NEUROBIOLOGY OF LEARNING AND MEMORY **67,** 259–263 (1997)
ARTICLE NO. NL963764

BRIEF REPORT

Reversal of Relative Thresholds for Synaptic Facilitation and Increased Excitability Induced by Serotonin and Tail Nerve Stimulation in *Aplysia* Sensory Neurons

Silvia A. Bunge, Juliane Mauelshagen, and Thomas J. Carew[1]

Departments of Biology and Psychology, Yale University, New Haven, Connecticut 06520

Tail shock induces reflex sensitization in *Aplysia* and, in parallel, induces a number of modulatory effects in central neurons, such as increased excitability in tail sensory neurons (SNs) and facilitation of synaptic transmission from SNs to motor neurons. Both of these modulatory effects are mimicked by exogenous application of serotonin (5HT) or electrical stimulation of the tail nerve P9. In the present study we examined the activation thresholds for increased excitability and synaptic facilitation induced by either 5HT or P9 stimulation. We found that the concentration of 5HT sufficient to produce a significant increase in excitability produced no significant synaptic facilitation and, conversely, that the intensity of nerve stimulation sufficient to produce significant synaptic facilitation produced no excitability changes. This reversal of relative thresholds for these modulatory effects may reflect the differential access of exogenous 5HT and endogenous 5HT (released by tail nerve stimulation) to the SN cell body and synaptic terminals, respectively.

A single tail shock induces short-term behavioral sensitization of the siphon and tail withdrawal reflexes in *Aplysia* (Walters, Byrne, Carew, & Kandel, 1983a,b; Marcus, Nolen, Rankin, & Carew, 1988) and, in parallel, induces several modulatory effects in tail sensory neurons (SNs): increased excitability, increased input resistance, spike broadening, and facilitation of synaptic transmission to the tail motor neurons (MNs); (Walters et al., 1983a,b). These modulatory effects are mimicked by stimulation of the tail nerve P9 (Klein, Hochner, & Kandel, 1986; Mercer, Emptage, & Carew, 1991) or by exogenous application of serotonin (5HT) (Brunelli, Castellucci, & Kandel, 1976; Walters et al., 1983a,b; Mercer et al., 1991). We have recently shown that the different modulatory effects induced by 5HT in the SNs have different activation thresholds (Stark, Mercer, Emptage, & Carew, 1996). To extend this general observation, in the present study we examined the activation thresholds for increased excitability and synaptic facilitation, induced either by 5HT or by P9 stimulation.

Standard procedures for isolated ganglion preparations of adult *Aplysia californica* were used for intracellular recording from monosynaptically connected tail SNs and MNs located in the pleural and pedal ganglia, respectively (e.g., Walters et al., 1983a; Emptage, Mauelshagen, & Carew, 1996). For nerve stimulation experiments, P9 was wrapped around silver chloride post electrodes connected to an external stimulator (Grass S88). The MN was hyperpolarized to −80 mV to prevent it from spiking.

For 5HT experiments, SN activation was achieved in two ways: (1) with a short (0.3 ms) depolarization that elicited one action potential in order to evoke a single EPSP in the MN and (2) with a long (200 ms) depolarization to assess excitability in the SN. After three baseline measures of EPSP amplitude (interstimulus interval (ISI) = 15 min), two 5-min pulses

[1] We thank Laura Stark for helpful comments on the manuscript. This work was supported by a DFG grant to J.M. and NSF Grant BNS831130 and NIH Grant R01-14-1083 to T.J.C. We also acknowledge here the loss of a coauthor of this paper, Juliane Mauleshagen, a valued friend and colleague, who died in a climbing accident on October 4, 1996.

Address reprint requests and correspondence to Thomas J. Carew, Department of Psychology, Yale University, P.O. Box 208205, New Haven, CT 06520-8205. Fax: (203) 432 7172.

