exposure to dieldrin. Additional mortality may occur away from roosts under conditions that cause rapid fat utilization and, consequently, residue mobilization (*2*). Such conditions include migratory stress (*16*) and initiation of flight by juveniles (*17*). Dieldrin mortality may also be common in other parts of the species' range where row crop agriculture is extensive, but it may be undetected because many maternity roosts are located over water where dead bats probably disappear rapidly.

Populations of the Indiana bat in the MPL area also may have experienced mortality caused by dieldrin. However, because maternity roosts are probably small and located in trees (only two have ever been found) (*18*), the impact of dieldrin may never be known. Populations of the Indiana bat in the MPL area declined an estimated 48 percent between March 1975 and March 1976, but human disturbance of caves was thought responsible (*19*).

Even though aldrin and dieldrin were banned by the U.S. Environmental Protection Agency effective 1 October 1974, the sale and use of existing stocks remains legal. Therefore, we do not know when the use of aldrin will cease, nor do we know how long dieldrin will persist in prey insects once the use of aldrin has stopped. Soil residues of dieldrin showed no significant reduction 6 years after application of aldrin on Missouri cornfields (*20*). We urge that the potential threat to these bats posed by the chemicals now being substituted for aldrin (heptachlor and toxaphene in Missouri) be considered before their use becomes widespread.

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3. Bats were recovered under authority of the Federal Endangered Species Permit PRT-8-31-C.
4. Cave 048, situated in a bluff just above the Bourbeuse River floodplain, was dry; its mouth was less than 100 m from a field, and most of the nearby floodplain was cultivated to row crops. Riparian forest was narrow or absent. The bats foraged up- and downstream over the river adjacent to the cultivated fields. Cave 054 was adjacent to the Meramec River and contained a large spring which emerged from the cave but did not pass beneath the bat roost. Fields of row crops were directly across the river from the cave. On 1 July 1976, bats from cave 054 were seen foraging over the river as far as 13 km downstream near cultivated fields and as far as 17 km upstream near forest [R. K. LaVal, R. L. Clawson, M. L. LaVal, W. Caire, *J. Mammal.* **58**, 592 (1977)]. Cave 036 was a dry cave situated in a bluff above the Meramec River. Nearby was a large pasture but no row crops. Most of the narrow floodplain both up- and downstream from the cave was densely forested, although some row crops were present.
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7. The brain and carcass of each individual were analyzed for *p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, dieldrin, heptachlor epoxide, oxychlordane, *cis*-chlordane, *trans*-nonachlor, endrin, hexachlorobenzene (HCB), toxaphene, mirex, and polychlorinated biphenyls (PCB's). The PCB that was recovered resembled Aroclor 1260 in all cases. Hexane extracts of samples were cleaned by Florisil column chromatography, and the eluate containing the pesticides and PCB's was fractionated by Silicar column chromatography [E. Cromartie, W. L. Reichel, L. N. Locke, A. A. Belisle, T. E. Kaiser, T. G. Lamont, B. M. Mulhern, R. M. Prouty, D. M. Swineford, *Pestic. Monit. J.* **9**, 11 (1975)]. Fractions were analyzed with a gas-liquid chromatograph (Hewlett-Packard 5753) equipped with a ^{63}Ni detector in a column containing OV-17 with 1.95 percent QF-1 at 195°C. The gas flow rate in the column was 60 ml/min; 5 percent methane in argon was used, with a purge rate of 40 ml/min. Average percentage recoveries from spiked tissues ranged from 89 to 104 except for HCB, which was 80. Residue data were not adjusted on the basis of these recoveries. All residues are reported as parts per million by weight of fresh (wet) tissue unless noted otherwise. The lower limit of sensitivity was 0.1 ppm for carcasses and 0.5 ppm for brains and milk. Residues in 10 percent of the samples were confirmed on a gas chromatograph–mass spectrometer (LKB 9000) with operating conditions as described in Cromartie *et al.*
8. The six bats without measurable dieldrin in their carcasses were excluded from this analysis.
9. Brains of bats from cave 048 contained as much as 4.9 ppm DDE, 1.5 ppm heptachlor epoxide, and 1.4 ppm oxychlordane. Brains of bats from cave 054 contained up to 3.0 ppm DDE, 1.8 ppm heptachlor epoxide, 2.7 ppm oxychlordane, 3.9 ppm *trans*-nonachlor, and 13 ppm PCB's. Brains of bats from cave 036 contained up to 2.9 ppm DDE, 4.8 ppm heptachlor epoxide, and 3.3 ppm oxychlordane.
10. This milk also contained 4.1 ppm heptachlor epoxide, 0.58 ppm oxychlordane, 0.90 ppm *cis*-chlordane, and 1.4 ppm *trans*-nonachlor.
11. Means and standard errors for the percentages of carcass as lipid were cave 048, 6.22 ± 0.82; cave 054, 3.02 ± 1.43; and cave 036, 4.34 ± 0.44.
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Dual Actions of Substance P on Nociception: Possible Role of Endogenous Opioids

