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Mechanosensory-induced behavioural gregarization in the desert locust *Schistocerca gregaria*

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Summary

Desert locusts show an extreme form of phenotypic plasticity, changing between a cryptic solitarious phase and a swarming gregarious phase that differ in many aspects of behaviour, physiology and appearance. Solitarious locusts show rapid behavioural phase change in response to tactile stimulation directed to the hind femora. Repeatedly touching as little as one quarter of the anterior (outer) surface area of a hind femur produced full behavioural gregarization within 4 h. Solitarious locusts have approximately 30% more mechanosensory trichoid sensilla on the hind femora than do gregarious locusts but have similar or fewer numbers of sensilla elsewhere on the legs. Tactile stimulation of a hind femur in solitarious locusts that had been restrained so that they could not move their legs failed to induce any behavioural gregarization. Patterned electrical stimulation of metathoracic nerve 5, which innervates the hind leg, however, produced full gregarization in restrained locusts. Our data show for the first time that the gregarizing signal combines both exteroceptive and proprioceptive components, which travel in both nerves 5B1 and 5B2, and provides us with a powerful experimental method with which to elicit and study neuronal plasticity in this system. Acetic acid odour, a strong chemosensory stimulus that activates the same local processing pathways as exteroceptive stimuli, failed to elicit behavioural gregarization, suggesting an early segregation in the central nervous system of the mechanosensory signals that leads to gregarization.

Key words: phenotypic plasticity, phase transition, exteroception, proprioception, solitary, solitarious, gregarious, grasshopper, *Schistocerca gregaria*.

Introduction

Locusts have the remarkable ability to change between two morphologically, physiologically and behaviourally distinct forms depending on their population density (Uvarov, 1966). This density-dependent phase polyphenism is an extreme form of variation common to many species that migrate, form seasonal herds or are otherwise subject to fluctuating population densities (Dingle, 1995). Locusts in the solitarious phase are cryptic in appearance and behaviour, occur at very low population densities and actively avoid each other. Conversely, locusts in the gregarious phase are brightly coloured as nymphs, more behaviourally active and aggregate together into bands, which may ultimately form migrating swarms consisting of millions of individuals (Uvarov, 1966; Simpson et al., 1999). The transition between the two extreme phases is a multistage process, with some changes in morphology and physiology taking several stadia or even generations to complete (Pener, 1991; Applebaum and Heifetz, 1999). By contrast, many behaviours change extremely rapidly. Solitarious locusts acquire most of the behavioural characteristics of gregarious phase locusts within only 4 h of crowding including, critically, the propensity to aggregate and thus reinforce the gregarizing stimuli provided by other locusts (Ellis, 1963; Gillett, 1973; Roessingh and Simpson, 1994; Bouaïchi et al., 1995). These rapid changes make locust phase change a potentially valuable tool in understanding neuronal plasticity and its behavioural consequences.

Olfactory and visual stimulation provided by other locusts promotes behavioural gregarization when presented together but has little effect individually. An extremely potent stimulus, however, is repeated physical contact produced by either touching or jostling, which elicits rapid and full behavioural gregarization in the absence of any other sensory stimuli (Roessingh et al., 1998; Hägele and Simpson, 2000). This mechanical stimulation must be directed to the middle or hind legs in order to have any effect, with the hind femur being by far the most effective site at which to elicit gregarization (Simpson et al., 2001). Here, we have analysed what constitutes an adequate gregarization-inducing

mechanosensory stimulus and developed a suitable preparation for future experimental investigations of processes in the central nervous system that drive or accompany phase change.

The five goals of the study were: (1) to determine how much of the surface of a hind femur needs to be mechanically stimulated in order to elicit behavioural gregarization; (2) to establish whether there is evidence for mechanosensory specialisation in the hind femur of solitarious locusts in terms of the number or distribution of mechanosensory hairs; (3) to develop a fixed preparation in which gregarization could be elicited in immobilised locusts, either through mechanical stimulation of a hind femur or electrical stimulation of hind leg nerves; (4) to analyse the relative roles of exteroceptive and proprioceptive signals carried in different leg nerves in eliciting gregarization; and (5) to determine whether gregarization is mediated *via* spiking local interneurones that receive convergent mechanosensory and chemosensory inputs from the hind leg (Newland, 1999) or through a separate mechanosensory pathway. To do this we used a chemosensory stimulus, acetic acid odour, that strongly activates chemosensory neurones without activating mechanoreceptors (Newland, 1998).

Materials and methods

Insects

All locusts (*Schistocerca gregaria* Forskål) originated from a colony maintained at the Department of Zoology, University of Oxford, UK reared under crowded conditions (500–1000 insects per 56 cm×76 cm×60 cm rearing bin). Solitarious phase locusts were derived from this colony but had been reared under physical, visual and olfactory isolation from other locusts for two or three generations as described in Roessingh et al. (1993). Unless otherwise stated, all experiments used final-instar nymphs.

Assaying behavioural phase state

The behavioural phase state of locusts was measured using an observation arena (41 cm×30 cm), flanked by two 7.5 cm×30 cm backlit chambers separated from the main arena by perforated clear plastic partitions. A group of 20 gregarious-phase final-instar nymphs was placed in one chamber, while the other chamber was left empty. An experimental locust was introduced into the arena *via* a central hole in its floor and the animal's behaviour recorded for the following 500 s using an event recorder in real time (Roessingh et al., 1993; Simpson et al., 1999, 2001). These data were analysed using the same statistical multiple logistic regression model in SPSS (version 10.0.07) as developed by Simpson et al. (2001), based on observations of 96 gregarious and 96 solitarious final-instar nymphs. A logistic algorithm was used to make an optimally fitting model, which correctly classified 92.7% and 91.7% of gregarious and solitarious locusts, respectively. This algorithm produced an index of solitariousness, termed P_{sol}, which is the probability that an assayed locust belonged to the solitarious model group. This provided an indicator of behavioural phase state, ranging from 1 (for locusts indistinguishable from the solitarious model group) to 0 (when locusts behaved fully like the gregarious model group). In the analyses, locusts with P_{sol} values of less than 0.2 were judged to have become fully behaviourally gregarious. The P_{sol} distributions obtained for each experiment were normalised rank transformed to make the data suitable for parametric analyses to compare the effects of different experimental treatments on phase state.

Localisation of sites sensitive to mechanosensory-induced gregarization

A hind femur was stroked in locusts in which different regions on the anterior surface had been coated with a water-based poster paint (Winsor and Newton, Harrow, UK), leaving only one region with mechanosensory hairs free to move. Ninety-six locusts isolated for three generations were divided between six treatments in which half or three-quarters of the femur was painted (Fig. 1) and two control treatments in which the whole femur was painted and either left unstimulated or stroked as in the other treatments. The painted femora were stroked for approximately 5 s once every 60 s for 4 h using a small paintbrush as described in Simpson et al. (2001). At the end of the 4 h stimulation period, behavioural phase state was assayed.

Counts of mechanoreceptors on the legs

The anterior faces of detached legs were drawn at 10× magnification using a *camera lucida* attached to a dissecting microscope, with the location of each tactile hair mechanoreceptor (60–780 μm long; Newland, 1991) marked on the drawing. The numbers of basiconic (bimodal chemosensory and mechanosensory) sensilla on the anterior distal hind femur were also counted, but as these are small (less than 40 μm long) a cast was made of the surface of the femur using transparent nail polish, which made a clear impression of the cuticle and all the sensilla. The casts were viewed and drawn under transmitted light at 100× magnification with the aid of a *camera lucida*.

