Introduction

The study of plasticity in simple reflexes has contributed greatly to the study of learning and memory. Simple reflexes offer the dual advantages of easily quantified behavior and relatively simple underlying neural circuits. For these reasons, the defensive withdrawal reflexes of the gill, siphon, and tail in the marine mollusk *Aplysia* have been studied extensively at the behavioral, cellular, and molecular level (Carew and Sahley 1986; Glanzman 1995).

The tail-elicited siphon withdrawal reflex (TSW) has been a useful preparation in which to study learning and memory in *Aplysia*. However, comparatively little is known about the neural circuitry that translates tail sensory input (via the P9 nerves to the pleural ganglion) to final reflex output by siphon motor neurons (MNs) in the abdominal ganglion. To address this question, we examined the functional architecture of the TSW circuit by selectively severing nerves of semi-intact preparations and recording either tail-evoked responses in the siphon MNs or measuring siphon withdrawal responses directly. We found that the neural circuit underlying TSW is functionally lateralized. We next tested whether the expression of learning in the TSW reflects the underlying circuit architecture and shows side-specificity. We tested behavioral and physiological correlates of three forms of learning: sensitization, habituation, and dishabituation. Consistent with the circuit architecture, we found that sensitization and habituation of TSW are expressed in a side-specific manner. Unexpectedly, we found that dishabituation was expressed bilaterally, suggesting that a modulatory pathway bridges the two (ipsilateral) input pathways of the circuit, but this path is only revealed for a specific form of learning, dishabituation. These results suggest that the effects of a descending modulatory signal are differentially “gated” during sensitization and dishabituation.

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possibility that network inhibition gates the contralateral modulation in this reflex circuit (Bristol et al. 2004).

Some of these results have been reported previously in abstract form (Bristol et al. 2000).

METHODS

Subjects

Adult Aplysia californica (100–300 g) were acquired commercially (Marinus, Long Beach, CA; Marine Specimens Unlimited, Long Beach, CA; Aplysia Resource Center, Coral Gables, FL) and housed individually in a 600-l aquarium with continuously circulating artificial sea water (ASW: Instant Ocean, Aquarium Systems, Mentor, OH) at ~15°C. Animals were fed dried seaweed twice weekly.

Experimental preparations

All animals were anesthetized with an injection of isotonic MgCl₂ into the body cavity.

SEMI-INTACT BEHAVIOR EXPERIMENTS. The reduced preparation used in behavioral experiments was similar to that used by Stopfer et al. (1996). Briefly, the tail and mantle were surgically removed along with the ring ganglia and abdominal, leaving the peripheral innervation of the tail (by both P9 nerves) and mantle (by siphon, branchial, and pericardial nerves) intact. To maintain normal turgor, the tail and mantle were pinned to the silicone-elastomer (Sylgard)-coated floor of separated chambers containing circulating tank seawater (15°C), whereas the ring ganglia were pinned ventral side up in a separate Sylgard-coated third chamber and perfused continuously at room temperature (20–22°C) with artificial seawater (ASW) containing (in mM) 460 NaCl, 55 MgCl₂, 11 CaCl₂, 10 KCl, and 10 Tris (pH 7.6). The P9 nerves and P-ACs exited the third chamber through small slits and sealed with petroleum jelly. Preparations were allowed ≥60 min to recover prior to testing.

SEMI-INTACT CELLULAR PHYSIOLOGY EXPERIMENTS. All peripheral nerves were cut except the P9 nerves, which innervate the tail. The CNS (abdominal ganglion and ring ganglia) and the tail were then excised from the animal and transferred to a recording dish coated with 4% paraformaldehyde to facilitate desheathing and to prevent contractions of the connective tissue in response to nerve stimulation. 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 slit in the separating barrier. The slit was then sealed using petroleum jelly to improve electrical isolation and to allow for independent manipulation of bath levels. The tail was pinned dorsal side up and cannulated for continuous perfusion of ASW 30 min prior to and throughout the experiment. In experiments examining lateralized learning, the tail was cut in half to ensure unilateral sensory input.

Procedures

BEHAVIORAL TESTING. In behavioral experiments, duration of siphon withdrawal was measured from stimulus onset to the start of siphon relaxation by a human observer. Siphon withdrawal was elicited by a water jet (0.5-s duration) to the dorsal tail (~1 cm from the...
tip), which elicited siphon withdrawal that typically lasted between 5 and 10 s in duration.

