

## Neural Circuit of Tail-Elicited Siphon Withdrawal in *Aplysia*. II. Role of Gated Inhibition in Differential Lateralization of Sensitization and Dishabituation

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Submitted 10 July 2003; accepted in final form 14 September 2003

**Bristol, Adam S., Stéphanie Marinesco, and Thomas J. Carew.** Neural circuit of tail-elicited siphon withdrawal in *Aplysia*. II. Role of gated inhibition in differential lateralization of sensitization and dishabituation. *J Neurophysiol* 91: 678–692, 2004. First published September 17, 2003; 10.1152/jn.00667.2003. In the preceding report, we observed that tail-shock-induced sensitization of tail-elicited siphon withdrawal reflex (TSW) of *Aplysia* was expressed ipsilaterally but that dishabituation induced by an identical tail shock was expressed bilaterally. Here we examined the mechanisms of this differential lateralization. We first isolated the modulatory pathway responsible for the induction of contralateral dishabituation by making selective nerve cuts. We found that an intact pleural-abdominal connective, the descending pathway connecting the ring ganglia with the abdominal ganglion, ipsilateral to the shock was required for contralateral dishabituation. We examined whether network inhibition suppresses the contralateral effects of tail shock in nonhabituated preparations. We found that blockade of inhibitory transmission in the CNS by the nicotinic ACh inhibitor d-tubocurarine (d-TC) rendered tail shock capable of inducing bilateral sensitization. We next asked whether serotonin (5-HT), a neuromodulator released in the CNS in response to tail shock, was affected by d-TC. We found that d-TC does not alter 5-HT processes in the ring ganglia: it had no effect on the lateralized pattern of tail nerve shock-induced changes in tail sensory neuron excitability, a 5-HT-dependent process, and it did not alter tail nerve shock-evoked release of 5-HT. By contrast, d-TC enhanced 5-HT release in the abdominal ganglion. Consistent with this observation, restricting d-TC to the abdominal ganglion rendered tail nerve shock capable of producing bilateral sensitization. Together with the results of the preceding paper, our results suggest a model in which TSW sensitization and dishabituation can be dissociated both anatomically and mechanistically.

### INTRODUCTION

The study of the neural mechanisms underlying learning and memory has been a major focus in neuroscience for more than a century. The use of cellular models of learning, such as hippocampal long-term potentiation (LTP) (e.g., Cain 1997; Malinow and Mainen 1996), synaptic facilitation in *Aplysia* (e.g., Byrne et al. 1991; Kandel 2001), and learning-induced neuronal changes in *Hermisenda* (e.g., Fryszak and Crow 1997; Gandhi and Matzel 2000), has contributed greatly to our current understanding of the molecular mechanisms of learning and memory. However, in most cases, it is unlikely that learning results from a single mechanism expressed at a single locus

with the CNS (Frost et al. 1988). Thus to understand how multiple mechanisms expressed at several loci can contribute to behavioral change, it is important to first identify where in a neural circuit plasticity occurs, then examine how these forms of plasticity are coordinated to give rise to learning.

The defensive reflexes of the marine mollusk *Aplysia* have proven useful for a cellular as well as a circuit-level analysis of learning. Both the siphon-elicited siphon withdrawal reflex (SSW) and the tail-elicited siphon withdrawal reflex (TSW) show multiple forms of learning (Carew et al. 1971, 1972; Hawkins et al. 1998; Pinsker et al. 1970, 1973; Stopfer et al. 1996; Sutton et al. 2002). Although a great deal is known about learning-related neural plasticity (e.g., Byrne and Kandel 1996; Glanzman 1995; Kandel et al. 1983; Lechner and Byrne 1998) and circuit dynamics (Cleary et al. 1995; Frost and Kandel 1995; Frost et al. 1988) underlying SSW, less is known about the cellular and molecular mechanisms of TSW learning. Understanding the TSW is important, for the vast majority of the molecular studies of learning in *Aplysia* have examined the sensory neurons (SNs) in the pleural ganglia (the ventrocaudal [VC] cluster, which contain the tail SNs), in either a culture system (e.g., Bartsch et al. 1995; Martin et al. 1997) or the intact CNS (e.g., Pollock et al. 1985; Purcell and Carew 2001; Sherff and Carew 1999; Sutton and Carew 2000; for reviews, see Byrne et al. 1991, 1993).

Plasticity of the tail SNs and their connections with tail motor neurons (MNs) has been studied extensively (Cleary et al. 1998; Mauelshagen et al. 1996, 1998; Scholz and Byrne 1987) and has recently been used to generate predictions regarding the roles of particular molecular cascades in various temporal stages of memory for sensitization in the TSW (Purcell et al. 2003; Sutton and Carew 1999). Although the tail SN-MN synapse has proven to be of considerable heuristic value in predicting mechanisms of TSW learning, it is not a direct component of the TSW neural circuit. That is, tail SNs do not make monosynaptic connections with siphon MNs, and the interneuronal linkages that translate tail sensory input (via the P9 nerves to the pedal ganglia) into siphon motor output (via siphon MNs in the abdominal ganglion) have not been identified.

This study is part of a larger program aimed at understanding the functional anatomy of the neural circuit underlying TSW. In the preceding paper (Bristol et al. 2004), we showed that the

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TSW circuit is lateralized: sensory processes are restricted to the ipsilateral half of the tail and sensory input follows an ipsilateral path through the ring ganglia to the siphon MNs in the abdominal ganglion. We also showed that although the TSW circuit is lateralized, not all forms of TSW learning are expressed ipsilaterally (Bristol et al. 2004). Specifically, habituation and tail-shock-induced sensitization are side-specific, but dishabituation (induced with an identical tail shock as used for sensitization) is expressed bilaterally. This latter result suggested the hypothesis that in untrained animals, tail shock induces ipsilateral sensitization that is functionally suppressed contralaterally but that prior habituation training somehow gates the contralateral inhibitory effects of a lateralized noxious stimulus, giving rise to contralateral dishabituation.

In the present study, we investigated the mechanisms underlying contralateral dishabituation. We first attempted to isolate the modulatory pathway responsible for the contralateral effects of shock by severing various crossing pathways in the *Aplysia* CNS. We found that an intact pleural-abdominal connective ipsilateral to the shocked side was necessary for contralateral dishabituation. We next asked whether network inhibition suppresses contralateral effects of shock blocking inhibitory synaptic transmission using the nicotinic acetylcholine receptor antagonist d-tubocurarine (d-TC) (Tauc and Gerschfeld 1962). Consistent with our hypothesis, disinhibition induced by d-TC enhanced baseline TSW and gave rise to bilateral tail-shock-induced sensitization of TSW. Several lines of evidence suggest that this disinhibitory effect is localized to the abdominal ganglion. First, d-TC exposed to the ring ganglia alone did not enhance baseline TSW nor did it have any effect on the expression of lateralized sensitization. In addition, d-TC exposed only to the ring ganglia had no effect on the release of a known facilitatory modulator, serotonin (5-HT), and it failed to reverse the normal lateralized effects of nerve shock in enhancing SN excitability. In contrast, d-TC exposed to only the abdominal ganglion both enhanced baseline TSW and resulted in bilateral TSW sensitization. d-TC also enhanced the release of 5-HT within the abdominal ganglion. Collectively, these data suggest a model explaining tail-shock-induced contralateral dishabituation by a mechanism in which habituation training reduces inhibitory processing in the SW network in the abdominal ganglia. A mechanism such as this could be of value for any form of lateralized plasticity that appears to result from lateralized neuronal processing.

Some of these data have been presented previously in abstract form (Bristol and Carew 2002).

## METHODS

### Subjects

Adult *Aplysia californica* (100–300 g) were acquired commercially (Marinus, Long Beach, CA; Marine Specimens Unlimited, Long Beach, CA) and housed individually in a 600-l aquarium with continuously circulating artificial sea water (ASW; Instant Ocean, Aquarium Systems, Mentor, OH) at  $\sim 15^{\circ}\text{C}$ . Animals were fed dried seaweed three times a week.

### Electrophysiology experiments

**EXPERIMENTAL PREPARATIONS.** Electrophysiology experiments utilized semi-intact preparations consisting an intact CNS connected to the tail. All animals were anesthetized with injections of isotonic

$\text{MgCl}_2$  into the body cavity. All peripheral nerves were cut except the P9 (tail) nerves. The CNS (abdominal ganglion and ring ganglia) and the tail were then excised from the animal and transferred to a recording dish coated with silicone elastomer (Sylgard; Dow-Corning, Midland, MI). The abdominal ganglion was briefly fixed (30-s immersion in 0.04% glutaraldehyde) to facilitate desheathing and to prevent contractions of the connective tissue in response to nerve stimulation. The left abdominal hemiganglion was desheathed in a 1:1 mixture of artificial seawater [ASW, containing (in mM) 460 NaCl, 55  $\text{MgCl}_2$ , 11  $\text{CaCl}_2$ , 10 KCl, and 10 Tris, pH 7.6] and isotonic  $\text{MgCl}_2$  to prevent synaptic transmission. The recording dish was fitted with two separate chambers: one contained the CNS, the other contained the tail. The P9 nerves were passed between the chambers via a small open slits in the separating barrier. The slits were then sealed using petroleum jelly to improve electrical isolation and to allow for independent manipulation of bath levels. In most experiments, the tail was cut in half (rostral to caudal) to ensure lateralized input. The tail halves were pinned dorsal side up and cannulated for continuous perfusion of ASW 30 min prior to and throughout the experiment.

In experiments examining tail SN excitability, isolated ring ganglia were excised from anesthetized animals, briefly fixed, and pinned to a silicone-elastomer-coated recording dish. One of the two pleural ganglia was desheathed to reveal the VC cluster of tail sensory neurons (Walters et al. 1983b). Throughout all experiments, ganglia were continuously perfused with ASW at room temperature ( $20^{\circ}\text{C}$ ) at  $\sim 6$  ml/min and illuminated from below through a dark field condenser.