1074-7427/97 $25.00

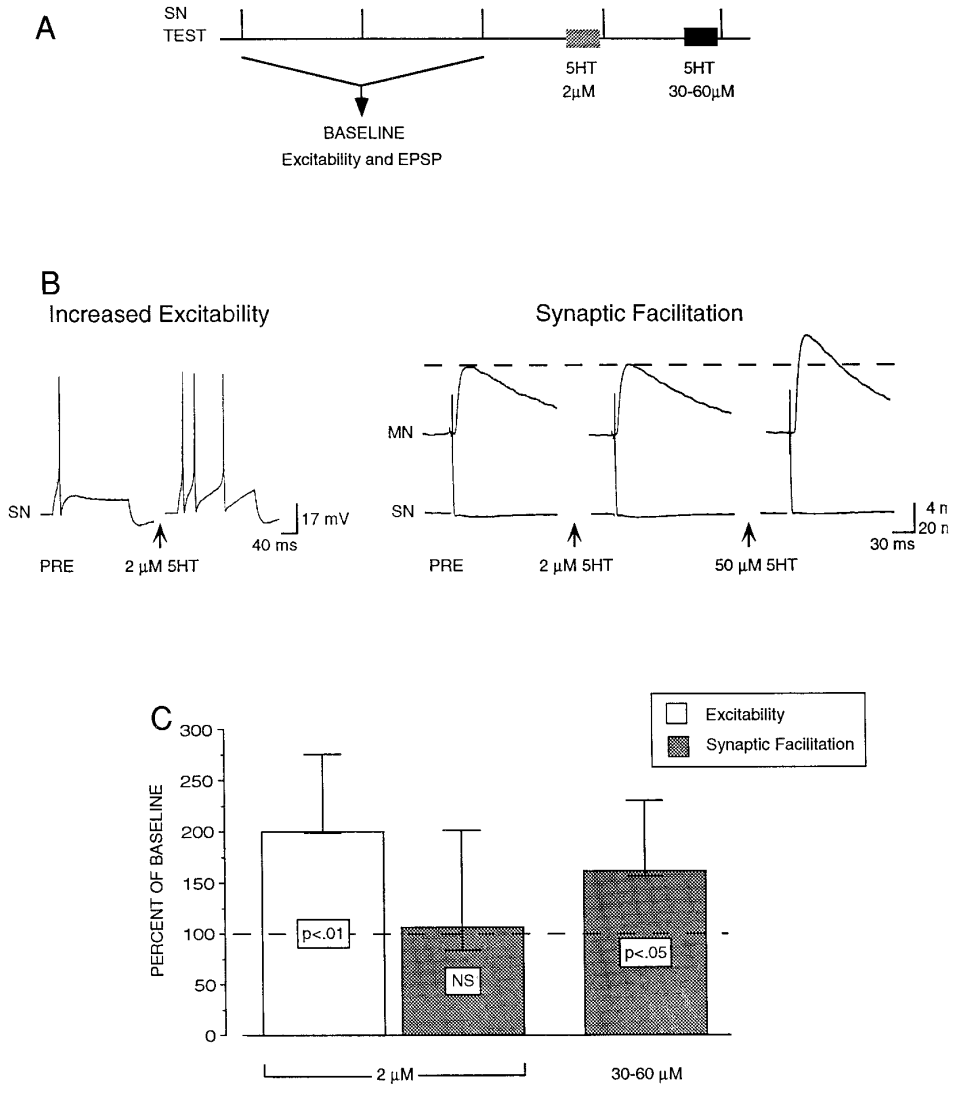

FIG. 1. Threshold concentration of 5HT induces excitability increases but no synaptic facilitation. (A) Outline of experimental procedure. (B) Representative intracellular traces from a SN and MN. The 2 μM 5HT induced increased excitability but not facilitation of the EPSP. The 50 μM subsequently induced facilitation at the same synapse. (C) Summary data from seven experiments showing that increased excitability is induced at a lower concentration of 5HT than is synaptic facilitation. Data are expressed as median percent of baseline ± interquartile ranges.

of 5HT were administered in 15-min intervals (the first with 2 μM, the second with 30–60 μM; Fig. 1A). One minute after the end of each 5HT pulse, facilitation of the EPSP and increased excitability (as measured by an increase in the number of spikes elicited in the SN) were assessed. In each test, facilitation of the EPSP was evaluated prior to SN excitability to avoid any interaction with possible homosynaptic effects (e.g., depression or PTP) resulting from activation of the SN during assessment of excitability.