Mechanical stimulation of restrained locusts

Locusts were immobilised ventral side uppermost, with all legs fixed out laterally but with the anterior surface of the hind femur directed upwards, and given the same mechanosensory stimulation regime as the free-moving animals described above.

Gregarization elicited by electrical stimulation of leg nerves

Locusts were restrained in modelling clay ventral side uppermost as described above. Stimulating electrodes, made from a pair of 50 μm coated steel wires, delivered 5 s of pulses once every 60 s for 4 h. Each 5 s burst consisted of 10 ms square pulses repeated at 50 Hz organised into 200 ms trains separated by 200 ms intervals. This electrical stimulation pattern was designed to simulate the temporal sequence of stimulation produced by stroking a femur with a paintbrush. The stimulating electrodes were placed within either the thorax or the hind femur according to treatment. In each location, a

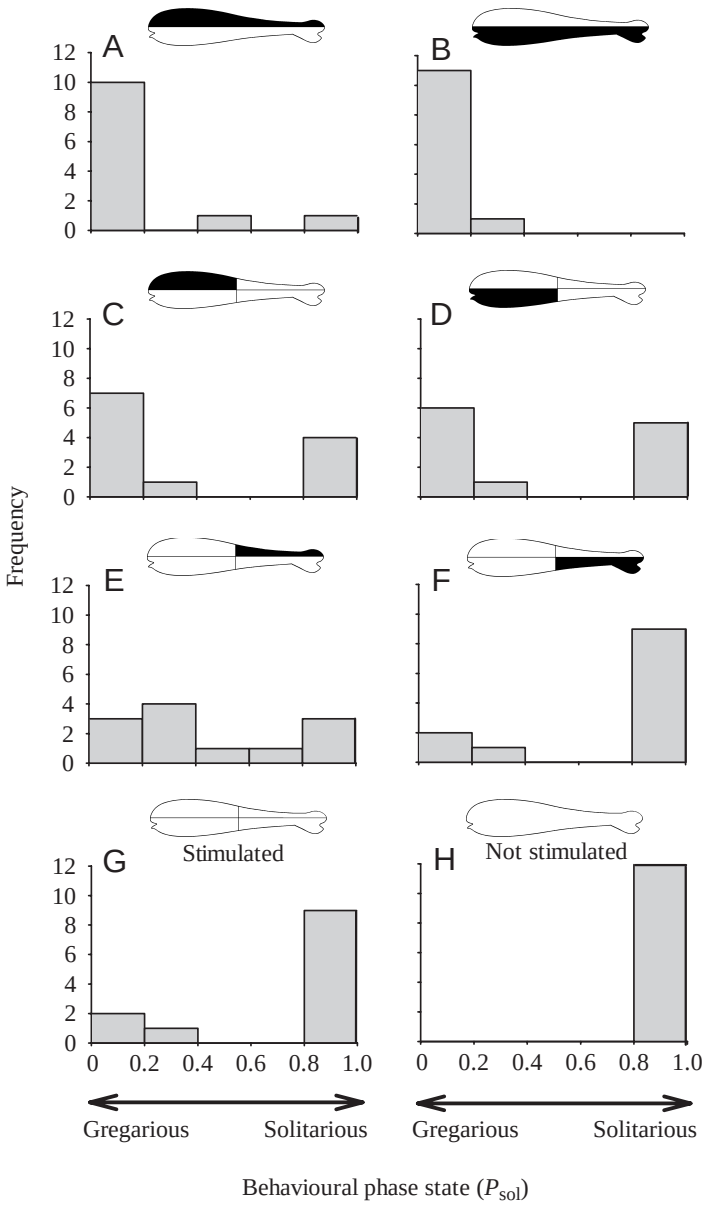

Fig. 1. Mechanosensory stimulation directed to just part of the hind femur elicited rapid behavioural gregarization. (A–F) Histograms showing the frequencies of locusts falling into different categories of behavioural phase state after 4 h of mechanosensory stimulation directed towards the regions indicated in black on a hind femur. The white areas were painted over to prevent the activation of mechanoreceptive hairs. (G,H) In two control treatments, a hind femur was entirely painted over and either stimulated as in the other treatments or left unstimulated. N=12 locusts per treatment.

flap was cut in the cuticle and overlying air sacs and trachea were removed or displaced to reveal the selected nerve. The bare tips of the stimulating electrodes were carefully wrapped around the nerve and insulated with a 10:1 petroleum jelly: paraffin oil mix. Nerve 5B1 in the femur was identified by the branches it made onto the extensor tibiae muscle and by the activity of the femoral chordotonal organ (Usherwood et al., 1968), monitored using an AC amplifier and oscilloscope. Nerve 5B2 was identified by the branches it made onto the flexor tibiae muscle (Heitler and Burrows, 1977). The cuticle was replaced as far as possible and the wounds sealed with more petroleum jelly. In one set of experiments, the extensor tibiae muscle was stimulated directly by electrodes inserted into the muscle through the cuticle. The voltage was adjusted so that it just elicited leg movements. Protracted electrical stimulation often led to leg autotomization, but the nerves remained undamaged provided that the hind coxa was immobilised. After 4 h of stimulation, the electrodes were carefully removed and the locusts were rested for 10 min before being assayed for behavioural phase state. Controls where made in which the electrodes were placed but no current was passed. In all experiments, lost haemolymph was replaced with locust saline as necessary.

Effect on gregarization of cutting selected leg nerves

The thorax was cut open and nerve 5A, 5B or the whole of nerve 5 cut using fine iridectomy scissors. The cuticle was resealed using beeswax and, after a 10 min recovery period, the unrestrained locusts were placed in experimental boxes and a hind femur stimulated with a paintbrush for 4 h as described above.

Gregarization by strong chemosensory stimulation of leg receptors

Locusts were placed in boxes under a fume hood and stimulated at 1 min intervals for 4 h with one of the following treatments: 5 cm^3 of the vapour taken from the headspace of a bottle of glacial acetic acid, which was slowly delivered over a hind femur for 5 s using a 5 ml syringe; a 10% dilution of the acetic acid vapour mixed with air; 5 cm^3 of air; stroking a hind femur with the fibres and ferrule of a fine paintbrush mounted on a 5 ml syringe containing 5 cm^3 of acetic acid vapour that was simultaneously discharged through the brush whilst stroking; or just stroking a hind femur with a brush. After stimulation, the locusts were removed and their behavioural phase state tested.