**INTRACELLULAR RECORDINGS.** Ganglia were visualized with a Zeiss dissecting microscope fitted with a recording stage. Standard intracellular recording techniques were used. Neurons were impaled with glass microelectrodes (resistance: 6–15 M $\Omega$ ) filled with 3 M KCl. Electrical potentials were amplified on a AxoClamp 2B amplifier (Axon Instruments, Union City, CA) recorded using a POWERLAB data acquisition unit (ADInstruments, Milford, MA) and accompanying Chart 3.6 software.

Siphon MNs were identified by their size and location and by their tonic firing activity (Belkin and Abrams 1998; Hickie and Walters 1995). MNs were hyperpolarized to approximately  $-70$  mV for the duration of the experiment to minimize spiking. Tail sensory neurons were localized to the VC cluster of the pleural ganglion (Walters et al. 1983b).

**EXPERIMENTAL PROCEDURES.** Each half of the tail could be stimulated independently using stimulator-driven “tappers” (a blunted low-gauge needle attached to an electrical relay) that delivered brief (50 ms) tactile taps to the dorsal surface. The area underneath the initial 500 ms of the evoked complex EPSP in the siphon MN was integrated ( $\Delta\text{mV} \cdot \text{s}$ ) for a quantitative measure of net activation.

In experiments examining the modulatory pathway underlying contralateral dishabituation, pedal commissure (Pd), cerebral connectives (Cr), and pleural-abdominal connectives (P-AC) were severed manually using fine microscissors 30 min prior to the start of the experiment. Nerve cuts were made in normal ASW. The dishabituation protocol consisted of two baseline TSW measures (5-min intertrial interval [ITI]) followed by habituation training (10 taps, 10-s interstimulus interval [ISI]) of one side of the tail. Immediately following, a single P8 shock (3 s, 15 V, 5-ms pulses at 20 Hz) was administered to the nonhabituated side. Posttests of evoked MN responses to stimulation of both sides were taken at 2 and 7 min after P8 stimulation.

The design of experiments examining the effects of network disinhibition on sensitization is shown in Fig. 4A. Tests of tap-evoked MN activation were taken at 5-min ITI alternating test order (e.g., L-R, R-L, etc.). The first two tests served as baseline measures of tap-evoked input into the MN. Immediately after the pretests, 40 ml 100  $\mu\text{M}$  d-TC (ICN Biochemicals, Costa Mesa, CA) was perfused into the whole CNS bath, a chamber containing only the ring ganglia, or a chamber containing only the abdominal ganglion. The exchange of

media bathing the CNS was stopped after d-TC perfusion (~6 min), and the drug was left in static bath for the duration of the experiment. Two additional pretests taken in the presence of d-TC were followed by a stimulation of a P8 nerve using a conventional suction electrode. P8 nerve shock, (3 s, 15 V, 20 Hz, 5-ms pulses) was used as an analog of body wall shock. Body wall shock has been used previously to induce sensitization in *Aplysia* (e.g., Cleary et al. 1998). Control experiments with no nerve shock showed that the reflex enhancing effects of d-TC reach asymptote by 10 min (see Fig. 3). Postshock test was conducted at 2 min after training.

In experiments examining the effects of P9 (tail) nerve shock on tail SN excitability, excitability measures were taken at 5-min ITI and consisted of a 300-ms constant depolarizing current pulse adjusted to elicit one to two spikes (range: 0.5–1.7 nA). In some experiments, the ipsilateral or contralateral P9 nerve was stimulated (3 s, 15 V, 20 Hz, 5-ms pulses) 3 min after a second baseline test. In other experiments, SNs were activated prior to P9 nerve shock in bursts of three action potentials (20 Hz) at 0.1 Hz for 10 iterations. This pattern of SN activation was chosen to simulate SN activation during TSW habituation (Stopfer and Carew 1994; Walters et al. 1983b). The effect of d-TC on nerve-shock-induced SN excitability changes was also examined. In these experiments, 100  $\mu$ M d-TC was perfused into the CNS bath chamber prior to P9 nerve shock. Posttests were taken 2 min after nerve shock.

### Chronoamperometry experiments

**ELECTROCHEMICAL DETECTION OF 5-HT RELEASE.** Serotonin release was directly measured using carbon-fiber electrodes covered with cellulose as described by Marinesco and Carew (2002a,b). Cellulose-coated electrodes allow a more sensitive detection of biogenic amines in the *Aplysia* CNS with a detection limit as low as 10–20 nM for 5-HT (Marinesco and Carew 2002a). The active part of the carbon fiber implanted in the ganglia was 7  $\mu$ m (diameter)  $\times$  150  $\mu$ m (length). Recordings were performed with a VA-10 voltammeter (NPI Electronic, Tamm, Germany) connected to a three-electrode potentiostat. Reference and auxiliary electrodes were both made of a chlorided silver wire (Medwire, Mount Vernon, NY).

A chronoamperometric technique was used to detect 5-HT release in the CNS. This electrochemical technique allows the detection of oxidation currents at high temporal resolution (2 Hz) (Marinesco and Carew 2002b). Chronoamperometry was performed with four successive voltage steps (80 mV, 40 ms; 230 mV, 15 ms; 250 mV, 40 ms; 400 mV, 15 ms) applied between the working and reference electrodes every 500 ms. 5-HT oxidation currents were estimated as the difference in current between the fourth and third pulse. We have previously shown that his technique allows reliable estimates of 5-HT concentrations in the *Aplysia* CNS (Marinesco and Carew 2002a,b). The recorded current was filtered with a 1-kHz low-pass filter and measured by averaging its value over the last 5 ms of each pulse to improve the signal-to-noise ratio.

Chronoamperometric recordings were performed in three different locations within the *Aplysia* CNS: in the pleural ganglion, underneath the tail SNs cell bodies, in the pedal ganglion, underneath the tail MN cell bodies, in the putative region of tail SN-MN synapses, and in the abdominal ganglion, underneath the LFS motor neurons cell bodies. These locations were determined visually using an Olympus SZH stereomicroscope (Melville, NY). The carbon fiber was inserted ~200  $\mu$ m deep into the neuropil, at a 15–20° angle.

Tail nerves were stimulated with a 2-s train of 5-ms pulses at 40 Hz (~30–50 V), a procedure known to induce significant 5-HT release in the CNS of *Aplysia* (Marinesco and Carew 2002b). Ipsilateral and contralateral tail-nerves were shocked at a 15-min interval to obtain stable electrochemical signals (30-min ISI for the same nerve). d-TC was then infused in the recording medium for  $\geq$ 10 min before the next shock was administered. At the end of the experiment, the carbon fiber electrode was calibrated in a flow-injection chamber, by injection

of a solution of 500 nM 5-HT for 1 min. Because the 5-HT oxidation current is linear with 5-HT concentration (Marinesco and Carew 2002a), we could estimate the maximal concentration released into the neuropil in each experiment, using the standard value obtained with 500 nM 5-HT.

**STATISTICAL ANALYSIS.** Summary data are presented graphically as means  $\pm$  SE. Differences between means were analyzed using ANOVAs and *t*-test with an alpha level of 0.05 adopted for all tests of statistical significance.

## RESULTS

### Characterization of modulatory pathway

Although the TSW is mediated by a lateralized reflex pathway (Bristol et al. 2004), the ability of shock to dishabituate contralateral responses indicates the presence of a bilateral modulatory pathway. This observation is consistent with the recent finding that in the pleural and pedal ganglia, shock-evoked release of 5-HT, a neuromodulator known to be involved in sensitization in *Aplysia*, occurs predominately on the shocked side, but a small amount of release also occurs contralaterally (Marinesco and Carew 2002b). Therefore, if a crossing modulatory pathway in the ring ganglia underlies the contralateral effects of shock, then severing this pathway should abolish contralateral dishabituation.

We examined this question by cutting the Pd commissure or the Cr connectives, the two possible pathways connecting the left and right sides of the ring ganglia, and testing for the occurrence of contralateral dishabituation (Fig. 1A). We found, however, that despite a cut Pd commissure, habituated MN responses were enhanced by contralateral P8 stimulation beyond that expected by recovery from habituation alone (Fig. 1; unpaired *t*-test of contralateral dishabituation with Pd cut at 2 min posttest,  $t_{(6)} = 2.61$ ,  $P < 0.05$ ). Similarly, we found that cutting the Cr connectives did not affect contralateral dishabituation (Fig. 1; 2 min posttest,  $t_{(5)} = 2.62$ ,  $P < 0.05$ ). Importantly, we previously found that Pd commissure and Cr connective cuts alone do not enhance tail tap-evoked responses in siphon MNs (Bristol et al. 2004). These data suggest that any modulatory pathway that may cross within the ring ganglia does not underlie the bilateral effects of shock.

An additional possibility is that the shock-induced modulation occurs within the abdominal ganglion, which is known to contain interneurons participating in the siphon withdrawal (Cleary et al. 1995). To address this possibility, we severed the P-AC, which connects the ring ganglia with the abdominal ganglion, ipsilateral to the shocked P8 nerve and tested for contralateral dishabituation. We found that after cutting the P-AC, shocking the ipsilateral P8 nerve did not enhance depressed MN responses as it did in previous dishabituation experiments. Rather, the MN response showed a recovery profile very similar to that observed after habituation alone (Fig. 1;  $t_{(10)} = 0.38$ ,  $P = \text{NS}$ ). Thus these data indicate that the descending P-AC pathway ipsilateral to the shocked P8 is required for the induction of contralateral dishabituation. This finding, consistent with previous results obtained by Advokat (1980), favors a model in which the locus of change underlying dishabituation is either interneurons within the ipsilateral ring ganglia that project to the abdominal ganglion via the ipsilat-

