References and Notes

1. V. G. Bruce, *Cold Spring Harbor Symp. Quant. Biol.* **25**, 29 (1960); J. Aschoff, Ed., *Circadian Clocks* (North-Holland, Amsterdam, 1965); M. Menaker, Ed., *Symposium on Biochronometry* (National Academy of Sciences, Washington, D.C., in press).
2. K. Adler, *Science* **164**, 1290 (1969).
3. H. Underwood and M. Menaker, *ibid.*, **170**, 190 (1970).
4. ———, *ibid.* **167**, 298 (1970).
5. J. W. Truman and L. M. Riddiford, *ibid.*, p. 1624; J. W. Truman, in *Symposium on Biochronometry*, M. Menaker, Ed. (National Academy of Sciences, Washington, D.C., in press); C. S. Pittendrigh and S. Skopik, *Proc. Nat. Acad. Sci. U.S.* **65**, 500 (1970).
6. W. F. Zimmerman and D. Ives in *Symposium on Biochronometry*, M. Menaker, Ed. (National Academy of Sciences, Washington, D.C., in press).
7. The circadian rhythms of congenitally blind and artificially blinded mice do not entrain to light : dark cycles [F. Halberg, M. B. Visscher, J. J. Bittner, *Amer. J. Physiol.* **179**, 229 (1954)]. Although the most likely explanation is that the eyes mediate light effects on the circadian rhythm, it is possible that degeneration in the central nervous system could interfere with the coupling between the circadian rhythm and some extraoptic photoreceptor.
8. J. W. Hastings and B. M. Sweeney, *J. Gen. Physiol.* **43**, 285 (1959); M. L. Sargent and W. Briggs, *Plant Physiol.* **42**, 1504 (1967).
9. C. F. Ehret, *Cold Spring Harbor Symp. Quant. Biol.* **25**, 149 (1960).
10. E. Bünning and G. Jorrenz, *Z. Naturforsch.* **15b**, 205 (1960); V. G. Bruce and D. H. Minis, *Science* **163**, 583 (1969).
11. K. D. Frank and W. F. Zimmerman, *Science* **163**, 688 (1969).
12. T. H. Goldsmith, R. J. Barker, C. F. Cohen, *ibid.* **146**, 65 (1964).
13. T. H. Goldsmith and H. Fernández, in *The Functional Organization of the Compound Eye*, C. G. Bernhard, Ed. (Pergamon, New York, 1966), p. 125.
14. J. H. Sang, *J. Exp. Biol.* **33**, 45 (1956).
15. W. F. Zimmerman, C. S. Pittendrigh, T. Pavlidis, *J. Insect Physiol.* **14**, 669 (1968).
16. D. M. Eichenbaum and T. H. Goldsmith, *J. Exp. Zool.* **169**, 15 (1968).
17. T. H. Goldsmith and H. R. Fernández, *J. Exp. Biol.* **49**, 669 (1968); M. I. Mote and T. H. Goldsmith, *J. Exp. Zool.* **173**, 137 (1970).
18. P. Karrer and E. Jucker, *Carotenoids* (Elsevier, Amsterdam, 1950); L. Zechmeister, *Cis-trans Isomeric Carotenoids, Vitamins A and Arylpolyenes* (Springer-Verlag, Vienna, 1962).
19. As was for *Drosophila*, Goldsmith *et al.* (*12*) found some visual sensitivity in *Musca* (housefly) grown aseptically for one generation on carotenoid-free diets. From the subsequent finding (*13*) that visual sensitivity continued to decrease in succeeding generations of aseptically grown flies, it was concluded that some carotenoids are transmitted through the egg and diluted out in succeeding generations.
20. D. H. Minis and C. S. Pittendrigh, *Science* **159**, 534 (1968).

21. Research supported by NSF grant GB 8303, PHS grant EY 00222, and a NIH special fellowship (W.F.Z.). We thank K. Frank and M. I. Mote for help and critical suggestions.