**ELECTROPHYSIOLOGY.** The left abdominal hemiganglion was desheathed in a 1:1 mixture of ASW and MgCl₂ to prevent synaptic transmission. Throughout the experiment, ganglia were continuously perfused with ASW at room temperature (20°C) at ~6 ml/min and illuminated from below through a dark field condenser. 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 MΩ) filled with 3 M KCl. Electrical potentials were amplified on a Getting 5-A (Getting Instruments, Iowa City, IA) or an Axoclamp 2B (Axon Instruments, Foster City, CA). Data analysis was conducted 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) and were hyperpolarized to ~70 mV to minimize action potential initiation during the experiment. LFS siphon MNs were used in this study, although LFS subtypes (head-directing, LFSa; tail-directing, LFSb) (Hickie and Walters 1995) were not distinguished. Thus occasional differences in MN responsiveness to tail stimulation could be due to the response properties of the different LFS MN subtypes. The tail was stimulated in two ways: using a stimulator-driven “tapper” (a blunted low-gauge needle attached to an electrical relay) to deliver a brief (50 ms) tactile taps to the dorsal tail or using mild electrical current (1–10 nA, 50 ms) passed through fine Teflon-coated silver wires implanted just under the dorsal skin. The location of the stimulus was systematically varied; in some experiments, a central location was used to recruit bilateral input equally. In other experiments, the stimulus was delivered to either the left or right side of the tail.

The area underneath the initial 500 ms of the evoked complex EPSP was integrated (ΔmV · s) for a quantitative measure of the net MN activation. This measure is sensitive to changes in both the amplitude and duration of the evoked cellular response. The 500-ms integration time was chosen because it adequately encompasses the duration of the evoked MN response. In many cases, the postsynaptic MN hyperpolarization was insufficient to completely prevent spiking during tail stimulation (likely due to the afferent input being too great to suppress all spikes). Therefore spikes were included in analyses of MN activation if they occurred within 500 ms of activation. Sensitization and habituation processes, which increase MN spiking, also increase the occurrence of afterhyperpolarizations (AHPs), which are much slower than spikes, thereby contributing more to the net PSP waveform. Thus the net effect of spikes coupled with their AHPs would decrease the overall activation waveform area. Therefore MN spiking would work against detecting increases in the evoked PSP measurements accompanying sensitization and dishabituation. Nonetheless, clear increases were consistently detected. In our studies, spike threshold was sufficiently high (given the continuous current clamp) and the effect of nerve shock sufficiently robust that the presence of action potentials in the MN activation traces did not contaminate our analyses of changes in the complex PSP.

**EXPERIMENTAL PROTOCOL.** In behavioral experiments, water jet stimuli to the central tail were delivered every 15 min. In physiology experiments, tail taps were administered once every 5 min. After pretests conducted with a fully intact nervous system, nerve cuts were performed manually using fine microscissors. Nerve cuts were performed in normal ASW. The cuts were made on either the left or right P9 nerves or the left or right pleural-abdominal connectives (P-AC), approximately half way along the length of the nerve. All data are expressed as percent change from precut baseline. In some experiments, the pedal-pedal commissure (Pd) or the cerebral-pleural/cerebral-pedal connectives (Cr) were cut. In all experiments, the cuts elicited a brief barrage of input into the MNs followed by a period of enhanced spontaneous input persisting for about two minutes. Electrophysiological control experiments were also conducted to rule out habituation to tail taps as an contributing factor to the decrease in the evoked responses observed after nerve cuts. In these experiments, when taps were administered at 5-min intervals but no cuts were made, no significant decrement in the tail tap-evoked MN response was observed (data not shown; F(4, 32) = 0.11, P = NS). A 15-min intertrial interval (ITI) does not produce habituation of semi-intact TSW (Sutton et al. 2001).

In experiments examining habituation, sensitization, and dishabituation, protocols were designed such that training and testing procedures could be directly compared across experiments. A qualitative comparison of the training protocols is shown in Fig. 1C. The timing of postraining tests (5 and 10 min in behavioral experiments; 2 and 7 min in physiology experiments) was chosen as the earliest possible times to obtain a response measure uncontaminated by shock-induced siphon contractions or P8 stimulation-induced activation of siphon MNs.

**HABITUATION TRAINING.** Habituation protocols were chosen for behavioral and physiological experiments that optimized response decrement lasting between 5 and 10 min. In behavioral experiments, stimuli were applied to the right or left side of the tail ~1 cm from the posterior tip of the tail (see Fig. 1). When testing both sides at a given time-point (i.e., pre- and posttests), tests on each side were separated by 30 s. Baseline TSW duration was the average of two pretests (ITI = 15 min). Ten minutes after the last pretest, habituation training was administered to one side with left and right side training counterbalanced across experiments. Habituation training consisted of five blocks of six stimuli with a 10-s IS1 and 1 min separating each block. Posttests were conducted at 5 and 10 min after the last habituation trial. Posttests were always given to the habituated side first, so that we could directly compare shocked and nonshocked preparations at the same time point (see next 2 sections).

