Plasticity of Synaptic Connections in Sensory-Motor Pathways of the Adult Locust Flight System

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Wolf, Harald and Ansgar Büschges. Plasticity of synaptic connections in sensory-motor pathways of the adult locust flight system. J. Neurophysiol. 78: 1276–1284, 1997. We investigated possible roles of retrograde signals and competitive interactions in the lesion-induced reorganization of synaptic contacts in the locust CNS. Neuronal plasticity is elicited in the adult flight system by removal of afferents from the tegula, a mechanoreceptor organ at the base of the wing. We severed one hindwing organ and studied the resulting rearrangement of synaptic contacts between flight interneurons and afferent neurons from the remaining three tegulae (2 forewing, 1 hindwing). This was done by electric stimulation of afferents and intracellular recording from interneurons (and occasionally motoneurons). Two to three weeks after unilateral tegula lesion, connections between tegula afferents and flight interneurons were altered in the following way. 1) Axons from the forewing tegula on the operated side had established new synaptic contacts with metathoracic elevator interneurons. In addition, the amplitude of compound excitatory postsynaptic potentials elicited by electric stimulation was increased, indicating that a larger number of afferents connected to any given interneuron. 2) On the side contralateral to the lesion, connectivity between axons from the forewing tegula and elevator interneurons was decreased. 3) The efficacy of the (remaining) hindwing afferents appeared to be increased with regard to both synaptic transmission to interneurons and impact on flight motor pattern. 4) Flight motoneurons, which are normally restricted to the ipsilateral hemiganglion, sprouted across the ganglion midline after unilateral tegula removal and apparently established new synaptic contacts with tegula afferents on that side. The changes on the operated side are interpreted as occupation of synaptic space vacated on the interneurons by the severed hindwing target neurons. However, if the hindwing receptors are retracted, the forewing organs are able to take over their function by sprouting of the central arborizations of the involved interneurons. In addition, the amplitude of compound excitatory postsynaptic potentials elicited by electric stimulation was increased, indicating that a larger number of afferents connected to any given interneuron.

INTRODUCTION

Establishment of the proper synaptic connections is of central importance for the function of an animal’s nervous system and correct behavioral responses. This topic has long attracted the attention of neuroscientists. The resulting studies provided a detailed picture of the cellular mechanisms that guide the development of nervous systems and determine the formation of synaptic contacts (e.g., Jessel and Goodman 1996). Target competition among growing axons (e.g., Dan and Poo 1994) and retrograde signaling from target neurons (Murfhey and Davis 1994) have been shown to play a major role in nervous system development. The situation is different with regard to the synaptic reorganization that occurs in mature nervous systems in response to injury and concomitant (partial) deafferentation. A considerable body of information has been accumulated on functional aspects of behavioral and systems recovery (Cotman 1978) and contributing neuronal mechanisms (Aubert et al. 1995; Jelsma and Aguayo 1994; Schachner 1994), but relatively little is known about the cellular processes that govern the reestablishment and reorganization of synaptic contacts (Cotman 1978; Flohr 1988; Stein et al. 1974). This is mainly due to the complexity of the neuronal pathways studied so far, predominantly in vertebrates (e.g., Kaas 1991).

We addressed these questions in the locust flight system, which may offer the opportunity to examine the cellular mechanisms that underlie lesion-induced neuronal plasticity in the adult. Previous studies have demonstrated that lesion of functionally important afferents elicits pronounced changes in the synaptic contacts between the remaining afferents and interneurons of the flight control network (Büschtges et al. 1992a). These changes are accompanied by sprouting of the central arborizations of the involved interneurons. To study the cellular mechanisms that guide this reorganization of synaptic contacts, and in particular to assess the possible involvement of retrograde signaling and target competition, we examined the effects produced by selective, partial deafferentation of the adult locust flight system.