Abstract. *Substance P produces analgesia when administered to mice in very small doses by the intraventricular route (1.25 to 5 nanograms per mouse). The analgesic effect can be blocked by naloxone. At higher doses (greater than 50 nanograms per mouse), this activity is lost. At these higher doses, however, substance P produced hyperalgesia when combined with naloxone and analgesia when combined with baclofen [β-(4-chlorophenyl)-gamma-aminobutyric acid]. Substance P may have dual actions in brain, releasing endorphins at very low doses and directly exciting neuronal activity in nociceptive pathways at higher doses.*

Substance P (SP), the undecapeptide which was discovered in brain and intestine by Von Euler and Gaddum (*1*) almost five decades ago, has been suggested to be a transmitter utilized by primary sensory afferents (*2–4*). Otsuka and colleagues (*4*) reported that the hexapeptide fragment of SP, pyro-Glu-Phe-Phe-Gly-Leu-Met-NH_2 (SP^{6-11}; < Glu, pyrrolidone carboxylic acid; Phe, phenylalanine; Gly, glycine; Leu, leucine; Met, methionine) is more active in spinal cord than SP itself. Hughes (*5*) originally reported a tentative amino acid content of the endogenous opioid peptides which was very similar to the amino acid composition of SP^{6-11}, and this prompted us to question whether this fragment of SP might be Hughes' peptide or a very closely related substance. Indeed, we observed that SP administration into the lateral ventricles of mouse had an analgesic effect antagonizable by naloxone as has been reported also by Stewart and colleagues (*6*). Our studies with the mouse vas deferens preparation, however, showed that neither SP nor its hexapeptide fragment, SP^{6-11}, had any direct action on opiate receptors. We describe these experiments here and provide evidence that SP has dual effects on pain perception and may interact with enkephalins in controlling nociceptive processes.

Substance P was tested for analgesic activity by the mouse hot-plate procedure (*7*), with the plate maintained at 52°C. Cox standard mice (20 to 23 g) were used in these studies. The test utilized an apparatus with an electrically heated, thermostatically controlled metal plate (Technilab Instruments, model 475 analgesiometer). A Plexiglas cylinder, 12 inches (1 inch = 2.54 cm) high, 4.75 inches in inner diameter, and open

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at the top, confined the mice to a defined area of the hot plate. SP was given intracerebroventricularly (ICV) while naloxone and baclofen [β-(4-chlorophenyl)-γ-aminobutyric acid were given subcutaneously. Hamilton microsyringes bearing 27-gauge needles with stops at 2.5 mm from the needle tip were utilized for intraventricular administration (*8*). The animals were gently restrained, the scalp was incised, and 5 or 10 μl of isotonic saline or drug solution were administered into the lateral ventricle at a rate of 0.5 to 1.0 μl/sec. The success rate of intraventricular hits was approximately 90 percent, as determined by dye injections in a separate group of animals. The time in seconds from contact with the plate until a hind-paw lick occurred was recorded as the response latency. The latency until an escape jump occurred was also recorded. Each mouse was used only once.

