Substantial changes in central nervous system neurotransmitters and neuromodulators accompany phase change in the locust

Stephen M. Rogers[1,2,*], Thomas Matheson[1,†], Ken Sasaki[1,‡], Keith Kendrick[3], Stephen J. Simpson[2] and Malcolm Burrows[1]

[1]*Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK*, [2]*Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK* and [3]*Laboratory of Cognitive and Developmental Neuroscience, Babraham Institute, Babraham, Cambridge CB2 4AT, UK*

*Author for correspondence (e-mail: Smr34@cam.ac.uk)

†Present address: Department of Biology, University of Leicester, University Road, Leicester LE1 7RH, UK

Present address: Human Information Systems, Kanazawa Institute of Technology, 3-1 Yakkaho, Matto, Ishikawa 924-0838, Japan

Summary

Desert locusts (*Schistocerca gregaria*) can undergo a profound transformation between solitarious and gregarious forms, which involves widespread changes in behaviour, physiology and morphology. This phase change is triggered by the presence or absence of other locusts and occurs over a timescale ranging from hours, for some behaviours to change, to generations, for full morphological transformation. The neuro-hormonal mechanisms that drive and accompany phase change in either direction remain unknown. We have used high-performance liquid chromatography (HPLC) to compare amounts of 13 different potential neurotransmitters and/or neuromodulators in the central nervous systems of final instar locust nymphs undergoing phase transition and between long-term solitarious and gregarious adults. Long-term gregarious and solitarious locust nymphs differed in 11 of the 13 substances analysed: eight increased in both the brain and thoracic nerve cord (including glutamate, GABA, dopamine and serotonin), whereas three decreased (acetylcholine, tyramine and citrulline). Adult locusts of both extreme phases were similarly different. Isolating larval gregarious locusts led to rapid changes in seven chemicals equal to or even exceeding the differences seen between long-term solitarious and gregarious animals. Crowding larval solitarious locusts led to rapid changes in six chemicals towards gregarious values within the first 4 h (by which time gregarious behaviours are already being expressed), before returning to nearer long-term solitarious values 24 h later. Serotonin in the thoracic ganglia, however, did not follow this trend, but showed a ninefold increase after a 4 h period of crowding. After crowding solitarious nymphs for a whole larval stadium, the amounts of all chemicals, except octopamine, were similar to those of long-term gregarious locusts. Our data show that changes in levels of neuroactive substances are widespread in the central nervous system and reflect the time course of behavioural and physiological phase change.

Key words: desert locust, *Schistocerca gregaria*, phase transition, HPLC, solitarious, gregarious, polymorphism.

Introduction

Many animals undergo profound changes in behaviour that adapt them to changing needs and conditions at different stages in their life histories. Neuromodulation of neuronal networks or intrinsic changes in the amounts of neurotransmitters within these networks are two means of effecting such behavioural change, as has been reported to accompany the onset of sexual maturity or reproductive status (Fabre-Nys et al., 1997; Broad et al., 2002), the development and metamorphosis of insects and amphibians (Homberg and Hildebrand, 1994; Kloas et al., 1997; Takeda, 1997; Sillar et al., 1998; Lehman et al., 2000a,b; Consoulas et al., 2000; Mercer and Hildebrand, 2002), and during caste differentiation (Sasaki and Nagao, 2001, 2002) and the division of labour between workers in social insects (Taylor et al., 1992; Wagener-Hulme et al., 1999; Schulz and Robinson, 1999). In the shorter term, neuromodulation by octopamine and serotonin in insects and Crustacea is associated in complex ways with social status arising from agonistic encounters (Kravitz, 2000; Sneddon et al., 2000; Stevenson et al., 2000), and serotonin has an important role in regulating the sensitivity of photoreceptors between night and day (Cuttle et al., 1995; Hevers and Hardie, 1995).

Locusts undergo an extreme form of phenotypic plasticity that is driven by population density, which results in extensive but reversible changes in many aspects of morphology, physiology and behaviour (Uvarov, 1966; Simpson et al.,

1999). Locusts in the wild usually exist in the solitarious phase under low population densities of less than 3 per 100 m^2. Solitarious locusts are cryptic in appearance and behaviour, fly mainly at night and actively avoid contact with each other. If environmental conditions force locusts together into high population densities, however, they transform to the gregarious phase. In this phase locusts are highly active, have bright warning colours as nymphs, are predominately day flying and, critically, are attracted towards other locusts, thus forming cohesive groups. The rates of change of phase characteristics vary over time scales ranging from hours to generations. Solitarious locusts behave fully gregariously within just 4 h of crowding, thereby increasing their propensity to move towards other locusts (Roessingh et al., 1993; Roessingh and Simpson, 1994). By contrast, gregarious-phase locust nymphs that are isolated only partially solitarise within 24 h, and then remain in this transitional behavioural state for the rest of the stadium. Further behavioural solitarisation requires isolation for several stadia (Roessingh and Simpson, 1994) or generations *via* a maternal influence over embryonic development (Islam et al., 1994a,b; Bouaichi et al., 1995).

The necessary sensory stimuli that trigger the initial behavioural gregarization of solitarious locusts have been characterised (Roessingh et al., 1998; Hägele and Simpson, 2000; Simpson et al., 2001; Rogers et al., 2003). There are also clear differences in specific neuronal circuits and muscular systems between the two extreme locust phases that can be related to these differences in behaviour (Matheson et al., 2003, 2004; Blackburn et al., 2003; Fuchs et al., 2003). The neuro-hormonal mechanisms that drive and maintain phase change, however, remain largely unknown (Pener, 1991; Pener and Yerulshami, 1998; Breuer et al., 2003). The peptide [His^7]-corazonin promotes gregarious colouration and morphometric changes in solitarious locusts (Tawfik et al., 1999; Hoste et al., 2002) but has no effect on phase-related behaviour (Hoste et al., 2002). Previous work has analysed amounts of octopamine in *Locusta migratoria* (Fuzeau-Braesch and David, 1978; Fuzeau-Braesch and Nicholas, 1981) and a partially phase-changing species, *Schistocerca americana* (Morton and Evans, 1983), with conflicting results. No previous study has monitored changes in neurochemicals during the phase change process from hours to generations as we show here. As the detailed time course of behavioural phase change is now well established, the present study analyses the accompanying changes in putative neuromodulators and neurotransmitters within the nervous system on a temporal scale that maximises the likelihood of discovering coincident and hence potentially causal relationships between changes in behaviour and neurochemistry. We used high performance liquid chromatography (HPLC) to analyse changes in 13 different potential neurotransmitters and neuromodulators in the central nervous system of desert locusts at nine key stages during solitarization and gregarization. We identify chemicals that differ quantitively between phases and track the time course of these differences as phase-change occurs.

Materials and methods

Insects

All locusts *Schistocerca gregaria* Forskål originated from a colony maintained at the department of Zoology, University of Oxford, and have been reared under crowded conditions for many generations (500–1000 insects per 56 cm×76 cm×60 cm rearing bin). Solitarious-phase locusts were derived from this colony but had been reared in a separate facility under physical, visual and olfactory isolation from other locusts. The husbandry procedures for the isolated locusts were the same as those used by Roessingh et al. (1993). Final larval instar locusts were used for the time course of phase-change experiments. A group of second-generation isolated adult locusts was compared with long-term gregarious adults in a separate experiment.

Experimental treatments

The time-course analysis of the effects of isolation and crowding was performed by taking gregarious-phase locusts from the stock culture, isolating different cohorts for sequentially longer periods, then taking third generation solitarious locusts and crowding them, as shown in Fig. 1. Nine stages of isolation/crowding were examined in final instar nymphs. These were: (1) long-term gregarious-phase locusts taken from the main culture, (2) gregarious-phase locusts isolated for 24 h, (3) gregarious-phase locusts isolated from the start of the penultimate nymphal stadium until their final nymphal stadium, (4) first-generation, isolated-reared locusts (i.e. locusts hatched from separated eggs and reared separately), (5) second-generation, isolated-reared locusts (i.e. offspring of locusts reared under the previous treatment conditions), (6) third-generation isolated-reared locusts, (7) third-generation, isolated-reared locusts crowded together (in a group of 12) in a standard solitarious locust-rearing cage (10 cm×10 cm×25 cm) for 4 h, (8) third-generation isolated-reared locusts crowded together as in (7) for 24 h and (9) third-generation isolated-reared locusts crowded together as in (7) from the start of the penultimate larval stadium until their final nymphal stadium.

Each treatment group initially consisted of 12 locusts, split approximately evenly between sexes, but some losses during the experiment meant that final sample sizes ranged from 10 to 12. All locusts were analysed 2–5 days from their previous moult.

For comparison of neurotransmitters and neuromodulators in adult solitarious and gregarious locusts, nine gregarious locusts taken from the gregarious culture were compared with nine locusts that had been reared in isolation for two generations. All adult locusts were in the pre-reproductive stage, 5–10 days after their final moult.