The afferent neurons that show the postlesion plasticity mentioned above are from the tegula, a mechanoreceptor at the base of the locust wing (Fig. 1). During flight, this receptor organ signals the downstroke movement of the wing to the CNS. Input from the pair of hindwing tegulae is essential for the generation of a functional flight motor command (Wolf 1993). The hindwing tegulae exert their influence through direct excitatory and disynaptic inhibitory connections with elevator and depressor interneurons of the flight control network, respectively (Pearson and Wolf 1988), most of which reside in the metathoracic ganglion. The two forewing tegulae are of little, if any, importance in flight pattern generation (Büschtges and Pearson 1991), a fact reflected by their sparse and variable synaptic contacts to flight interneurons. However, if the hindwing receptors are removed, the forewing organs are able to take over their function, leading to reestablishment of the normal flight motor pattern (Büschtges et al. 1992b). This is achieved through...
three populations of afferent neurons. This situation contrasts with systems possessing single pairs of sensory structures (cerci, tympanal organs, etc.) and may render the locust tegula particularly useful for studies on neural plasticity.

In the present study we observed that, after unilateral removal of a hindwing tegula, rearrangement of afferent synaptic connections occurs not only on the operated side of the animal but also in the contralateral hemiganglion. This implies the action of retrograde signals, considering the strictly ipsilateral projection of tegula afferents (Fig. 1D). This result represents the first evidence for retrograde signaling in neuronal plasticity of adult insects. The overall pattern of changes in afferent connections suggests that competitive interactions among tegula afferents regulate their contact with flight interneurons.

METHODS

Animals and preparation procedure

Locusta migratoria were taken from crowded breeding colonies established at the University of Konstanz and the University of Kaiserslautern. The tegula of one hindwing was severed in experimental animals after the imaginal moult. Animals were used when mature, that is, at ages of 14–21 days. Control animals were at the same age as the respective experimental locusts. The wings of a locust were unfolded, exposing the tegulae at all four wing bases, and one hindwing tegula was seized with a pair of fine forceps and excised from the soft cuticle of the wing hinge area with microsurgery scissors. Alternatively, the tegula was pinched off with a custom-made pair of forceps with spoon-shaped tips. The resulting wound was soon sealed by setting hemolymph, and the animals were marked with a color code and released into the breeding colony. Experiments were carried out no sooner than 2 wk after the operation.

Backfill of motor nerves

Legs, wings, and neck connectives of a locust were severed and the animal was opened by a dorsal midline incision. The nervous system was exposed by removing gut, trachea, fatty tissue, and ventral muscles overlying the nerve cord (details in Robertson and Fig. 1. Locust tegula: morphology and experimental arrangement. A–C: morphology and location of right hindwing tegula at successively lower magnifications. A and B: raster electron microscopic photographs of (A) tegula and (B) wing hinge area between forewing (fw) and hindwing (hw). C: drawing of flying locust with right pair of wings removed (wing stumps indicated in black). Arrows in B and C: right hindwing tegula. Upper margin of photograph in B: animal’s dorsal midline. Knob-shaped tegula comprises ~35 hair sensilla and a few more chordotonal receptors (Kutsch et al. 1980). D: drawing of locust thoracic CNS and experimental situation. Central nervous arborizations of the 3 unlesioned tegulae are indicated (metathoracic arborizations of left forewing receptor omitted). Arrows: stimulation sites on nerves 1C1. All parts of figure in dorsal view, except C, which shows dorsolateral view. Anterior is to right. meta, metathoracic ganglion; meso, mesothoracic ganglion; pro, prothoracic ganglion.

new synaptic connections between forewing tegulae and flight interneurons (Btschges et al. 1992a).

In studying the cellular mechanisms that produce these changes in synaptic connections in the adult locust, we took advantage of the fact that there are four wings, and tegulae, controlled by a common flight motor network (Robertson and Pearson 1985). We investigated the synaptic rearrangement elicited by removal of just one hindwing tegula. Possible interactions among the tegula afferents are not abolished after removal of a single receptor but continue among the remaining

sections were drawn and reconstructed into thick sections to examine the relationship of motoneuron arborizations to known anatomic landmarks in the metathoracic ganglion (Tyrer and Gregory 1982).

