

The Neurobiology of a Transformation from Asocial to Social Life During Swarm Formation in Desert Locusts

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Abstract Many species display complex social interactions and for such animals other members of their species can form a major or even dominant part of their environment. Social interactions between these animals can induce long-lasting changes in brain function and behaviour that in turn alter the ways in which they respond to each other. Locusts are insects that can change reversibly between an asocial solitarious phase and a social gregarious phase that is driven by changes in population density. Phase change encompasses both a socially driven mechanism and multifaceted changes in behaviour, physiology, neurochemistry, brain morphology and even appearance. At low densities, locusts occur in the solitarious phase. Their biology is governed by the need to be inconspicuous and they actively avoid other locusts, thus maintaining their low population density. When sheer population size and scarce resources force solitarious locusts together despite their aversion to each other, a transformation is triggered that results in the gregarious phase. The stimuli responsible for starting this transformation are provided by other locusts, notably mechanosensory stimulation resulting from inadvertent jostling of each other. After just a few hours these stimuli induce changes in behaviour, including, critically, a change toward a propensity to be attracted towards other locusts. This attraction initiates a positive feedback loop whereby the continual presence of other locusts provides the necessary stimuli to drive the process towards the extreme gregarious phenotype and eventually to swarming. The biology of gregarious locusts is then dominated by the demands of group living. There is intense competition for resources and considerably greater sensory complexity in the environment brought about by living in a constantly moving throng of other animals. These behavioural demands are reflected in the substantially larger brains of gregarious locusts compared with solitarious locusts. Phase differences can also be detected at the level of identified neurons and circuits and in dramatic changes in

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neurochemistry, but only serotonin shows a substantial increase during the critical 1–4 h window during which gregarious behaviour is established. Blocking the action of serotonin or preventing its synthesis prevents behavioural gregarization. Applying serotonin or its agonists induces gregarious behaviour even in locusts that have never encountered other locusts. The analysis of phase change in locusts provides insights into a feedback circuit between the environment and the neurobiology of social interaction. Remarkably, there is emerging evidence that the neuronal mechanisms underlying this transformation in locusts show similarities with those underlying social behaviours in other animals.

Introduction

Locusts are grasshoppers (Insecta: Orthoptera: Acrididae) that are most well known for their propensity to undergo periodic mass swarming events, where billions of individuals covering up to hundreds of km² erupt from their desert retreats and consume all the available vegetation. Whilst various locust species remain a considerable agricultural problem in many parts of the world, it is perhaps not immediately clear what they have to offer as models for understanding the neurobiology of social interaction. Their usefulness comes from the realisation that swarming is not an inevitable part of the life history of locusts and that they can spend many generations in a form that not only does not swarm but actively avoids other locusts. The swarming and non-swarming forms of the locust are known as the gregarious and solitarious phases (Fig. 1), and the two phases differ so extensively in behaviour, physiology, colouration and morphology (Fig. 1) that until the 1920s they were thought to be different species (Uvarov 1921). All locust species, of which there are only approximately 20 out of >10,000 Acridid species, however, are able to transform reversibly from the completely asocial solitarious phase to the highly social gregarious phase, depending upon environmental cues.

Remarkably, there is strong evidence that the neuronal mechanisms underlying the transformation from solitarious to gregarious behaviour in locusts show pronounced similarities to those underlying social behaviours in other animals. We are just beginning to understand the molecular and neuronal mechanisms driving the early stages of this transformation (Rogers et al. 2004; Anstey et al. 2009; Ma et al. 2011; Guo et al. 2011; Wu et al. 2012; Ott et al. 2012). The sheer extent of behavioural change provides a rich substrate with which to perform comparative analyses of how neuronal circuits organise social behaviour. Such research is considerably facilitated by the detailed information already available about the structure and function of the nervous systems of locusts, down to the cellular physiology of individual neurons and circuits (Burrows 1996). The brain and nervous system of the asocial solitarious locust make the ideal control for comparison with the brain and nervous system of the social gregarious locust. Such a facultative switch, between asocial and social forms within the same animal,