For tail nerve stimulation experiments, after three baseline measures of EPSP amplitude (ISI = 15 min; Fig. 2A), a 200-ms depolarizing pulse adjusted to elicit one action potential was delivered to the SN to determine baseline excitability. To achieve functionally comparable intensities of nerve stimulation between different preparations, we established a nerve stimulus threshold (NST), defined as the minimum voltage (5-ms pulse delivered to P9) required to cause an increase in the frequency and/or amplitude of EPSPs in the MN. Across all experiments, the NST ranged from 7 to 45 V. P9 was subsequently stimulated with a 3-s train of 5-ms pulses at 10 Hz, at voltages corresponding to fixed increments of the NST (1.2× to

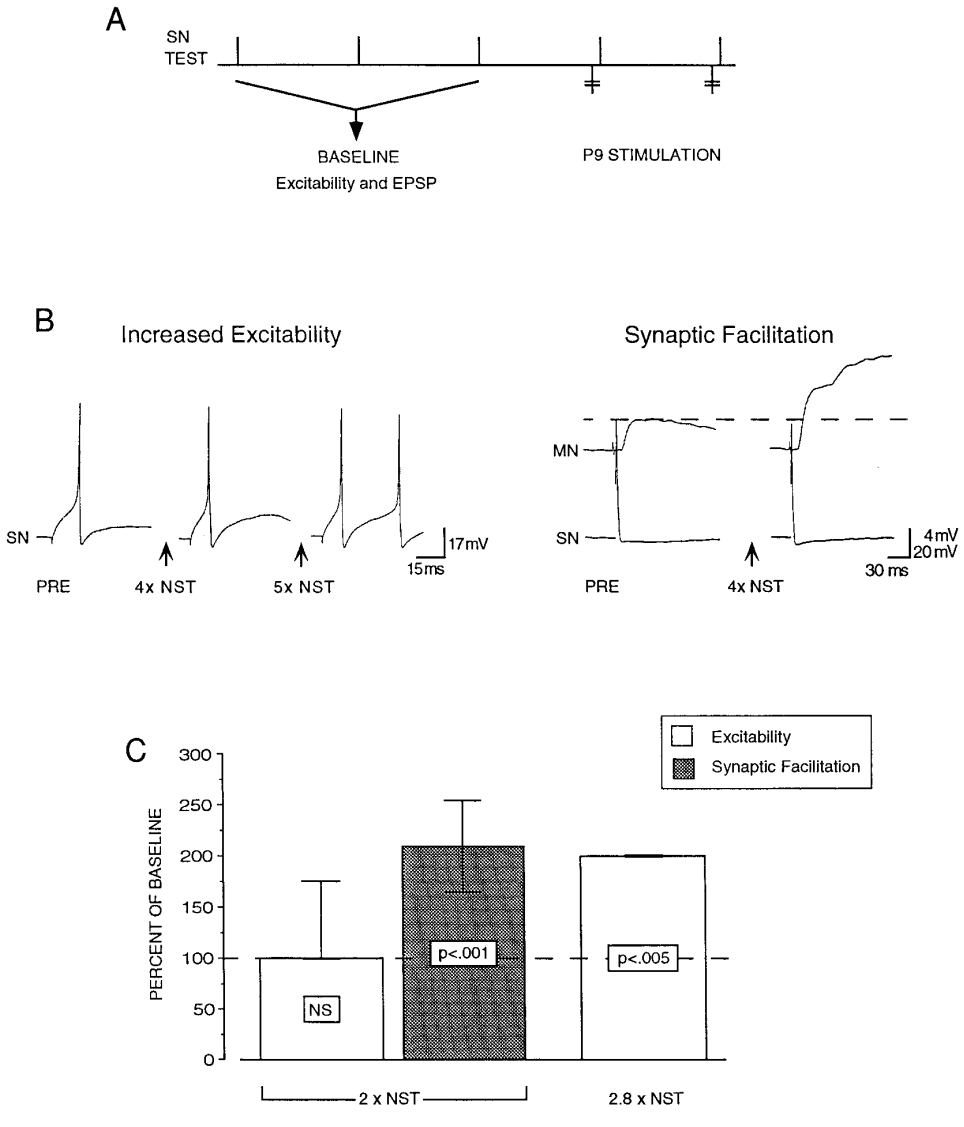

FIG. 2. Threshold tail nerve stimulation induces synaptic facilitation but no increase in excitability. (A) Outline of experimental procedure. (B) Representative intracellular traces from a SN and a MN. Synaptic facilitation was induced by a stimulus 4× NST, but at this same intensity, no excitability changes were induced. Excitability changes were subsequently induced with a stimulus 5× NST. (C) Summary data from 15 experiments showing that, unlike with 5HT application (see Fig. 1C), synaptic facilitation was observed at lower stimulus intensities (mean = 2× NST) than increased excitability (mean = 2.8× NST). Data expressed as in Fig. 1C.