In semi-intact physiology experiments, two pretests of MN responses elicited by left and right side tail stimulation were taken at 5-min ITI. Immediately after, one side was subject to habituation training which consisted of 10 taps delivered on a 10-s IS1. Posttests of evoked MN responses to stimulation of both sides were taken at 2 and 7 min after training. In previous studies, we have observed that the trials necessary to produce optimal physiological decrement and optimal behavioral habituation can differ (e.g., Stopfer and Carew 1996; Stopfer et al. 1996). The difference likely reflects the fact that synaptic decrement observed in a single motor neuron (in physiological experiments) provides a reasonable estimate, but not a perfect match, to the network decrement that ultimately produces behavioral habituation.

**SENSITIZATION TRAINING.** Behavioral experiments examining sensitization consisted of two pretests (15-min ITI) followed by a single tailshock administered to the lateral edge of the tail using a hand-held bipolar electrode. The shock (1.5-s duration, AC current) was applied 30 s after the last habituation trial through a hand-held electrode. The nominal current across the electrode was 100 mA, but much of this current is shunted by the seawater. Postshock tests of TSW duration were taken at 5 and 10 min after the shock. Experiments were conducted blind such that the behavioral observer did not know whether the animal was shocked (or on which side).

In semi-intact physiology experiments, two pretests of each side (5-min ITI) were followed by a sensitizing stimulus that was a 3-s shock to a P8 nerve (15 V, 5-ms pulses at 20 Hz), an analog of body wall shock. Body wall shock has been used previously to induce sensitization in *Aplysia* (e.g., Cleary et al. 1998). P8 was shocked using a suction electrode into which the cut end of the P8 nerve was drawn. Posttests of tap-evoked MN responses were taken 2 and 7 min after P8 shock.

**DISHABITUATION TRAINING.** Dishabituation experiments combined the protocols used in the habituation and sensitization experiments.
Following baseline TSW measures and subsequent habituation training, a single tail shock was delivered to either the habituated or nonhabituated side of the tail. Posttests were taken 5 and 10 min after the shock. In physiology experiments, a single P8 shock was administered to the nonhabituated side after habituation training. Posttests were taken at 2 and 7 min after P8 stimulation.

Data analysis

Summary data are presented graphically as means ± SE. Differences between means (percent change from the appropriate baseline) were analyzed using ANOVAs and paired t-test with an alpha level of 0.05 (2-tailed) adopted for all tests of statistical significance.

RESULTS

Like all opisthobranch mollusks, A. californica possess a bilaterally symmetrical nervous system (Kandel 1979). The tail is innervated by the distal processes of the two P9 nerves that emanate from the left and right pedal ganglia. The pedal ganglia are part of a network of interconnected ganglia, together with the pleural and cerebral ganglia, which collectively make up the circumsophogeal ring ganglia (see Fig. 1). Importantly, it is at the level of the ring ganglia where commissures are present that connect the left and right halves of the CNS. These include the pedal commissure and a potential path through the cerebral ganglion via the cerebral-pleural and cerebral-pedal connectives. With regard to the TSW, output from the ring ganglia projects to the abdominal ganglion via the two pleural-abdominal connectives (P-ACs). Thus information flow through the TSW circuit must pass from the tail via the P9 nerves to the ring ganglia, wherein an unknown amount of neural processing occurs, and then is transferred to the abdominal ganglion via the P-ACs.

Tactile sensory fields of the dorsal tail are lateralized

To determine the topographic organization of sensory input to the TSW circuit, we first investigated the receptive fields of single P9 nerves. Siphon MNs can be activated by input from either side of the tail, allowing a single MN to serve as a measure of left or right side tail stimulation. Using brief stimuli applied through wire electrodes implanted into the left and right sides of the tail (see METHODS), we assessed the evoked response in siphon MNs before and after a single P9 nerve was cut. As shown in Fig. 2, we found that cutting a P9 nerve abolished the response to ipsilateral tail stimulation (Fig. 2C; average precut MN response = 10.26 ± 6.68 ΔmV · s, average postcut MN response = 0.27 ± 0.72 ΔmV · s; mean change = −96.4%; t(8) = 3.83, P < 0.01). In contrast, the evoked MN response to stimulation of the side opposite of the cut P9 was not reduced, but slightly enhanced (Fig. 2C; average precut MN response = 18.50 ± 10.19 ΔmV · s, average postcut MN response = 21.30 ± 11.70 ΔmV · s; mean change = 12.1%; t(7) = 2.40, P < 0.05). This modest enhancement probably reflects activation of modulatory pathways during nerve transection. Examples showing experiments in which the left and right P9s were cut are shown in Fig. 2, A and B, respectively. It is unlikely that the absence of an evoked MN response after ipsilateral P9 transection was due to insufficient stimulation strength since a strong MN response was observed with contralateral side tail stimulation (the side with an intact P9). Thus any crossing projections from the intact P9 would likely have been activated. Collectively, these data suggest that the sensory processes derived from a single P9 nerve are restricted to the ipsilateral half of the tail. Similar results were obtained by Jahan-Parwar and Fredman (1978), who noted lateralized P9 sensory fields when probing the posterior foot. Thus sensory input into the TSW network is lateralized.