Results

Localisation of sites effective in eliciting gregarization

Mechanical stimulation of different regions on the anterior surface of the femur produced behavioural gregarization in the majority of locusts after 4 h (Figs 1, 2). An analysis of variance (ANOVA) indicated significant differences between the treatment and control groups ($F_{7,88}$=10.55, P<0.001; Fig. 2). The control locusts in which the whole femur was painted and then left unstimulated remained highly solitarious (median P_{sol}=0.99, which was comparable with the model solitarious group; Fig. 1H). The paint formed an effective barrier to the mechanical stimulation of tactile hairs, as most locusts remained solitarious when the entire hind femur was painted and then stroked (median P_{sol}=0.90; not

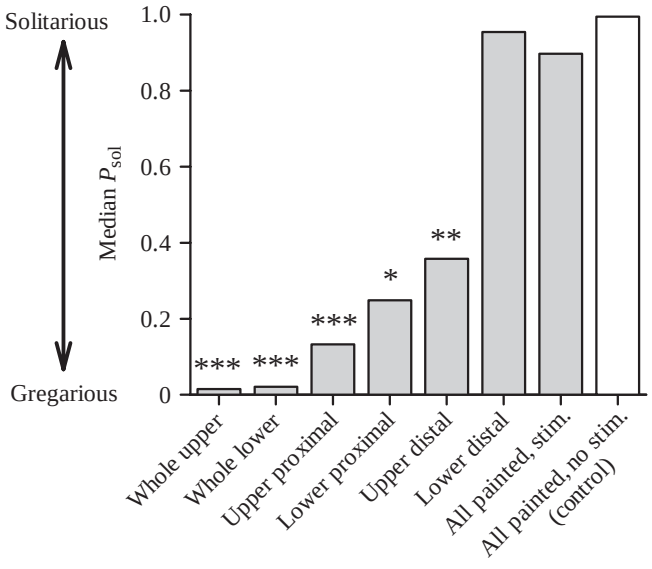

Fig. 2. Median P_{sol} values of the data shown in Fig. 1, obtained after locusts were mechanically stimulated on different regions of the femur as indicated on the *x*-axis. Values close to 0 indicate that the locusts behaved in a gregarious manner in the assay; values near to 1 indicate that they remained solitarious. Asterisks indicate degree of significance of Dunnet's two-tailed *post-hoc* tests against the unstimulated controls (white bar): *, $P<0.05$; **, $P<0.01$; ***$P<0.001$.

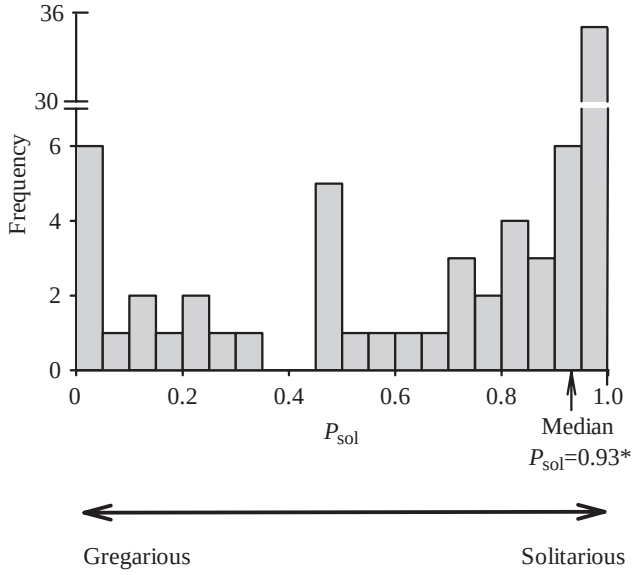

Fig. 3. Mechanical stimulation of a hind femur of locusts unable to move their legs produced only a small change in behavioural phase state. Distribution of P_{sol} values obtained after 4 h of mechanical stimulation in restrained locusts (N=77). 45% of restrained locusts remained highly solitarious ($P_{sol}>0.95$) after mechanical stimulation, but more animals showed some increased gregariousness compared with unstimulated controls. The asterisk indicates the degree of significance (*t*-test) of the rank normalised data against the controls in the previous experiment. *, $P<0.05$.

significantly different from the painted and unstimulated controls; Figs 1G, 2). Conversely, strong behavioural gregarization was elicited by stimulating free mechanoreceptors on either just the upper or lower halves of the femoral surface [median $P_{sol}<0.025$ (Fig. 2), with 83–92% of the experimental locusts having $P_{sol}<0.2$ (Fig. 1A,B)]. Stimulation of a smaller region, one-quarter of the femoral surface, also produced significant behavioural gregarization for three of the four tested regions (Figs 1C–E, 2) but was not as strong a stimulus: a greater proportion of locusts failed to show gregarious behaviour; fewer locusts (<60%) had $P_{sol}<0.2$ (Fig. 1), and median P_{sol} values were consequently higher (0.1–0.36; Fig. 2). The lower distal quadrant alone stood out as a wholly ineffective site for tactile gregarization (Figs 1F, 2).

Mechanical stimulation of restrained locusts

Mechanical stimulation of the hind femur in locusts that were unable to move failed to elicit behavioural gregarization after 4 h (Fig. 3). There was only a small, though significant, change in P_{sol} values in immobilised stroked locusts, where the median P_{sol} value was 0.93, compared with 0.98 for the control treatments in the painted leg experiments described above (*t*-test; equal variance not assumed; d.f.=32.04, N=101, $P<0.05$). P_{sol} values of <0.2, consistent with fully gregarious behaviour, were found in only 13% of stroked restrained locusts.

Counts of mechanoreceptors on the legs

Solitarious final-instar nymphs had 29% more sensilla on the anterior (outward) surface of a hind femur than did gregarious nymphs (mean ± S.E.M., 135±6.1 compared with 105±5.4; Fig. 4; significant interaction term of ANOVA shown in Table 1). Both phases had similar numbers of tactile hairs on the hind tibiae and tarsi as well as on all the segments of the front and middle legs (Fig. 4A). Adult solitarious locusts had 26% more tactile hairs on their hind femora than did gregarious adults (177±4.6 and 140±6.3 sensilla, respectively; Fig. 5A; significant interaction term of ANOVA shown in Table 1). Adult solitarious locusts had significantly fewer tactile hairs on the hind tarsus, front femur and front tarsus than gregarious phase adults (Fig. 5A), in contrast to final-instar nymphs.

Table 1. *Results of analysis of variance of the number of tactile hair sensilla on the legs of solitarious and gregarious phase locusts*

	Nymphs		Adults	
Factor	d.f.	*F*	d.f.	*F*
Leg region	8	146.12*	5	451.58*
Phase	1	2.57	1	3.15
Phase × leg region	8	4.74*	5	12.48*
Error	161		96	

Sensilla were counted on the outward faces of a femur, tibia and tarsus of a front, middle and hind leg of final-instar locusts and on a femur, tibia and tarsus of a front and hind leg of adult locusts.

*Significant at $P<0.001$.