Preparation of samples

Experimental locusts were removed from their rearing cages and placed in 7.5 cm diameter plastic plant pots, either individually if previously isolated, or as a group if previously crowded. Some cut wheat seedlings were added and the pots
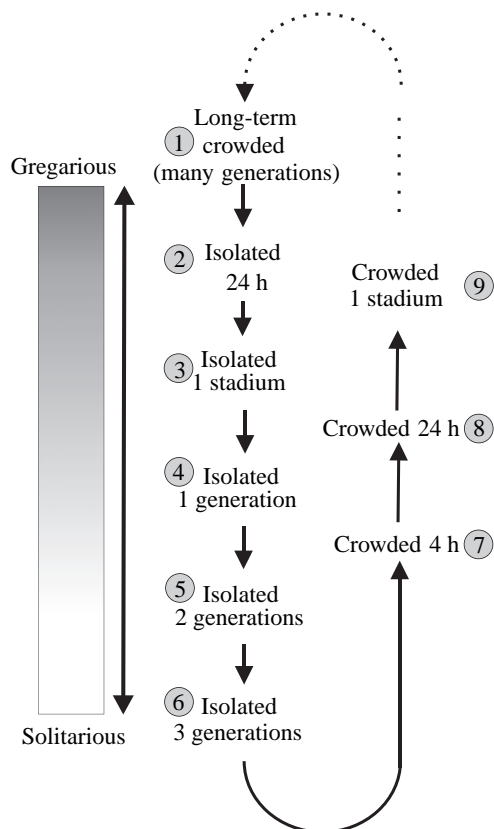
Fig. 1. Schematic of the nine stages of phase change analysed. Shown descending on the left-hand side, cohorts of long-term crowded (gregarious-phase; stage 1) locusts are taken and isolated for increasing periods, becoming increasingly solitarious. Locusts that have been isolated for three whole generations (long-term solitarious; stage 6) are then taken and crowded for increasing periods, causing a progressive change to the gregarious phase, shown rising on the right-hand side. Locusts that are crowded for long enough revert to the gregarious phase state.

covered with pierced cling-film. The locusts were then left undisturbed for 1 h. At the end of this resting period the pots were gently lifted with 30 cm long forceps and plunged into liquid nitrogen; the holes in the base of the plant pots allowed rapid access of the freezing liquid. The frozen locusts were removed individually, decapitated, and the head and body placed on pre-chilled dissecting blocks kept on ice. The whole brain including the optic lobes was dissected from the head, and the complete thoracic ganglion chain of final instar locusts, or the pro- and metathoracic ganglia only of adult locusts, was dissected from the thorax. Ice-cold ultrapure locust saline prepared using Analar™ quality reagents and ultrapure deionised water was used as necessary during the dissection. The optic lobes were detached from the rest of the brain and the heavily pigmented retina removed and discarded. The two optic lobes were combined to make one sample. The central region of the brain constituted another sample and in final instar nymphs the thoracic ganglion chain the third. In adult locusts the pro- and metathoracic ganglia only were made

into separate samples. Individual tissue samples were placed in chilled 100 μl micro-homogenisers with 50 μl of 150 mmol l^{-1} perchloric acid containing 100 ng ml^{-1} 3,4-dihydroxybenzylamine hydrobromide (DHBA; Aldrich, Poole, Dorset, UK) as an internal standard for the HPLC and homogenised for 2 min. The samples were then transferred to 1.5 ml Eppendorf tubes and centrifuged for 30 min at 17 500 ***g***. The supernatant was measured using a 50 μl Hamilton syringe, transferred to another Eppendorf tube and then stored at –80°C until the HPLC analysis (for approximately 2 weeks).

Preparation for HPLC

Samples were analysed using three different HPLC systems designed to measure either amino acids, monoamines or acetylcholine/choline. For the amino acid analysis, 2 μl of the sample solution was mixed with 48 μl of ultrapure locust saline (25× dilution); for the monoamine system, 13 μl of the sample was used undiluted, and for the acetylcholine system, 5 μl of the sample was mixed with 95 μl of ultrapure saline (20× dilution). Standard solutions were used to calibrate each of the HPLC systems, for both the identification and quantification of different peaks. Standard solutions for the amino acid system contained aspartate, glutamic acid, citrulline, glycine, arginine, taurine and γ-amino butyric acid (GABA), all 250 nmol l^{-1}; for the monoamine system, octopamine (OA, 50 ng), tyramine (TA, 40 ng), DHBA (internal standard; 0.25 ng), *N*-acetyldopamine (NADA, 0.2 ng), dopamine (DA, 0.5 ng) and serotonin (5-hydroxytryptamine, 5-HT, 0.75 ng), all measured in mass per 10 μl injected sample; and for the acetylcholine system, choline and acetylcholine both 200 nmol l^{-1}.

Amino acid analytical system

Amino acids were analysed using an HPLC gradient system at a flow rate of 520 μl min^{-1} (125 gradient pump; Beckman, Fullerton, CA, USA) with a C18 reversed phase column (3 μm SphereClone column, Phenomenex, Macclesfield, Cheshire, UK; 15 cm length×3.2 mm i.d., heated at 35°C) and fluorescence detection (CMA/280) as previously described (Kendrick et al., 1996). A Gilson (Villiers-le-Bel, France) model 231/401 auto-injector was used with programmable pre-column derivatisation using OPA (*o*-pthaldialdehyde). Injection volumes were 13 μl including both sample and OPA. Gradients and data collection were controlled using Beckman 32 Karat HPLC software. Detection limits were 1–5 nmol l^{-1}.

Monoamine analytical system

Monoamines were analysed using an isocratic HPLC system (M480 pump; Gynkotek, Germering, Germany; flow rate 200 μl min^{-1}) with electrochemical detection (Waters M469, Waters Milford, MA, USA, using a BAS 6 mm Unjet cell at +0.65 V) as previously described (Kendrick et al., 1996). A reversed phase C18 column was used (Phenomenex 3 μm SphereClone; 15 cm length×2.0 mm i.d., heated at 35°C). A cooled autoinjector (CMA/200) was used to load samples (10 μl sample volume injected). Data were integrated using a

Table 1. *The amounts of 13 different chemicals in the three regions of the central nervous system of long-term gregarious and long-term solitarious final instar locust nymphs*

Chemical	Long-term gregarious		Third generation solitarious		Change	Solitarious value as % of gregarious value
	Mean	S.E.M.	Mean	S.E.M.		
Aspartate	2.36	0.370	7.44	1.201	+	315
Glutamate	13.54	1.768	26.63	2.329	+	197
Glycine	12.37	2.933	36.54	5.663	+	295
GABA	2.97	0.285	3.95	0.203	+	133
Arginine	15.37	2.068	19.98	0.951	+	130
Taurine	3.13	0.472	3.81	0.306	+	121
Acetylcholine	7288.63	224.881	6076.67	537.389	−	83
Tyramine	0.096	0.0225	0.046	0.0125	−	48
Citrulline	1.08	0.375	0.09	0.016	−	8
Dopamine	0.10	0.035	0.12	0.028	+	118
Serotonin	0.07	0.015	0.09	0.026	+	131
Octopamine	4.48	1.355	4.17	1.184	=	93
N-acetyldopamine	1.41	0.289	1.15	0.232	−	81

Amounts are expressed as nmol per nervous system.

Gynkosoft (Dionex, Sunnyvale, CA, USA) integration package. Detection limits were 5–25 pg ml^{-1}.

Acetylcholine analytical system

Acetylcholine/choline were analysed using an isocratic HPLC system (CMA 250 pump, 120 μl min^{-1}) with electrochemical detection (BAS LC4C with 6 mm Unijet cell at +0 V coated with peroxidase to produce a 'wired enzyme detector') as previously described (Kendrick et al., 1996). A Unijet analytical column was used (BAS, ACh/Ch column, 52 cm length×1 mm i.d.). Data were integrated using a Gynkosoft (Dionex) integration package. Detection limits were 0.5 nmol l^{-1}.

Statistical analyses of the data were made using SPSS (version 11). Outlying data points lying more than 2.5 standard deviations from the sample mean (in practice corresponding to values more than twice that of the next closest data point) were excluded from the analyses. Data from different chemicals were square root or natural log (ln) transformed as necessary to render them suitable for parametric analyses.

Results

Long-term gregarious and long-term solitarious (third-generation isolated) locust nymphs contained different amounts of 11 of the 13 tested chemicals (see Table 1, which lists total amounts measured in the three regions of the central nervous system). Eight substances were more abundant in solitarious locusts than in gregarious locusts. The amounts of glutamate, glycine and aspartate were double or more in solitarious locusts than in gregarious locusts, whilst GABA, taurine, serotonin and dopamine increased by a more modest 20–35%. Three chemicals, acetylcholine, tyramine and citrulline, were less abundant in long-term solitarious locusts than in long-term gregarious ones. The decreases ranged from

17% for acetylcholine to 50% for tyramine, whilst citrulline underwent a substantial 90% decrease on solitarization. Only octopamine showed a mean difference of less than 10% between phases, and whilst solitarious locusts had 17% less *N*-acetyldopamine than gregarious locusts, the standard errors of the mean (S.E.M.) overlapped between phases.

The detailed analysis of the phase change process, taking long-term gregarious locusts and then cohorts of insects isolated for periods of hours to three generations and then crowding third-generation solitarious locusts for periods of 4 h to one stadium, revealed significant changes in 12 of the 13 tested chemicals in at least one of the nine stages of isolation or crowding, (multivariate analysis of variance, MANOVA; Table 2, based on the total amounts present in the sampled regions of the central nervous system). Only *N*-acetyldopamine (Fig. 2M) showed no significant change with any of the treatments, or between regions of the central nervous system as either final instar nymphs (Table 2) or adults (Table 3). The data for final instar nymphs are divided into three patterns of response following isolation and crowding: amino acids that increased on isolation throughout the central nervous system (Figs 2A–F, 3); chemicals that decreased on isolation throughout the central nervous system (Figs 2G–I, 4) and the monoamines dopamine (Figs 2J, 5A), serotonin (Figs 2K, 5B) and octopamine (Figs 2L, 5C), which showed large regional changes, particularly during the early stages of isolation and crowding.

Qualitatively, the differences between long-term gregarious adults and second-generation solitarious adults were similar to those of final instar nymphs for most chemicals (Table 3, Fig. 6). Aspartate, glutamate and glycine were present in approximately double the quantity in solitarious compared to gregarious adults, as in the final instar nymphs. GABA, arginine, taurine, dopamine and serotonin were also present in greater amounts in solitarious adults but had more extreme

Table 2. *Results of a MANOVA on the measured amounts of 13 different neurochemicals in the central nervous systems of final larval instar locusts subjected to different conditions of crowding or isolation*

(A) Effect	Pillai's trace value	F	Hypothesis d.f.	Error d.f.	P
Intercept	0.995	1063.2	13	73	<0.001
Treatment	2.649	3.047	104	640	<0.001
Sex	0.252	1.889	13	73	0.045
Treatment×Sex	1.504	1.425	104	640	0.006

(B) Between subject effects				
Chemical	Model $F_{17, 85}$	Treatment $F_{8, 85}$	Sex $F_{1, 85}$	Treatment×Sex $F_{8, 85}$
Aspartate	3.44***	5.14***	10.23**	0.55
Glutamate	3.53***	4.69***	10.66**	1.18
Citrulline	3.54***	5.09***	0.48	2.21*
Glycine	2.91***	4.73***	1.77	0.58
Arginine	3.35***	2.76**	12.26***	2.17*
Taurine	3.81***	5.38***	5.96*	1.92
GABA	3.13***	4.58***	3.42	1.17
Acetylcholine	1.35	2.08*	2.60	0.47
Octopamine	5.24***	6.75***	3.55	6.24***
N-Acetyldopamine	1.11	0.81	1.34	0.87
Dopamine	1.94*	2.46*	0.11	1.00
Tyramine	1.77*	2.88**	0.025	0.73
Serotonin	1.571	2.29*	0.69	0.66

*P<0.05, **P<0.01 and ***P<0.001.
MANOVA, multivariate analysis of variance.

relative increases compared to nymphs, with solitarious adults also having approximately double the quantities of these chemicals compared to the 20–35% increases seen for the same chemicals in final instar nymphs. There was less citrulline in gregarious adults but the difference was smaller than in nymphs (50% compared to >90% decrease, respectively), whilst there were no significant differences in the amounts of *N*-acetyldopamine, tyramine or octopamine. The acetylcholine analysis was not performed on the adult samples.