Nerve cuts reveal lateralized circuit architecture

We extended our analysis further downstream in the TSW circuit by asking whether the lateralized input is maintained at the level of the output of the ring ganglia to the abdominal ganglia via the P-ACs. Using the same lateralized input evoked by stimulating electrodes implanted in the sides of the tail, we tested whether cutting a single P-AC would also abolish input from the tail ipsilateral to the cut (as it did in the case of P9 cuts; Fig. 2). If so, then it would indicate that sensory input carried by a P9 is not transferred to the contralateral P-AC. Alternatively, if cutting a single P-AC did not abolish the MN response to same side tail stimulation, this would imply that a “crossing over” of tail sensory information occurs at the level of the ring ganglia. We found that after severance of a single P-AC, stimulation of the side of the tail ipsilateral to the cut (e.g., cut left P-AC and stimulate left half of the tail) no longer evoked input to the siphon MNs (Fig. 3; average precut MN response = 7.71 ± 6.65 ΔmV · s, average postcut MN response = 0.19 ± 0.33 ΔmV · s; mean change = −95.6%; t(6) = 3.00, P < 0.05). In contrast, when we stimulated the side contralateral to the cut P-AC, the response was not abolished, but was modestly enhanced (Fig. 3; average precut MN response = 7.86 ± 9.55 ΔmV · s, average postcut MN response = 12.52 ± 9.96 ΔmV · s; mean change = 112%, t(6) = 2.99, P < 0.05). Again, the enhancement was likely due to facilitatory modulation of TSW circuit elements after the nerve cut. Taken together, these data suggest that sensory processes emanating from the P9 nerves are largely restricted to the ipsilateral side of the tail and that the sensory information carried by a P9 nerve is conveyed to the P-AC on the same side and does not cross to the contralateral P-AC.

Next we confirmed and extended our results by combining behavioral measures, in which siphon contractions were recorded from semi-intact preparations that retained the siphon and gill, with electrophysiological measures of TSW output (see METHODS). In behavioral experiments, the tail was stimulated along the dorsal mid-line using a water pik stimulus, which recruited bilateral input. Because the vigorous water pik stimulus could not easily be used in electrophysiological experiments, a brief cutaneous tap to the medial tail was used (see METHODS). The cutaneous tap was chosen over the subcutaneous stimuli through implanted wire because the tap more closely corresponded with the stimulus used in behavioral experiments. Moreover, using a centrally located tail stimulus allowed us to test the extent to which sensory projections of P9 nerves are laterally restricted and further examine the circuit lateralization by testing unique predictions based on bilateral tail input. Thus in contrast to the previous experiments in which we used a lateralized tail input, in these experiments we employed a tail stimulus that we expected to recruit bilateral input. We predicted that if the TSW circuit was indeed lateralized, then bilateral input would require that cuts to the both sides of the circuit would be required to eliminate tap-evoked
MN responses. In contrast, cuts to only one side of the TSW circuit would reduce but not abolish tap-evoked responses.

First we examined the effects of cutting a single P9 nerve on TSW (Fig. 4A). We found that severing either the left or right P9 nerves reduced but did not abolish the TSW in response to central stimulation of the tail (mean change duration after left P9 cut = −12.2%; mean change duration after right P9 cut = −20.6%). Likewise, the tail-evoked complex PSP in siphon MNs persisted after single P9 cuts (mean change after left P9 cut = 14.72%; mean change after right P9 cut = −52.9%). Occasionally, cuts of the left P9 resulted in an enhancement of the tap-evoked MN response (4/8 experiments), resulting in an overall increase in the average percent change. In no cases did cuts of the right P9 result in an enhanced MN response. The reason for this discrepancy is unknown. Nevertheless, the persistence of the mid-line tap-evoked response indicates that the receptive fields of both P9 nerves extend across the dorsal mid-line and that a central tail stimulus recruited bilateral input. When we cut either the left or right P-AC, we found that both the TSW and the MN complex PSP evoked by central tail stimulation were reduced but not abolished (Fig. 4B; mean change in TSW duration after left P-AC cut = −59.08%, after right P-AC cut = −22.48%; mean change in MN complex PSP after left P-AC cut = −28.1%, after right P-AC cut = −33.2%). In earlier experiments, however, we did not observe a reduction in the tail-evoked MN response after P-AC cuts (see Fig. 3C). This difference can be accounted for by the difference in stimulation procedures and stimulus intensities used across the two experiments. The experiments in Fig. 3 used implanted silver wires, whereas the experiments depicted in Fig. 4 used the cutaneous tail tapper. Repeated stimulation with implanted silver wires at higher stimulus intensities has been shown to result in an incremental sensitization effect. Because the experiments in Fig. 3 were designed to examine whether cutting single P-ACs would abolish the response, we intentionally used a higher intensity stimulus (e.g., Fig. 2B) to adequately test this hypothesis.