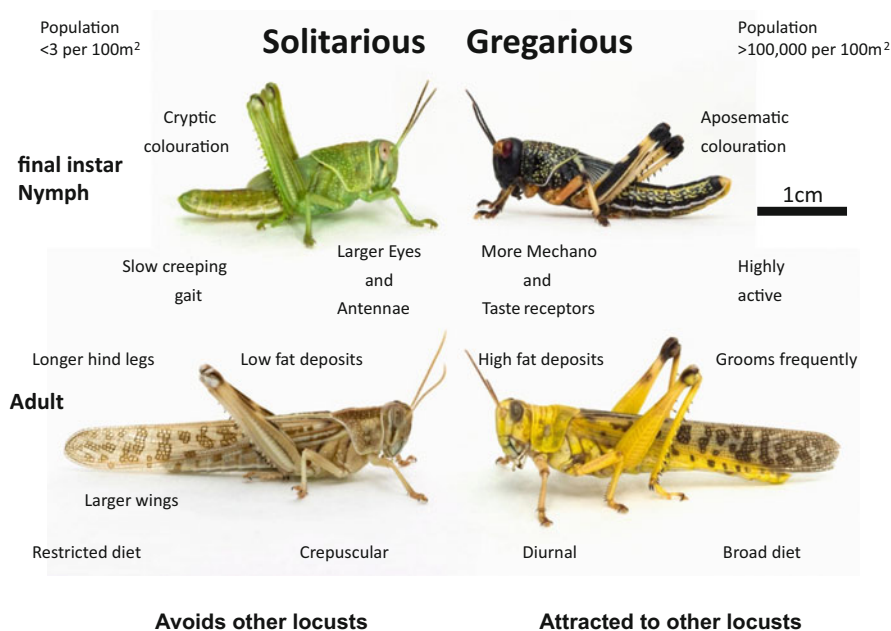


Fig. 1 Last larval instar (*top*) and adult (*bottom*) solitary (*left*) and gregarious (*right*) phase desert locusts, *Schistocerca gregaria* showing their characteristic differences in appearance and posture. Some of the other key behavioural and physiological features of each phase are listed around the photographs

contrasts strongly with more familiar social insects such as bees and ants, where the degree of sociality is typically fixed for individuals within each species.

Locust Phase Change: Phenotypic Plasticity and Sociality

Locusts show some of the most dramatic examples of phenotypic plasticity, the environmentally driven expression of alternative phenotypes from the same genotype shown by any animal (West-Eberhard 2003). Phase change is driven entirely by changes in population density and is mediated by a socially driven mechanism (Uvarov 1966, 1977; Simpson et al. 1999). At low densities, locusts occur in the solitary phase. Solitary locusts are typical grasshoppers in that they rely on being inconspicuous to avoid predators; they are cryptic in both colouration and behaviour. They walk with a slow, creeping gait, fly predominantly at night, have narrow dietary preferences and actively avoid other locusts (unless seeking a mate), thus spreading themselves out thinly in the environment and maintaining their low population density. By contrast, gregarious desert locusts (*Schistocerca gregaria*) walk with a rapid, upright gait, fly during the day, have a broad diet and, most

importantly, are attracted to other locusts such that they may eventually aggregate into vast migratory swarms. With inconspicuousness no longer an option, they have bright warning colours, which advertise that they eat small quantities of poisonous plants to make themselves distasteful to predators (Sword 1999). They also have shorter wings and hind legs and smaller eyes and antennae but more close-proximity touch and taste receptors.