Amino acids that increased on isolation

All the measured amino acids, except for citrulline, were present in greater amounts throughout the central nervous systems in long-term solitarious locusts than in long-term gregarious locusts (Figs 2A–F, 3). Overall two patterns of change with increasing isolation and then crowding could be seen (Fig. 3A,B). One group, consisting of aspartate, glutamate and glycine, showed large increases (doubling or more) throughout the central nervous system following isolation for a 24 h period (Fig. 3A; all significant at P<0.05 in a Dunnet *post hoc* test against the gregarious controls). Levels of all three chemicals, however, then fell back towards gregarious values on isolation for an entire stadium (Fig. 3A). Locusts that were isolated for longer, i.e. 1–3 generations, had increasing amounts of these amino acids with each generation of isolation, so that eventually the amounts present after three generations were similar to the high levels occurring after the initial 24 h period of isolation (Fig. 3A).

Phase change in the opposite direction, gregarization produced by crowding third generation solitarious locusts for as little as 4 h, led to dramatic decreases (Fig. 3A) towards values found in long-term gregarious locusts. By 24 h of

Table 3. *Results of a MANOVA on the measured amounts of 12 different neurochemicals in the brain and optic lobes of adult long-term gregarious and second generation solitarious locusts*

(A) Effect	Pillai's trace value	$F_{12, 5}$	P
Intercept	0.979	19.19	0.002
Phase	0.977	17.41	0.003

(B) Between subject effects	
Chemical	Phase $F_{1, 16}$
Aspartate	5.65*
Glutamate	13.36**
Citrulline	12.13**
Glycine	14.08***
Arginine	14.88***
Taurine	17.07***
GABA	7.49*
Octopamine	0.303
N-Acetyldopamine	3.56 (P=0.07)
Dopamine	7.43*
Tyramine	0.254
Serotonin	6.90*

*P<0.05, **P<0.01 and ***P<0.001.
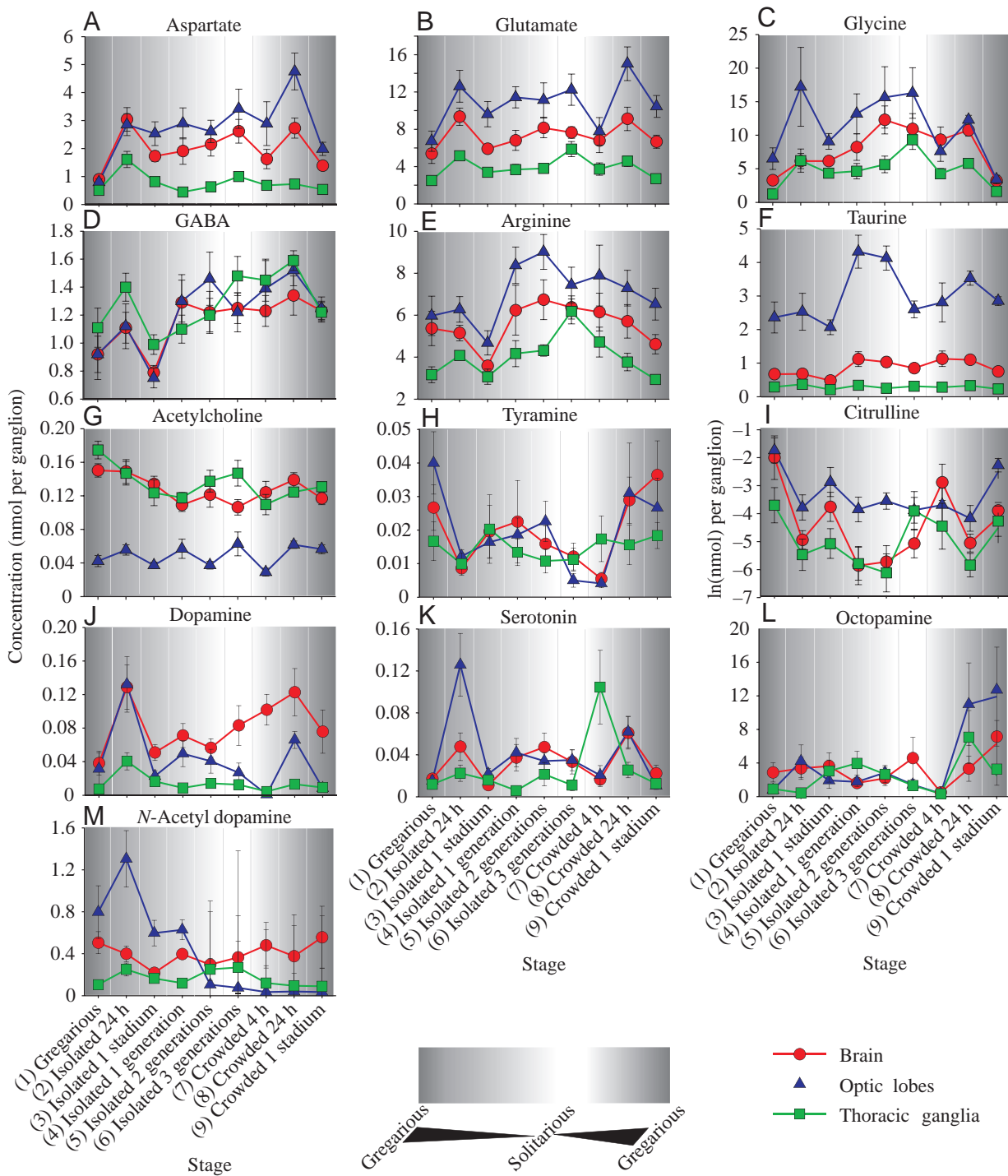MANOVA, multivariate analysis of variance.

Fig. 2. (A–M) Changes in the amounts of 13 chemicals in three different regions of the central nervous system during successive stages (1–9) in the isolation of long-term gregarious phase locusts (stage 1; darkest grey background) and the gregarization of third generation solitarious locusts (stage 6; white background). Values (mean ± S.E.M.) are the amounts found in the brain (red circles) excluding optic lobes, optic lobes (blue triangles) and the thoracic ganglia (green squares) for final larval instar nymphs at the stages of isolation or crowding indicated on the *x*-axis. *N*=9–12 for each point. Note that the values for citrulline are ln transformed. Stages as in Fig. 1.

crowding, however, the amounts had increased again and were again similar to those of long-term isolated locusts. In the second group of chemicals, consisting of GABA, taurine and arginine, there were smaller (20%) or no increases following

24 h isolation of long-term gregarious locusts (all non-significant; Fig. 3B). On longer isolation, for one stadium, the mean amounts of all three amino acids even fell below those detected in long-term gregarious locusts.
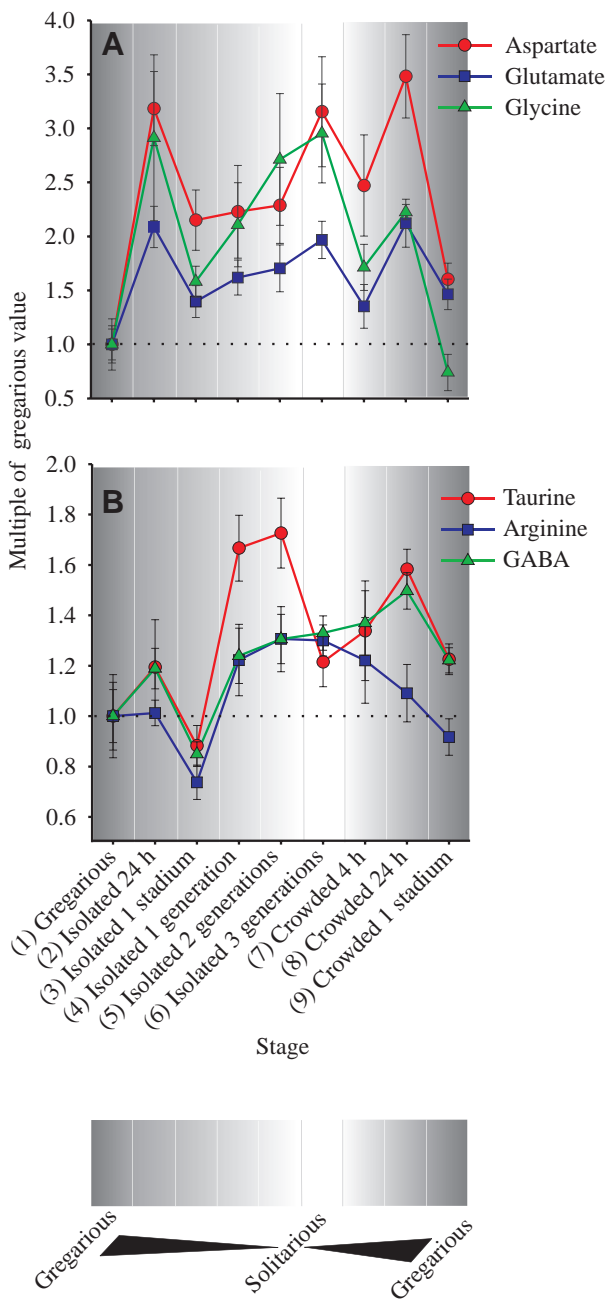
Fig. 3. Amino acids that increased on solitarization in final instar nymphs. (A) Amino acids that exhibited large (over 2×) rapid changes. Aspartate, glutamate and glycine showed rapid increases on the initial 24 h isolation of gregarious locusts, before declining again in the first stadium of isolation. Amounts increased again over a further 1–3 generations of isolation. Crowding third generation isolated locusts led to a rapid decline in the first 4 h, but amounts had increased third generation isolated values by 24 h of crowding. Values returned towards long-term gregarious values after 1 crowded stadium. (B) Amino acids that exhibited smaller (maximum 1.8×) and slower changes. Taurine, arginine and GABA did not change significantly during the first 24 h of crowding or isolation, but increased only after 1 generation of isolation. Values (mean ± S.E.M.) are sum amounts in the three sampled regions of the central nervous system, standardized to give the mean long-term gregarious value as 1. Stages as in Fig. 1.
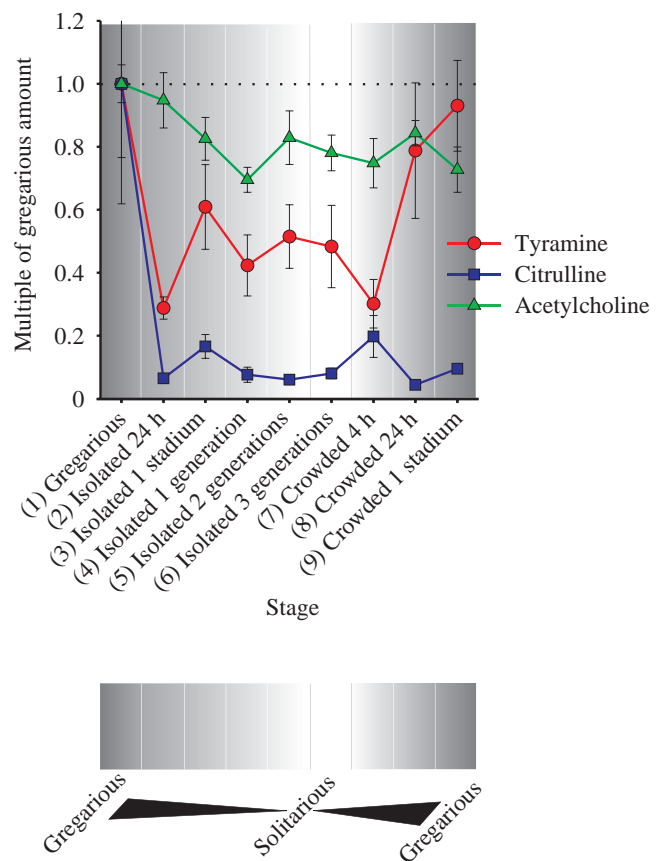