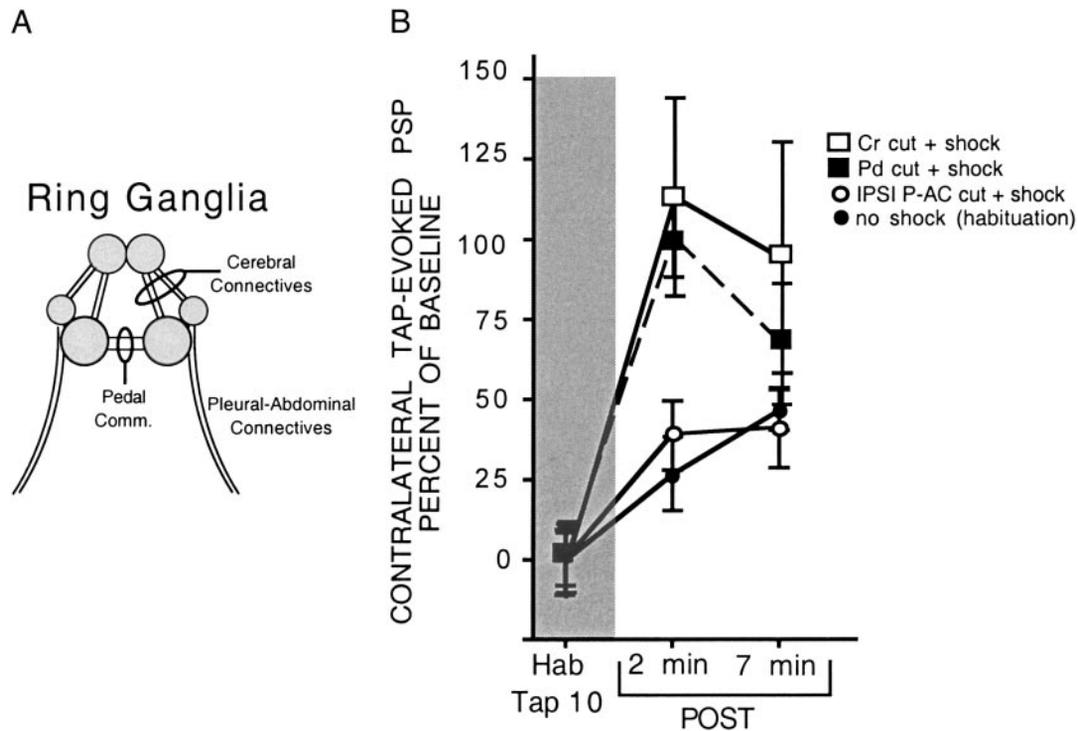


FIG. 1. Postshock enhancement of tap-evoked motor neuron (MN) responses after ipsilateral pleural-abdominal connective (P-AC) cut resembles habituation-like recovery. *A*: schematic diagram of the position of the cerebral and pleural-abdominal connectives and the pedal commissure within the ring ganglia of *Aplysia*. *B*: direct comparison of postshock enhancement under conditions of severed cerebral connectives (□), pedal commissure (■), and P-AC ipsilateral to the shock (○) with the recovery from habituation alone (no shock, ●). Whereas preparations with cerebral connectives (Cr) connective or pedal commissure (Pd) commissure cuts still showed marked postshock enhancement (dishabituation), preparations with the ipsilateral P-AC severed showed postshock enhancement similar to that due to habituation recovery alone.

eral P-AC or interneurons within the abdominal ganglion, upstream of siphon MNs.

#### *Inhibition gates contralateral effects of nerve shock*

In the next experiments, we sought to examine alternative properties of the neural circuit for TSW that could account for the differential lateralization of sensitization and dishabituation. We hypothesized that the contralateral effects of shock are normally suppressed by network inhibition in the untrained animal (Fig. 2*A*) but that prior habituation training gives rise to a form of activity-dependent disinhibition of modulatory processes on that side of the circuit, thereby allowing the contralateral effects of shock to be expressed (Fig. 2*B*).

This hypothesis leads to the prediction that if network inhibition suppresses the contralateral effects of nerve shock (thereby giving rise to lateralized sensitization), then blocking network inhibition (disinhibition) should give rise to bilateral sensitization. Because most fast inhibitory transmission in *Aplysia* is attributable to an increase in  $Cl^-$  conductance via nicotinic acetylcholine receptors (nACh) (Blankenship et al. 1971; Kehoe 1972; Trudeau and Castellucci 1993b), we tested this prediction using the nACh antagonist d-tubocurarine (d-TC). Importantly, the 100  $\mu$ M concentration we used was previously determined to block inhibitory effects of Ach but had no effect on excitatory transmission (Trudeau and Castellucci 1993b). Consistent with a report by Trudeau and Castellucci (1993b), we noted

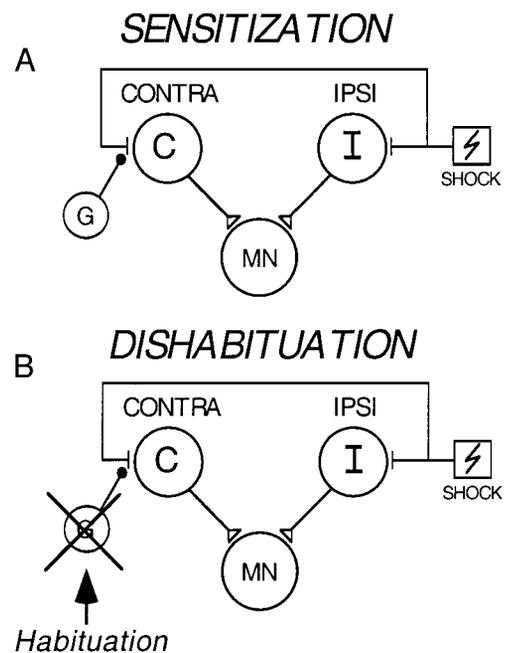


FIG. 2. Hypothesized mechanism underlying lateralized sensitization and bilateral enhancement of tail-elicited siphon withdrawal reflex (TSW). *A*: the contralateral effects of nerve shock (shock, ⚡) are suppressed by network inhibition (*G*) in the untrained animal. *B*: prior habituation training drives a form of activity-dependent disinhibition thereby allowing the contralateral effects of shock to be expressed. *C* and *I*, reflex pathways contralateral and ipsilateral, respectively, to the shock.

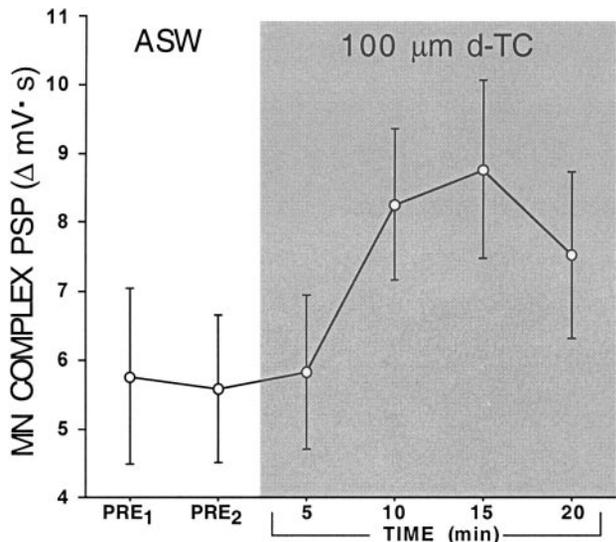


FIG. 3. d-Tubocurarine (d-TC) enhances baseline response of siphon MNs to tail stimulation. Summary of 10 experiments showing that bath application of 100 μM d-TC enhanced baseline tap-evoked response of siphon MNs. The reflex-enhancing effects of d-TC reached asymptote within 10 min of perfusion.

that bath application of 100 μM d-TC to the entire CNS reliably enhanced baseline responses evoked by tail taps (see METHODS) in siphon MNs (10-min test,  $t_{(9)} = 2.36, P < 0.05$ ). As can be seen in Fig. 3, the reflex-enhancing effects of d-TC reached their maximal value within 10 min. This observation allowed us to examine the effects of d-TC on nerve-shock-induced sensitization after 10 min exposure to d-TC because any additional increase in the tap-evoked response could not be attributed to the cumulative effects of the drug alone.

We next asked whether disinhibiting the CNS via d-TC would render the circuit capable of bilateral sensitization.

Consistent with our earlier observations (Bristol et al. 2004), we found that a single P8 nerve shock in normal ASW (see METHODS) produced a significant enhancement of MN responses evoked by ipsilateral tail stimulation but had no effect on MN responses evoked by contralateral side stimulation [Fig. 4B; 2-way ANOVA,  $F(1,10) = 5.18, P < 0.05$ ; contralateral vs. ipsilateral enhancement,  $t_{(10)} = 2.28, P < 0.05$ ]. However, when the CNS was disinhibited using d-TC, P8 nerve shock resulted in enhancement of both ipsilateral and contralateral tap-evoked MN response [Fig. 4C; 2-way ANOVA,  $F(1,16) = 0.22, P = \text{NS}$ ; ipsilateral enhancement,  $t_{(8)} = 3.06, P < 0.05$ , contralateral enhancement,  $t_{(8)} = 2.95, P < 0.05$ ]. These results reaffirm the side-specificity of sensitization and further indicate that cholinergic inhibition normally prevents the contralateral enhancement of TSW after lateral nerve shock.