Recording and staining of identified interneurons; stimulation of tegula afferents

Locusts were opened and the ventral nerve cord was exposed as described above. The meta- and mesothoracic ganglia were supported on a stainless steel spoon to stabilize them for intracellular recording. Sometimes the peripheral nerves, except nerve 1, were severed to reduce movement of the animal. Saline according to Clements and May (1974) was used to keep the ganglia submerged.
Double hook electrodes in the bipolar configuration were placed under the three nerves 1C1 supplying the two forewing and the one remaining hindwing tegulae in operated locusts (Fig. 1). Four hook electrodes were used in control animals. The electrodes were insulated from the hemolymph with silicone grease. Before the intracellular penetration of central neurons was started, the threshold for activation of tegula afferents was adjusted for each of the electrodes with the use of the distal pair of hooks as stimulation electrodes and the proximal pair as recording electrodes. This arrangement allowed selective stimulation of the axons arising from the tegula (hindwing: Pearson and Wolf 1988; forewing: Büschges et al. 1992a). Stimulus voltages ranging from 1.0 to 1.3 times threshold were used to examine whether or not an interneuron received synaptic input from the afferents of one of the tegulae. Response amplitudes typically saturated at stimulus strengths of 1.3 times threshold; that intensity was thus used to assess lesion-related changes in the amplitudes of synaptic potentials (e.g., Fig. 2E).

Microelectrodes with tip resistances of 40–80 MΩ when filled with 5% Lucifer yellow in distilled water were used for intracellular recording. On completion of physiological experiments, interneurons were filled with Lucifer yellow if they received input from tegula afferents. Interneurons were identified according to their morphological characteristics under an epifluorescence microscope after the thoracic ganglia had been removed from the locust body, fixed, dehydrated, and cleared according to standard procedures (Robertson and Pearson 1982). Filled cells were always previously described flight interneurons (Pearson and Robertson 1987; Robertson and Pearson 1983–1985), although the criterion for dye injection had been the presence of tegula input.

**Determination of reset characteristics**

The thoracic nerve cord was accessed from the ventral side in semi-intact locusts suspended in front of a wind tunnel. The experimental procedure is described in detail by Wolf and Pearson (1987). Briefly, all six legs of the locust were severed and the animal was glued to a fork-shaped holder by the thoracic pleurae. A small flap of cuticle was removed above the metathoracic ganglion and the ganglion and the roots of peripheral nerves were exposed. Bipolar hook electrodes were placed on nerves 1C1 of the hindwing tegulae, or of the one remaining hindwing tegula in operated animals, and were insulated with silicone grease. Stimulus intensity was adjusted to activate the afferent axons from the tegula as described above, well below voltages that evoked twitches in metathoracic elevator muscles. Flight was elicited by stroking the animal’s abdomen or by activating the wind tunnel. During flight, tegula afferents were stimulated with bursts of 0.1-ms voltage pulses (4 or 5 pulses delivered at 200 Hz in each burst). Stimulus bursts were applied at rates of 2–3 Hz, thus occurring at random phases of the wingbeat cycle. Electromyograms recorded from one of the first basalar and tergesternal muscles (Snodgrass 1929) served as a monitors for the wingbeat rhythm (Pearson and Wolf 1987) and as the basis for quantitative evaluation.

The phase of the stimulus was determined in relation to the wingbeat rhythm before stimulus presentation on the basis of the three periods preceding the period of stimulation (Wolf and Pearson 1988). Phase values were sorted into bins of 0.1. This stimulus phase, or “old phase”, is plotted on the abscissa in Fig. 3B (Fig. 3Aa). Similarly, the phase of stimulus presentation was determined in relation to the wingbeat rhythm after the stimulus had been delivered on the basis of the three wingbeat periods following the stimulus period. This “new phase” is plotted on the ordinates in Fig. 3B (Fig. 3Ab).

**Data acquisition and evaluation**

Electrophysiological data were recorded on magnetic tape (digital audio tape recorder Biologic DTR 1800 and Racal Store 4DS) and displayed on a storage oscilloscope (Yokogawa OR2300 and Tektronix 5223) or a chart recorder (Gould ES 1000) for subsequent evaluation. Stimulus-triggered averaging (Fig. 2, B and C) was performed on a digital oscilloscope with averaging facility (Gould 1425). Central synaptic delays were calculated assuming a conduction velocity of 1.5 m/s for tegula afferents, a value repeatedly verified during the experiments. Student’s t-test and Fisher’s test were used for statistical analyses (Sachs 1978).