Fig. 4. Chemicals that decreased on solitarization in final instar nymphs were tyramine, citrulline and acetylcholine. Tyramine decreased to 30% of its gregarious value within 24 h of isolation and remained low during all stages of solitarization, but increased rapidly within 24 h of crowding. Citrulline decreased to less than 10% of gregarious amounts after 24 h isolation and remained low during all stages of isolation and gregarization monitored. Acetylcholine declined slowly over a period of 24 h to 1 generation of isolation and remained at approximately 80% of the gregarious amount during further generations of isolation. It did not recover following 1 stadium of crowding. Values (mean ± S.E.M.) are sum amounts in the three sampled regions of the central nervous system, standardized to give the mean long-term gregarious value as 1. Stages as in Fig. 1.

For arginine and GABA, first-generation isolated locusts had increased amounts relative to locusts isolated for one stadium, but this was followed by only modest or no increases on each subsequent generation of isolation (Fig. 3B). Taurine differed somewhat from this pattern; there was a substantial increase (60%) relative to gregarious in the first and second generations of isolation but then a decrease to only 25% more than the gregarious level in the third generation of isolation (Fig. 3B). No other chemical showed the same change from the second to third generation of isolation. The amounts of arginine, taurine and GABA did not change when third-generation solitarious locusts were crowded for a 4 h period, but subsequently the amounts of taurine and GABA increased significantly after 24 h crowding.
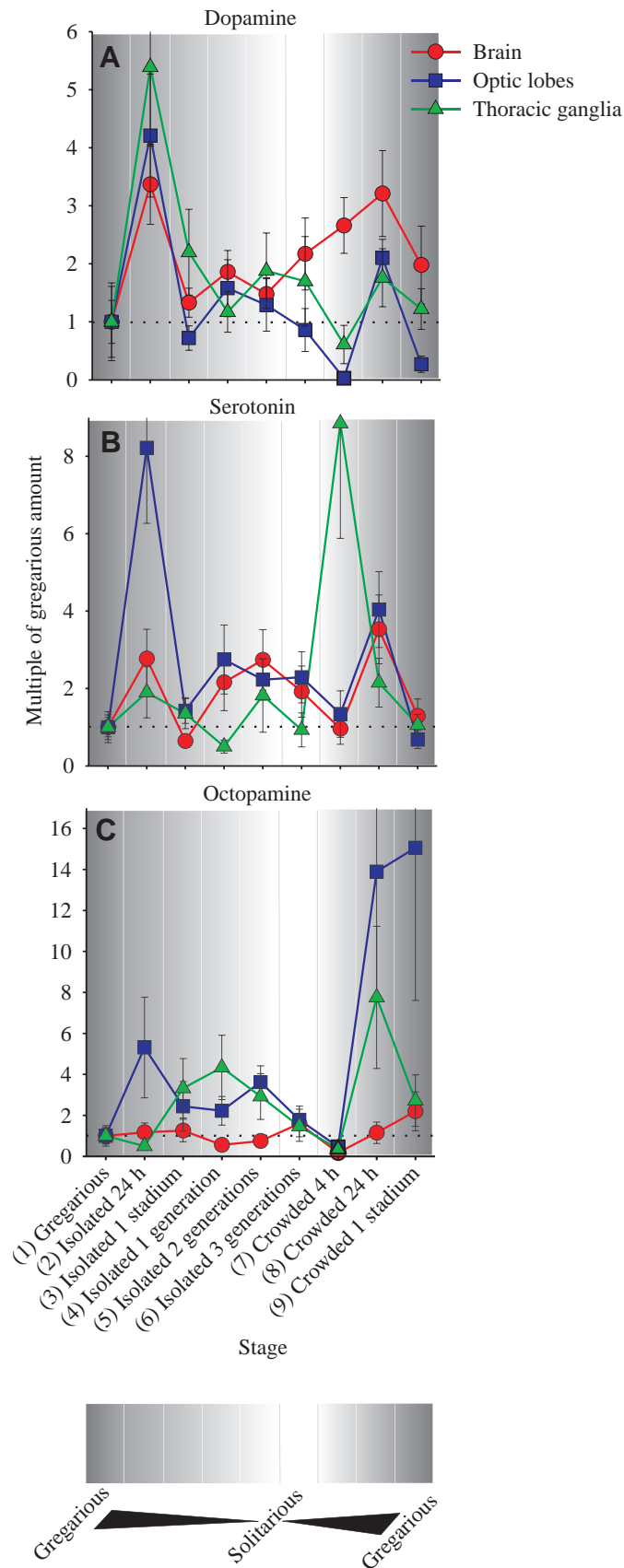
Fig. 5. Changes in the monoamines dopamine, serotonin and octopamine during phase change. Dopamine (A) increased three- to fivefold throughout the central nervous system following a 24 h period of isolation of gregarious locusts, but declined to near gregarious values after 1 stadium of isolation. Locusts isolated for longer periods had amounts just above those of gregarious locusts. There was an increase in brain dopamine levels 4–24 h following crowding, but a decline in the optic lobes and thoracic ganglia. Mean amounts of optic lobe serotonin increased eightfold on initial isolation, accompanied by more modest changes in the brain and no change in the thorax. Amounts declined to near gregarious levels throughout the central nervous system on further isolation. 4 h of crowding produced a ninefold increase in thoracic serotonin, followed after 24 h by smaller (fourfold) increases in the brain and optic lobes. There were no significant changes in octopamine (C) during the entire isolation process, but crowding third generation solitarious locusts for 24 h caused a 13-fold increase in the optic lobes and a sevenfold increase in the thoracic ganglia. The amounts of octopamine in the optic lobes remained high after 1 stadium of crowding. Values (mean ± S.E.M.) are the amounts in the three sampled regions of the central nervous system, standardized to give the mean long-term gregarious value as 1. Stages as in Fig. 1.

of all amino acids in both groups towards gregarious values in the final larval instar (Fig. 3), and only the amounts of aspartate remained significantly different from those of long-term gregarious animals.

Chemicals that decreased on isolation of gregarious locusts

Three chemicals, citrulline, tyramine and acetylcholine, decreased significantly following isolation of gregarious phase locusts, (Fig. 4). The relative decrease after three generations of isolation ranged from over 90% for citrulline, to 50% for tyramine and 20% for acetylcholine. Acetylcholine decreased progressively with increasing periods of isolation, reaching a minimum on the first generation of isolation and staying at a constant level thereafter. By contrast there was a rapid decrease in citrulline and tyramine following isolation for 24 h, but the amounts had increased somewhat (in the direction of the gregarious amounts) after 1 stadium of isolation. They subsequently decreased again during 1–3 generations of isolation. This pattern of change shown by citrulline and tyramine (Fig. 4) mirrored that of the amino acids that increased on isolation, as described above.

Crowding third-generation solitarious locusts for 4 h to 1 stadium had no effect on the amount of acetylcholine, which remained consistently lower than in long-term gregarious locusts. Crowding third-generation solitarious locusts caused an increase in citrulline within 4 h, but by 24 h of crowding amounts had decreased again towards fully solitarious values. Crowding had no significant effect on the amount of tyramine after 4 h of crowding, but after crowding for 24 h there was a dramatic increase up to near gregarious levels, which was sustained after 1 stadium of crowding.