*d-TC has no effect on modulatory processes in the ring ganglia*

In additional experiments, we attempted to localize the actions of d-TC. We reasoned that d-TC may act within the ring ganglia to give rise to contralateral effects of nerve shock by relieving inhibition of the serotonergic (5-HT) system. It is known that 5-HT is released in the CNS in response to noxious stimulation and contributes importantly to both sensitization and synaptic plasticity in *Aplysia* (Glanzman et al. 1989; Levenson et al. 1999; Mackey et al. 1989; Marinesco and Carew 2002b). Recently, Marinesco and Carew (2002b) showed that 5-HT release evoked by tail-nerve shock was four to five times greater in the pleural and pedal ganglia ipsilateral to the side of stimulation compared with the contralateral ganglia. Because lateralized 5-HT release in the ring ganglia provides a plausible mechanism for lateralized sensitization in the intact animal, we reasoned that bilateral sensitization after d-TC (Fig. 4) could be

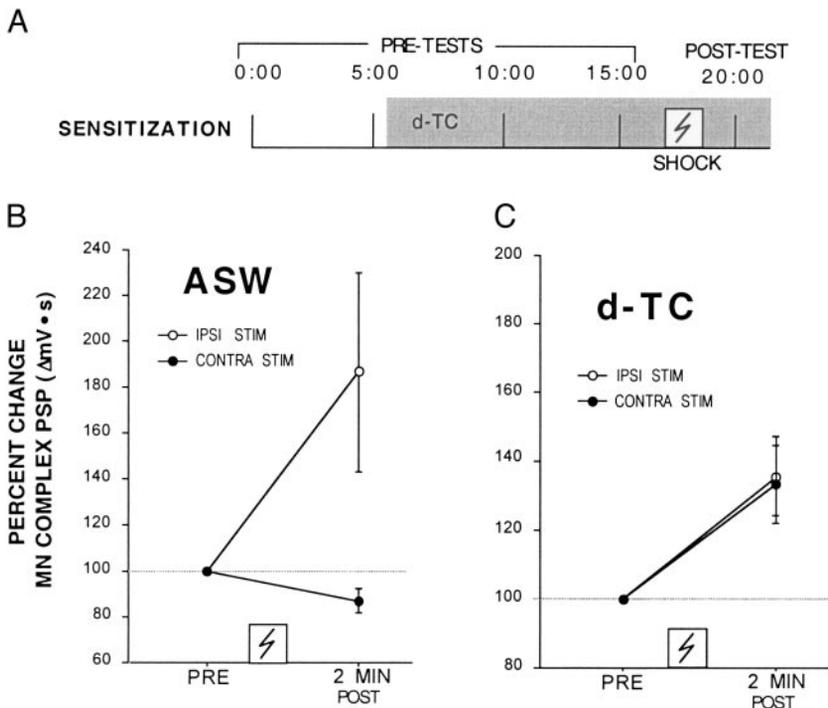


FIG. 4. Network disinhibition with d-TC converts lateralized sensitization to bilateral sensitization. A: diagram of experimental protocol. B: summary of 11 control experiments in which a P8 (body wall) nerve was shocked in the presence of bath artificial seawater (ASW). After shock, only the ipsilateral tap-evoked MN response was enhanced. C: summary of 17 experiments in which a P8 nerve was shocked in the presence of d-TC. After shock, both the ipsilateral and contralateral tap-evoked MN response was enhanced.

due to a change in the lateralization of 5-HT release in this region of the CNS. Therefore we investigated the effect of d-TC on two 5-HT-dependent processes within the ring ganglia: the ability of tail nerve shock to increase pleural (tail) SN excitability and the lateralized release of 5-HT.

Numerous studies have demonstrated that 5-HT increases tail SN excitability (e.g., Bunge et al. 1997; Critz et al. 1991; Mercer et al. 1991; Purcell and Carew 2001; Wright and Kirschman 1995). Excitability increases also occur after peripheral nerve stimulation and correlates well with direct measurements of 5-HT release (Marinesco and Carew 2002b). Consistent with previous reports (Marinesco and Carew 2002b; Mercer et al. 1991; Walters et al. 1983a), we found that a single P9 shock produced reliable increases in the excitability of tail SNs ipsilateral to the shocked nerve (Fig. 5A;  $t_{(4)} = 3.14$ ,  $P < 0.05$ ). In contrast, P9 shock failed to produce an increase in excitability of tail SNs located contralateral to the shocked nerve (Fig. 5B;  $t_{(4)} = 2.44$ ,  $P = \text{NS}$ ). In separate experiments, when d-TC was added locally to the bath, P9 shock still had no effect on excitability of contralateral tail SNs (Fig. 6A;  $t_{(2)} = 1.00$ ,  $P = \text{NS}$ ). We also asked whether previous repeated activation of a tail SN, using a protocol that simulates repeated tactile input that gives rise to habituation (Bristol et al. 2004; Stopfer et al. 1996), would render it sensitive to the effects of contralateral P9 shock. To explore this possibility, tail SNs were activated in short bursts (3 action potentials, 20 Hz) to mimic their response to cutaneous stimulation (Walters et al. 1983b) in the same manner previously used to produce re-

sponse habituation (10 bursts, 0.1 Hz) (Bristol et al. 2004). Despite prior activation, tail SNs showed no change in excitability after contralateral P9 nerve shock (Fig. 6B;  $t_{(4)} = 1.00$ ,  $P = \text{NS}$ ).

Both the ring ganglia and the abdominal ganglion contain neurons responsible for the TSW reflex (Frost and Kandel 1995; Kupfermann and Kandel 1969). 5-HT is known to be an important neuromodulator in tail-shock-induced sensitization. Thus the disinhibitory effects we observe in response to d-TC could be due to changes in the release of 5-HT in either (or both) of these regions of the CNS. To examine this question, we directly measured 5-HT release evoked by tail-nerve shock using electrochemical techniques (Marinesco and Carew 2002a,b) in three ganglia involved in the TSW reflex, both before and after d-TC application. 5-HT release was measured in the vicinity of tail SN cell bodies in the pleural ganglion, near synapses with tail MNs in the pedal ganglion, and in the vicinity of the LFS motor neurons in the abdominal ganglion (left hemiganglion, Fig. 7A).

In the ring ganglia, the maximal 5-HT concentration after tail-nerve stimulation was greater in the pleural ganglion ( $113 \pm 15$  nM,  $n = 4$ ) than in either the pedal ganglion ( $60 \pm 12$  nM,  $n = 4$ ) or in the abdominal ganglion ( $22 \pm 2$  nM,  $n = 10$ ). Confirming earlier results (Marinesco and Carew 2002b), 5-HT release in the pleural and pedal ganglia was highly lateralized to the side ipsilateral to the stimulation (Fig. 7, B and C). The maximal 5-HT concentration after tail-nerve shock was 4.7 times greater on the ipsilateral side compared with the

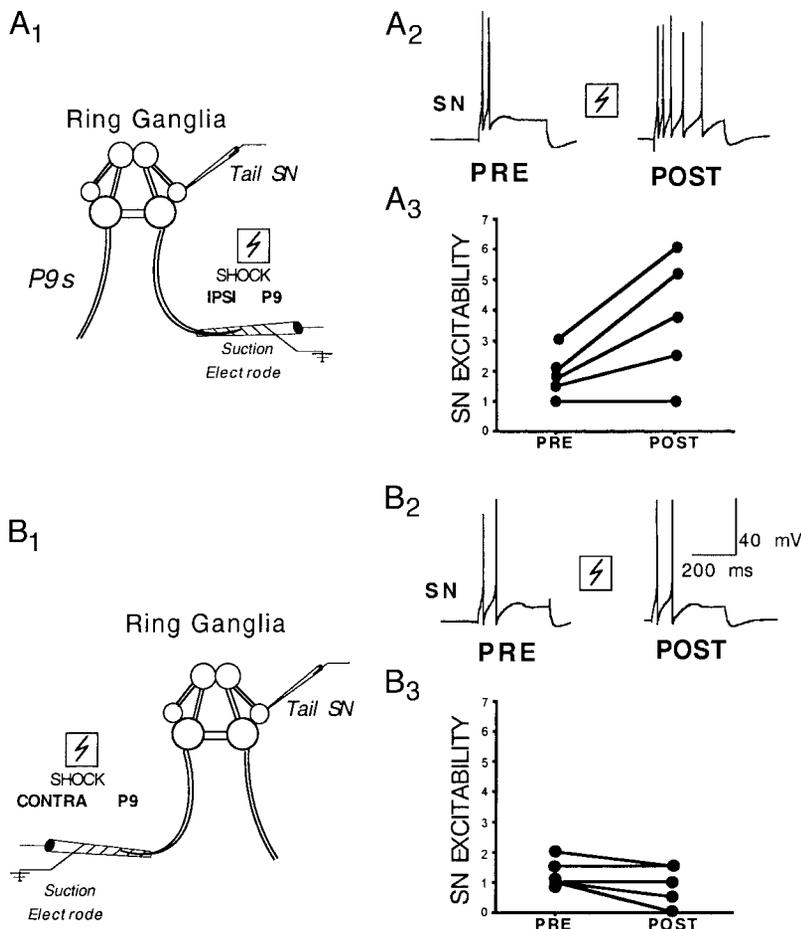


FIG. 5. Excitability of tail sensory neurons (SNs) is enhanced by ipsilateral, but not contralateral, tail nerve shock. *A1*: diagram of ring ganglia illustrating position of recording electrode used to measure excitability of a tail SN and suction electrode used to stimulate ipsilateral P9 nerve. *A2*: sample recordings from a single tail SN. After ipsilateral P9 shock, a 300-ms current pulse that had evoked 2 spikes subsequently evoked 5 spikes. *A3*: summary data of 5 experiments showing that ipsilateral P9 shock increases tail SN excitability. *B1*: diagram of ring ganglia illustrating position of recording electrode used to measure excitability of a tail SN and suction electrode used to stimulate contralateral P9 nerve. *B2*: sample recordings from a single tail SN. *B3*: summary of 5 experiments showing that shocking the contralateral P9 nerve has no effect on tail SN excitability.