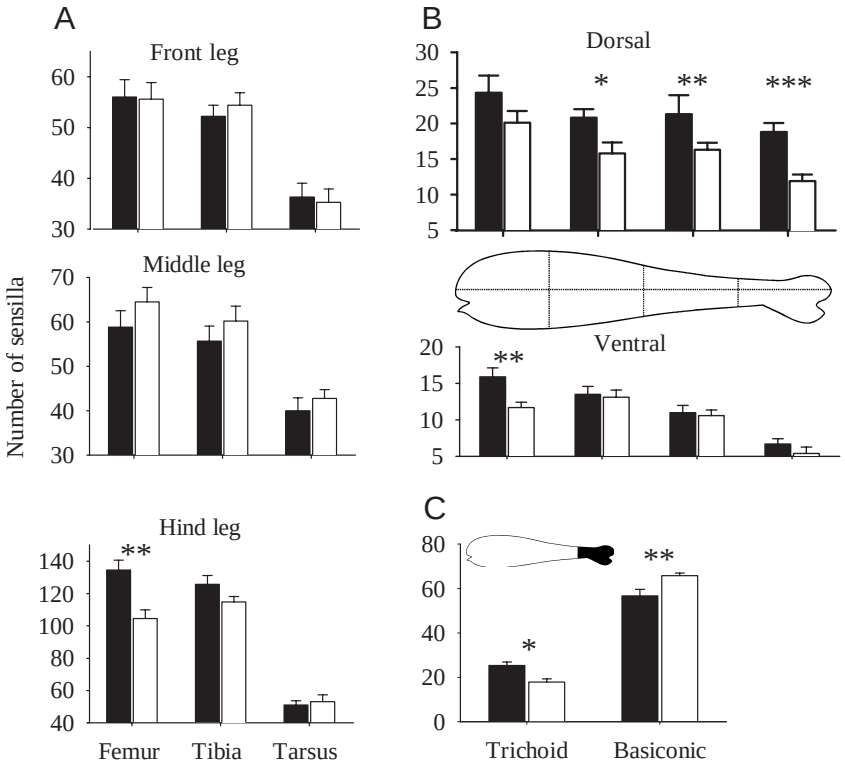

Fig. 4. Numbers of tactile hairs and basiconic sensilla on the hind femur of final-instar nymphs differed between phases. (A) Numbers of tactile hairs on the outward faces (viewed laterally) of the front, middle and hind legs. Solitarious locusts (black bars) had significantly more tactile hairs on the hind femur than did gregarious locusts (white bars). (B) These additional tactile hairs occurred on the dorsal distal and most ventral proximal regions of the anterior face of the hind femur. (C) On the distal anterior hind femur (black region of inset), solitarious locusts had significantly fewer basiconic sensilla but significantly more tactile hairs than did gregarious locusts. Significance (*t*-test) is indicated by asterisks: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; $N=10$ locusts per group throughout.

Solitarious adult locusts also had fewer sensilla on the front tibiae, but this was marginally non-significant (Fig. 5A). Adult locusts of both phases had similar numbers of sensilla only on the hind tibiae.

The hind femora of solitarious locusts are proportionately longer than those of gregarious locusts (Dirsh, 1953), but this was not significantly correlated with the number of sensilla in either nymphs or adults [General Linear Model (GLM) analyses using femur length or length^2 (proportionate to area) as covariate; data not shown].

The additional sensilla of solitarious locusts were not evenly distributed over the hind femora (Figs 4B, 5B,C). There were 60% more sensilla on the most distal dorsal region and 36% more sensilla on the most proximal ventral region, but similar numbers on the distal ventral and proximal dorsal surfaces in final-instar nymphs (*post-hoc t*-tests; significance as in Fig. 4B). A similar distribution of sensilla was found on the hind femora of adults (Fig. 5B). Most hairs were located on the longitudinal ridges along the dorsal, latero-dorsal, latero-ventral and ventral surfaces of the femur and thus formed dense

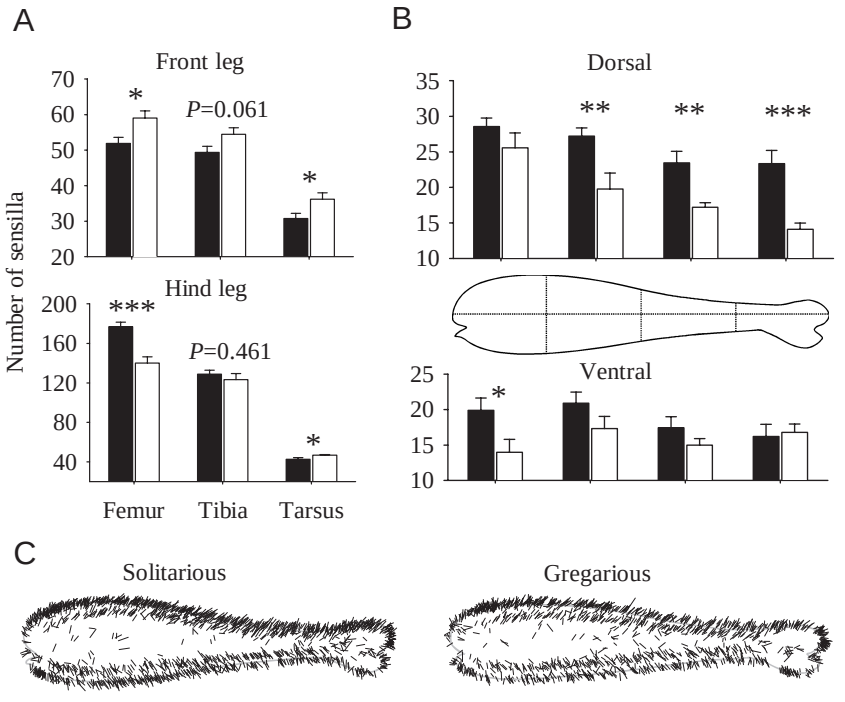

Fig. 5. Numbers of tactile hairs on the front and hind legs of adult locusts differed between phases. (A) Numbers of tactile hairs on the front and hind legs. Solitarious locusts had fewer sensilla on the front leg and on the hind tarsus but had more sensilla on the hind femur than did gregarious locusts. (B) The distribution of tactile hairs on the hind femur was similar to that of final-instar nymphs (see Fig. 4B). (C) Maps showing the positions of the hairs from nine individuals overlaid onto averaged femur outlines; most hairs were confined to the longitudinal ridges on the femur. Significance (*t*-test) is indicated by asterisks: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; $N=9$ locusts per group throughout.

aggregations in solitarious locusts (Fig. 5C shows locations of hairs in adults). Solitarious locusts had significantly fewer bimodal basiconic sensilla on the distal femur than did gregarious locusts (57±2.9 and 66±1.1 sensilla, respectively; Fig. 4C).

Gregarization elicited by electrical stimulation of leg nerves in immobilised locusts

Electrical stimulation of the whole of metathoracic nerve 5 within the thoracic cavity produced full gregarization in most immobilized locusts (Fig. 6A; median P_{sol}=0.13, with 62% of assayed locusts having P_{sol}<0.2). Electrical stimulation of nerve 5A alone was wholly ineffective at eliciting gregarization (Fig. 6B; median P_{sol}=0.98). While stimulation of nerve 5B (Fig. 6C) elicited some change in phase state, it was less effective than stimulating the whole of nerve 5 (median P_{sol}=0.51, with just 37% of locusts having P_{sol}<0.2). Sham-operated controls, in which stimulating wires were placed around nerve 5 in the thorax but no current passed, had a median P_{sol} of 0.86, and only 0.6% of control locusts had a P_{sol}<0.2 (Fig. 6H). An ANOVA of all the thoracic nerve 5-stimulated data and sham-operated controls indicated a significant effect of treatment (normalized ranked data, $F_{3,54}$=4.56, P=0.006; N values for each group and the results of Dunnet's two-tailed *post-hoc* tests against the control group are given in Fig. 6).