Locusts are typically insects of arid environments that have infrequent and unpredictable rainfalls, leading to sparse vegetation growth only capable of supporting low population densities of locusts. Occasionally, perhaps once every decade, more sustained rains lead to brief periods of superabundant vegetation growth, leading to a concomitant rapid rise in the locust population. Sheer population size and increasingly patchy food resources brought about by consumption and the cessation of rain will act to force solitary locusts together despite their natural aversion to one another (Collett et al. 1998). This forced contact triggers a far-reaching transformation that results in the gregarious phase. This process begins with a rapid change of behaviour, such that after just a few hours of forced crowding, previously solitary locusts behave in many respects like locusts that have been in the gregarious phase their entire lives (Roessingh et al. 1993; Simpson et al. 1999). This change sets up conditions for a positive feedback loop in which the continual presence of other locusts provides the necessary stimuli to drive the process on to completion with further accumulating changes in behaviour, physiology and colouration. Some of these changes will occur within the life span of an individual; others develop over the course of several generations through epigenetic mechanisms (Uvarov 1966, 1977; Pener and Simpson 2009). Phase change is a fully reversible process that is the product of changes that occur with many different time scales (Roessingh and Simpson 1994; Simpson et al. 1999; Bouaichi et al. 1995; Gray et al. 2009).

The stimuli responsible for starting this transformation are provided entirely by other locusts and not any other indicator of environmental stress. In the Desert Locust, *Schistocerca gregaria*, there are two distinct sensory pathways through which the initial transformation in behaviour can be induced: first, mechanosensory stimulation specifically to the hind femora brought about by locusts jostling each other (Simpson et al. 2001; Rogers et al. 2003); and second, the combined sight and smell of other locusts with neither stimulus on its own being effective (Roessingh et al. 1998; Anstey et al. 2009). In the distantly related Australian Plague Locust (*Chortoicetes terminifera*), the change from solitary to gregarious behaviour is induced by mechanosensory stimulation of the antennae, not the hind legs (Cullen et al. 2010). The stimuli responsible for inducing behavioural gregarization in the Migratory Locust (*Locusta migratoria*), another major pest species, are still not fully characterised. Other aspects of phase transformation, notably the longer term changes in colouration and morphology, appear to be mediated by separate, parallel pathways with independent induction mechanisms and hormonal control (Lester et al. 2005). For example, the neuropeptide $[His^7]$ -Corazonin mediates the melanisation of gregarious locusts and some morphometric changes in body

proportions but has little effect on behaviour (Tawfik et al. 1999; Hoste et al. 2002; Maeno and Tanaka 2004).

Social Behaviour of Gregarious Locusts

The modifications shown by gregarious locusts are not primarily concerned with adapting them for sustained long-distance travel to new feeding areas, because solitary locusts can also fly for many hundreds of kilometres (Pener and Simpson 2009). Instead, it seems that the extensive adaptations shown by gregarious locusts occur to enable them to cope with the demands of living within a large group of conspecifics. To what extent, therefore, can gregarious locusts be considered social? Locusts display a different kind of social behaviour to that characterising eusocial insects such as bees, ants and termites; there is no reproductive or other division of labour between different individuals in the ‘society.’ All locusts in a swarm have the same behavioural phenotype, so their sociality arises from the need to engage with and respond to the large numbers of other locusts around them. In a swarm, other locusts form a substantial, even dominant part of the environment of each individual, and they constitute an ever-changing and potentially unpredictable element of it.

The most characteristic manifestation of social interaction displayed by locusts is the formation of coherent marching cohorts of wingless nymphs or flying swarms of adults, with the great majority of individuals moving in the same direction. These highly ordered migratory bands arise spontaneously when local population density exceeds a critical threshold (Buhl et al. 2006). From small nucleation events, hopper bands can expand and recruit all the locusts they encounter until they cover areas measuring kilometres across (Uvarov 1977). Even at their largest expanse, group structure is maintained entirely by local interactions and the implementation of simple behavioural rules of alignment and walking speed between near neighbours in the band (Buhl et al. 2006, 2011). In the Australian Plague Locust, *Chortoicetes terminifera*, the alignment between individuals that determines band cohesion and movement only operates if the distance between locusts is less than 13.5 cm (Buhl et al. 2011).