**RESULTS**

We tested the hypothesis put forward in the INTRODUCTION, namely, that retrograde signals and competitive interactions determine the pattern of lesion-induced plasticity in the adult locust flight system. This was done by removing the tegula organ of one hindwing (Fig. 1) in adult locusts with a matured flight motor system (see Kutsch 1974) ranging in age from 2 to 3 wk after the imaginal moult. Between 2 and 3 wk after that operation, the connectivity from the three remaining tegulae to metathoracic flight interneurons was examined and compared with the normal, intact situation.

We concentrated our analysis on elevator interneurons because these receive direct, that is, monosynaptic, excitatory input from the tegula afferents in the metathoracic ganglion (Pearson and Wolf 1988; Wolf and Pearson 1989). Moreover, elevator interneurons, and the elevator motoneurons ipsilateral to the respective tegula, are the only neurons known to receive monosynaptic input from the tegulae. The frequency of these monosynaptic connections differs with regard to hind- and forewing tegulae. The hindwing tegulae make direct contact with any given elevator interneuron in the metathorax. The forewing tegulae, by contrast, connect to only ~50% of metathoracic elevator interneurons. In the intact locust, all these connections are bilaterally symmetrical (that is, input from right and left tegulae is indistinguishable for both mirror-image individuals of an identified interneuron) (Büschges et al. 1992a).

**Connectivity of tegula afferents with flight interneurons on the side of tegula lesion**

Interneurons were connected to the forewing afferents ipsilateral to the lesioned tegula more frequently in operated animals than in intact locusts (sample recordings in Fig. 2, B and C; pooled data in Fig. 2D, compare black and hatched bars). On the average, the probability that a connection was observed (i.e., connectivity) was increased from 49% in control animals to 66% after removal of the ipsilateral hindwing organ (significant with \( P < 0.001 \)). Considering individual interneuron types, the increase could be as small as from 67% to 71% (interneuron 511) or as large as from 31% to 89% (interneuron 567). As a rule, the increase in connectivity was more pronounced in those interneurons that, in the intact animal, received input from the forewing tegula with lower frequency (compare interneurons 511, 515, 566, 567, and 513 in Fig. 2D).

Not only was connectivity between ipsilateral forewing afferents and interneurons increased, but so was the amplitude of the compound excitatory postsynaptic potentials (EPSPs) elicited in the interneurons by tegula nerve stimulation at saturating intensities (Fig. 2E). It was \( 3.34 \pm 0.68 \)
(SD) mV ($n = 18$), on the average, in locusts recovered from unilateral lesion of a hindwing tegula but only $1.65 \pm 1.1$ mV ($n = 16$) in intact controls (difference significant with $P < 0.001$; amplitudes of unitary synaptic events appeared unchanged) (Büsches et al. 1992a). As in the case of the all-or-none assessment of connectivity (Fig. 2D), the increase in EPSP amplitude varied among individual interneuron types. It was most pronounced in neuron types that received smaller EPSPs in the intact locust (compare interneurons 511, 515, 566, and 567 in Fig. 2E).

In depressor interneurons, stimulus-related inhibitory synaptic potentials were often elicited by stimulation of the forewing afferents ipsilateral to the site of lesion, whereas there was no response to contralateral stimulation (data not shown). In intact locusts, by contrast, input from the forewing tegulae is hardly ever recorded in depressor interneurons (Büsches et al. 1992a). Inhibitory postsynaptic responses in depressor interneurons are mediated via polysynaptic pathways, which involve the elevator interneurons (Pearson and Wolf 1988; Wolf and Pearson 1989).

Connectivity of tegula afferents with flight interneurons on the side contralateral to tegula lesion

Even more pronounced than the increase in connectivity on the ipsilateral side was a decrease in connectivity between contralateral forewing afferents and elevator interneurons (Fig. 2, B and C; Fig. 2D, black and open bars). On the average, forewing afferents decreased their connection to elevator interneurons from the 49% probability observed in intact locusts to 12% after lesion of the contralateral hindwing tegula (significant with $P < 0.01$). A decrease in connectivity was noted for all types of elevator interneurons, but it varied between 41% and 38% in interneuron 566 and between 31% and 0% in interneuron 567.