Dopamine

Dopamine increased three- to fivefold in all three regions

Third-generation solitarious locusts that had been crowded for an entire stadium showed a strong decrease in the amounts

Fig. 6. Relative differences in the amounts of 12 chemicals in the brains and thoracic ganglia of second generation solitarious and long-term gregarious adult locusts. Values are standardized to give a mean gregarious value of 1; the grey bars indicate ± S.E.M. of the gregarious values. The circles shown the mean multiple of the gregarious value found in second-generation isolated locusts (± S.E.M.). Asterisks indicate significance of *post hoc* comparisons, as detailed in Table 2. NS, not significant.
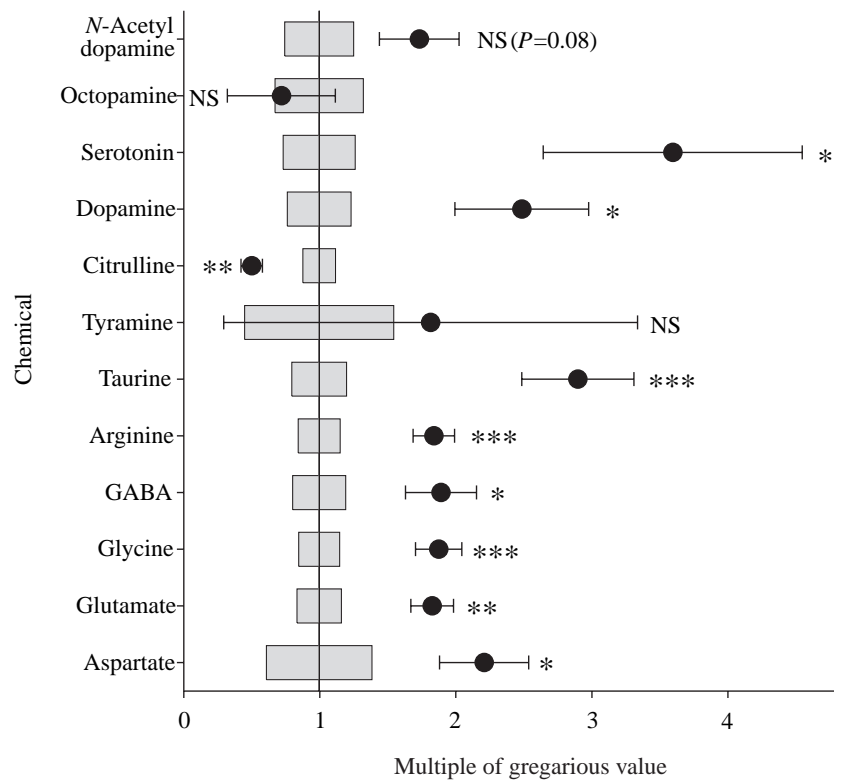
of the central nervous system following 24 h isolation of gregarious locusts (Fig. 5A). This was followed by a decline back towards gregarious values following 1 stadium of isolation, a pattern similar to some of the amino acids (cf. Fig. 3A). Isolation for 1–3 generations led to some increase in the amount of dopamine in the brain and thoracic ganglia but measured amounts were highly variable between samples and the differences were not significant in final instar nymphs. In adults, however, crowding for two generations caused a significant elevation of dopamine (Fig. 6, Table 3). Amounts of dopamine fell in the optic lobes and thoracic ganglia during 4 h of crowding of third-generation solitarious nymphs to levels below those found in long-term gregarious nymphs. Indeed, no dopamine was detected in any of the optic lobe samples after 4 h of crowding (Fig. 5A). In the brain, dopamine levels after a 4 h period of crowding remained similar to those of third-generation solitarious locusts, approximately 2.5 times greater than that of long-term gregarious animals. After 24 h of crowding, amounts of dopamine in the brain and optic lobes were significantly greater than in long-term gregarious locusts, but after 1 stadium of crowding there was a decrease towards long-term gregarious values in all three regions.

Serotonin

The mean amount of serotonin in the brain doubled and there was an eightfold increase in the optic lobes following 24 h isolation of long-term gregarious locusts (Fig. 5B). This was followed by a decrease so that animals isolated for 1 stadium had amounts of serotonin similar to those in long-term gregarious locusts. There were increases in the mean amounts present in the brain and optic lobes of one- to three-generation isolated locusts but, as with dopamine, these changes were non-significant in nymphs whilst significant in second-generation solitarious adults (Fig. 6, Table 3). Isolation for 24 h to three generations caused no change in the amount of serotonin present in the thoracic ganglia. Crowding third-generation solitarious nymphs for 4 h led to a small decrease in the amounts of serotonin found in the brain and optic lobes, but to a ninefold increase in the thoracic ganglia. After 24 h crowding there were fourfold increases in the brain and optic

lobes, but amounts present in the thoracic ganglia had declined again to near their gregarious level. After 1 stadium of crowding, amounts of serotonin in all three regions of the central nervous system were similar to those of long-term gregarious locusts.

Octopamine

There were no statistically significant changes in the amount of octopamine present throughout the central nervous system during isolation (from 24 h to three generations). There was, however, considerable variation in the amounts detected in the optic lobes and thoracic ganglia of locusts isolated for 24 h to two generations, with the mean amounts well above those of long-term gregarious nymphs (Fig. 5C). There was no significant difference between amounts detected in long-term gregarious and second generation solitarious adult locusts (Fig. 6). Crowding long-term solitarious locusts, however, produced large changes in the amount of octopamine. Crowding for 4 h caused octopamine to decrease to an undetectable level throughout the central nervous system (Fig. 5C), paralleling changes seen in dopamine levels in the optic lobes and thoracic ganglia (Fig. 5A). After 24 h of crowding, however, octopamine levels increased by a mean of eightfold in the thoracic ganglia, and by a mean of 14-fold in the optic lobes, although there was great variability between samples. After 1 stadium of crowding, amounts of octopamine present in the thoracic ganglia and brain were near those of gregarious locusts, but the amount present in the optic lobes remained 15 times larger than in long-term gregarious locusts.
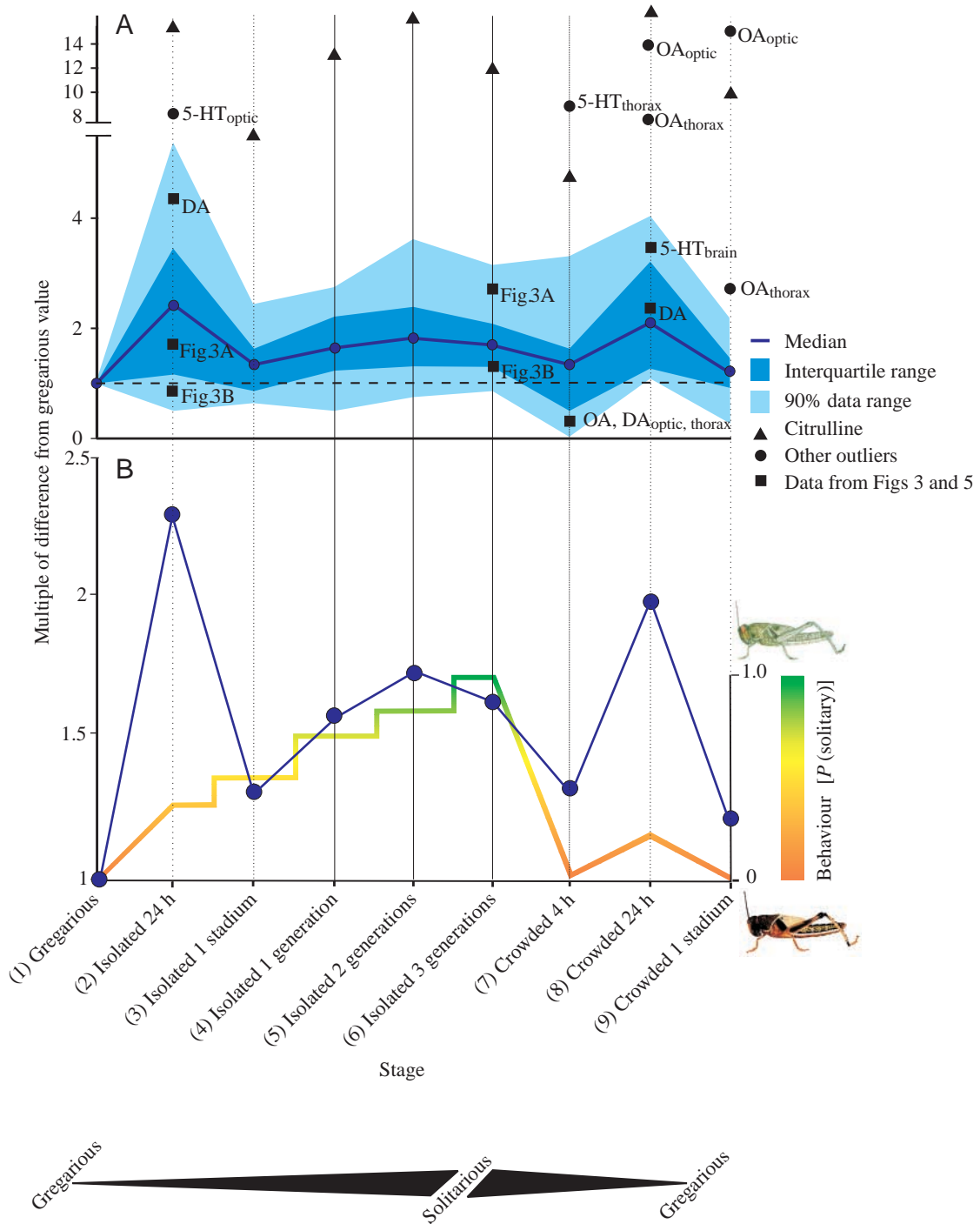
Fig. 7. Summary of the neurochemical changes that occur on isolation of gregarious and crowding of solitarious locusts. (A) Median values (dark blue circles and line), interquartile range (dark blue region) and 95% data range (light blue region) for all chemicals analysed, expressed as multiple of the difference from long-term gregarious values. The inverse values were used for chemicals that declined in solitarious locusts (cf. Fig. 3). Outliers (data that lie more than two interquartile ranges from the median) are shown as circles and, for citrulline, as triangles. Selected data from Figs 3 and 4 (squares) are also plotted for comparison with the overall pattern. Optic, optic lobes; thorax, thoracic ganglia; OA, octopamine; 5-HT, serotonin; DA, dopamine. (B) Median changes in the amounts of neurochemicals as in A (blue line), plotted against the change in behavioural phase state associated with the same degrees of isolation and crowding (stages 1–9; see Fig. 1), expressed as P (solitary) in a logistic regression behavioural assay (orange, gregarious; green, solitarious). Data for behavioural phase state taken from Roessingh et al. (1993) and Roessingh and Simpson (1994).

Discussion

This study demonstrates that the wide-ranging modifications of behaviour that characterise phase change are also accompanied by extensive and specific changes in many neurotransmitters and neuromodulators (Fig. 7). Long-term solitarious and gregarious locusts differed in 11 of the 13 chemicals sampled; only *N*-acetyldopamine and octopamine were not significantly different either as adults or final instar nymphs. Furthermore, amounts of many of the chemicals underwent significant changes within 24 h of either the isolation of gregarious locusts or the crowding of solitarious locusts. In several instances the magnitude of these rapid changes exceeded the long-term differences in amounts between phases. Discussion of the possible effects of these various substances is divided into long-term and short-term differences.