SP proved to have slight but statistically significant analgesic activity (increase in latency to hind-paw lick; there was no significant effect on latency to jump) at very low doses administered by the intraventricular route (Fig. 1A). The threshold dose for this response appeared to be of the order of 1 ng per mouse into the lateral ventricle. The effect peaked at a dose of 2.5 ng per mouse and could be antagonized by prior treatment with a dose of naloxone (0.2 mg/kg, subcutaneously), which had no effect alone. The analgesic effect did not increase with higher doses. In fact, doses of 10 ng per mouse and higher (up to 1000 ng per mouse) were without effect, except for a small barely significant increase in response latency after a dose of 50 ng per mouse. Tests with 2.5 ng of SP per mouse were run at 15, 30, 60, and 90 minutes after injection (Fig. 1B).

SP and the short-chain fragments were studied on the mouse vas deferens preparation (*9*, *10*) to characterize their action on opioid receptors. Single vas deferens from mature mice (Cox standard, Harlan Industries, 30 to 40 g) were suspended in 3 ml of modified Krebs solution (*9*) aerated with 95 percent O_2 and 5 percent CO_2 and maintained at 37°C. The field-stimulated twitch (0.15 Hz, 1 msec, 40 V) was recorded on a polygraph by an isometric transducer. Met-enkephalin and a [D-Ala]^2-met-enkephalin (synthesized in the Lilly Laboratories by E. Smithwick and R. Shuman) inhibited the field-stimulated twitch of this preparation while SP and its hexapeptide fragment (SP^{6-11}, Beckman) potentiated the twitch and caused the quiescent tissue to contract (Fig. 2). The stimulant actions of SP and SP^{6-11} on this preparation were not mediated by classical opiate receptors since they could not be blocked by naloxone. SP or SP^{6-11} could restore a twitch reduced by enkephalin but only at a concentration which by itself had a direct stimulant action (Fig. 2). Adding enkephalin to the bath in the presence of SP or its fragment did not alter the size of the subsequent depression by enkephalin. Thus, the apparent ability of SP to reverse a depression induced by enkephalin on this in vitro opiate receptor model system must be considered a physiological rather than a pharmacological antagonism. Similar results were obtained with the octapeptide fragment of SP (SP^{4-11}, Beckman).

We suspected that the bell-shaped dose-response curve for SP in the hot-plate test might be due to dual opposing actions of this peptide in brain. Since low doses produced analgesia that could be antagonized by naloxone and yet SP itself did not bind to opiate receptors, we presumed that the analgesia at low doses was most likely due to a release of the endogenous opioid peptides. We hypoth-