In addition to alignment and mass migration, there is a more general social dimension within a locust swarm that is generated by the intense competition for resources, particularly food, and necessitates interactions between individuals. At its most extreme, this manifests itself as a propensity to cannibalism, with vulnerable conspecifics being devoured to compensate for protein deprivation (Bazazi et al. 2008, 2011; Hansen et al. 2011). Whilst swarms provide safety in numbers by using a herd strategy to protect against predation, this protection is offset by the risk of falling victim to their own conspecifics. Nevertheless, living in a group, even with the risk of cannibalization, provides survival benefits. This benefit has been termed a ‘lifeboat mechanism,’ where individuals in the group are on average able

to survive longer and travel farther compared to individuals attempting to migrate on their own (Hansen et al. 2011).

Adaptations in the Brain for Social Life in a Swarm

Gregarization requires the acquisition of new behaviours and negotiating new risks, such as the behavioural demands of generalist foraging and intense intraspecific competition. Furthermore, when locusts become gregarious, there is an inherently much greater sensory complexity in the environment brought about by living in the midst of a dense and highly mobile swarm of other animals. How is this reflected by the structure and function of the nervous system (Fig. 2)? The brains of gregarious locusts have very different proportions and are 30 % larger overall than in solitary locusts (Ott and Rogers 2010). Phase-related differences in brain proportions arise from a combination of allometric growth of different brain regions relative to each other as overall size increases and discrete changes in specific brain regions (grade shifts). In the visual system, gregarious locusts have proportionately larger second (medulla) and third visual neuropiles (lobula) compared to the first (lamina). This expansion of the higher visual centres in gregarious locusts occurs despite the fact that they have smaller eyes than solitary locusts (Dirsh 1953; Rogers et al. 2010). The increased size of these higher centres is possibly linked to the complex optic flow-fields and increased risk of collision inherent in a swarm (Matheson et al. 2004; see below).

In the olfactory system, gregarious locusts have fewer olfactory sensilla on the antenna than solitary locusts (*Locusta migratoria*: Greenwood and Chapman 1984; *S. gregaria*: Ochieng' et al. 1998). The antennal lobe, where the olfactory receptor neurons first project, is 10 % smaller in gregarious locusts than in solitary locusts of matching brain size. This contrasts with the massive expansion of the mushroom bodies, a higher olfactory and multi-modal integration centre, in gregarious locusts. The primary calyx in particular, which is composed mainly of axon terminals of projection neuron from the antennal lobes and intrinsic Kenyon cell dendrites, showed the most dramatic phase-related difference (50 % absolute) of all brain regions investigated. Overall it seems that, in both the visual and olfactory systems as well as other important integration centres such as the central complex, the larger brains of gregarious locusts prioritize higher levels of integration. Solitary locusts invest more in low-level sensory processing, having disproportionately larger primary visual and olfactory neuropiles. The reason for this difference may lie in the need to increase sensitivity, since the solitary lifestyle entails greater activity at lower light levels, and the need to detect stimuli over greater distances, since they are subject to increased individual predation risk.

Phase-related differences in brain function can be shown to occur at the level of individual identified neurons and neuronal circuits, for example, in a circuit that signals the approach of an object on a collision course with the locust (Simmons and Rind 1992; Rind and Simmons 1992). The Descending Contralateral Movement