Connectivity of the remaining (contralateral) hindwing tegula was not changed in operated locusts, a result not too surprising in view of the fact that, in the intact situation, this receptor organ is connected to all known elevator interneurons with high efficacy (Pearson and Wolf 1988; Wolf and Pearson 1989). However, we observed an increase of the maximum EPSP amplitudes elicited in elevator interneurons from $5.3 \pm 2.6$ (SD) mV ($n = 28$) in controls to $7.4 \pm 2.4$ mV ($n = 13$) when stimulating the remaining hindwing afferents of operated individuals. Although of relatively low

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**Fig. 2.** Changes in connectivity of forewing tegula afferents after unilateral lesion of hindwing tegula. A: schematic diagram of experimental situation. Arrows: stimulation sites on nerves 1C1 of intact tegula organs. Heavy U-shaped structure represents bilateral dendritic arborizations of typical metathoracic flight interneuron (e.g., Pearson and Robertson 1987; Robertson and Pearson 1984); bilaterally symmetric somata and ascending axons indicate that connectivity data are similar for both mirror-image individuals of interneuron type (Pearson and Wolf 1988) (compare morphology of afferent projections in Fig. 1D). Removal of 1 hindwing tegula is indicated. B and C: sample recordings of synaptic potentials elicited in interneuron 515 by stimulation of tegula afferents in intact locust (B) and in animal with 1 hindwing tegula removed (C). Ipsil and contra refer to side of lesion (right side in A); these labels and scale bars are valid for both B and C. Note missing response and increased amplitude of excitatory postsynaptic potentials (EPSPs), respectively, when contra- and ipsilateral forewing afferents were stimulated in operated animal (C). Stimulus artifacts mark time of stimulation at respective nerve branches; between 15 and 25 responses were averaged for each trace. D: frequency of synaptic connections between forewing tegula afferents and elevator interneurons 511 ($n = 29/15$ recordings in intact/operated animals), 515 ($n = 14/13$), 566 ($n = 16/8$), 567 ($n = 13/9$), and 513 ($n = 6/2$). Frequency of synaptic contacts is compared for (intact) control animals (black columns), tegula contralateral to site of lesion ("contralesional" open columns), and tegula ipsilateral to severed hindwing receptor ("operated side," hatched columns). Averages for all 62 interneurons examined (85 interneurons in controls) are shown in right set of columns (altogether 7/15 interneurons of types 504, 510, and 514 add to numbers given above). E: amplitudes of compound EPSPs recorded at saturating stimulus intensities (Pearson and Wolf 1988) (ipsilateral forewing tegula afferents stimulated in nerve 1C1; see A and Fig. 1D) are compared in intact (open bars) and operated (hatched bars) locusts. All elevator interneurons received synaptic input with increased amplitude after operation. Altogether 18 interneurons were evaluated in intact locusts and 16 in operated animals, and $n = 8$ EPSP amplitudes were measured in each neuron. SDs are indicated for averages derived from all recordings.
statistical significance \( P < 0.02 \), this result is notable because it indicates a tendency for the hindwing afferents to signal to the flight interneurons with increased efficacy after the lesion.

A behavioral test for the efficacy of tegula input substantiated these electrophysiological data. In tethered, semi-intact locusts flying in front of a wind tunnel (Wolf and Pearson 1987), bilateral stimulation of the hindwing tegula afferents resets the wingbeat rhythm to the elevation phase (Fig. 3B, ○) (Wolf and Pearson 1988), an event related to the role of these receptor organs in the control of wing movement (Wolf 1993). This reset is also observed after complete elimination of sensory feedback from wing receptors (deafferentation, data not shown). Unilateral stimulation of hindwing afferents has little or no effect (Fig. 3B, Δ, \( n = 7 \) locusts) (see also Neumann 1985). In animals that had recovered from unilateral removal of a hindwing tegula, however, stimulation of the remaining hindwing receptor was always \( (n = 7) \) able to reset the wingbeat rhythm (Fig. 3B; ○; sample recording in Fig. 3A), even though the shape of the observed reset characteristic differed from that determined for bilateral stimulation in intact individuals. This demonstrates that, as a result of the lesion, the afferents from the remaining hindwing tegula had indeed increased their impact on the interneurons of the flight oscillator (and because elevator interneurons are the only known direct targets of the tegula afferents, this signifies an improved connection here).