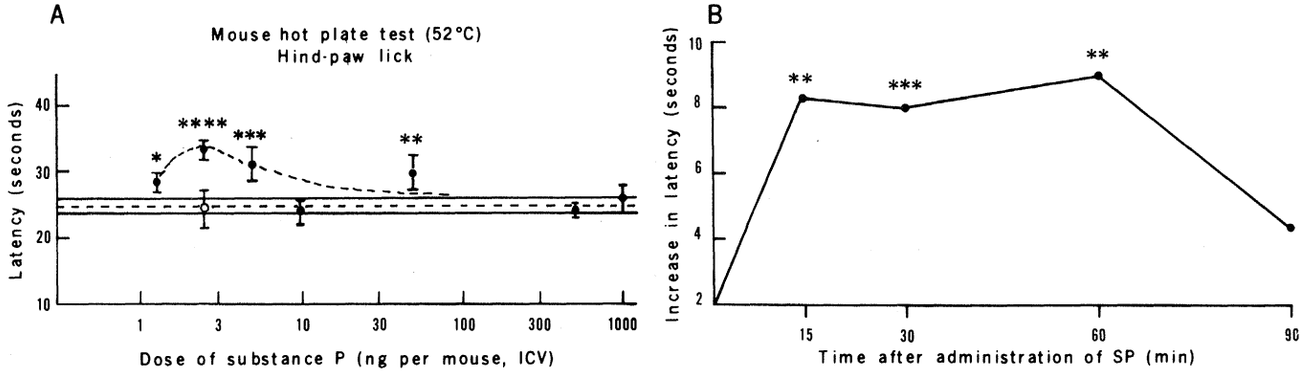

Fig. 1. (A) Dose-related analgesia produced by SP after intraventricular (ICV) administration. The horizontal lines give the mean control (saline-treated mice) latency (± standard error) in seconds. The filled circles give the mean latencies (± standard error) at 30 minutes after treatment with various doses of SP (1 to 1000 ng per mouse). The open circle shows the antagonism of the analgesic effect of SP at 2.5 ng per mouse by naloxone given at 0.2 mg/kg, subcutaneously, 15 minutes before testing. Substance P produced statistically significant analgesia at 1.25, 2.5, 5, and 50 ng per mouse. (B) Time course of the analgesic effect of intraventricular SP (2.5 ng per mouse). The points represent the increase in latency (seconds) over the control level as a function of time after administration. (*, $P < .05$; **, $P < .025$; ***, $P < .01$; ****, $P < .0005$, Student's *t*-test; *N*, ten mice per treatment.

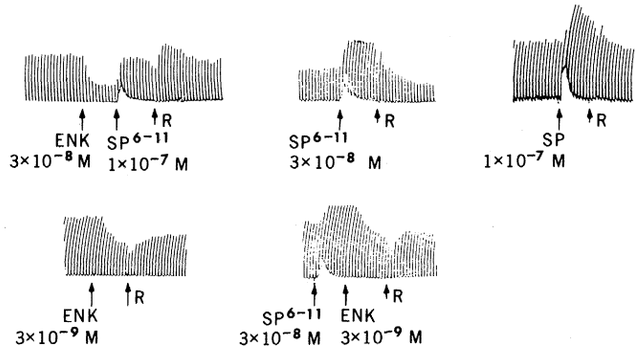

Fig. 2. Physiological antagonism by SP^{6-11} of enkephalin-induced depression of electrically induced twitches of mouse vas deferens. (Upper row) An analog of [Met^5]enkephalin with D-Ala in the 2 position (ENK) at $3 \times 10^{-8}M$ depressed the twitch and SP^{6-11} at $1 \times 10^{-7}M$ reversed this depression. Substance P will do likewise (not shown). However, SP^{6-11} alone ($3 \times 10^{-8}M$) potentiated to the same extent the twitch of a control tissue not exposed to enkephalin and SP did likewise. R, rinse. (Lower row) Lack of pharmacological antagonism of enkephalin-induced depression of vas deferens by prior treatment with SP^{6-11}.

esized that at higher doses this presynaptic effect of SP might be opposed by direct excitatory postsynaptic actions. SP is known to excite, and enkephalins to inhibit, neurons involved in transmission of nociceptive information (*11–13*). If such a dual action were indeed the explanation for the lack of analgesic effect of SP at the higher doses, then it should be possible to reveal each of these actions separately by antagonizing the other. Thus, we treated mice with a combination of either a high dose of SP (500 ng per mouse) plus naloxone to block the analgesic effect of any released enkephalins, or a high dose of SP (500 ng per mouse) plus baclofen in an attempt to block the postsynaptic excitatory actions of SP (*4*).

We observed the predicted separation of activities. After treatment with a dose of naloxone that had no effect alone (0.2 mg/kg, subcutaneously), a high dose of SP (500 ng per mouse, ICV) with no resultant effect alone produced a significant hyperalgesic response (decreased latency to the hot-plate jump response) (Fig. 3A). In the presence of baclofen, the same high dose of SP (500 ng, ICV), which had no effect alone and a hyperalgesic effect when combined with naloxone, produced an analgesic effect (increased latency to both hind-paw lick and jump) (Fig. 3B). Baclofen itself, as has been observed (*14*), produced a slight analgesia partially antagonizable by naloxone, and this effect was potentiated by SP given intraventricularly. This might have been due to the blockade by baclofen of the postsynaptic excitatory activity of SP, leaving the inhibitory activity of the released enkephalins unopposed. There is, however, controversy concerning the ability of baclofen to antagonize SP (*15*); baclofen may act instead on γ-aminobutyric acid (GABA) receptors to produce postsynaptic inhibition. Indeed, such a direct GABA-like inhibitory action opposing the excitatory activity of SP, that is, a physiological antagonism rather than a pharmacological antagonism of SP, could explain its potentiation of SP analgesia.