Stimulating both branches of nerve 5B together in the femur (Fig. 6D,J) produced a similar degree of gregarization to that produced by stimulating nerve 5B within the thorax (median P_{sol}=0.66; Fig. 6D), but stimulating either nerve 5B1 (Fig. 6E)

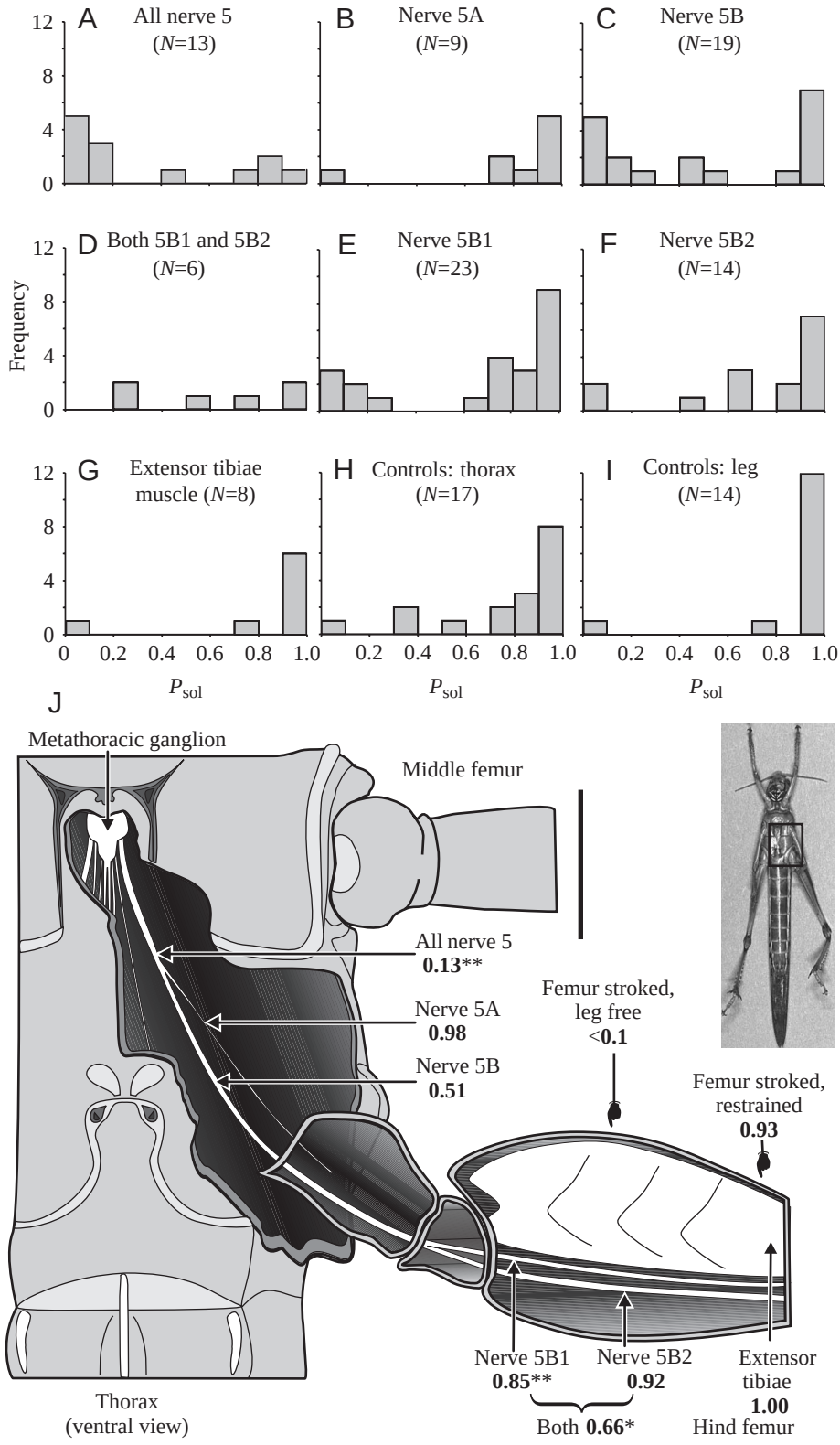

Fig. 6. Patterned electrical stimulation of metathoracic nerve 5 can elicit behavioural gregarization. (A–I) Histograms showing the distributions of P_{sol} values obtained after immobilised locusts were electrically stimulated for 4 h by electrodes attached to the indicated branches of metathoracic nerve 5 or the extensor tibiae muscle. (J) Ventral view of the thorax and proximal hind leg dissected to show the metathoracic ganglion, nerve 5 and its principal branches and the extensor tibiae muscle. Values in bold are median P_{sol} values obtained after electrical stimulation at each location. Nerve 5A runs between two muscles in the coxa, hence its apparent sudden termination. Significance (Dunnet's *post-hoc* tests of ANOVA) is indicated by asterisks: *, P<0.05; **, P<0.01; ***, P<0.001. Scale bar, 2 mm.

or nerve 5B2 (Fig. 6F) alone was much less effective. Stimulation of nerve 5B1 alone produced a small significant behavioural change, but the median P_{sol} was still 0.85 compared with a median value of 1.00 for sham-operated femoral controls (Fig. 6E,I,J), whereas electrical stimulation of nerve 5B2 (Fig. 6F) or of the extensor tibiae muscle alone (Fig. 6G) was entirely ineffective at eliciting any behavioural change. The small change in behavioural phase state produced by stimulation of nerve 5B1 was similar to the effect seen when femora of restrained animals were stimulated mechanically. An ANOVA of the normalized ranked data for animals stimulated in the femur indicated a significant effect of the different stimulation regimes on phase state ($F_{4,61}$=4.02, P=0.006; N values for each group and the results of Dunnet's two-tailed *post-hoc* tests against the sham-operated control group are given in Fig. 6). Autotomization of the hind leg was a common occurrence when nerves either in the leg or thorax were repeatedly stimulated. We could see no relationship between the incidence of autotomization and change in behavioural phase state, which depended solely on the nerve stimulated.

Gregarization following the cutting of selected leg nerves

The data from the electrical stimulation experiments suggested that sensory inputs from more than one branch of metathoracic nerve 5 may be needed to elicit gregarization, as stimulating the whole of nerve 5 was more effective than stimulating nerve 5B. Free-moving locusts in which nerve 5A had been cut, however, gregarized normally following 4 h of stroking the hind femur on the operated side (Fig. 7B; median P_{sol}=0.18). Conversely, cutting nerve 5B completely prevented mechanosensory-induced gregarization from occurring (Fig. 7C), and the data were similar to a control group in which the whole of nerve 5 had been severed prior to mechanical stimulation (Fig. 7A). A second control group, in which the femur contralateral to an entirely cut nerve 5 was stroked, showed normal gregarization (Fig. 7D). These data indicate that the operation on the insect itself did not prevent, or promote, the propensity for phase transition. An ANOVA of normalized ranked data showed a significant effect of the different treatments on the resulting behavioural phase state ($F_{3,32}$=4.00, P=0.016; the significances of Dunnet's two-tailed *post-hoc* tests against the ipsilaterally stimulated control group are shown in Fig. 7).

