sensory impact of the habituated stimulus. *J. comp. physiol. Psychol.* **88:** 665–669, 1975.
13. Vander Wende, C. and S. Margolin. Analgesic tests based upon experimentally induced acute abdominal pain in rats. *Fedn Proc.* **15:** 494, 1956.
14. Weiss, B. and V. G. Laties. The psychophysics of pain and analgesia in animals. In: *Psychophysics: The Design and Conduct of Sensory Experiments*, edited by W. C. Stebbins. New York: Appleton-Century-Crofts, 1970, pp. 185–209.
15. Woolfe, G. and A. MacDonald. The evaluation of the analgesia action of pethidine hydrochloride (Demerol). *J. Pharmac. exp. Ther.* **80:** 300–307, 1944.
16. Young, R. D., B. E. Thorn, R. A. Levitt and M. J. Weyant. Use of the flinch-jump technique to study narcotic analgesia in the rat. *Physiol. Psychol.* **6:** 226–228, 1978.