As predicted, combined cuts of the P9s and P-ACs revealed a lateralized circuit architecture (Fig. 4, C and D). For example, cutting a P9 and the ipsilateral P-AC (e.g., right P9 and right
P-AC) reduced, but did not abolish, the TSW (Fig. 4C; mean change in TSW duration after left P9 and left P-AC cuts = −44.84%, after right P9 and right P-AC cuts = −31.38%) or the tail-elicited complex PSP in the MN (Fig. 4C; after left P9 and left P-AC cuts = −10.96%, after right P9 and right P-AC cuts = −25.35%). Note that the reduction after ipsilateral P9 and P-AC cuts is comparable to the reduction obtained after single cuts of P9 or P-AC alone (see Fig. 4, A and B). However, cutting a P9 and the contralateral P-AC (e.g., right P9 and left P-AC) completely eliminated the TSW in seven preparations (Fig. 4D). Similarly, the evoked MN response was eliminated in seven preparations (Fig. 4D). In the electrophysiological experiments, we confirmed that the elimination of the tail-evoked MN response was complete (i.e., could not be evoked in other MNs) by impaling several other siphon MNs in the same preparation. No tail-elicited response was observed in the sample of other MNs (range = 2–10 additional MNs tested per experiment; data not shown). Thus combined cuts of a P9 and the opposite side P-AC completely blocks bilateral tail sensory input to siphon MNs even though potential paths connecting the two sides of the nervous systems remained intact within the ring ganglia. This pattern of results can be accounted for by positing a lateralized circuit architecture, one in which tail input does not cross within the ring ganglia, that is accessed by bilateral stimulus input.

Consistent with this view, severing the pedal (Pd) commissure had no effect on TSW (mean change TSW duration = 2.5%) or on the evoked PSP in the MN (mean change in tail-elicited MN complex PSP = 4%). Likewise, cutting the connectives to the cerebral ganglion (Cr) had no effect (mean change TSW duration = −2.2%). Thus neither pathway appears to be utilized in the reflex circuit.

**Differential lateralization of sensitization and dishabituation**

The results of the previous experiments showed that the neural circuit underlying the TSW is lateralized, such that sensory input is processed ipsilaterally in the CNS. Therefore, we hypothesized that if the functional architecture of the TSW circuit constrains the form of TSW learning, then habituation, dishabituation, and sensitization of TSW should be expressed in a side-specific fashion. We tested these predictions both behaviorally and physiologically. As shown in Fig. 5A, the tail was cut in half in the following experiments to ensure lateralized input to the TSW circuit.

**SENSITIZATION.** We first examined sensitization of TSW. In behavioral experiments, we found that a single shock administered to the lateral edge of the tail produced a significant enhancement of TSW elicited by stimuli applied to the unshocked side [Fig. 5B; 2-way ANOVA, F(1,12) = 11.13, P < .05; 5-min test, t(6) = 4.48, P < 0.05; 10-min test, t(6) =
These data are consistent with previous reports that behavioral sensitization in *Aplysia* can be expressed in a side-specific fashion (e.g., Cleary et al. 1998). Similar results were obtained in physiological experiments. We found that stimulating a P8 nerve (which innervates the body wall) enhanced the evoked response to stimulation of the ipsilateral tail (Fig. 5 D; average percent change = ~ +350%), whereas the response to stimulating the tail contralateral to the shocked P8 was only modestly changed (mean percent change = ~ +140%); 2-way ANOVA, F(1, 24) = 4.72, P < 0.05; 2-min test, t(13) = 4.45, P < 0.05; 7-min test, t(121) = 20.73, P < 0.05). Figure 5 C depicts one experiment in which the MN response evoked by tapping the side of the tail ipsilateral to the stimulated P8 is considerably greater than the response than evoked by contralateral side tail tap. Thus in both behavioral and physiological experiments, sensitization showed a lateralized expression, consistent with the lateralized structure of the TSW circuit.

**HABITUATION.** We next examined habituation. Consistent with previous reports (Stopfer et al. 1996), we found that habituation of the TSW can be side-specific. Repeated stimulation of one side of the tail resulted in a significant reduction in TSW duration that did not generalize to the contralateral side [Fig. 6A; 2-way ANOVA, F(1,4) = 7.57, P = 0.05; 5-min test, t(4) = 10.40, P < 0.05]. Attenuated TSW was still observed 10 min after training, indicating that our habituation protocol produced a robust behavioral decrement (t(4) = 10.65, P < 0.05).

Physiological experiments also revealed that habituation can be lateralized: repeated stimulation of one half of the tail reduced the tap-evoked response in siphon MNs but did not affect MN responses evoked by taps to the other half of the tail [Fig. 6, B and C; 2-way ANOVA, F(1,7) = 9.96, P < 0.05; 2-min test, t(7) = 4.95, P < 0.05]. The response to taps of the trained side recovered gradually, returning to baseline level when measured 7 min after training (7-min test, t(7) = 0.82, NS). Together, these data indicate that the lateralized TSW circuit architecture yields side-specific habituation.