2 November 1970

Narcotic Tolerance and Dependence: Lack of Relationship with Serotonin Turnover in the Brain

Abstract. *Serotonin turnover was measured in mouse brain by means of the conversion of radioactivity from labeled tryptophan into serotonin. Animals with a high degree of tolerance to and physical dependence on morphine did not differ from control mice.*

Recent studies have suggested that serotonin pathways in the central nervous system might be involved in the mediation of the opiate effect, even though brain serotonin concentrations do not change during the development of tolerance and physical dependence (*1*). Way and his co-workers (*2*) showed that, after treatment with pargyline, there is a significant increase of brain serotonin in morphine-tolerant mice, which they interpreted as an indication of increased serotonin turnover. *p*-Chlorophenylalanine (PCPA), which inhibits brain tryptophan hydroxylase (*3*), was found to antagonize the acute analgesic effect of morphine in rats (*4*) and to inhibit the development of tolerance and physical dependence in mice (*2*).

In the experiments described here tolerance and physical dependence were produced in mice by the implantation and retention for 3.7 days of a specially prepared 75-mg morphine pellet (*5*). Control mice were subjected to the same operative procedure without pellet implantation. Tolerance to opiate-induced running activity was demonstrated by placing ten implanted mice and ten control mice in cumulative counter cages for 1 hour, then injecting them with levorphanol (20 mg/kg). The running activity (*6*), measured for 30 minutes after the injection, was found to be 11.9 ± 2.6 beam crossings per minute in the control mice and 0.05 ± 0.03 beam crossing per minute in the implanted mice. We determined whether mice were physically dependent on morphine by using the narcotic antagonist, naloxone, to precipitate the jumping syndrome described by Maggiolo and Huidobro (*7*). The degree of dependence was quantitated from the number of mice jumping off a platform within 10 minutes of the naloxone injection, as described by Way *et al.* (*8*). Control mice would not jump at any dose of naloxone up to the ED_{50} (median effective dose) for convulsions (150 ± 13 mg/kg). The ED_{50} of naloxone for jumping in the implanted mice at 3.7 days was 0.75 ± 0.25 mg/kg.

We measured serotonin turnover in male Swiss-Webster mice (National Institutes of Health, Bethesda, Md.) (24 to 26 g) by a direct method employ-

Table 1. Conversion of [^{3}H]tryptophan to serotonin in brains of tolerant-dependent mice (3.7 days after implantation with 75-mg morphine pellets) and in control (sham-implanted) mice; L-[^{3}H]tryptophan (1 c/mmole, 500 μc/kg) was injected intravenously at zero time. Four mice were used in each group. Data are the means ± the standard errors of the means; dpm, disintegrations per minute. See text for criteria of tolerance and dependence.

Treatment	Minutes after injection	Tryptophan content of brain (nmole/g)	Brain tryptophan specific radioactivity (dpm/nmole)	Serotonin content of brain (nmole/g)	Brain serotonin specific radioactivity (dpm/nmole)	Conversion index* (nmole g^{-1} min^{-1})
Control	10	22.0 ± 0.8	6322 ± 207	2.80 ± 0.27	3365 ± 102	0.12 ± 0.007
Tolerant-dependent	10	20.1 ± 2.2	7434 ± 613	2.50 ± 0.25	4344 ± 335	0.12 ± 0.005
Control	20	21.1 ± 1.6	2412 ± 247	3.15 ± 0.13	2305 ± 372	0.12 ± 0.05
Tolerant-dependent	20	21.2 ± 1.6	2742 ± 143	3.08 ± 0.27	2827 ± 114	0.14 ± 0.025
Control	40	17.7 ± 3.2	764 ± 109	2.55 ± 0.25	1613 ± 220	0.12 ± 0.03
Tolerant-dependent	40	23.4 ± 1.0	1285 ± 82	3.15 ± 0.26	2526 ± 200	0.13 ± 0.01
Control	80	16.3 ± 1.8	361 ± 28	2.78 ± 0.31	1014 ± 34	0.083 ± 0.013
Tolerant-dependent	80	18.3 ± 1.6	438 ± 41	2.85 ± 0.06	1231 ± 461	0.10 ± 0.01
Control	160	18.6 ± 1.2	286 ± 32	2.58 ± 0.15	937 ± 88	0.046 ± 0.007
Tolerant-dependent	160	21.9 ± 2.2	313 ± 15	2.88 ± 0.27	744 ± 24	0.04 ± 0.003