Stewart and colleagues (*6*) reported an analgesic action of SP, administered either intracerebrally or intraperitoneally to mice. This analgesia could be antagonized by naloxone. Naloxone-reversible analgesia has also been observed after administration of SP into the region of the periaqueductal gray in rat (*16*). Oehme *et al.* (*17*), to the contrary, have reported that SP produces hyperalgesia and Stern and colleagues (*18*) have suggested that SP may be a natural antagonist of morphine in the central nervous system. Our results may explain these apparently conflicting data. Thus, SP has dual actions in the central nervous system and may produce either analgesia or hyperalgesia, depending on the dose and other conditions of the experiment. Substance P might act under certain conditions as a natural antagonist of morphine, but this would be a physiological rather than pharmacological antagonism (*19*).

We recorded the latencies to two responses (hind-paw lick and escape jump) in our use of the hot plate to assess analgesic activity. The two responses seem to reflect somewhat different behavioral phenomena, and only the jump response shows a diurnal rhythm in latency and a susceptibility to the hyperalgesic activity of naloxone (*14, 20*). In the present experiments, intraventricular SP was analgesic (increased latency) on the hind-paw lick measure alone, whereas the combination of SP and naloxone was hyperalgesic (reduced latency) on the jump response alone. The reason for this separation of effects is unclear, but may indicate that there are different neural systems or receptors (or both) mediating the two responses. Furthermore, the long duration of the analgesic effect after very small doses of SP is surprising since SP is reported to have a very short half-life in rodent brain (*21*). This long duration was observed also in the experiments of Stewart *et al.* (*6*). The analgesia may be due to a metabolite of SP.

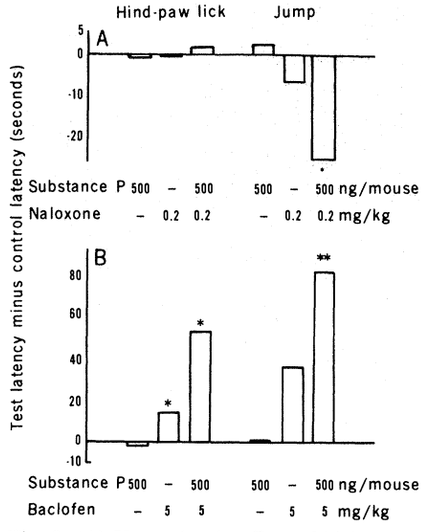

Fig. 3. (A) Hyperalgesic effect of high doses of SP (ICV) plus naloxone (subcutaneously). The dose at 500 ng per mouse did not influence latency to hind-paw lick when given alone or with naloxone but did significantly decrease the latency to jump response when combined with naloxone (0.2 mg/kg, subcutaneously). Neither SP nor naloxone alone at these doses had a significant effect on jump latency. (B) Analgesic effect of high doses of SP plus baclofen. SP alone at 500 ng per mouse did not alter latency to either hind-paw lick or jump. Baclofen alone at 5 mg/kg, subcutaneously, slightly increased response latencies but only the effect on hind-paw lick was statistically significant. The combination of SP plus naloxone produced a much greater increase in the latencies to both responses. The analgesia produced by SP can be totally blocked by naloxone while the effect of higher doses of baclofen alone can be blocked only partially by naloxone.

In summary, SP in very small doses (1.25 to 5 ng per mouse) given by the intraventricular route produced a slight but significant analgesia. This effect was lost at higher doses (> 10 to 50 ng per mouse). In the presence of baclofen, these higher doses produced analgesia, but in the presence of naloxone they produced hyperalgesia. The analgesia produced by low doses of SP or high doses of SP plus baclofen were antagonized by naloxone. We conclude that SP has dual actions in brain, releasing, at very low doses, endogenous opioids and producing, at higher doses, postsynaptic excitatory activity which counteracts the inhibitory activity of the endogenous opioids. These data are compatible with the possibility that SP and the enkephalins play dual opposing roles in regulating neuronal activity in nociceptive pathways. The data also suggest that there may be more than one receptor type for SP such as has been suggested also for the enkephalins.