Chemosensory stimulation of leg receptors

Puffing a stream of air over a hind femur elicited little behavioural response in the locusts. By contrast, the odour of acetic acid, particularly at the stronger concentration, evoked vigorous withdrawal reflexes similar to those evoked by tactile stimulation but with less adaptation. Neither air (Fig. 8A) nor acetic acid odour (Fig. 8B,C), however, elicited any behavioural gregarization; median P_{sol} values were 0.99 for air-treated animals, 0.97 for the 10% strength acetic acid odour and 0.96 for the full-strength acetic acid odour. Fewer than 12.5% of locusts had P_{sol} values less than 0.2. Stroking a hind femur in conjunction with acetic acid odour (Fig. 8E),

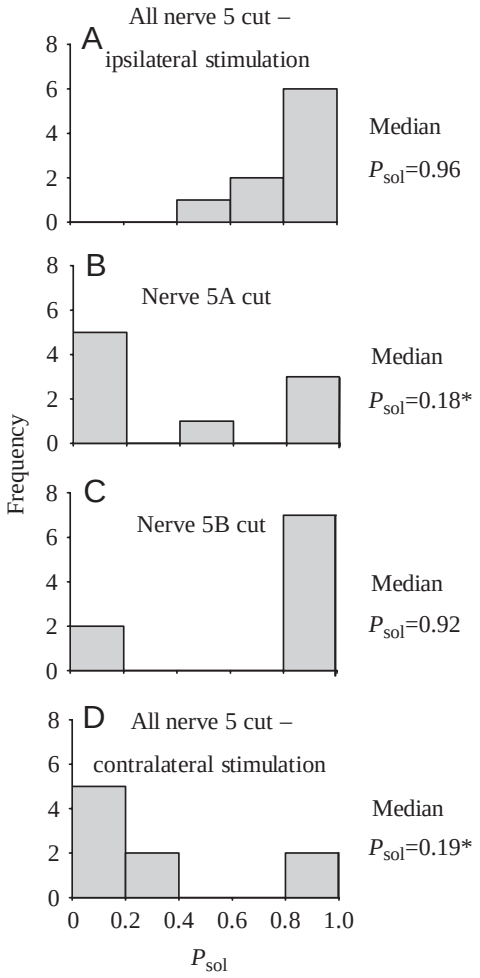

Fig. 7. Frequency histograms showing the distribution of P_{sol} values of free-moving locusts in which metathoracic nerve 5 had been cut before the hind femur was mechanically stimulated for 4 h. Either (A) the whole of nerve 5, (B) nerve 5A or (C) nerve 5B was cut, or (D) the whole of nerve 5 was cut but the insect was stimulated on the contralateral (intact) femur. The median P_{sol} values are indicated next to each graph; significance is indicated by an asterisk (Dunnet's *post-hoc* tests of ANOVA; P<0.05, N=9 locusts for all groups).

however, produced strong behavioural gregarization, with a median P_{sol} value of 0.10 compared with a value of 0.15 for those just mechanically stimulated (Fig. 8D). The acetic acid odour therefore did not inhibit or enhance gregarization. An ANOVA of normalized ranked data confirmed the significance of the results ($F_{4,35}$=4.60, P=0.004; the significances of Dunnet's *post-hoc* tests using the air-stimulated animals as the control group are indicated in Fig. 8).

Discussion

Touch-induced gregarization and the distribution of mechanosensory sensilla

Tactile stimuli provide a potent drive for behavioural phase transition in locusts, but their efficacy differs depending on the site that is stimulated. The most effective location is the

anterior (outward-facing) surface of a hind femur (Simpson et al., 2001). We show that mechanical stimuli directed to most regions on this surface in free-moving solitarious locusts can elicit full behavioural gregarization.

Stimulation of half of the femoral surface is more effective at inducing gregarization than stimulating just one quarter. This suggests that in order to elicit gregarization reliably there may be a minimum area over which stimulation must occur but that there is no specific region on a femur that must be touched. The only ineffective site is the ventral distal region, which has few tactile hairs.

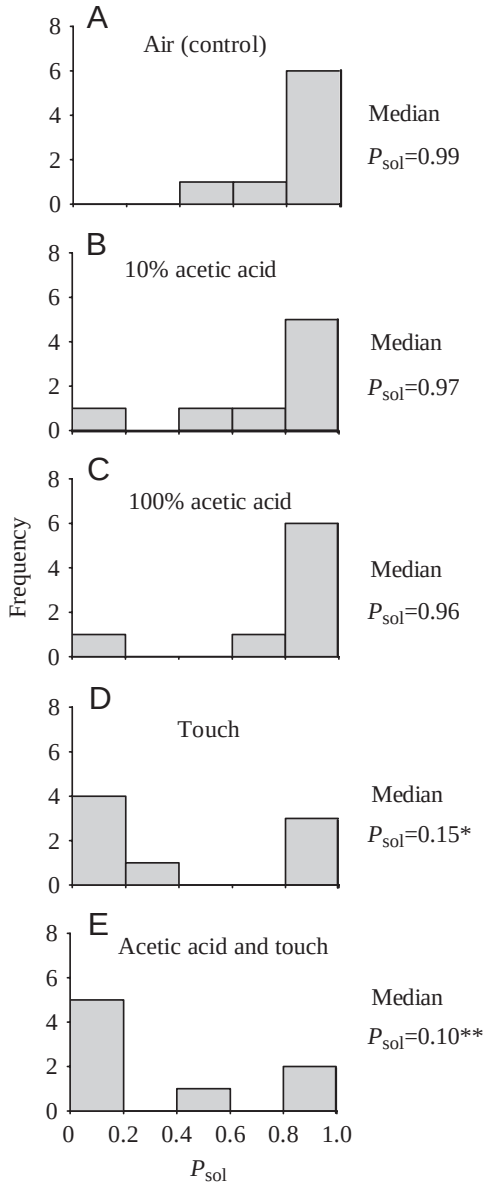

Fig. 8. Frequency histograms showing the distribution of P_{sol} values obtained in the behavioural assay after locusts were stimulated at 1 min intervals with either (A) 5 cm^3 air, (B) 0.5 cm^3 acetic acid odour mixed with 4.5 cm^3 air, (C) 5 cm^3 acetic acid odour drawn from the headspace of a bottle of glacial acetic acid, (D) stroking with a small paintbrush or (E) simultaneously stroking with a paintbrush whilst 5 cm^3 acetic acid odour was applied. Median P_{sol} values for each distribution are indicated next to each graph; significance is indicated with asterisks (Dunnet's *post-hoc* of ANOVA analyses): *, P<0.05; **, P<0.01, N=8 locusts for all groups.

A striking finding of this study is that solitarious phase locusts have, on average, 30% more tactile hairs on their hind femora than do gregarious locusts. By contrast, solitarious locusts have similar numbers or fewer tactile hairs on other hind leg segments and on the front and middle legs. The hind femora have a considerably larger surface area than any other leg segment and because of their orientation during walking or standing present themselves as large targets for mechanosensory stimuli. The additional tactile hairs of solitarious locusts are concentrated on the most prominent or protruding parts of the hind femur, in the regions most likely to be contacted or jostled by other locusts. These data suggest that there is some functional specialization of the hind femora of solitarious locusts towards detecting mechanosensory gregarizing stimuli. Nevertheless, although touch-induced gregarization is largely restricted to the hind femora, this cannot simply be a consequence of the number of mechanoreceptors present on different legs. A hind femur has more tactile hairs than any other leg segment, but it has fewer tactile hairs on one quarter of its anterior surface than on the whole of the front or middle femora; yet, stimulating just these hind leg sensilla is sufficient to produce gregarization, whereas stroking the whole of the front or middle femora has little gregarizing effect.

The legs of locusts also possess dual modality basiconic sensilla, which contain a single mechanosensory and several chemosensory afferent neurones. The mechanosensory afferents from basiconic sensilla are more sensitive to touch than those from tactile hairs, responding best to short, intermittent stimuli and adapting strongly on repeated stimulation (Newland and Burrows, 1994). There are 14% fewer basiconic sensilla on the distal femora of solitarious locusts than gregarious locusts. Despite this, solitarious locusts still have 27% more mechanosensory afferents overall in this region because they have 41% more tactile hairs.