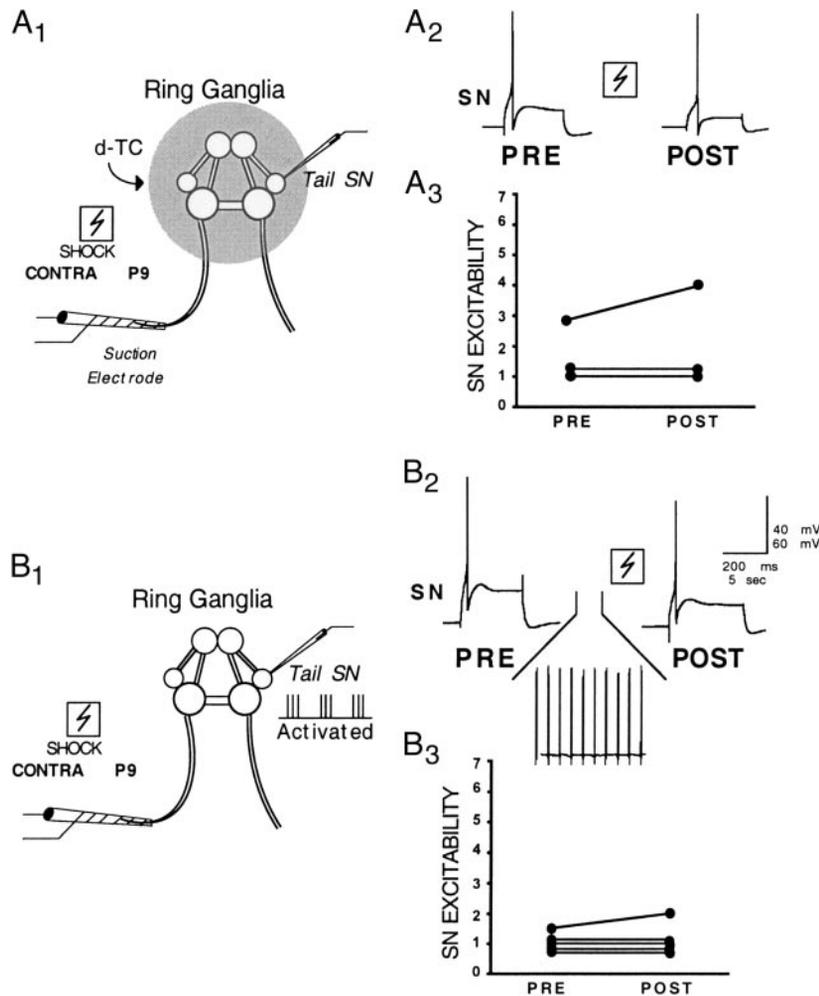


FIG. 6. Effect of d-TC exposure and previous SN activity on the inability of contralateral P9 shock to increase SN excitability. *A1*: diagram of ring ganglia illustrating position of recording electrode used to measure excitability of a tail SN, stimulated P9 nerve, and region of d-TC exposure. *A2*: sample recordings from a single tail SN. In the presence of 100  $\mu$ M d-TC, contralateral P9 shock did not enhance SN excitability. *A3*: summary data from 3 d-TC experiments. *B1*: diagram of ring ganglia illustrating position of recording electrode used to measure excitability of a tail SN and the stimulated contralateral P9 nerve. As indicated in the inset, the SN was activated in a habituation-like pattern prior to P9 shock (see METHODS). *B2*: sample recordings from a single tail SN. Activating the SN prior to the contralateral P9 shock had no effect on excitability. *B3*: summary data from 5 SN activation experiments.

contralateral side in the pleural ganglion (113 vs. 24 nM) and 12 times in the pedal ganglion (60 vs. 5 nM).

After d-TC 100  $\mu$ M was applied for  $\geq 10$  min, 5-HT release in the pleural and pedal ganglion was unchanged (Figs. 7, *B* and *C*, and 8*A*): 112 nM in the pleural ganglion (99% of control) and 61 nM in the pedal ganglion (102% of control). Thus 5-HT release during d-TC application was still lateralized to the side ipsilateral to the stimulation. Again, 5-HT release was more than four times greater on the ipsilateral side than on the contralateral side when measured in both the pleural and pedal ganglion.

The effects of d-TC on 5-HT release in the abdominal ganglion produced different results. Reliable 5-HT release could be measured in the left hemiganglion in the region of the LE siphon SNs and the LFS siphon MNs. In contrast to the ring ganglia, 5-HT release in the abdominal ganglion was not lateralized:  $22 \pm 2$  nM after left tail-nerve shock,  $20 \pm 2$  nM after right tail-nerve shock ( $n = 10$ ,  $P = 0.65$ , Figs. 7*D* and 8*B*). Interestingly, in the presence of d-TC, 5-HT release was enhanced in the abdominal ganglion by  $\sim 40\%$ , regardless of which tail-nerve was stimulated ( $P = 0.03$  left nerve,  $P = 0.02$  right nerve, Figs. 7*D* and 8*B*).

These results indicate that bilateral sensitization after d-TC (Fig. 4) cannot be explained by a change in the lateralization of 5-HT release within the CNS because d-TC did not produce any detectable effects on 5-HT release in the ring ganglia nor

did it alter the pattern of P9 shock-induced increases in tail SN excitability. However, d-TC significantly enhanced 5-HT release in the abdominal ganglion, suggesting that neural circuits involved in TSW in this region of the CNS may be the site of d-TC-induced disinhibition.

#### *Inhibition within the abdominal ganglion suppresses the contralateral effects of nerve shock*

Because the entire CNS (ring ganglia and abdominal ganglion) was exposed to d-TC, it was not possible in earlier experiments (Fig. 4) to determine the anatomical locus of the reflex-enhancing effects. As described in the preceding text, we noted that d-TC exposure increased levels of P9 shock-induced 5-HT release in the abdominal ganglion but had no effect on evoked 5-HT release in the pleural or pedal ganglia. Therefore we carried out the next series of experiments to investigate whether the effects of d-TC could be localized to the ring ganglia or abdominal ganglion by restricting d-TC exposure to either the ring or abdominal ganglia alone. We examined the ring ganglia despite the lack of effect of d-TC on 5-HT release (Figs. 7 and 8) because it is possible that d-TC may exert its effect in the ring ganglia independent of the 5-HT system.

We first observed that d-TC exposure restricted to the abdominal ganglion produced an enhancement of the baseline tap-evoked MN responses, whereas a comparable experiment

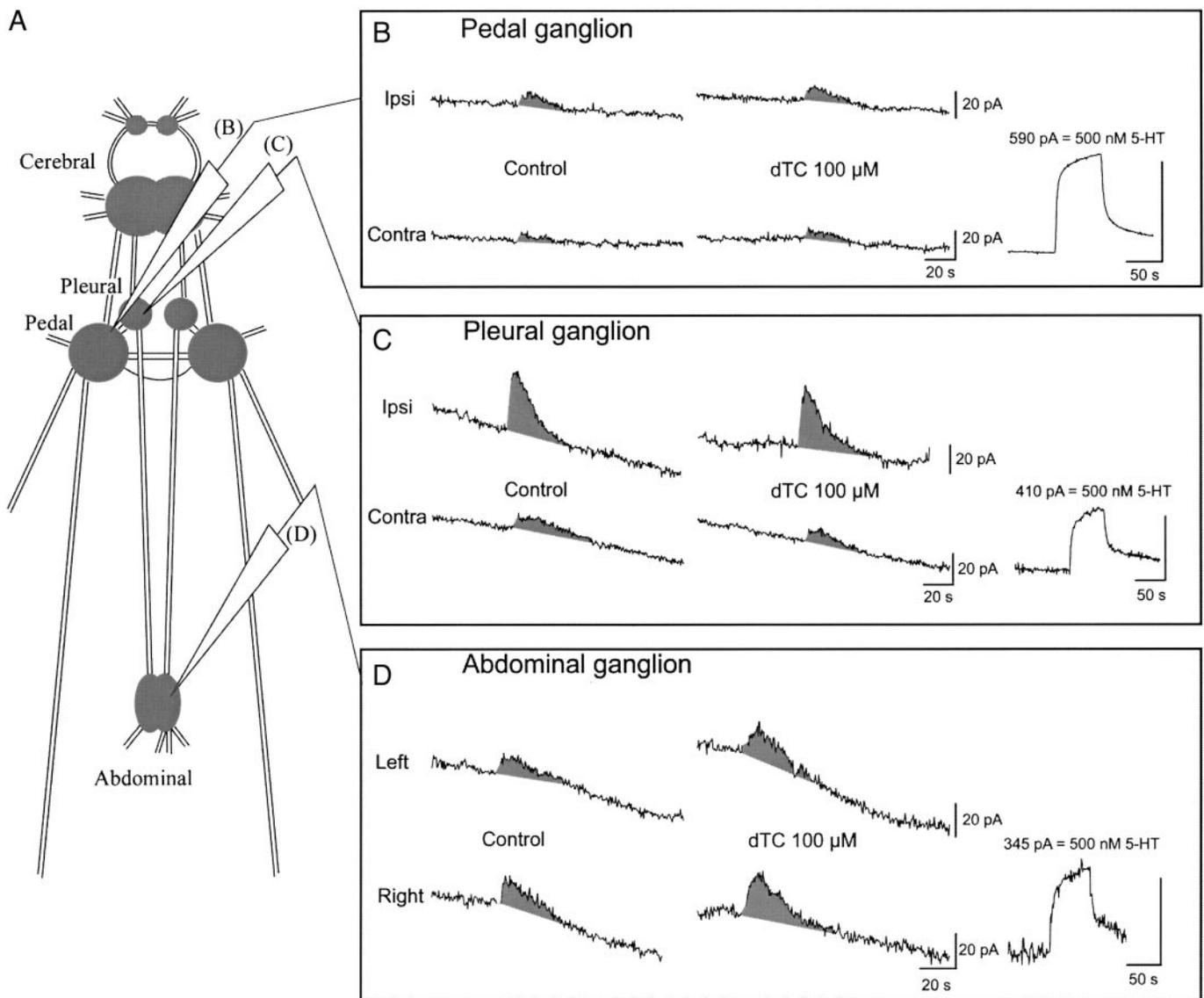


FIG. 7. d-TC differentially affects 5-HT release in the *Aplysia* CNS. **A**: 5-HT release was measured with carbon fiber electrodes in the *Aplysia* CNS. Examples of recordings obtained in the pedal (**B**), pleural (**C**), and abdominal ganglia (**D**), before and after infusion of 100  $\mu$ M d-TC. Serotonin release appears as an increase in the 5-HT oxidation current recorded from the carbon fiber electrodes [area shaded ( $\blacksquare$ )]. The maximal 5-HT concentration in the extracellular space ( $\{5\text{-HT}\}_{\text{ext}}$ ) can be estimated using a calibration curve obtained by injecting 500 nM 5-HT during 1 min in a flow-injection chamber immediately after the experiment (right side).

in the ring ganglia produced no change (Fig. 9;  $F(2,41) = 3.17$ ,  $P = 0.05$ ; ASW vs. abdominal d-TC post hoc  $t$ -test,  $t_{(27)} = 2.51$ ,  $P < 0.05$ ). This suggests that the reflex enhancement observed during whole CNS exposure to d-TC (see Fig. 4) was due to effects specifically within the abdominal ganglion. In addition, we found that a single P8 shock in the presence of d-TC restricted to the abdominal ganglion gave rise to bilateral sensitization (Fig. 10; ipsilateral enhancement,  $t_{(4)} = 3.11$ ,  $P < 0.05$ ; contralateral enhancement,  $t_{(4)} = 3.12$ ,  $P < 0.05$ ). In contrast, a single P8 shock with d-TC restricted to the ring ganglia still produced only ipsilateral sensitization (Fig. 11; ipsilateral enhancement,  $t_{(8)} = 2.81$ ,  $P < 0.05$ ; contralateral enhancement,  $t_{(6)} = 1.06$ ,  $P = \text{NS}$ ). These data indicate that d-TC allows for the expression of bilateral sensitization through disinhibition of the components of the TSW circuit within the abdominal ganglion.