5× NST). 1 min after each P9 stimulation, synaptic facilitation and increased excitability were assessed as in the 5HT experiments. In both 5HT and nerve stimulation experiments, if both facilitation and increased excitability were observed at the lowest 5HT concentration or tail nerve stimulus intensity, the experiment was not continued. Statistical comparisons were made by means of a Wilcoxon test.

Typical results from a 5HT experiment are shown in Fig. 1B. A concentration of 2 μM 5HT was sufficient to produce increased excitability in the SN, but not facilitation of the EPSP. Subsequently a higher concentration of 5HT (50 μM) produced both facilitation and even more excitability (excitability data not shown). The results are summarized in Fig. 1C. The 2 μM 5HT produced a significant increase in excitability (from one to two spikes, $T(7) = 0$; $p < .02$) but no significant synaptic facilitation ($T(7) = 9$; N.S.). Significant synaptic facilitation was subsequently observed with (empirically determined) higher concentrations of 5HT (30–60 μM; $T(5) = 0$; $p < .05$).

Typical results from a P9 experiment are shown

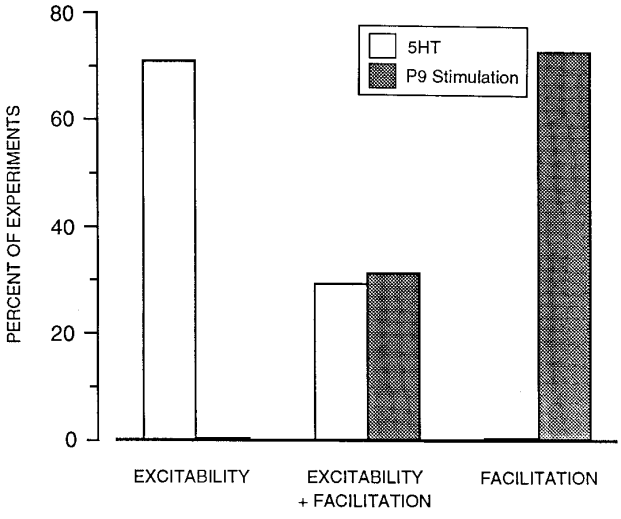

FIG. 3. Relative distribution of increased excitability and synaptic facilitation in experiments employing 5HT or P9 stimulation. Histograms provide a comparison of the relative distribution of experiments in which (1) excitability alone, (2) excitability and synaptic facilitation, or (3) synaptic facilitation alone was observed at the lowest concentration of 5HT or the lowest nerve stimulus intensity. These results demonstrate the reversal of relative thresholds for synaptic facilitation and increased excitability under these two different modes of inducing comparable modulatory effects in the SNs.

in Fig. 2B. A stimulus intensity of 4× NST produced facilitation of the EPSP but no excitability changes in the SN. Subsequently, a higher intensity stimulation (5× NST) produced both increased excitability and facilitation of the EPSP (facilitation data not shown). The results are summarized in Fig. 2C. A mean stimulus intensity of 2× NST ± 0.21 induced significant synaptic facilitation ($T(15) = 0$; $p < .001$), but no significant increase in excitability. At a higher nerve stimulus intensity (mean = 2.8× NST ± 0.35), significant increased excitability was observed ($T(11) = 0$; $p < .005$).

As shown in Fig. 3, a comparison of the proportion of 5HT and nerve stimulation experiments in which increased excitability and/or synaptic facilitation (each defined as any increase over baseline) was observed at the lowest 5HT concentration or the lowest nerve stimulus intensity reveals opposite trends: excitability in the absence of synaptic facilitation was observed in 5 of 7 5HT experiments; this was never seen in the P9 experiments. Conversely, synaptic facilitation in the absence of excitability was observed in 11 of 15 P9 experiments, but was never observed in the 5HT experiments. In the remaining experiments, the lowest 5HT concentration or lowest nerve shock intensity produced both increased excitability and synaptic facilitation. Importantly, significant facilitation was induced with a higher 5HT concentration (Fig. 1C), and significant excitability was induced with increased nerve stimulation (Fig. 2C), indicating that both forms of modulation could be induced in either experimental condition.