**DISHABITUATION.** Dishabituation and sensitization have been thought to be due to the same (or similar) underlying mechanisms (Groves and Thompson 1970; Kandel 1979). Thus our finding that the effects of tail shock (and P8 shock) are laterally restricted leads to the prediction that tail shock should dishabituate only ipsilateral, but not contralateral, tail responses. We tested this hypothesis by habituating the TSW and shocking either the trained or nontrained sides of the tail. When we...
shocked the side of the tail that had been habituated, we found that it exhibited an enhanced TSW that was significantly greater than the habituated baseline (Fig. 7A; mean change 5-min test = 26.01%; t(5) = 16.48, P < 0.05). In contrast, the contralateral, nonhabituated side showed no change (mean change 5-min test = -2.65; t(4) = 1.28, P > 0.05). Thus, consistent with our prediction, these data indicate that dishabitation is lateralized when the dishabituating stimulus is administered to the habituated side. Surprisingly, in preparations where we shocked the nonhabituated side, we found that both habituated and nonhabituated sides exhibited a significantly enhanced TSW (Fig. 7B; mean change habituated side at 5-min test = +30.23, t(5) = 3.16, P < 0.05; mean change nonhabituated side at 5-min test = +44.06%, t(5) = 16.22, P < 0.05).

FIG. 5. Sensitization of TSW is expressed ipsilateral to the shocked side. A: the terms “ipsi” and “contra” are relative to the shocked side (see METHODS). B: summary data from 7 behavioral experiments showing that sensitization of TSW is restricted to the side ipsilateral to the shock. C: sample traces from a single siphon MN in response to tail stimulation ipsilateral (top) and contralateral (bottom) to the shocked P8 nerve. P8 shock increased the MN response to ipsilateral tail stimulation only. D: summary from 14 physiology experiments showing that P8 stimulation enhancement the MN response to ipsilateral, but not contralateral, tail stimulation.

FIG. 6. Habituation of the TSW is side-specific. A: summary of 5 behavioral experiments showing that habituation of one side of the tail does not generalize to the contralateral side. B: sample recordings from a single siphon MN in response to stimulation of the left (top) and right (bottom) side of the tail. Note that the traces correspond to the time points indicated on the line graph in part C. Habituation of the right side of the tail did not generalize to the left side of the tail. C: summary of 8 physiology experiments showing that habituation of one side of the tail does not generalize to the other side of the tail.
Thus contrary to our predictions, TSW enhancement occurs bilaterally when the dishabituating stimulus is administered to the nonhabituated side. This counterintuitive result was confirmed physiologically. Shocking the P8 nerve on the side opposite to the habituated side enhanced the evoked MN response to both the habituated and nonhabituated side taps (Fig. 7, C and D; 2-min posttest, t(11) = 20.20, P < 0.05). Importantly, in both the behavioral and physiological experiments, the response magnitude at the first posttest was facilitated beyond that produced by recovery from habituation alone (compare with Fig. 6, A and C). These data suggest that TSW modulation produced by shock is not laterally restricted, as it appears in instances of ipsilateral sensitization, but is bilateral, as revealed by the potential of shock (or P8 stimulation) to dishabituate contralateral responses.

DISCUSSION

The purpose of this study was to examine the functional architecture of the neural circuit underlying the TSW reflex. To determine the overall organization of the circuit, we used reduced preparations that allowed us to make selective nerve cuts and thereby interrupt the path of sensory information flow throughout the CNS. We found that the TSW circuit is functionally lateralized. First, we found that the organization of sensory processes from the P9 nerves innervating the dorsal tail are largely confined to the ipsilateral side of the tail. Second, we found that sensory input carried by a tail nerve (P9) does not cross to the contralateral side of the CNS as it passes through the ring ganglia and P-AC to the siphon MNs. This conclusion is based on three observations. First, the response of siphon MNs to unilateral input (generated by stimulating one side of the tail) can be abolished by severing the ipsilateral but not the contralateral P-AC. Second, TSW and siphon MN responses evoked by bilateral input (generated by stimulating the mid-line of the tail) are abolished by severing a P9 and an opposite side P-AC but not by severing a P9 and a P-AC on the same side. Third, TSW and siphon MN responses are not affected by severing either the Pd commissure or Cr connectives. These results enable us construct of general functional model of the TSW circuit (Fig. 8B). In this model, the neural circuit underlying the TSW is lateralized, such that input entering via the P9 nerves does not cross to the contralateral P-ACs. Because MNs are excited by input from both sides of the tail surface, convergence of left and right tail sensory
information must occur within the abdominal ganglion, either at the level of the MNs or at upstream excitatory interneurons.