Morphological changes associated with recovery from unilateral hindwing tegula lesion

Are morphological changes associated with the changes in synaptic contact between flight interneurons and tegula afferents? The sample morphologies shown in Fig. 4 illustrate that we did not observe any altered branching patterns of flight interneurons (Fig. 4A) or tegula afferents (Fig. 4B) on the level of analysis achieved with camera lucida drawings (drawings of interneurons in Fig. 4A show 3rd- and sometimes 4th-order neurites only, so as not to obscure the overall pattern of arborization). Although individual variability may mask minor modifications in the arborization patterns, it is clear that there are no major morphological changes like those reported by Büschges and coworkers (1992b) as a result of bilateral removal of the hindwing tegulae.

Unlike the situation in interneurons and afferents, sprouting was observed in metathoracic elevator motoneurons after unilateral removal of a hindwing tegula. This is illustrated in Fig. 5 for the motoneurons supplying the posterior tegocoxal muscle (muscle 119; Snodgrass 1929). In intact locusts, the arborizations of most flight motoneurons are restricted to the ipsilateral ganglion half (Fig. 5, left), and the dendrites of many neurons do not even reach the ganglion midline (Fig. 5A; motoneurons innervating the dorsal longitudinal muscles 81 and 112 are an exception to this rule) (Tyrer and Altman 1974). Two to three weeks after tegula lesion, motoneuron sprouts were observed to cross the ganglion midline in individual neurons marked with Lucifer yellow (Fig. 5Bi) as well as in backfill preparations showing the complete motoneuron supply of the posterior tegocoxal muscle (Fig. 5D). Sprouts occurred mainly in two areas: anteriorly, just posterior to the roots of nerve 1, and in medial positions, just anterior to the roots of nerve 3. Cross sections through these regions (Fig. 5, Eii and Fi) revealed that the two main sprouting areas correspond to dorsal commissures II and V, respectively (Tyrer and Gregory 1982). This is also the area of the metathoracic ganglion where the afferents of fore- and hindwing tegulae have their dorsal branches (Büschges et al. 1992a; Tyrer and Altman 1974).

In keeping with these anatomic data, synaptic potentials were recorded in elevator motoneurons in response to stimu-
lation of the afferents from all three remaining tegulae (Fig. 5Bi). Response latencies indicated that monosynaptic connections might exist between the posterior tergocoxal motoneuron and contralateral hindwing afferents. The central delays were just over 1 ms in both cases, but ∼4.5 ms when the contralateral forewing tegula was stimulated, indicating exclusively polysynaptic contacts in the latter case. These observations contrast with the situation in intact locust, where metathoracic flight motoneurons make (monosynaptic) connections only with the ipsilateral hindwing afferents (Pearson and Wolf 1988).

DISCUSSION

The changes in synaptic connectivity observed in the locust flight system after the removal of one hindwing tegula are summarized in Fig. 6. 1) Two weeks after the operation, connectivity between forewing afferents and elevator interneurons was significantly increased on the operated side and decreased on the contralateral side (Fig. 2, B±D). 2) Compound EPSPs elicited by stimulation of ipsilateral forewing afferents were enlarged in operated animals (Fig. 2E). An increase in the efficacy of afferents from the remaining hindwing tegula is indicated, first, by a 40% increase in the amplitude of compound EPSPs and, second, by altered reset characteristics (Fig. 3). Expansion of the number of hindwing afferents contacting the examined interneurons is the candidate mechanism for these changes. Together with the data from the forewing afferents listed above, this indicates not just that the presence or absence of input from a particular (forewing) tegula to an interneuron was affected by the operation but that the number of afferents contacting a given interneuron changed in parallel with the connectivity patterns. Figure 6 illustrates both the changes in the probability that a particular tegula makes contact to flight interneurons (1 synaptic terminal represents 10% connectivity) and the changes in EPSP amplitudes, presumably reflecting altered numbers of synapsing afferents (indicated by larger sizes of synaptic terminals). Finally, flight motoneurons on the operated side of the animal sprouted across the ganglion midline (Fig. 5) to establish new synaptic contacts with tegula afferents on that side (Fig. 5Bi).
Changes on the operated side of the locust nervous system; dynamic patterns of afferent synaptic connections