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Eye Movements of Monkeys During Learning-Set Formation

Abstract. *Eye movements of stump-tailed monkeys were measured during learning of a long series of two-choice pattern discrimination problems. The amount of scanning per trial (shifts in visual fixation from one pattern to the other) and the duration of individual fixations on the patterns increased during the course of learning-set formation and (except for the amount of scanning by some animals) remained high during the prolonged training following learning-set formation. Some of the changes in eye movements were different from those seen during the learning of single discrimination problems, a difference that possibly reflects cognitive processes specific to the learning-set task.*

Primates—both human and nonhuman—depend on vision for most of their information about the environment. This is surely one of the reasons experimental psychologists using animals as subjects have made such extensive use of visual discrimination tasks in studying learning and memory (*1*). One might expect eye movements to play an important role in the discrimination process, but little is known about them in this regard because of the technical problems involved in their measurement. During the past few years, we have been measuring the changes in eye movements of monkeys during discrimination learning, using a computerized method that we developed for the purpose. We now describe such changes during the formation of discrimination learning sets. Learning-set formation is of particular interest because of evidence suggesting that the mechanism involved in this kind of learning (which has been variously referred to as "hypothesis," "strategy," or "concept" development) is qualitatively different from that involved in the learning of individual discrimination problems (*2*), although the question is still debated (*3*).

Four wild-born, stump-tailed monkeys (*Macaca arctoides*), approximately 2.5 to 3.5 years old, were each given a series of 435 two-choice, dot-pattern, discrimination problems. These were generated on an oscilloscope under computer control and were displayed just behind two circular, clear plastic panels, each 2.5 cm in diameter (*4*). The monkeys sat in commercial restraining chairs facing the panels. Each animal was trained with the particular pair of patterns that composed a problem until it made 19 correct responses during 20 consecutive trials (criterion trials). Then a new pair of patterns was presented until the same criterion was met. There were 300 training trials per test day, and the intertrial interval was 5 seconds. A correct choice (pressing the panel behind which was displayed the pattern that had been selected at random as the positive one for the particular problem) was reinforced with a 190-mg, banana-flavored, whole-diet food pellet (P. J. Noyes), and an incorrect choice terminated the trial. The details of the eye-movement recording and related training techniques have been described (*5–7*).

All four animals showed a marked improvement in the rate of learning of the dot-pattern problems over the course of approximately the first 100 problems [F (19,57) = 15.4, $P < .001$] (Fig. 1). Thereafter, there was virtually no improvement; learning-set formation had essentially reached asymptote. Hence, the training period would appear to have been of sufficient length to establish any possible permanent changes in eye movements as a consequence of learning-set formation.

Amount of scanning, the number of shifts in visual fixation from one pattern to the other, was one eye-movement variable measured (*8*). The amount of scanning by all animals increased sharply to a maximum shortly before learning-set formation was complete and then decreased somewhat (Fig. 1) (*9*). The persisting high level of scanning following learning-set formation would seem to be a major exception to the pattern of scanning that we have observed following the learning of single discrimination problems. In such cases, the amount of scanning also increased to a high level during learning, but then decreased to the minimum necessary for the animal to observe the positive stimulus before making the choice response (*7*). For a simple, two-choice problem, this minimum is 0.5 scans per trial. In the present instance, however, the four animals fell into two distinct classes of two animals each in terms of amount of scanning, especially during

Table 1. Mean results on criterion trials of discrimination learning during different stages of the experiment: (i) the first problem when initially presented, (ii) the last five problems of the learning-set series, and (iii) the last 5 days of the repeated presentation of the first problem, at the end of the learning-set series. Data were analyzed with t-tests of the difference between correlated means.

Problem	Scans (No.)	Duration of fixations (msec)	
		Preceding	Last
Number 1	0.65	189	287
Last five	1.04	294*	461*
Number 1 repeated	0.72	243	321*

*Significantly different from the mean in the row above.

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