This study also investigated the consequences of the lateralized circuit architecture underlying the Aplysia TSW on three simple forms of learning: habituation, sensitization, and dishabituation. We asked whether the lateralized neural circuit would yield side-specific expression of these forms of learning. We found that repeated stimulation of one side of the tail resulted in a marked habituation in both behavioral and physiological measures of TSW. However, repeated stimulation of one side of the tail did not affect TSW elicited by stimulating the other side of the tail. Thus TSW habituation showed side-specificity. We also found that sensitization was side-specific; a shock to one side enhanced TSW measures when elicited on the same, but not the opposite, side. Surprisingly, we found that the same shock that produced unilateral sensitization, produced contralateral dishabituation; i.e., it produced enhancement if the side opposite of the shock had been previously habituated. Thus the nonhabituated side ipsilateral to the shock showed reflex enhancement (ipsilateral sensitization), as did the habituated side contralateral to the shock (contralateral dishabituation; Fig. 8A). The occurrence of contralateral dishabituation showed that unilateral shock can, in fact, give rise to bilateral effects. These findings suggest a crossing pathway linking the two sides of the TSW circuit. Figure 8B indicates three possible pathways: one across the cerebral connectives, one across the pedal commissure, and one within the abdominal ganglion.

**Organization of sensory input**

Our finding that the sensory fields of dorsal tail are lateralized is consistent with previous work on Aplysia. For example, Jahan-Parwar and Fredman (1978) found that the sensory fields of the ventral foot (caudal tail), also innervated by the P9 nerves, are lateralized. Correspondingly, the receptive fields of tail sensory neurons found in the pleural ganglia are limited to the ipsilateral tail surface (Walters et al. 1983b). Innervation of the anterior tentacles and the rhinophores of Aplysia by the cerebral nerves also show an ipsilateral organization; projections from the left n2, n3, n4, and n6 cerebral nerves innervate the left anterior tentacle, whereas the left n1 and n5 cerebral nerves innervate the left rhinophore and vice versa for the right side (Jahan-Parwar 1972).

Lateralized sensory fields have also been noted in other gastropod species. Kennekes (1994) retrogradely labeled sensory neurons innervating the lip nerves of the snail *Helix* and showed that only ipsilateral cells were labeled. The receptive fields of cerebral mechanoreceptors innervating the ventral oral veil of *Tritonia* and in *Pleurobranchaea* also show ipsilateral specificity (Audesirk and Audesirk 1980; Bicker et al. 1982). Thus although we did not undertake a systematic mapping of the dorsal tail sensory fields of individual P9 nerves, our data suggest P9 sensory fields are predominately lateralized. This is consistent with a wider body of evidence indicating a general topographical principle of lateralization in the sensory projections of bilateral nerves.

**Neural organization of discrete reflexes**

The TSW has a clear stimulus-evoked onset, is graded as a function of stimulus intensity, and has a duration that correlates with the duration of the eliciting stimulus, all of which are characteristic features of a discrete response reflex. Thus it is instructive to compare the inferred TSW circuit architecture we propose with that of other discrete response reflexes. The basic
neural circuitry mediating mammalian limb flexion and eyelid closure have been well-studied and are known to be lateralized, using the ipsilateral spinal cord and ipsilateral cranial nuclei, respectively (Sherrington 1906; Thompson 1988). However, in the case of the spinal crossed extension reflex, the basic lateralyzed flexion circuit is utilized to move the ipsilateral limb, but flexion of the contralateral limb is simultaneously inhibited by contralateral inhibition of MNs (Haines et al. 1997). Thus although there appears to be general principle of lateralization of discrete response reflexes across species with bilateral nervous systems, it is important to consider that contralateral inhibitory processing may not always be obvious when examining an evoked reflex response. For example, in Aplysia, behavioral evidence exists for specific changes in the direction of siphon withdrawal after noxious stimulation to the head or tail (Illich et al. 1994; Nolen and Johnson 2001; Walters and Erickson 1986). The inhibition of incompatible siphon movements is likely to occur within the abdominal ganglion circuitry, perhaps by inhibiting different classes of siphon MNs (Hickie and Walters 1995) or upstream excitatory interneurons (Bristol et al. 2001).

Lateralized learning

Several previous studies have noted lateralized expression of TSW learning in Aplysia. For example, Cleary et al. (1998) reported side-specific induction of behavioral sensitization with repeated shocks to one side of the animal as well as cellular biophysical and physiological changes in pedal and pleural ganglia ipsilateral to the trained side. Similar results were obtained by Scholz and Byrne (1987), who observed biophysical changes in tail SNs ipsilateral, but not contralateral, to the side that received an aversive shock. Finally, a study by Stopfer et al. (1996) showed that behavioral habituation of TSW could also be side-specific.