Long-term differences

The different patterns of changes amongst the sampled chemicals argue against phase change causing a single overarching adjustment in metabolic rate, or even overall growth of the central nervous system. During isolation relative increases in chemicals ranged from 30% to over 300% for the amino acids, and some chemicals decreased in the same period. There were no simple correlations between changes in the amount of one chemical and changes in a related chemical compound. Thus, for example, although GABA is synthesised from glutamate, the observed doubling of glutamate in solitarious locusts was accompanied by only a 30% increase in GABA. Tyramine is a precursor of octopamine and is possibly a neuromodulator in its own right (Downer et al., 1993; Roeder et al., 2003) but, whilst amounts of tyramine decreased in long-term solitarious locusts, amounts of octopamine were no different between phases. NADA is a metabolite of dopamine (Sasaki and Nagao, 2000), but whilst amounts of dopamine differed between phases, those of NADA did not.

The monoamines analysed play important roles in the regulation and modulation of insect nervous systems and may control or modify many physiological processes and behaviours (Burrows, 1996; Homberg, 2002). Only a small number of neurones in the fully gregarious locust show antibody staining to serotonin, but many of these neurones have extensive ramifications throughout the central nervous system, including most of the major neuropiles of the brain (Klemm and Sundler, 1983; Homberg, 1991). In each of the thoracic ganglia there are two large and up to three small pairs of cell bodies associated with each neuromere (Tyrer et al., 1984). There are about 3000 neurones showing dopamine-like immunoreactivity in the peripheral optic lobes of locusts, with a further 110 pairs of dopamine-containing neurones in the central part of the brain, with notable absences of staining in the mushroom bodies, olfactory regions of the antennal lobes and most of the lobula (Wendt and Homberg, 1992). In the thoracic ganglia, there are three pairs of cells showing dopamine-like immunoreactivity in the prothoracic ganglion, one pair in the mesothoracic and none in the metathoracic.

Serotoninergic, dopaminergic and octopaminergic fibres all supply the storage lobes of the corpora cardiaca (Konings et al., 1988).

By contrast with the monoamines, the roles of some of the amino acids are largely unknown. Although absolute amounts of amino acids detected differed considerably in different regions, the relative changes they underwent during phase change were generally consistent throughout the whole of the central nervous system, suggesting that there are some universal changes in neuronal function, in addition to specific modification of key neuronal networks.

Aspartate, glutamate, glycine and arginine are all structural amino acid monomers used in protein synthesis and would therefore be expected to be found in any tissue. The rapidity of the changes in the amounts of three of these amino acids in locusts subjected to short-term crowding or isolation argues against the differences being explained by changes in the relative size of the brain between phases. Nor can the differences be explained by changes in dietary protein intake, which remains unchanged between phases in locusts fed a nutritionally optimal diet, as in the present study (Simpson et al., 2002). An additional set of samples taken from another body tissue might suggest whether the changes in the amounts of structural amino acids are a general consequence of phase change or more specific to the central nervous system. The fat body, however, differs in extent and composition between phases (Ayali et al., 1996; Pener et al., 1997), suggesting that different organs may undergo their own characteristic modifications during phase change. By contrast, the muscles are innervated by neurones using many of the same substances that we have analysed, and therefore differences are likely to reflect those found in the central nervous system.

Glutamate, GABA and acetylcholine are widespread and important neurotransmitters in the central and peripheral nervous systems of insects. Glutamate is thought to be the principal excitatory transmitter at neuromuscular junctions, and is therefore strongly associated with motor neurones, but it also acts as a central neurotransmitter (Wafford and Sattelle, 1989; Parker, 1994). There are 350–600 cell bodies displaying strong glutamate immunoreactivity in each of the thoracic ganglia (Watson and Seymour-Laurent, 1993). The inhibitory neurotransmitter GABA has a wide distribution in the central nervous system, as demonstrated by immunocytochemistry (Breer and Heilgenberg, 1985). In locust thoracic ganglia, each ganglion has approximately 250 cell bodies exhibiting GABA-like immunoreactivity including inhibitory motor neurones, a population of spiking local interneurones (Watson, 1986) and non-spiking local interneurones (Wildman et al., 2002). Acetylcholine is thought to be an important excitatory neurotransmitter of locust mechanosensory (Lutz and Tyrer, 1988; Parker and Newland, 1995; Gauglitz and Pflüger, 2001) and chemosensory afferents (Python and Stocker, 2002). The decrease in the amount of acetylcholine on increasing solitarization would be congruent with the observed decrease in the numbers of exteroceptive and chemoreceptive sensilla,

and therefore sensory afferents, on most leg segments in solitarious locusts (Rogers et al., 2003).

Arginine is the precursor of nitric oxide (NO) and citrulline is a metabolic byproduct of NO formation (Palmer et al., 1988). Citrulline is produced in stoichiometric amounts to NO and has been shown previously to be a reliable index of NO release (Kendrick et al., 1996). Whereas the differences in the amounts of arginine could arise from a number of causes, the reciprocal changes in the amounts of citrulline on increasing solitarization provide an indication, albeit indirectly, that NO signalling differs between phases throughout the central nervous system. NO is a rapidly diffusing intercellular signalling molecule synthesised by many neurones in the locust central nervous system (Ott et al., 2001). These include neuronal populations in the optic lobes (Elphick et al., 1996), the antennal lobes, which show the highest concentration of nitric oxide synthase in the locust brain (Müller and Bicker, 1994) and mechanosensory processing neuropiles in the thoracic ganglia (Ott and Burrows, 1998).

The remaining amino acids, glycine, aspartate and taurine, have as yet largely undefined roles in the central nervous system of insects. Glycine is an important inhibitory neurotransmitter in vertebrates (Kuhse et al., 1995), but has no known neuronal function in invertebrates. Despite this, glycine showed some of the strongest differences during phase change, paralleling the pattern of change seen in glutamate and aspartate. Immunocytochemical staining has revealed regions of strong aspartate staining in the lamina of the optic lobe that are widely conserved in phylogenetically distant groups of insects (Sinakevitch and Strausfeld, 2004). There are distinct aspartate-rich laminas in the mushroom bodies of bees and cockroaches that alternate with taurine-rich regions (Ehmer and Gronenberg, 2002; Sinakevitch et al., 2001). Aspartate is also an agonist of one class of cation-selective glutamate receptor (Usherwood, 1994) and may therefore have a functional role in signalling of some glutaminergic neurones. Taurine is abundant in the central nervous system of insects (Schafer et al., 1988) and the mushroom bodies contain discrete laminas that stain strongly for taurine, hinting at specific functional roles (Sinakevitch and Strausfeld, 2004).

Rapid changes on crowding or isolation

Behavioural gregarization is fully established within 4 h of crowding (Roessingh and Simpson, 1994; Fig. 6B), and over this period locusts shift from being repelled by other locusts to being strongly attracted (Simpson et al., 1999). Subsequently, other locusts provide gregarizing stimuli, continuously reinforcing the gregarious phase state in a positive feedback loop. Therefore, the changes that occurred within just hours of crowding third-generation solitarious locusts might have a particular functional significance in the initiation of phase change. Eight of the twelve chemicals that showed significant differences with phase state dropped towards or even below gregarious values with 4 h crowding of third-generation solitarious locusts (Fig. 6B). The amount of thoracic serotonin, however, stood out from this trend in that it increased ninefold

during the first 4 h of crowding. This massive change in serotonin in just the thoracic ganglia may be particularly significant to the early stages of gregarization, as only sensory receptors on the middle and particularly the hind legs can detect key mechanosensory gregarizing stimuli (Simpson et al., 2001; Rogers et al., 2003).

The rapid chemical changes that occurred on initial crowding were not sustained; after crowding for a 24 h period, the amounts of most amino acids were more similar to those of long-term solitarious locusts than those that had been crowded for just 4 h. Amounts of serotonin (in the brain only), dopamine and octopamine (optic lobes and thoracic ganglia), however, having decreased in the first 4 h of crowding, subsequently increased above the levels of long-term solitarious locusts during this period. Data from Roessingh and Simpson (1994) suggest that there is a small degree of reversion to a more solitarious behavioural state in animals crowded for 24 h relative to their values after 4 h (Fig. 6B). This was possibly due to the fact that 12 h of the crowding period was spent in the dark, during which time activity levels, and thus interactions among individuals, would have been greatly reduced. This could also account for some of the reversion to solitarious levels of neurochemicals, but the degree of neurochemical change is more extreme than that suggested by the behavioural reversion.

Locusts crowded for a longer period, the final larval stadium, showed a strong shift in levels of most chemicals towards amounts characteristic of long-term gregarious locusts, with octopamine in the optic lobes, acetylcholine and citrulline being the notable exceptions. These changes largely concur with the robust behavioural gregarization shown by locusts after this time.

The expression of solitarious behaviour follows a different pattern from that of gregarization. In locusts that have been gregarious for generations there is an initial, rapid phase of solitarization over the first 4 h of isolation but this stabilizes at an intermediate level of solitarious behaviour [median P (solitary)$\approx$0.4; Roessingh and Simpson, 1994; Simpson et al., 1999; Fig. 6B]. Locusts can only become more solitarious if kept isolated across stadia and generations. By contrast, when locusts that have been solitarious for generations are briefly crowded for 24–96 h they become fully behaviourally gregarious. When reisolated, they return to a fully solitarious state within a few hours (Roessingh and Simpson, 1994). This suggests that solitarization is at least a two-stage process.

The rapid change in the amounts of many neurochemicals that occurred during the initial (24 h) isolation period of gregarious locusts has a partial correlate in the rapid partial behavioural solitarization that occurs over this period (Fig. 6B). Many chemicals, however, increased to levels equal to those of long-term solitarious locusts, and thus exceeded the equivalent behavioural change. Amounts of eight of the twelve chemicals were either more than doubled or halved in quantity within 24 h of isolating gregarious locusts. Changes that occurred in dopamine and levels of serotonin in the brain and particularly optic lobes in the first 24 h of isolation exceeded

those of long-term solitarious locusts. Large changes in serotonin are therefore implicated in both the early stages of gregarization and solitarization, but occur in a different location within the central nervous system, i.e. the thoracic ganglia during gregarization and the brain during solitarization. As with the effect of crowding, these large initial changes were not sustained in the medium term and amounts of most chemicals found in locusts isolated for one stadium were more similar to those in long-term gregarious locusts than to those in animals that had been isolated for just 24 h, and it required a generation or more of isolation for more stable chemical differences to appear.