The 5HT experiments confirm earlier results showing that increased excitability of the SNs is induced at a lower concentration than is synaptic facilitation (Emptage et al., 1996; Stark et al., 1996). However, the P9 experiments show the unexpected opposite result: synaptic facilitation is induced at lower stimulus intensities than is increased excitability. One explanation for the reversal of thresholds with P9 stimulation compared to 5HT may be differential access of exogenous and endogenous 5HT to the SN somata and synaptic terminals. While increased excitability is most likely a somatic process, synaptic facilitation requires that 5HT act directly at the SN–MN synapse (see, e.g., Emptage & Carew, 1993). From this perspective, exogenous 5HT would increase excitability via 5HT receptors on the SN soma (to which bath-applied 5HT should have immediate access), but to induce synaptic facilitation, 5HT would have to diffuse into the neuropil, perhaps giving rise to a modest concentration gradient. In contrast, endogenous 5HT released by P9 stimulation (see Mercer et al., 1991) may be more effectively released onto SN nerve terminals than onto somata. In considering this explanation of our overall results, we should emphasize that we cannot exclude additional possibilities, such as procedural differences between the two conditions (e.g., whereas the duration of 5HT bath exposure can be well controlled, the actual duration of the presumed 5HT exposure induced by nerve stimulation cannot be) or mechanistic differences between the modulatory effects induced by exogenously applied 5HT and tail nerve stimulation, including the possible involvement of other neuromodulators known to affect SNs, such as small cardioactive peptide (Abrams et al., 1984) and FMRF-amide (Belardetti et al., 1987), which may also be released by tail nerve stimulation.

In conclusion, our results reveal interesting differences between modulatory effects induced in SNs by 5HT application compared to tail nerve stimulation. Many different preparations, ranging from semi-intact animals to isolated SNs and MNs in cell culture, are used to examine cellular and molecular mechanisms of sensitization in *Aplysia.* The results of the present paper highlight the importance, at each level of analysis, of attempting to determine the relation-

ship between cellular observations and their potential behavioral significance.

REFERENCES

Abrams, T. W., Castellucci, V. F., Camardo, J. S., Kandel, E. R., & Lloyd, P. E. (1984). Two endogenous neuropeptides modulate the gill and siphon withdrawal reflex in *Aplysia* by presynaptic faciulitation involving cAMP-dependent closure of a serotonin-sensitive potassium channel. *Proceedings of the National Academy of Science USA,* **81,** 7956–7960.

Bellardetti, F., Kandel, E. R., & Siegelbaum, S. A. (1987) Neuronal inhibition by the peptide FMRFamide involves opening of S K+ channels. *Nature,* **325,** 153–156.

Brunelli, M., Castellucci, V., & Kandel, E. R. (1976). Synaptic facilitation and behavioral sensitization in *Aplysia*. Possible role of serotonin and cyclic AMP. *Science,* **194,** 1178–1180.

Emptage, N. J., & Carew, T. J. (1993). Long-term synaptic facilitation in the absence of short-term facilitation in *Aplysia* neurons. *Science,* **262,** 253–256.

Emptage, N. J., Mauelshagen, J., & Carew, T. J. (1996). Threshold serotonin concentration required to produce synaptic facilitation differs for depressed and nondepressed synapses in *Aplysia* sensory neurons. *Journal of Neurophysiology,* **75,** 843–854.

Klein, M., Hochner, B., & Kandel, E. R. (1986). Facilitatory transmitters and cAMP can modulate accommodation as well as transmitter release in *Aplysia* Sensory Neurons: Evidence for parallel processing in a single cell. *Journal of Neurobiology,* **83,** 7994–7998.

Marcus, E. A., Nolen, T. G., Rankin, C. H., & Carew, T. J. (1988). Behavioral dissociation of dishabituation, sensitization and inhibition in *Aplysia. Science,* **241,** 210–213.

Mercer, A. R., Emptage, N. J., & Carew, T. J. (1991). Pharmacological dissociation of modulatory effects of serotonin in *Aplysia. Science,* **254,** 1811–1813.

Stark, L. L., Mercer, A. R., Emptage, N. J., & Carew, T. J. (1996). Pharmacological and kinetic characterization of two functional classes of serotonergic modulation in *Aplysia* sensory neurons. *Journal of Neurophysiology,* **75,** 855–866.

Walters, E. T., Byrne, J. H., Carew, T. J., & Kandel, E. R. (1983a). Mechanoafferent neurons innervating tail of *Aplysia.* I. Response properties and synaptic connections. *Journal of Neurophysiology,* **50,** 1522–1542.

Walters, E. T., Byrne, J. H., Carew, T. J., & Kandel, E. R. (1983b). Mechanoafferent neurons innervating tail of *Aplysia.* II. Modulation by sensitizing stimuli. *Journal of Neurophysiology,* **50,** 1543–1559.