## DISCUSSION

In this study, we sought to examine the mechanisms determining the expression of shock-induced enhancement of the TSW of *Aplysia*. We were motivated by our previous observation that an identical tail shock gives rise to ipsilateral sensitization but contralateral dishabituation (Bristol et al. 2004). Specifically, we explored the possibility that crossing modulatory pathways mediate contralateral dishabituation. We found that only severance of the P-AC ipsilateral to the shocked side abolished contralateral dishabituation. We next investigated the effects of network disinhibition using the nACh antagonist d-TC on the lateralized expression of nerve shock-induced sensitization of TSW. We first found that exposure of d-TC to the entire CNS produced a persistent enhancement of tail tap-evoked responses in siphon MNs that reached asymp-

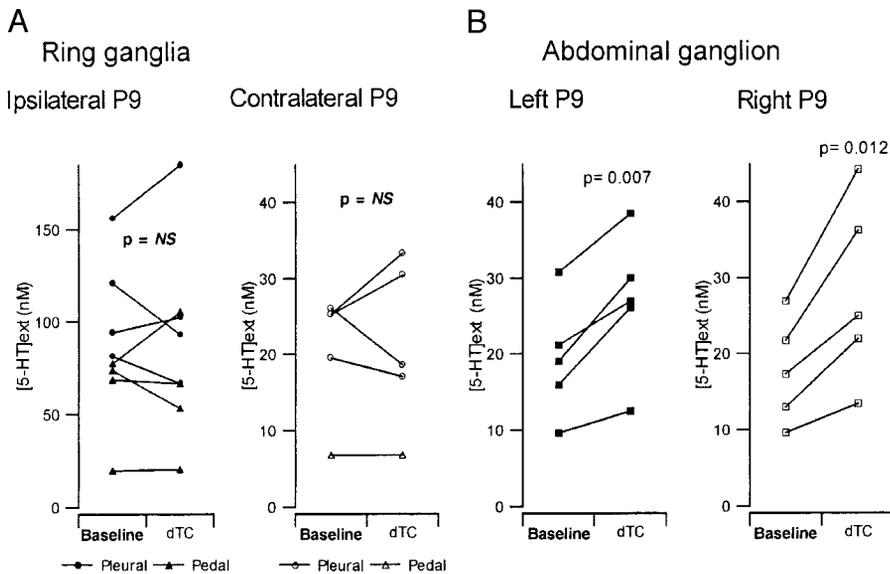


FIG. 8. d-TC enhances 5-HT release in the abdominal ganglion. Summary results of all experiments obtained from the ring ganglia (pleural + pedal, A) and the abdominal ganglion (B). 5-HT release is increased after infusion of 100  $\mu$ M d-TC in the abdominal ganglion, but not in the ring ganglia.

tote within 10 min of drug exposure. We next showed that a single shock of the P8 nerve (which innervates the body wall), which normally produces only an ipsilateral enhancement of tap-evoked MN responses (Bristol et al. 2004), results in bilateral enhancement when the entire CNS was exposed to d-TC. Similar effects were observed when d-TC was restricted to only the abdominal ganglion. However, application of d-TC to only the ring ganglia had no effect either on baseline MN responses or on lateralized sensitization. In addition, experiments in which 5-HT release was directly measured using electrochemical techniques revealed that d-TC exposure increased nerve shock-evoked 5-HT release in the abdominal ganglion but not in the ring ganglia. Finally, contralateral nerve shock was incapable of increasing pleural SN excitability in the ring ganglia even under conditions of d-TC disinhibition or

prior SN activation. Collectively, our results indicate that cholinergic inhibition suppresses the contralateral effects of shock and that the disinhibitory effects of d-TC are produced in neural circuits within the abdominal ganglion.

#### Effect of d-TC on 5-HT release

We initially hypothesized that bilateral sensitization produced under d-TC disinhibition was due to an alteration in the lateralized pattern of 5-HT release within the pleural and pedal ganglia (the site of tail SNs and tail MNs, respectively). However, we found that d-TC treatment had no effect on the side-specificity of P9 shock-induced changes in tail SN excitability, a 5-HT-dependent process. In addition, when 5-HT release was measured directly, d-TC had no effect on the lateralized pattern of release in the ring ganglia, although 5-HT release measured in the abdominal ganglion was increased.

The role of 5-HT in contralateral dishabituation TSW is as yet unclear. Although a large body of work has demonstrated facilitatory effects of 5-HT on tail and siphon SNs (e.g., Byrne and Kandel 1996; Wright and Kirschman 1995), several studies have noted inhibitory effects of 5-HT on various types of interneurons. For example, Trudeau and Castellucci (1993a) showed that exposure to exogenous 5-HT resulted in inhibition of synaptic connections between unidentified excitatory INs and siphon MNs in the abdominal ganglion. More recently, Bristol, Fischer, and Carew (2001) found that 5-HT inhibited the output of L29s, tail-responsive INs located in the abdominal ganglion that are known to be important mediators of feed-forward excitation to the siphon MNs (Fischer and Carew 1993; Hawkins et al. 1981). Thus it appears unlikely that increases in 5-HT in the abdominal ganglion produced by d-TC could give rise to enhanced TSW, considering that 5-HT has been shown to be inhibit excitatory INs in the abdominal ganglion.

Several possibilities may account for the pattern of 5-HT-related findings described in the preceding text. First, the increase in abdominal 5-HT release under d-TC disinhibition we observed may indicate the parallel enhanced release of other modulators that have facilitatory effects. Numerous neuromodulators have been identified in *Aplysia*, such as FMRFamide (Small et al. 1989, 1992), dopamine (Flinn et al. 2001;

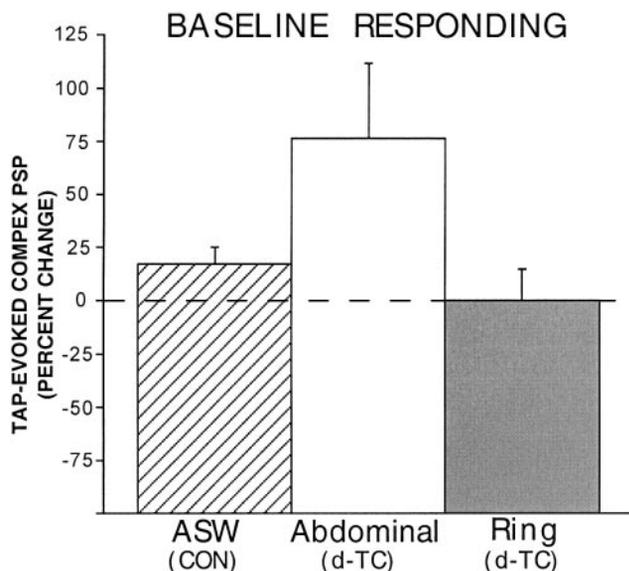


FIG. 9. d-TC restricted to the abdominal ganglion, but not the ring ganglia, enhances baseline tap-evoked MN responses. Summary of experiments in which ASW or 100  $\mu$ M d-TC was exposed to either the abdominal or ring ganglia. Ten-minute exposure of d-TC to the abdominal ganglion enhanced tap-evoked MN responses ( $n = 8$ ). ASW control preparations ( $n = 21$ ) and d-TC exposure to the ring ganglia ( $n = 15$ ) showed no change.