Lateralized learning has been examined in other preparations as well. In eyelblink conditioning in rabbits, lesions of the cerebellar cortex and/or interpositus nucleus abolish conditioned responses by the ipsilateral eye and prevents relearning with the ipsilateral eye but does not affect the contralateral eye (Lavond et al. 1985; McCormick and Thompson 1984; Thompson 1986; Thompson and Krupa 1994). This suggests that although the conditioned and unconditioned stimulus pathways cross the mid-line and back again (Thompson and Krupa 1994), neural structures crucial to behavioral memory are ipsilateral to the trained eye. In addition, Sandoz and Menzel (2001) recently examined the side-specificity of olfactory conditioning in honeybees. Using the proboscis-extension reflex as a behavioral measure, they found that olfactory learning could be side-specific or bilateral, depending on the training protocol. That is, even though certain conditions yielded lateralized learning, such as when the two sides were counter-conditioned (one side being trained to A+/B− and the other trained to A−/B+), the data suggested that bilateral operations underlie olfactory learning despite training being restricted to one side.

Our data are consistent with previous findings in Aplysia. Our finding that the underlying neural circuit is lateralized provides an anatomical basis for several reports of side-specific effects of TSW learning. Importantly, we extend previous work by reporting a novel aspect of TSW learning, the bilateral effects of lateral shock resulting in contralateral dishabituation. The findings that the same shock gives rise to unilateral sensitization in untrained preparations and bilateral enhancement in preparations shocked contralateral to the habituated side require a mechanism that can account for the effects of shock when superimposed on different learning states within the reflex pathway.

Mechanisms of TSW plasticity

In the cases of discrete reflexes, habituation is commonly thought to be due to synaptic decrement occurring within the reflex circuit (Bristol et al. 2003). Our data are consistent with this view and further suggest that the lateralized aspects of the TSW circuit make use of unshared elements. Moreover, Stopfer and Carew (1996) showed that habituation of TSW resulted in a facilitation of tail sensory neuron synaptic transmission onto follower cells, suggesting that synaptic decrement at interneuronal sites may be the primary underlying mechanism. This hypothesis contrasts with the observations of Hawkins and colleagues (e.g., Antonov et al. 1999; Cohen et al. 1997) that implicate decrement of primary siphon neurons in habituation of the siphon-elicited gill withdrawal. Thus the site(s) of decrement underlying habituation may be different in different reflex systems in Aplysia.

Our results examining sensitization and dishabitation, however, suggest that these forms of learning rely on additional neural components extrinsic to the reflex circuit. The biogenic amine serotonin (5-HT) has long been known to be involved in behavioral sensitization and to cause facilitation of sensorimotor synapses in Aplysia (see e.g., Byrne and Kandel 1996; Carew and Sahley 1986). As mentioned in the preceding text, several studies have noted lateralized effects of shock, suggesting that lateralized sensitization relies, at least in part, to cellular changes induced in the TSW pathway ipsilateral to the shocked side (Cleary et al. 1998; Scholz and Byrne 1987). Recent chronooamperometric recordings have shown that 5-HT release in the pleural and pedal ganglia evoked by tail nerve shock is also lateralized; that is, P9 shock (an analog of aversive cutaneous stimulation) resulted in 5-HT release predominantly in the ipsilateral pleural and pedal ganglia (Miresnesco and Carew 2002). Thus our present report of a lateralized circuit pathway, coupled with recent findings on patterns of 5-HT release, provide a plausible explanation for the observation of side-specific sensitization: it arises due to restricted 5-HT exposure to an ipsilateral circuit pathway. A more complete characterization of interneurons participating in the TSW is certainly needed to understand the mechanisms underlying reflex plasticity.

The mechanisms underlying contralateral dishabituation are as yet unclear. One possible model extends the role of 5-HT-induced facilitation of tail SN that underlies sensitization. Marcus et al. (1988) demonstrated that sensitization of siphon withdrawal reflexes in Aplysia required a higher intensity stimulus than did dishabituation. Ehrlich et al. (1992) found that depletion of SHT in the leech Hirudo abolished sensitization of the touch-elicted shortening reflex but only reduced dishabituation. These results can be interpreted as indicating that, at least for these withdrawal reflexes, sensitization has a higher threshold than does dishabituation, which fits nicely with previous work indicating that the threshold for 5-HT-induced synaptic facilitation is lower (and has different biochemical mechanism) for depressed synapses than for
nondepressed synapses (for a review, see Byrne and Kandel 1996; Emptage et al. 1996) and that relatively low levels of 5-HT are released in ganglia contralateral to the shocked side (Marinesco and Carew 2002). Collectively, these findings suggest that contralateral dis habitation could be due to a permissive state in which repeatedly activated SNs (due to habituation training), and perhaps interneurons downstream of the SNs, are more functionally sensitive to the effects of 5-HT exposure, given that modest 5-HT release contralateral to shock. The general question of the neural mechanisms contributing to the differential lateralization of sensitization and dishabitation is addressed in the companion paper that follows (Bristol et al. 2004).

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