Only octopamine has previously been measured with regard to phase change and the results were unclear. Morton and Evans (1983) found no phase-related difference in octopamine in *Schistocerca americana gregaria*, whereas Fuzeau-Braesch and colleagues (Fuzeau-Braesch and David, 1978; Fuzeau-Braesch and Nicholas, 1981), using *Locusta migratoria*, suggested there were strong differences, with solitarious phase locusts containing more octopamine than gregarious insects. It should be noted, however, that although *S. americana* changes its colour with rearing density, behavioural phase change is much weaker in this species (Sword, 2003). We found no differences between amounts of octopamine in long-term solitarious and gregarious desert locusts, but the large increase we saw after the initial stages of gregarization (when levels fell) may account for the data obtained by Fuzeau-Braesch and colleagues if their husbandry or handling procedures inadvertently led to behavioural gregarization of their solitarious animals.

Octopamine is often loosely characterised as a 'stress hormone' (Davenport and Evans, 1984a; Birmingham and Tauck, 2003), and levels have been shown to increase in the haemolymph by up to eightfold in response to conditions such as food deprivation (Davenport and Evans, 1984b) or heat stress (Hirashima et al., 2000). Indeed, the widespread changes in brain chemicals we report here, whilst clearly correlated with both solitarization and gregarization, do not necessarily have a causative role. These rapid changes may instead arise from an aversive response to being subjected to a novel situation, i.e. being placed in or out of a group unexpectedly, and should perhaps be seen as another kind of phase characteristic.

We have previously demonstrated specific neurophysiological differences between the two extreme locust phases (Matheson et al., 2004), which may in part be underpinned by differences in synaptic transmission and neuromodulation, and this will become a focus of future analyses at the cellular and network level. Previous immunocytochemical analyses of neurones containing the neurotransmitters and neuromodulators analysed in this study have been performed only on long-term gregarious locusts. Perhaps phase change may alter the branching patterns and numbers of neurones expressing different neurochemicals, and this will be pursued in future studies. Our data demonstrate the extensive neurochemical changes in the central nervous system that accompany phase change in locusts and lay the foundation for more critical examinations of the causative role these substances may have in either gregarization or solitarization.

List of abbreviations

5-HT	serotonin/5-hydroxytryptamine
DA	dopamine
DHBA	3,4-dihydroxybenzylamine hydrobromide
GABA	γ-aminobutyric acid
HPLC	high performance liquid chromatography
NADA	*N*-acetyldopamine
OA	octopamine
OPA	*o*-pthaldialdehyde
TA	tyramine

References

Ayali, A., Golenser, E. and Pener, M. P. (1996). Flight fuel related differences between solitary and gregarious locusts (*Locusta migratoria migratorioides*). *Physiol. Entomol.* **21**, 1-6.

Birmingham, J. T. and Tauck, D. L. (2003). Neuromodulation in invertebrate sensory systems: from biophysics to behavior. *J. Exp. Biol.* **206**, 3541-3546.

Blackburn, L., Rogers, S. M. and Burrows, M. (2003). Phase differences in the walking behaviour of the desert locust *Schistocerca gregaria. Comp. Biochem. Physiol.* **134A**, S73.

Breer, H. and Heilgenberg, H. (1985). Neurochemistry of GABAergic activities in the central nervous system of *Locusta migratoria. J. Comp. Physiol.* **157A**, 343-354.

Breuer, M., Hoste, B. and De Loof, A. (2003). The endocrine control of phase transition: some new aspects. *Physiol. Entomol.* **28**, 3-10.

Bouaichi, A., Roessingh, P. and Simpson, S. J. (1995). An analysis of the behavioural effects of crowding and re-isolation on solitary-reared adult desert locusts (*Schistocerca gregaria*) and their offspring *Physiol. Entomol.* **20**, 199-208.

Broad, K. D., Hinton, M. R., Keverne, E. B. and Kendrick, K. M. (2002). Involvement of the medial prefrontal cortex in mediating behavioural responses to odour cues rather than olfactory recognition memory. *Neurosci.* **114**, 715-729.

Burrows, M. (1996). *The Neurobiology of an Insect Brain*. Oxford, UK: Oxford University Press.

Consoulas, C., Duch, C., Bayline, R. J. and Levine, R. B. (2000). Behavioral transformations during metamorphosis: remodeling of neural and motor systems. *Brain Res. Bull.* **53**, 571-583.

Cuttle, M. F., Hevers W., Laughlin S. B. and Hardie, R. C. (1995). Diurnal modulation of photoreceptor potassium conductance in the locust. *J. Comp. Physiol.* **176A**, 307-316.

Davenport, A. P. and Evans, P. D. (1984a). Stress-induced changes in the octopamine levels of insect haemolymph. *Insect Biochem.* **14**, 135-143.

Davenport, A. P. **and Evans, P. D.** (1984b). Changes in hemolymph octopamine levels associated with food-deprivation in the locust, *Schistocerca gregaria. Physiol. Entomol.* **9**, 269-274.

Downer, R. G., Hiripi, L. and Juhos, S. (1993). Characterization of the tyraminergic system in the central nervous system of the locust, *Locusta migratoria migratoides. Neurochem. Res.* **18**, 1245-1248.

Ehmer, B. and Gronenberg, W. (2002). Segregation of visual input to the mushroom bodies in the honeybee (*Apis mellifera*). *J. Comp. Neurol.* **451**, 362-373.

Elphick, M. R., Williams, J. R. D. and O'Shea, M. G. (1995). New features of the locust optic lobe – evidence of a role for nitric oxide in insect vision. *J. Exp. Biol.* **199**, 2395-2407.

Fabre-Nys, C., Ohkura, S. and Kendrick, K. M. (1997). Male faces and odours evoke differential patterns of neurochemical release in the mediobasal hypothalamus of the ewe during oestrus: an insight into sexual motivation? *Eur. J. Neurosci.* **9**, 1666-1677.

Fuchs, E., Kutsch, W. and Ayali, A. (2003). Neural correlates to flight-related density-dependent phase characteristics in locusts. *J. Neurobiol.* **57**, 152-162.

Fuzeau-Braesch, S. and David, J. C. (1978). Etude du taux d'octopamine chez *Locusta migratoria* (Insecte: Orthoptere) comparison entre insects gregaires, solitaires et traite au gaz carbonique. *C.R. Acad. Sci. Paris D* **286**, 697-699.

Fuzeau-Braesch, S. and Nicholas, G. (1981). Effect of carbon dioxide on subsocial insects. *Comp. Biochem. Physiol.* **68A**, 289-297.

Gauglitz, S. and Pflüger, H. J. (2001). Cholinergic transmission via central synapses in the locust nervous system. *J. Comp. Physiol.* **187A**, 825-836.

Hägele, B. F. and Simpson, S. J. (2000). The influence of mechanical, visual and contact chemosensory stimulation on the behavioural phase state of solitarious locusts *Schistocerca gregaria. J. Insect Physiol.* **46**, 1295-1301.

Hevers, W. and Hardie, R. C. (1995). Serotonin modulates the voltage dependence of delayed rectifier and Shaker potassium channels in *Drosophila* photoreceptors. *Neuron* **14**, 845-856.

Hirashima, A., Sukhanova, M. J. and Rauschenbach, I. Y. (2000). Biogenic amines in *Drosophila virilis* under stress conditions. *Biosci. Biotech. Biochem.* **64**, 2625-2630.

Homberg, U. (1991). Neuroarchitecture of the central complex in the brain of the locust *Schistocerca gregaria* and *S. americana* as revealed by serotonin immunocytochemistry *J. Comp. Neurol.* **303**, 245-254.

Homberg, U. (2002). Neurotransmitters and neuropeptides in the brain of the locust. *Microsc. Res. Tech.* **56**, 189-209.

Homberg, U. and Hildebrand, J. G. (1994). Postembryonic development of gamma-aminobutyric acid-like immunoreactivity in the brain of the sphinx moth *Manduca sexta. J. Comp. Neurol.* **339**, 132-149.

Hoste, B., Simpson, S. J., Tanaka, S., De Loof, A. and Breuer, M. (2002). A comparison of phase-related shifts in behavior and morphometrics of an albino strain, deficient in [His(7)]-corazonin, and a normally colored *Locusta migratoria* strain. *J. Insect Physiol.* **48**, 791-801.

Islam, M. S., Roessingh, P., Simpson, S. J. and McCaffery, A. R. (1994a). Effects of population density experienced by parents during mating and oviposition on the phase of hatchling desert locusts, *Schistocerca gregaria. Proc. R. Soc. Lond. B* **257**, 93-98.

Islam, M. S., Roessingh, P., Simpson, S. J. and McCaffery, A. R. (1994b). Parental effects on the behavior and coloration of nymphs of the desert locust *Schistocerca gregaria. J. Insect Physiol.* **40**, 173-181.

Kendrick, K. M., Guevara-Guzman, R., de la Riva C., Christensen, J., Ostergaard, K. and Emson P. C. (1996). NMDA and kainate-evoked release of nitric oxide and classical transmitters in the rat striatum: *in vivo* evidence that nitric oxide may play a neuroprotective role. *Eur. J. Neurosci.* **8**, 2619-2634.

Klemm, N. and Sundler, F. (1983). The organisation of catecholamine and serotonin immunoreactive fibres in the corpora pedunculata of the desert locust, *Schistocerca gregaria* Forsk. (Insecta, Orthoptera). *Neurosci. Lett.* **36**, 13-17.

Kloas, W., Reinecke, M. and Hanke, W. (1997). Stage-dependent changes in adrenal steroids and catecholamines during development in *Xenopus laevis. Gen. Comp. Endocrinol.* **108**, 416-426.

Konings, P. N. M., Vullings, H. G. B., Siebinga, R., Diederen, J. H. B. and Jansen, W. F. (1988). Serotonin-immunoreactive neurons in the brain of *Locusta migratoria* innervating the corpus cardiacum. *Cell Tissue Res.* **254**, 147-153.

Kravitz, E. A. (2000). Serotonin and aggression: insights gained from a lobster model system and speculations on the role of amine neurons in a complex behavior. *J. Comp. Physiol. A* **186**, 221-238.