* From Eq. 1 in text.

ing an intravenous injection of [^{3}H]-tryptophan, as described by Costa and his co-workers (*9*). Table 1 shows a comparison of the specific radioactivity of tryptophan (Trp*) and serotonin (S*) in tolerant-dependent (3.7-day morphine pellet implants) and sham-implanted control mice at various times after the intravenous injection of [^{3}H]tryptophan into the tail vein. The specific radioactivities of both tryptophan and serotonin are consistently somewhat higher in the tolerant-dependent mice than in the control mice. This result implies a more rapid transport of the amino acid from the blood into the brain, but it has no bearing on the question of serotonin turnover. The conversion index, CI (*10*), was calculated from

$$CI = \frac{S^* \times S}{Trp^* \times t} \quad (1)$$

where S is the brain serotonin content and t is the time interval (in minutes) from the injection of labeled tryptophan. No significant difference in the conversion index between tolerant-dependent mice and sham-implanted control mice was found at any time. Since Eq. 1 does not correct for serotonin efflux, the values obtained tend to decrease during the later time periods.

The fractional rate constant (k_S) for the turnover of brain serotonin was calculated (*9*) from a smoothed curve of the data in Table 1 for morphine-implanted mice (2.04 hr^{-1}) and sham-implanted mice (2.28 hr^{-1}) from the specific radioactivities of tryptophan and serotonin between 30 and 50 minutes after the injection of the labeled amino acid.

$$k_S = \frac{(S^*_{t_2} - S_{t_1})/(t_2 - t_1)}{[Trp^* - S^*)_{t_1} + (Trp^* - S^*)_{t_2}]/2}$$

where t_1 and t_2 equal 30 and 50 minutes, respectively, after the injection of the labeled amino acid. The rates of serotonin turnover calculated from the k_S for morphine-implanted and sham-implanted mice were 6.18 and 6.48 nmole g^{-1} hr^{-1}, respectively.

These studies demonstrate by direct measurements of serotonin turnover that the rate of serotonin synthesis and degradation remains unchanged in mice that have a high degree of tolerance to and physical dependence on morphine. However, Way and his co-workers (*2*) reported that, after treatment with the monoamine oxidase inhibitor pargyline, mice with morphine tolerance and physical dependence showed a much greater increase in serotonin accumulation than mice receiving only pargyline. The fact that there is a discrepancy in the results obtained by the two methods suggests that under some conditions the indirect pargyline method does not serve as a valid measure of serotonin turnover.

Findings by Way and his co-workers (*2*) suggested that PCPA inhibited the development of tolerance and physical dependence in mice. In extensive additional studies involving PCPA, details of which are being published elsewhere (*11*), we have been unable to confirm these findings. In our experience PCPA, in doses sufficient to reduce brain serotonin to one-third of its normal value, has no effect whatsoever upon the development of tolerance or physical dependence produced by opiate narcotics in mice.