After removal of one hindwing tegula, the afferents from the ipsilateral forewing receptor establish new synaptic contacts with flight interneurons to which they had previously been connected only sparsely or not at all (Fig. 2D), and the amplitude of compound EPSPs elicited in the interneurons increases (Fig. 2, B, C, and E). If forewing afferents connect to flight interneurons in the metathoracic ganglion with ~50% probability, this does not mean that only 50% of the ~75 afferent axons originating in a tegula (Kutsch et al. 1980) make synaptic contact in the metathorax, nor does it imply that more afferent neurons grow into the posterior ganglion if connectivity increases to 70% after hindwing tegula lesion. Backfill preparations indeed suggest that the number of afferent fibers in the meso-metathoracic connective remains constant and that all forewing afferents enter the metathoracic ganglion in the intact locust. Thus a given afferent neuron must be assumed to change the number of synaptic contacts it makes with the pool of metathoracic elevator interneurons, which comprises ~40 identified cells. A connectivity value of 50% thus implies that, although every afferent contacts an interneuron, only about half of the interneurons receive input from any of the ~75 afferents from a particular tegula. This interpretation explains the changes in connectivity and the changes in EPSP amplitude, reflecting altered numbers of synapsing afferents, as the result of the same cellular mechanisms, that is, expansion of the number of synaptic contacts the individual afferent neurons maintain (or decreased number of synaptic contacts when considering the contralateral forewing, see next section).

It must remain open whether or not such changes in the size of the pool of postsynaptic neurons contacted by an afferent involve any specificity with regard to particular interneuron types. A shift of synaptic contacts from one population of interneurons to another, as observed in the cricket cercal system (Murphey and Lemere 1984), is unlikely in the present case because elevator motoneurons and interneurons are the only known targets of the tegula afferents.

The changes observed on the operated side of the locust are in perfect agreement with the results of bilateral hindwing tegula removal reported previously (Büsches et al. 1992a,b). In those experiments, both forewing tegulae established new synaptic contacts with flight interneurons and increased the size of the compound postsynaptic potentials they elicited. This resulted in functional replacement of the hindwing tegulae by the forewing organs and recovery of the flight pattern within ~2 wk of the operation. After both unilateral and bilateral hindwing tegula lesion, forewing afferents appear to colonize the synaptic space vacated on the interneurons by the severed hindwing axons.

Changes on the side of the locust nervous system contralateral to tegula lesion, and possible cellular mechanisms

The changes observed on the side contralateral to the lesioned hindwing tegula are more complex and appear the most interesting. Connectivity between forewing afferents and flight interneurons was decreased (Fig. 2D), whereas afferents from the remaining hindwing tegula elicited larger compound EPSPs in elevator interneurons and had a stronger impact on flight pattern generation (Fig. 3) after the operation. How are these changes on the side contralateral to the lesion mediated? It is significant that although all the flight interneurons branch extensively on both sides of the ganglion midline (Pearson and Robertson 1987; Robertson and Pearson 1983, 1984), afferent projections of the tegulae are strictly ipsilateral (Figs. 1D and 4Bi) (Tyrer and Altman 1974), and even several weeks after unilateral lesion of a hindwing tegula they show no indication of contralateral sprouts (Fig. 4Bi). The mere fact that changes occur on the side contralateral to the lesion thus suggests that retrograde signals from flight interneurons mediate the simultaneous decrease of forewing tegula connectivity and increase of hindwing tegula efficacy. Flight interneurons, rather than contralateral afferents, are immediately affected by the loss of afferent input or presynaptic contact sites after the operation. Their bilateral arborization pattern makes them candidate elements that signal across the ganglion midline and produce retrograde messengers that affect synaptic contact with the contralateral afferent terminals. Unspecific responses to injury are also ruled out by the fact that the observed changes are specific and concern only neurons of the flight system (see METHODS) (see Büsches et al. 1992a).