Kuhse, J., Betz, H. and Kirsch, J. (1995). The inhibitory glycine receptor: architecture, synaptic localization and molecular pathology of a postsynaptic ion-channel complex. *Curr. Opin. Neurobiol.* **5**, 318-323.

Lehman, H. K., Klukas, K. A., Gilchrist, L. S. and Mesce, K. A. (2000a). Steroid regulation of octopamine expression during metamorphic development of the moth *Manduca sexta. J. Comp. Neurol.* **424**, 283-296.

Lehman, H. K., Murgiuc, C. M. and Hildebrand, J. G. (2000b). Characterization and developmental regulation of tyramine-beta-hydroxylase in the CNS of the moth, *Manduca sexta. Insect Biochem. Mol. Biol.* **30**, 377-386.

Lutz, E. M. and Tyrer, N. M. (1988). Immunohistochemical localization of

serotonin and choline acetyltransferase in sensory neurones of the locust. *J. Comp. Neurol.* **267**, 335-342.

Matheson, T., Basu Roy, R., Copeland, M., Krapp, H. and Rogers, S. M. (2003). Plasticity in the visual system of solitarious and gregarious locusts. *J. Physiol.* **547**, 91.

Matheson, T., Rogers, S. M. and Krapp, H. G. (2004). Plasticity in the visual system is correlated with a change in lifestyle of solitarious and gregarious locusts. *J. Neurophysiol.* **91**, 1-12.

Mercer, A. R. and Hildebrand, J. G. (2002). Developmental changes in the electrophysiological properties and response characteristics of *Manduca* antennal-lobe neurons. *J. Neurophysiol.* **87**, 2650-2663.

Morton, D. B. and Evans, P. D. (1983). Octopamine distribution in solitarious and gregarious forms of the locust *Schistocerca Americana gregaria. Insect Biochem.* **13**, 177-183.

Müller, U. and Bicker, G. (1994). Calcium-activated release of nitric oxide and cellular distribution of nitric oxide-synthesizing neurons in the nervous system of the locust. *J. Neurosci.* **14**, 7521-7528.

Ott, S. R. and Burrows, M. (1998). Nitric oxide synthase in the thoracic ganglia of the locust: distribution in the neuropiles and morphology of neurones. *J. Comp. Neurol.* **395**, 217-230.

Ott, S. R., Burrows, M. and Elphick, M. R. (2001). The neuroanatomy of nitric oxide-cyclic GMP signalling in the locust: Functional implications for sensory systems. *Amer. Zool.* **41**, 321-331.

Palmer, R. M., Ashton, D. S. and Moncada, S. (1988). Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* **333**, 664-666.

Parker, D. (1994). Glutamatergic transmission between antagonistic motor-neurons in the locust. *J. Comp. Physiol. A* **175**, 737-748.

Parker, D. and Newland, P. L. (1995). Cholinergic synaptic transmission between proprioceptive afferents and a hind leg motor neuron in the locust. *J. Neurophysiol.* **73**, 586-594.

Pener, M. P. (1991). Locust phase polymorphism and its endocrine relations. *Adv. Insect Physiol.* **23**, 1-79.

Pener, M. P., Ayali, A. and Golenser, E. (1997). Adipokinetic hormone and flight fuel related characteristics of density-dependent locust phase polymorphism: A review. *Comp. Biochem. Physiol.* **117B**, 513-524.

Pener, M. P. and Yerushalmi, Y. B. (1998). The physiology of locust phase polymorphism: an update. *J. Insect Physiol.* **44**, 365-377.

Python, F. and Stocker, R. F. (2002). Immunoreactivity against choline acetyltransferase, gamma-aminobutyric acid, histamine, octopamine, and serotonin in the larval chemosensory system of *Drosophila melanogaster. J. Comp. Neurol.* **453**, 157-167.

Roeder, T., Seifert, M., Kahler, C. and Gewecke, M. (2003). Tyramine and octopamine: antagonistic modulators of behavior and metabolism. *Arch. Insect Biochem. Physiol.* **54**, 1-13.

Roessingh, P., Bouaïchi, A. and Simpson, S. J. (1998). Effects of sensory stimuli on the behavioural phase state of the desert locust, *Schistocerca gregaria. J. Insect. Physiol.* **44**, 883-893.

Roessingh, P. and Simpson, S. J. (1994). The time-course of behavioural phase change in nymphs of the desert locust, *Schistocerca gregaria. Physiol. Entomol.* **19**, 191-197.

Roessingh, P., Simpson, S. J. and James, S. (1993). Analysis of phase-related changes in the behaviour of desert locust nymphs. *Proc. R. Soc. Lond. B* **252**, 43-49.

Rogers, S. M., Matheson, T., Despland, E., Dodgson, T., Burrows, M. and Simpson, S. J. (2003). Mechanosensory-induced behavioural gregarization in the desert locust *Schistocerca gregaria. J. Exp. Biol.* **206**, 3991-4002.

Sasaki, K. and Nagao, T. (2001). Distribution and levels of dopamine and its metabolites in brains of reproductive workers in honeybees. *J. Insect Physiol.* **47**, 1205-1216.

Sasaki, K. and Nagao, T. (2002). Brain tyramine and reproductive states of workers in honeybees. *J. Insect Physiol.* **48**, 1075-1085.

Schafer, S., Bicker, G., Ottersen, O. P. and Storm-Mathisen, J. (1988). Taurine-like immunoreactivity in the brain of the honeybee. *J. Comp. Neurol.* **268**, 60-70.

Schulz, D. J. and Robinson, G. E. (1999). Biogenic amines and division of labor in honey bee colonies: behaviorally related changes in the antennal lobes and age-related changes in the mushroom bodies. *J. Comp. Physiol. A* **184**, 481-488.

Sillar, K. T., Reith, C. A. and McDearmid, J. R. (1998). Development and aminergic neuromodulation of a spinal locomotor network controlling swimming in *Xenopus* larvae. *Ann. N.Y. Acad. Sci.* **860**, 318-332.

Simpson, S. J., Despland, E., Hägele, B. F. and Dodgson, T. (2001). Gregarious behavior in desert locusts is evoked by touching their back legs. *Proc. Natl. Acad. Sci. USA* **98**, 3895-3897.

Simpson, S. J, Raubenheimer, D., Behmer, S. T., Whitworth. A. and Wright, G. A. (2002). A comparison of nutritional regulation in solitarious- and gregarious-phase nymphs of the desert locust *Schistocerca gregaria*. *J. Exp. Biol*. **205**, 121-129.

Simpson, S. J., McCaffery, A. R. and Hägele, B. F. (1999). A behavioural analysis of phase change in the desert locust. *Biol. Rev*. **74**, 461-480.

Sinakevitch, I., Farris, S. M. and Strausfeld, N. J. (2001). Taurine-, aspartate- and glutamate-like immunoreactivity identifies chemically distinct subdivisions of Kenyon cells in the cockroach mushroom body. *J. Comp. Neurol*. **439**, 352-367.

Sinakevitch, I. and Strausfeld, N. J. (2004). Chemical neuroanatomy of the fly's movement detection pathway. *J. Comp. Neurol.* **468**, 6-23.

Sneddon, L. U., Taylor, A. C., Huntingford, F. A. and Watson, D. G. (2000). Agonistic behaviour and biogenic amines in shore crabs *Carcinus maenas*. *J. Exp. Biol.* **203**, 537-545.

Stevenson, P. A., Hofmann, H. A., Schoch, K. and Schildberger, K. (2000). The fight and flight responses of crickets depleted of biogenic amines. *J. Neurobiol.* **43**, 107-120.

Sword, G. A. (2003). To be or not to be a locust? A comparative analysis of behavioural phase change in nymphs of *Schistocerca americana* and *Schistocerca gregaria*. *J. Insect Physiol.* **49**, 709-717.

Takeda, N. (1997). The metabolism of biogenic monoamines during embryogenesis and metamorphosis in two anuran species. *Gen. Comp. Endocrinol.* **106**, 361-373.

Tawfik, A. I., Tanaka, S., De Loof, A., Schoofs, L., Baggerman, G., Waelkens, E., Derua, R., Milner, Y., Yerushalmi, Y. and Pener, M. P. (1999). Identification of the gregarization-associated dark-pigmentotropin in locusts through an albino mutant. *Proc. Natl. Acad. Sci. USA* **96**, 7083-7087.

Taylor, D. J., Robinson, G. E., Logan, B. J., Laverty, R. and Mercer, A. R. (1992). Changes in brain amine levels associated with the morphological and behavioural development of the worker honeybee. *J. Comp. Physiol. A* **170**, 715-721.

Tyrer, N. M., Turner, J. D. and Altman, J. S. (1984). Identifiable neurons in the locust central nervous-system that react with antibodies to serotonin. *J. Comp. Neurol*. **227**, 313-330.

Usherwood, P. N. R. (1994). Insect glutamate receptors. *Adv. Insect Physiol.* **24**, 309-341.

Uvarov, B. (1966). *Grasshoppers and Locusts.* Vol. 1. Cambridge, UK: Cambridge, University Press.

Wafford, K. A. and Sattelle, D. B. (1989). L-glutamate receptors on the cell body membrane of an identified insect motor neuron. *J. Exp. Biol.* **144**, 449-462.

Wagener-Hulme, C., Kuehn, J. C., Schulz, D. J. and Robinson, G. E. (1999). Biogenic amines and division of labor in honey bee colonies. *J. Comp. Physiol. A* **184**, 471-479.

Watson, A. H. D. (1986). The distribution of GABA-like immunoreactivity in the thoracic nervous-system of the locust *Schistocerca gregaria*. *Cell Tissue Res.* **246**, 331-341.

Watson, A. H. D. and Seymour-Laurent, K. J. (1993). The distribution of glutamate-like immunoreactivity in the thoracic and abdominal ganglia of the locust (*Schistocerca gregaria*). *Cell Tissue Res.* **273**, 557-570.

Wendt, B. and Homberg, U. (1992). Immunocytochemistry of dopamine in the brain of the locust *Schistocerca gregaria*. *J. Comp. Neurol.* **321**, 387-403.

Wildman, M., Ott, S. R. and Burrows, M. (2002). GABA-like immunoreactivity in nonspiking interneurons of the locust metathoracic ganglion. *J. Exp. Biol.* **205**, 3651-3659.