D. L. CHENEY, A. GOLDSTEIN
Department of Pharmacology, Stanford University, Stanford, California 94305
S. ALGERI, E. COSTA
Laboratory of Preclinical Pharmacology, National Institute of Mental Health, St. Elizabeths Hospital, Washington, D.C. 20032

References and Notes

1. E. W. Maynert and G. I. Klingman, *J. Pharmacol. Exp. Ther.* **135**, 285 (1962); J. W. Sloan, J. Brooks, A. Eisenman, W. R. Martin, *Psychopharmacologia* **4**, 26 (1964); L. M. Gunne, *Acta Physiol. Scand.* **58**, 204 (1963).
2. E. L. Way, H. H. Loh, F. Shen, *Science* **162**, 1290 (1968); F. Shen, H. H. Loh, E. L. Way, *J. Pharmacol. Exp. Ther.*, in press.
3. E. Jequier, W. Lovenberg, A. Sjoerdsma, *Mol. Pharmacol.* **3**, 274 (1967).
4. S. S. Tenen, *Psychopharmacologia* **12**, 278 (1968).
5. The morphine pellets were formulated by Dr. R. D. Gibson as described by Way and his co-workers (*2*). Except as otherwise noted, all injections were given intraperitoneally in physiological saline solution. Stated doses of all drugs refer to the free bases. Morphine sulfate was purchased from Mallinckrodt Chemical, and naloxone hydrochloride was a gift from Endo Laboratories; levorphanol tartrate was generously furnished by Hoffmann La-Roche Inc.; L-[^{3}H]tryptophan was obtained from New England Nuclear (specific activity, 1 c/mmole).
6. A. Goldstein and P. Sheehan, *J. Pharmacol. Exp. Ther.* **169**, 175 (1969).
7. C. Maggiolo and F. Huidobro, *Acta Physiol. Latinoamer.* **11**, 70 (1961).
8. E. L. Way, H. H. Loh, F. Shen, *J. Pharmacol. Exp. Ther.* **167**, 1 (1969).
9. N. H. Neff, P. F. Spano, A. Groppetti, C. T. Wang, E. Costa, *ibid.* **176**, 701 (1971); E. Costa, P. F. Spano, A. Groppetti, S. Algeri, N. H. Neff, *Atti Accad. Med. Lombarda* **23**, 1 (1969).
10. E. C. Azmitia, Jr., S. Algeri, E. Costa, *Science* **169**, 201 (1970); G. C. Sedvall, V. K. Weise, I. J. Kopin, *J. Pharmacol. Exp. Ther.* **159**, 274 (1968).
11. D. L. Cheney and A. Goldstein, *J. Pharmacol. Exp. Ther.*, in press.
12. Work supported by research grant MH13963 from the National Institute of Mental Health and training grant GM322 from the National Institute of General Medical Sciences.

12 October 1970; revised 30 November 1970

Oscillator Neurons in Crustacean Ganglia

Abstract. *The motor rhythm of ventilation in hermit crabs and lobsters appears to be controlled by a pair of neurons, one in each half of the subesophageal ganglion. Their membrane potentials oscillate and upon depolarization and hyperpolarization elicit spiking in two pools of motor neurons on each side, without spikes in the oscillator neurons themselves. The fact that higher order (command) interneurons can control the rate of the oscillator by means of a smoothly graded input lends support to the idea that oscillator neurons respond periodically to a constant ionic stimulus.*

A local, rhythmically active system in the subesophageal ganglion of decapod crustacea innervates the muscles of the coxal and basal segments of the scaphognathite by means of 11 motor neurons divided into levator and depressor pools and distributed between two branches of the second maxilla root. Alternating bursts of motor action potentials produce an up-and-down sculling motion that drives a stream of water through the gill chambers (*1*). The motor axon discharge pattern from the two branches on one side of a totally isolated ganglion of the hermit crab, *Pagurus pollicarus,* is shown in Fig. 1A. Such rhythmic, well-organized activity may persist in vitro for up to 48 hours when ganglia are superfused with cold saline containing hemoglobin (*2*). Although none of the visible cell bodies penetrated on the surface of the desheathed ganglion display electrical activity, it is possible, with fine-tipped (80 to 150 megohm) microelectrodes, to record within the neuropil from motor neurons and interneurons of the ventilation control system. The membrane potentials of the cells may be altered by current passed through either the recording electrode (by means of a bridge circuit) or the second barrel of a double-barreled electrode.

Oscillator interneurons are very hard