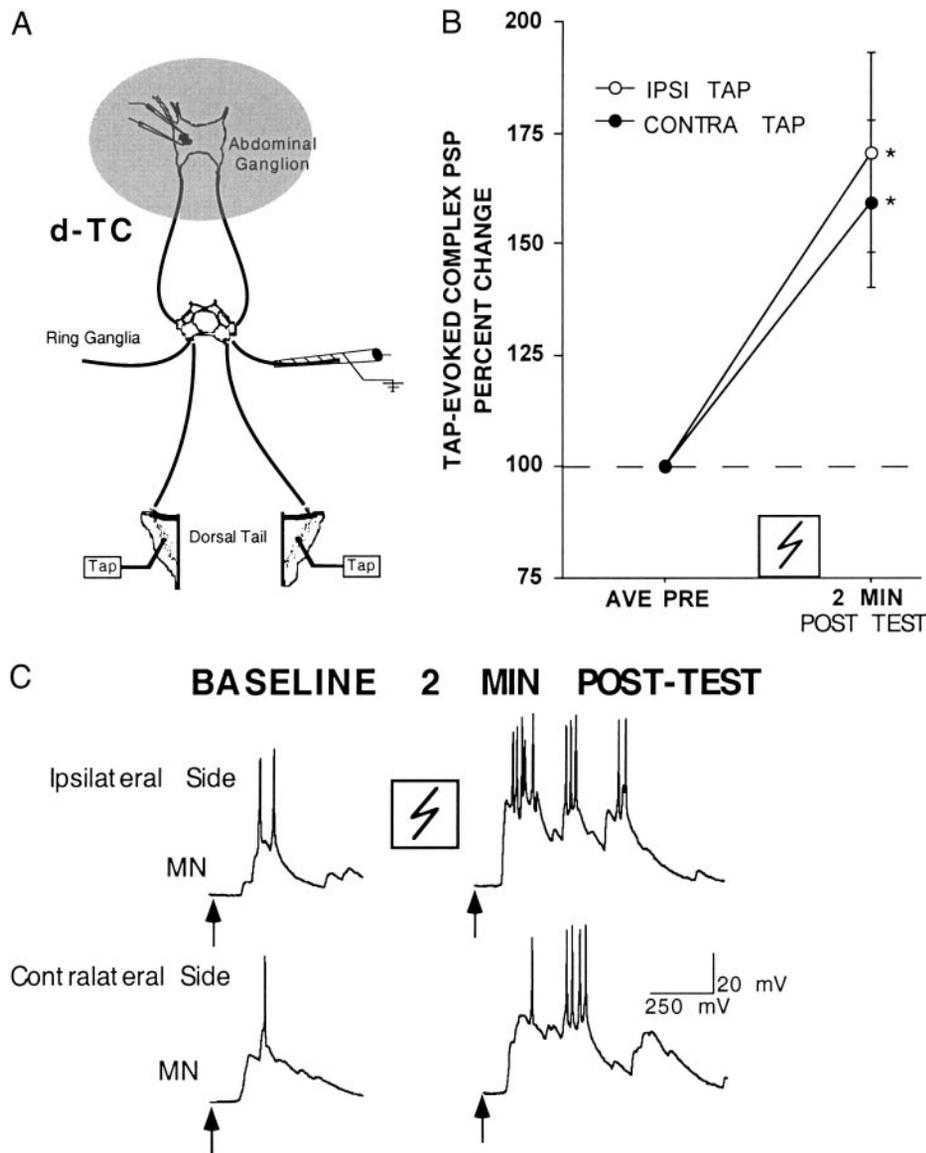


FIG. 10. P8 shock during d-TC disinhibition of the abdominal ganglion produces bilateral sensitization. *A*: diagram of preparation illustrating position of recording electrode used to measure tap-evoked MN responses, stimulated P8 nerve, and region of d-TC exposure. *B*: summary of 5 experiments showing bilateral sensitization during disinhibition of the abdominal ganglion. \*, statistical significance. *C*: sample recordings from a single MN in response to activation of the tail ipsilateral and contralateral to the shocked P8 nerve. Disinhibiting the abdominal ganglion using 100  $\mu$ M d-TC, P8 shock enhanced MN responses to both ipsilateral and contralateral side tail taps.

Nargeot et al. 1999), and small cardiac peptide (Fox and Lloyd 2002; Trudeau and Castellucci 1993a), several of which are likely to be released on noxious stimulation. Thus the contralateral effects of nerve shock may be due to the actions of neuromodulators other than 5-HT. Second, 5-HT may have facilitatory effects on as-yet unidentified abdominal INs in the TSW circuit (see following text). Third, it may be that network disinhibition may allow abdominal INs specific to the contralateral pathway to be activated by nerve shock at a rate sufficient for activity-dependent synaptic potentiation to offset depressive effects of 5-HT (Bristol et al. 2001). This mechanism supposes that contralateral reflex enhancement results from activity-dependent synaptic plasticity and not from the actions of 5-HT or other extrinsic modulators. Last, it may be that 5-HT effects are concentration dependent. As noted in the preceding text, 5-HT modulates multiple elements in the TSW circuit, and these different forms of modulation likely depend on different subtypes of 5-HT receptors, which in turn, may have different affinities for 5-HT (Barbas et al. 2003). Further experiments will be necessary to distinguish among these possibilities or reveal alternative explanations.

#### Mechanisms of sensitization and dishabituation

The monosynaptic connections between the tail SNs and tail MNs, and between the siphon SNs and siphon MNs, have served as useful cellular models of sensitization, habituation, and dishabituation of SW reflexes of *Aplysia*. Early analyses led to the view that homosynaptic depression of SN-MN connections was the mechanism of habituation (Castellucci and Kandel 1974; Castellucci et al. 1970; Jacklet and Lukowiak 1975), and heterosynaptic facilitation of SN-MN connections served as a common mechanism underlying sensitization and dishabituation (Byrne and Kandel 1996; Carew et al. 1979; Castellucci et al. 1986; Frost et al. 1985). For sensitization, however, it was soon appreciated that plasticity occurs at several sites in the SWR neural circuit after noxious stimulation and that these additional forms of plasticity work in concert to enhance siphon withdrawal (Fischer et al. 1997; Frost et al. 1988). In addition, a study by Stopfer and Carew (1996) showed that tail SN-MN synapses were enhanced after behavioral TSW habituation, suggesting that the critical mechanism underlying reflex decrement involved downstream IN

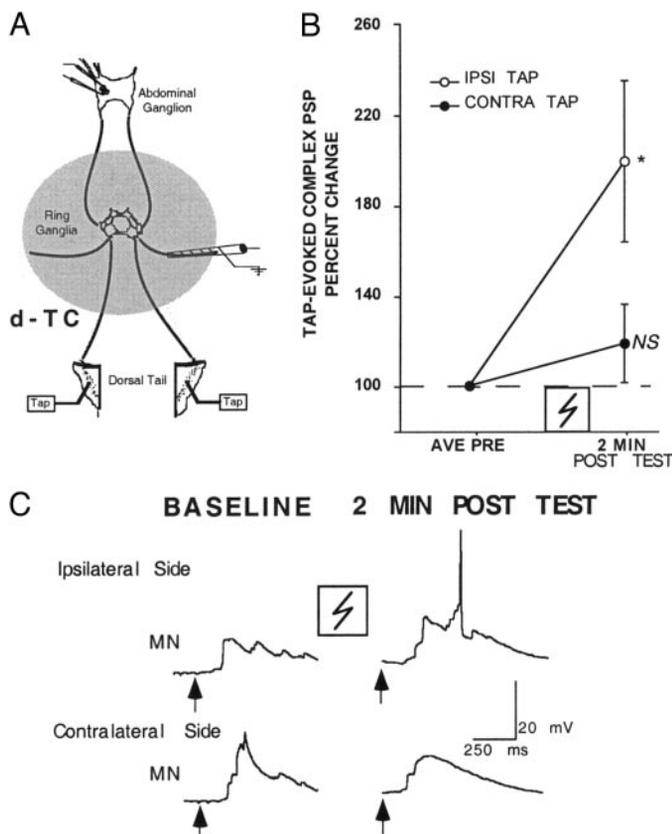


FIG. 11. P8 shock during d-TC disinhibition of the ring ganglia produces lateralized sensitization. *A*: diagram of preparation illustrating position of recording electrode used to measure tap-evoked MN responses, stimulated P8 nerve, and region of d-TC exposure. *B*: summary of 7 experiments showing lateralized sensitization during disinhibition of the ring ganglia. \*, statistical significance. *C*: sample recordings from a single MN in response to activation of the tail ipsilateral and contralateral to the shocked P8 nerve. During disinhibition of the ring ganglion using 100  $\mu$ M d-TC, P8 shock enhanced MN responses to ipsilateral, but not contralateral, side tail taps.

processing. Comparable studies by Hawkins and colleagues (e.g., Antonov et al. 1999; Cohen et al. 1997) examining siphon SN-MN synapses revealed a different result: habituation of the S SW reflex was accompanied by progressive decrement at the siphon SN-MN synapses. Thus studies by Stopfer and Carew (1996) and Cohen et al. (1997) show that, although the TSW and S-SW reflexes share many behavioral and cellular commonalities, they do not always utilize common mechanisms to produce all forms of learning.

The current study provides a new insight into the neural mechanisms of these simple forms of learning. In particular, our results provide preliminary evidence, at least for the TSW reflex, for an anatomical dissociation between the mechanisms of sensitization and dishabituation with sensitization mechanisms occurring in the ring ganglia and dishabituation mechanisms occurring in the abdominal ganglion. This model is suggested by two principal findings. First, lateralized tail nerve shock results in ipsilateral, but not contralateral increases in tail SN excitability, even after prior repeated SN activation (the cellular analogue of habituation training) or after d-TC disinhibition. These data, together with previous studies (e.g., Cleary et al. 1998; Marinesco and Carew 2002b; Scholz and Byrne 1987), suggest that the neural correlates of sensitization for the TSW occur in the ipsilateral ring ganglia and involve

changes in synaptic efficacy and biophysical properties of tail SNs. Second, when the actions of d-TC are localized to the abdominal ganglion, they mimic the effect of contralateral habituation training in rendering nerve shock capable of producing bilateral TSW enhancement. Insofar as habituation training and local d-TC are both permissive for bilateral enhancement, these data suggest the hypothesis that the disinhibitory mechanism produced by TSW habituation is localized to circuits within the abdominal ganglion.

A neural circuit like that depicted in Fig. 12 could account for the differential lateralization of sensitization and dishabituation of TSW. It does so by incorporating heterosynaptic facilitation with activity-dependent gating of contralateral inhibition. In the model, the basic reflex pathway consists of tail SNs in the ring ganglia, which drive interneurons (INs) in the abdominal ganglion, including excitatory INs that activate siphon MNs. The central position of the MN reflects bilateral input not the MN's anatomical position within the abdominal ganglion. The model utilizes two forms of plasticity: heterosynaptic facilitation due to the activation of modulatory cells (Mod) in the ring and abdominal ganglia and activity-dependent, homosynaptic depression (HSD), which occurs at IN synapses in the abdominal ganglion (see Fig. 12, \*).

According to the model, habituation of TSW occurs because repeated tail input induces HSD at IN-MN synapses in the abdominal ganglion (Fig. 12*B1*, site C). Concurrently, sensory input induces HSD at the inhibitory IN-Mod synapse, although this plasticity has no apparent behavioral relevance in the induction of habituation (see site B in Fig. 12*B*, 1 and 3). It is assumed, based on the data of Stopfer et al. (1996), that SN synapses do not depress during habituation training, therefore allowing for the induction of activity-dependent plasticity downstream of SNs.

Sensitization of TSW is restricted to side ipsilateral to the shock by heterosynaptic facilitation of ipsilateral tail SNs (Fig. 12*B2*, site A) and inhibitory control of contralateral heterosynaptic facilitation in the abdominal ganglion (Fig. 12*B2*, site B). Contralateral dishabituation occurs because, after habituation of the recently activated pathway, the inhibitory control of the abdominal Mod cell is relieved (HSD of IN-Mod synapse in Fig. 12*B3*, site B). Subsequent shock to the contralateral side, in addition to inducing heterosynaptic facilitation of tail SNs (Fig. 12*B3*, site A), is capable of activating the abdominal Mod cell and facilitate the contralateral pathway (Fig. 12*B3*, site B). Thus bilateral enhancement utilizes the mechanism for ipsilateral sensitization as well as an additional mechanism, heterosynaptic facilitation of abdominal INs in the contralateral reflex pathway. The model in Fig. 12 illustrates the operation of the modulatory processes on one side of circuit, but we assume a parallel reciprocal circuit on the other side.