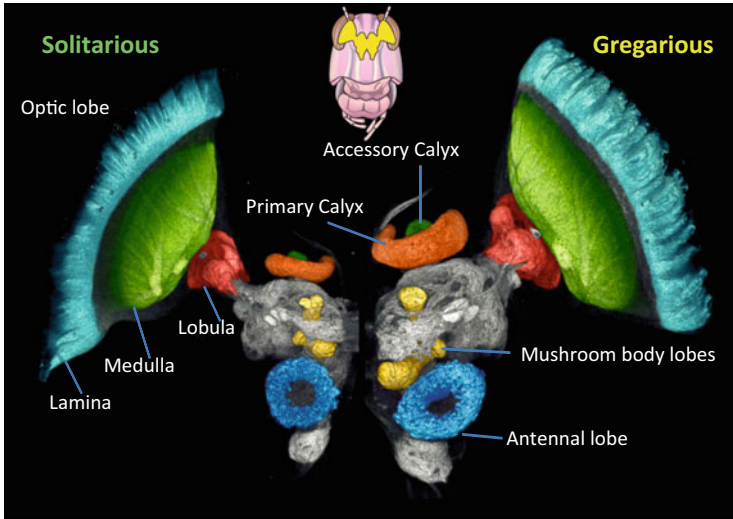


Fig. 2 Solitary and gregarious locusts differ profoundly in overall brain size and relative development of different brain regions. A composite frontal view of the brain (shown *in situ* in the head in the inset locust head), solitary on the *left* and gregarious on the *right*, was created from confocal microscope images of brains labelled with fluorescent antibodies against the synaptic protein synapsin. The locusts from which these images were taken had heads of the same size. Different neuropiles in the visual and olfactory processing pathways have been colour-coded (Data are from Ott and Rogers (2010))

Detector (DCMD) is a large intersegmental interneuron that originates in the brain and descends to the motor centres controlling locomotion in the thorax (Fig. 3a; Rowell 1971). It receives a monosynaptic input (1:1 gain) from a large-field visual interneuron, the Lobula Giant Motion Detector (LGMD). The receptive field organization of this neuron is broadly similar in both phases, spanning an entire visual hemisphere (Rogers et al. 2010), and it contains a large central region spanning $120^\circ \times 60^\circ$ that produces equally strong responses to looming stimuli originating from anywhere within it. The LGMD/DCMD of gregarious locusts, however, generates more action potentials at a higher frequency than those of solitary locusts when challenged by the same visual stimulus (Fig. 3b). Moreover, gregarious locusts have a small caudolateral focus of even greater responsiveness.

The response of DCMD in gregarious locusts habituates much less to repeated presentations of the same stimulus than in solitary locusts (Fig. 3b; Matheson et al. 2004). This maintenance of a strong response to repeated stimuli is presumably an adaptation to enable gregarious locusts to avoid colliding with other locusts in a swarm. By contrast, solitary locusts need to produce infrequent escape behaviours and then effectively disappear, relying on their camouflage and stillness.

The DCMD makes a monosynaptic connection onto an identified leg motor neuron, the fast extensor tibiae (FETi; Fig. 3c; Simmons 1980). The total synaptic