Action of retrograde signals, mostly of as yet unknown molecular identity, has been demonstrated in numerous invertebrate and vertebrate nervous systems (brief review in
Retrograde signals may be responsible for regulating a variety of presynaptic properties, such as terminal growth, transmitter release properties, or even choice of transmitter. The present data indicate that a retrograde signal activates the terminals of the tegula afferents on the contralateral side and mediates the observed redistribution of synaptic contacts and synaptic efficacy. It may indeed even be that same signal that mediates the changes in connectivity and postsynaptic potential amplitude observed with regard to the forewing afferents on the operated side.

In summary, opposite changes were observed with regard to the contralateral fore- and hindwing afferents after the operation. This is in accordance with the idea that tegula afferents compete for synaptic space on the elevator interneurons. The interpretation is supported by a large body of literature on the topic of competitive interactions (e.g., Shepherd and Murphey 1986; recent reviews in Dan and Poo 1994; Goodman and Shatz 1993; Murphey and Chiba 1989). On the lesioned side such competition would cause the forewing afferents to take over the synaptic sites vacated by the lesioned hindwing neurons, because they are the only tegula afferents present in that neuropil area. On the contralateral side, by contrast, competitive interaction of forewing and hindwing afferents would lead to increased dominance of the hindwing neurons, owing to their more numerous contacts and stronger influence on interneurons from the outset (Büschor et al. 1992a; Shatz 1995), which is in agreement with the experimental data.

Competition among tegula afferents may indeed explain the sparse and variable contacts between forewing afferents and metathoracic interneurons observed in normal animals. During development, hindwing afferents have the advantage of originating in a position much closer to the metathoracic target interneurons than the forewing afferents, which have to grow into an adjacent segmental ganglion. Hindwing afferents may already occupy much of the synaptic space on the interneurons at a time when the forewing afferents only just arrive (compare with Altman et al. 1978).

Sprouting of flight motoneurons

Lesion-induced central nervous sprouting of motoneurons has not been reported previously. The changes in central motoneuron morphology, and sometimes in synaptic connections, that have been studied in invertebrates, such as those that occur during insect metamorphosis (Truman and Reiss 1976; Weiss and Levine 1990; see also Pfüger et al. 1994 regarding hemimetabolous insects), were always associated with developmental rearrangement. The motoneuron sprouting (Fig. 5) observed in the present study conforms with the observation of apparently new synaptic contacts with the respective tegula afferents (Fig. 6).

The sprouting of flight motoneurons reported here is reminiscent of the sprouting of afferent neurons and interneurons observed in other insects after injury (e.g., Lakes-Harlan and Pfahler 1995; Schmitz 1989) or in the locust under different circumstances. In the locust, sprouting of both afferent and interneurons occurred after bilateral elimination of the hindwing tegulae (Büschor et al. 1992a) and altered the neurons’ connectivity patterns in a way similar to that reported here. The conspicuous lack of major morphological changes in the present study (Fig. 4) may be attributable to the smaller extent of the applied lesion, and particularly to the fact that one set of hindwing afferents was still present after injury and provided synaptic input to the flight interneurons. Along this line of argument, flight motoneurons, rather than interneurons, sprout after unilateral tegula removal because the ipsilateral motoneurons lose input from the hindwing tegula completely, because of the strictly ipsilateral projections of both afferent and motor neurons (Figs. 4, Bii and Biii, and 5, A and C). In the cricket cercal system, renowned for the initial demonstration of neural plasticity (Murphey 1986; Murphey and Lemere 1984) and retrograde signaling (Davis and Murphey 1994) in invertebrates, lesion-induced changes in the connectivity of cercal hair afferents occur without alteration in interneuronal arborization fields (review in Murphey and Chiba 1989). However, in this case there is extensive overlap of the arborizations of pre- and postsynaptic neurons from the outset.

The present results concern the topic of long-term stability of the adult locust flight circuitry and its maintenance. Considering the evident capacity for structural change, the mature flight motor control network appears as a state of equilibrium, not permanence. The connectivity patterns of sensory afferents seem to be under continuous control and always capable of adaptive modification. The same applies to the morphological properties and synaptic connections of interneurons (Büschor et al. 1992a,b) and motoneurons.

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