Tactile hairs fall into at least two response classes, with distinct angular deflection activation thresholds: low-threshold hairs have phaso-tonic response characteristics that enable them to respond to repeated stimuli, whereas high-threshold hairs adapt completely within a few cycles of stimulation (Newland, 1991). It is unknown whether the change in mechanoreceptor numbers affects one class of tactile hair more than the other and it is possible that the physiological properties of the mechanosensory neurones themselves could also differ between phases. We would predict that phaso-tonic mechanosensory neurones, whose long-lasting response properties mean that they are able to signal repeated contacts, should be more effective in inducing gregarization. This suggestion is supported by the reduction in the number of basiconic sensilla on the femora of solitarious locusts. The highly phasic response properties and rapid adaptation of basiconic mechanosensory afferents makes it unlikely that they could provide a suitable gregarizing signal.

The reduction in the number of basiconic sensilla also means

a reduction in the population of contact chemosensory neurones, since these sensilla are bimodal. This decrease in the number of contact chemosensory neurones contrasts with an increase in the number of antennal olfactory sensilla reported in solitarious *Locusta migratoria* (Greenwood and Chapman, 1984). Contact chemosensory stimuli are not thought to be essential in the early stages of gregarization (Hägele and Simpson, 2000), although the olfactory stimulation provided by other locusts in conjunction with appropriate visual stimulation can induce rapid behavioural gregarization (Roessingh et al., 1998). Our use of a strong chemosensory stimulus in the acetic acid odour experiment, which activates contact chemosensory neurones on the legs, was designed to test another hypothesis, specifically whether the gregarizing stimulus from tactile hairs passes through spiking local interneurones in the metathoracic ganglion. The experiment exploited the known convergence of contact chemosensory neurones and mechanosensory neurones from both basiconic sensilla and tactile hairs onto these interneurones (Burrows and Newland, 1994; Newland, 1999; Newland et al., 2000). The great majority of spiking local interneurones that receive an exteroceptive mechanosensory input also receive monosynaptic chemosensory inputs (Newland, 1999; Rogers and Newland, 2002). If the signal to gregarize passes solely from the tactile hair afferents to these spiking local interneurones it should have been possible to produce behavioural phase change by activating the same interneurones through a purely chemosensory stimulus (by using the acetic acid odour). As this stimulus had no effect on behavioural phase state, we conclude that either there must be a separate pathway that processes the tactile signals responsible for eliciting gregarization or that there must be a convergence with other signals, not provided by chemosensory stimulation, downstream from the spiking local interneurones. Tactile hair afferents are known to make parallel monosynaptic connections onto some classes of intersegmental interneurones (Laurent and Burrows, 1988a; Newland, 1990), non-spiking interneurones (Laurent and Burrows, 1988b), motor neurones (Laurent and Hustert, 1988) and perhaps onto as yet unidentified interneurones. Spiking local interneurones are important components in the pathway organising the leg avoidance reflexes elicited by mechanical or chemosensory stimuli, but we show that this pathway cannot by itself drive behavioural phase change.

Behavioural gregarization could not be induced by mechanical stimulation of restrained locusts that were unable to move their hind legs, indicating that the effective gregarizing stimulus is not purely exteroceptive. It must combine exteroceptive stimuli with another mechanosensory signal that is produced when free-moving animals are stimulated with a paintbrush. To help determine the nature of this additional signal we carried out electrical nerve stimulation and ablation experiments.

Effectiveness of electrical stimulation in eliciting gregarization

Electrical stimulation of metathoracic nerve 5, which carries nearly all the sensory and motor neurones that innervate the hind leg distal to the coxa, reliably elicited behavioural gregarization in fully restrained locusts. This argues against motivational state (e.g. ‘stress’) being a factor in the failure to elicit behavioural gregarization in restrained locusts through mechanical stimulation. It suggests instead that electrical stimulation of nerve 5 activates a necessary component of the normal mechanosensory stimulus that is missing when restrained locusts are touched. Electrical stimulation of proximal nerve 5 was generally more effective than stimulation applied more distally. One possible explanation for this could be that current leak from the electrodes placed around the whole of nerve 5 reached the metathoracic ganglion and directly stimulated central neurones integral to initiating phase change. This seems unlikely, however, because in other experiments the electrodes on nerves 5A and 5B were similar distances from the ganglion, yet stimulation of nerve 5A was entirely ineffective at eliciting gregarization. Electrical stimulation of nerve 5 provides us with a powerful fixed preparation with which to initiate and monitor the effect of behavioural gregarization in the central nervous system.

Are both exteroceptive and proprioceptive inputs needed to produce gregarization?

It seems likely that the component necessary to induce behavioural phase change that is missing during mechanical stimulation of restrained locusts or during chemosensory stimulation by acetic acid odour is proprioceptive input from the basal leg joints. This could relate either to the resting position of the leg or could signal the medial displacement of a hind leg towards the body caused by pressure from a paintbrush or another locust. If the latter, this suggests that for gregarization to occur locusts need to be not just touched but jostled strongly enough to produce limb or body displacement. This jostling, if it pushes the femur towards the body, will produce a movement about the complex thoraco-coxal joint with mostly remotion and levation components. The femoro-tibial joint moves in a plane oblique to the direction of the major force produced by touching with a paintbrush and is unlikely to be strongly stimulated, but there may be twisting forces out of the movement plane about the femoro-tibial joint. Movements of the leg that are part of the usual movement pattern of locomoting locusts should not produce proprioceptive stimuli that elicit gregarization, otherwise locusts would risk gregarizing during usual activity.

Moving the leg in the absence of tactile exteroceptive input, for example by stroking a femur when all the exteroceptors have been immobilised with paint, fails to elicit gregarization, ruling out the possibility that the gregarizing stimulus is entirely proprioceptive. Similarly, gregarization induced by sensory feedback from motor responses elicited by the mechanical stimulus in free-moving animals (e.g. avoidance reflexes; Siegler and Burrows, 1986) can be excluded because locusts do not gregarize when the extensor tibiae muscle is stimulated electrically so that the tibia moves or when

basiconic sensilla are stimulated with acetic acid odour, which elicits vigorous leg withdrawal responses.

The femur and basal leg joints contain many proprioceptors that could provide the necessary signal (Hustert et al., 1981; Bräunig et al., 1981; Bräunig, 1982; Mücke, 1991). Fig. 9 summarises the major sense organs of the hind leg served by different nerves. As full behavioural gregarization could be elicited by stimulation of nerve 5 alone, signals from leg proprioceptors whose axons run in nerves 2 and 3B (Bräunig and Hustert, 1985) can be excluded from a critical role in monitoring gregarizing stimuli, unless the relevant signals can be carried through several redundant pathways. The greater efficacy of electrically stimulating the whole of nerve 5 in driving gregarization compared with stimulating any of its branches separately initially suggested a role for proprioceptive neurones with axons in nerve 5A, such as from