This model posits that sensitization is mediated primarily by neuronal plasticity, such as heterosynaptic facilitation, within the ring ganglia occurring ipsilateral to the shock. In contrast, dishabituation involves an additional mechanism occurring in circuits within the abdominal ganglion, namely, gating of inhibitory control over contralateral facilitation. This model leads to the prediction that activation of circuits within the abdominal ganglion is required both for TSW habituation and for contralateral dishabituation but not for TSW sensitization. Preliminary experiments have confirmed this notion: we find that activation of the circuits within the abdominal ganglion is

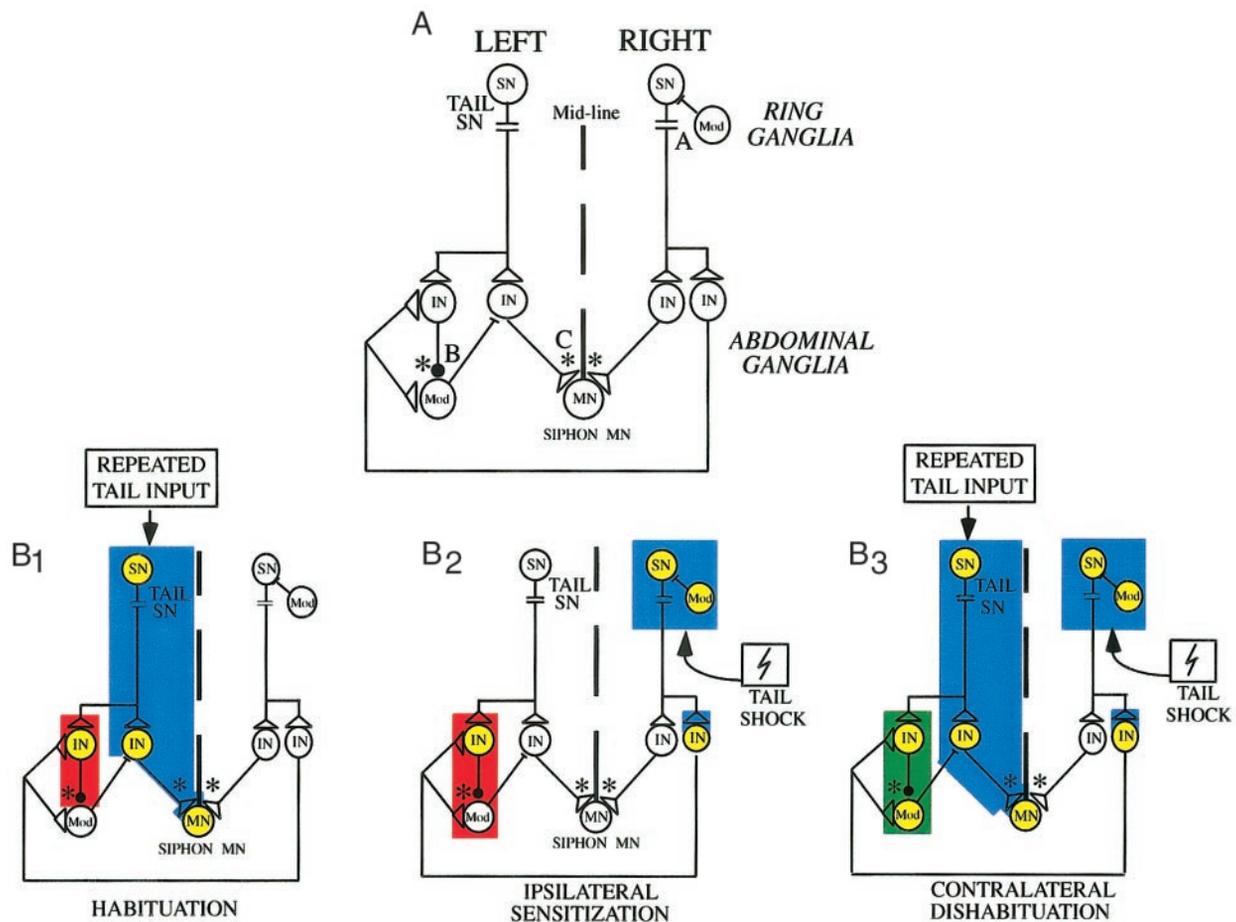


FIG. 12. Model neural circuit underlying TSW. *A*: a hypothesized neural circuit underlying TSW. The basic reflex pathway consists of tail SNs in the ring ganglia, excitatory drive onto interneurons (INs) in the abdominal ganglion, and activation of siphon MNs. Modulatory cells (Mod) in the ring and abdominal ganglia can heterosynaptically facilitate follower cells. \*, synapses at which activity-dependent, homosynaptic depression (HSD) occurs. In B1–B3, yellow cells indicate activation. *B1*: habituation of TSW occurs because repeated tail input induces homosynaptic depression at IN–MN synapses in the abdominal ganglion (site C). Concurrently, sensory input induces HSD at the inhibitory IN–Mod synapse, although this plasticity has no behavioral relevance in a habituation paradigm. *B2*: sensitization of TSW is restricted to side ipsilateral to the shock by heterosynaptic facilitation of ipsilateral tail SNs (site A) and inhibitory control of contralateral heterosynaptic facilitation in the abdominal ganglion (site B). *B3*: contralateral dishabitation occurs because, after habituation of the recently activated pathway, the inhibitory control of the abdominal Mod cell is relieved. Subsequent shock to the contralateral side, in addition to inducing heterosynaptic facilitation of tail SNs, is capable of activating the abdominal Mod cell and facilitate the contralateral pathway. Thus dishabitation utilizes the mechanism for ipsilateral sensitization as well as an addition mechanism, heterosynaptic facilitation of abdominal INs in the contralateral reflex pathway. A reciprocal circuit is presumed to be present for the other side of the circuit although, for simplicity, only one side is illustrated.

required for TSW habituation (Bristol and Carew 2003). Additional experiments indicate that plasticity within the ring ganglia alone is sufficient for TSW sensitization (Sutton and Carew, unpublished data). However, several elements in the abdominal SWR are known to be modified by either tail or mantle shock. For example, tail shock inhibits L30 INs (Fischer et al. 1997), inhibits or facilitates L29 INs depending on the level of activation (Bristol et al. 2001; Fang and Clark 1996), and increases the tonic firing rate of siphon MNs (Frost et al. 1988). Thus additional sensitization mechanisms within the abdominal ganglion may contribute to lateralized sensitization of TSW. Although additional experiments will be necessary to test these predictions, they are consistent with our finding that severing the p-AC ipsilateral to the shocked side blocks the expression of contralateral dishabitation.

An additional prediction derived from our model is that the interval during which contralateral dishabitation can occur will be governed by the recovery kinetics of the depression at synapses of the posited inhibitory INs (site B). It is not yet clear which INs in the abdominal ganglion are viable candidates for the inhibitory gating process. The fact that d-TC restricted to the ring ganglia does not enhance baseline TSW suggests that relatively little interneuronal processing in the baseline TSW circuit occurs there. Thus an analysis of previously identified inhibitory INs in the abdominal ganglion (for reviews, see Cleary et al. 1995; Frost and Kandel 1995) together with identification of new inhibitory INs will provide potentially important advances in understanding the complexity of simple forms of learning mediated by the total neural circuit underlying TSW.

### Plasticity in context

At the level of neural networks, synaptic plasticity provides a highly flexible mechanism for modulation of circuit output (Fischer and Carew 1997; Harris-Warrick et al. 1992). Recently, it has become clear that synaptic plasticity is not a static function of a synapse but can be modulated, a phenomenon known as “metaplasticity” (Abraham and Bear 1996; Abraham and Tate 1997). Metaplasticity, in its simplest form, refers to an alteration in the ability of a synapse to induce or maintain synaptic plasticity. For example, it has been shown in the hippocampus that previous activation history (Barrionuevo et al. 1980; Christie and Abraham 1992; Fujii et al. 1991; Huang et al. 1992; O’Dell and Kandel 1994; Wexler and Stanton 1993) or the presence of various neuromodulators (Kim and Yoon 1998; Terman et al. 1994; Villani and Johnston 1993) can have profound effects on the subsequent induction of plasticity. Such studies illustrate how the expression of synaptic plasticity depends crucially on the “context” in which it occurs. We use the term “context” to refer to the numerous factors that can influence the induction or maintenance of plasticity, such as the pattern of prior synaptic activation or the presence of neuromodulators.

Our results provide an additional example of how context determines the expression of plasticity. We show that the induction of one form of learning (habituation) becomes the context for another (contralateral dishabituation). Mechanistically, our results indicate that a reduction in network inhibition, perhaps via homosynaptic depression of inhibitory INs during habituation training (Fig. 12), provides the necessary context for reflex facilitation and dishabituation. Consequently, the same sensitizing stimulus that, in one instance, produces lateralized reflex enhancement, in another instance results in bilateral enhancement. Although future experiments must determine what forms of plasticity are subject to modulation, our results support the general argument that a complete understanding of learning (and its mechanisms) must consider the physiological context within which it occurs. The adaptive significance of this form of circuit plasticity remains to be fully elucidated. It may be that our results provide at least preliminary insights into the type of neural mechanisms that may be utilized in more complex forms of learning, such as context- or state-dependent recall (e.g., Bouton et al. 1999) and occasion setting (e.g., Schmajuk and Holland 1998), in which the processes underlying acquisition or expression are modulated by contextual variables present during training.

### ACKNOWLEDGMENTS

We thank Drs. Mike Sutton and Bill Wright for insightful discussions.

### GRANTS

This work was supported by National Institute of Mental Health Grant RO1 MH-141083 and National Science Foundation Grant IBN-0049013 to T. J. Carew.

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