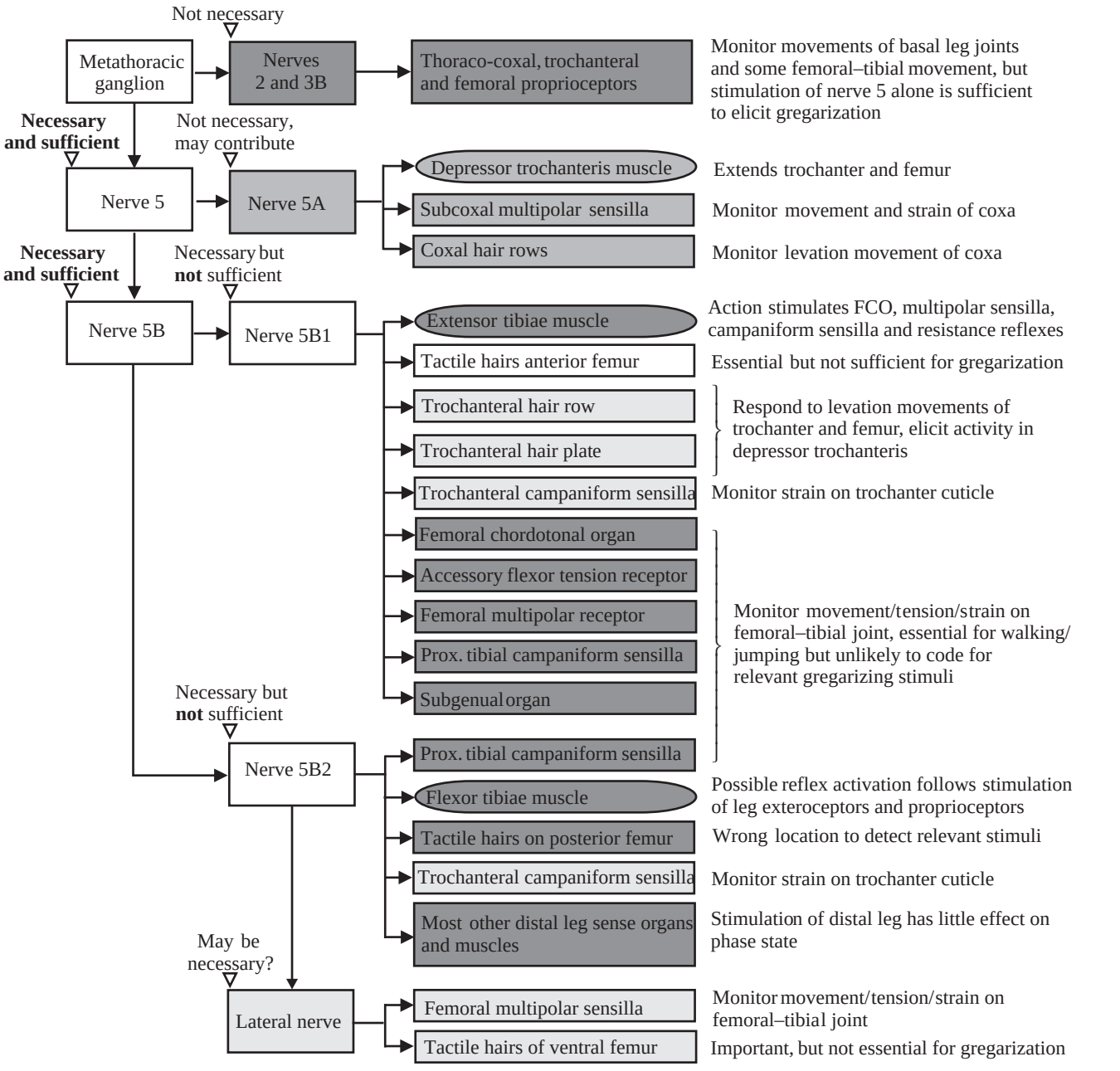

Fig. 9. Summary of the innervation of the exteroceptors, proprioceptors and muscles of the hind leg and their possible roles in eliciting behavioural phase change. We have shown that the nerves or sense organs in white boxes need to be stimulated in order to elicit phase change. Nerves or sense organs in the lightest grey boxes are implicated in signalling appropriate mechanosensory gregarizing stimuli. Nerve 5A and its innervated structures, in mid-grey boxes, may contribute to signalling gregarizing stimuli, but it is not necessary to stimulate this nerve for phase change to occur. We have either demonstrated or can infer that those nerves, muscles and sense organs in the dark grey boxes have no role in mechanosensory-elicited phase change. FCO, femoral chordotonal organ.

two (sub)coxal multipolar sensilla and hair rows sensitive to movements of the coxa (Bräunig et al., 1981; Bräunig, 1982; Fig. 9). Cutting nerve 5A, however, does not reduce the efficacy of touch stimulation, excluding a necessary role for these particular proprioceptors. It is possible, however, that the medial displacement of a hind leg during stimulation with a paint brush, which should strongly stimulate nerve 5A sensory neurones, could contribute to the proprioceptive component of the gregarizing signal and explain the slightly stronger gregarizing effect of electrical stimulation of the whole of nerve 5.

Electrical stimulation of nerve 5B is an effective gregarizing stimulus, yet stimulation of either nerve 5B1 or 5B2 alone is not effective in eliciting robust gregarization despite there being no sensory structures innervated by nerve 5B prior to its bifurcation into 5B1 and 5B2. Stimulating both nerve 5B1 and 5B2 together is almost as effective as stimulation of the whole of nerve 5B, suggesting that the relevant signals must be carried by neurones within nerves 5B1 and 5B2. Nerve 5B1 innervates tactile hairs on the anterior face of the femur, nerve 5B2 innervates those on the posterior face of the femur and, *via* its first branch (the lateral nerve; Heitler and Burrows, 1977), the row of tactile hairs on the ventral ridge of the femur (Fig. 9). It would be expected therefore that the major exteroceptive input driving gregarization would come *via* nerve 5B1. This exteroceptive input must be combined with proprioceptive signals, at least part of which should come from nerve 5B2. Both nerves 5B1 and 5B2 supply proprioceptive sensilla on the trochanter, including two campaniform sensilla fields each (Hustert et al., 1981; Bräunig, 1982), which monitor strain on the cuticle. Nerve 5B1 also innervates the trochanteral hair plate and hair row, which monitor movements of the femur. Any of these proprioceptors, alone or in combination, could monitor the displacement of the leg that accompanies appropriate touch stimulation and thus contribute to the gregarizing stimulus. We expect that at least one of the proprioceptive afferents must travel in nerve 5B2, which would explain why only stimulation of both nerves, or the conjoined nerve 5B, is effective in eliciting gregarization. Trochanteral campaniform sensilla are the only known basal leg joint proprioceptors in nerve 5B2, strongly implicating them in mediating proprioceptive phase change stimuli. There are a number of multipolar sensilla innervated *via* the lateral nerve branch of nerve 5B2, some of which are sensitive to twisting movements of the femoro-tibial joint (Bässler, 1977; Williamson and Burns, 1978; Matheson and Field, 1995), which could also respond to medial displacement of the hind femur (Fig. 9). As with exteroceptors, it is also possible that long-term solitarization is accompanied by changes in the number or physiological properties of proprioceptors.

The neuronal circuits that integrate mechanosensory gregarizing stimuli should combine exteroceptive signals from the anterior surface of a hind femur with a specific proprioceptive signal that naturally results from the inward displacement of the leg on contact with another locust. These neuronal circuits are the first central elements of the pathway that initiates the rapid and widespread neuronal plasticity that underlies behavioural phase change. We show that the inputs to these neuronal circuits come from tactile hairs on the hind femora and from other leg receptors and that this input system is itself modified by the process of phase change.

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