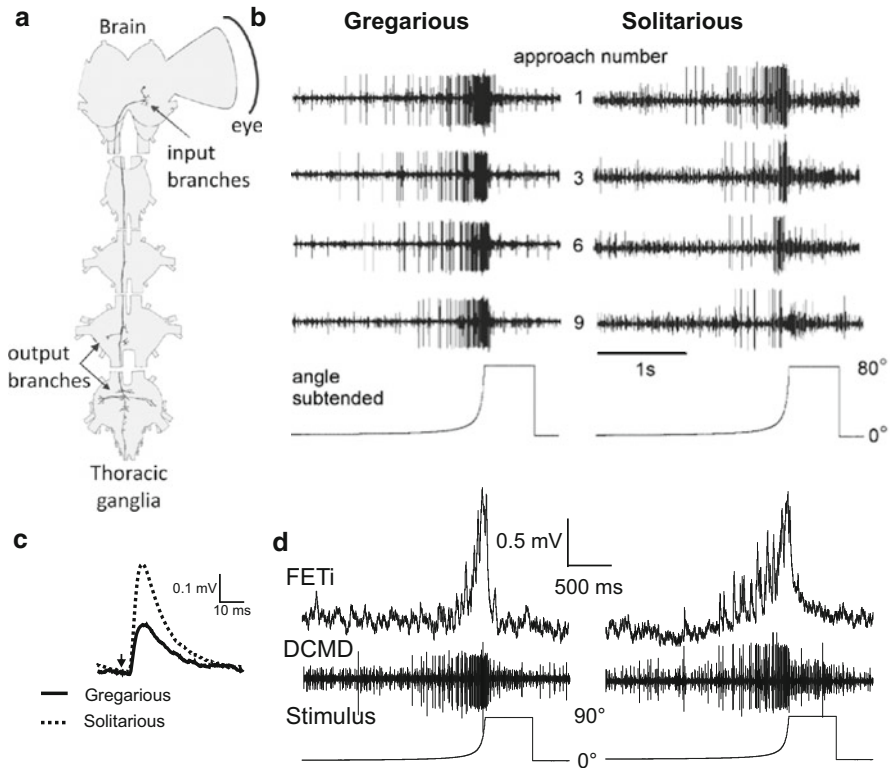


Fig. 3 The Descending Contralateral Motion Detector (DCMD) is a visual interneuron that detects objects on a collision course. **(a)** Anatomy of DCMD; it receives input from the optic lobe of the brain and descends down the nerve cord to the motor control centres located in the thoracic ganglia. **(b)** DCMD habituates to repeated presentations of the same stimulus, but the response in gregarious locusts remains robust. Extracellular recording from DCMD in a gregarious (*left*) and solitary (*right*) locust repeatedly stimulated by the same looming stimulus (*bottom row* shows the angular expansion of the approaching object) at 1-min intervals. The number and frequency of spikes evoked in DCMD decrease markedly by even the third approach in the solitary locust but remain strong in the gregarious locust even after nine repeated presentations. **(c)** EPSPs evoked by individual DCMD spikes in FETi are twice the amplitude in solitary locusts (*dashed line*) compared to those in gregarious locusts (*solid line*) (Data from Matheson et al. (2004)). **(d)** Compound EPSP response in FETi (*upper trace*) evoked by activity in the DCMD (*middle trace*) following exposure to a looming stimulus (angular subtense to 90°, *lower trace*); the total synaptic drive is similar in both phases, aided by the higher firing rates of DCMD in gregarious locusts and facilitation of the DCMD-FETi synapse, which emphasises the response to true looming stimuli (Data are from Rogers et al. (2007))

drive onto FETi produced by a looming stimulus has the same maximum amplitude in both phases, even though the DCMD spike pattern is stronger in gregarious locusts (Fig. 3d). The equalisation of the postsynaptic response arises in part because the individual excitatory postsynaptic potentials (EPSPs) in FETi evoked by each DCMD spike are only half the amplitude of those in solitary locusts

(Fig. 3c; Rogers et al. 2007). A sequence of DCMD spikes produced by a looming stimulus produces much stronger facilitation of EPSPs in the FETi of gregarious locusts than in solitary locusts (Fig. 3d), amplifying the strength of response to genuine looming stimuli over those produced by visual stimuli not on a direct collision course with the locust. This is especially important when dealing with the visually cluttered environment characteristic of a swarm. This nonlinear synaptic transformation of EPSP amplitude also tunes the DCMD-FETi synapse so that gregarious locusts respond earlier than solitary locusts to small moving objects.

The Tritocerebral Commissure Giant neurons (TCGs) are a pair of neurons that convey signals from wind-sensitive hairs on the head to thoracic flight motor centres (Bacon and Tyrer 1978). A current of air to the head will cause locusts to initiate flight if their tarsi are not in contact with the ground. Gregarious locusts will do this at a lower threshold than solitary locusts, despite having fewer sensory hairs on their heads (Fuchs et al. 2003). The increased sensitivity instead arises in part from the higher spike frequencies evoked in the sensory neurons by wind stimuli, which offset the lesser number of sensory receptors. There is, however, no difference in the signalling of the TCG neurons in the two phases preceding flight initiation, so that the actions of other interneurons must act to the lower behavioural threshold in gregarious locusts.

The Mechanism of Phase Change

Measuring Behaviour

If we are to understand the underlying mechanisms driving the rapid transformation from solitary to gregarious behaviour, it is essential to quantify that behavioural change in a controlled setting. We have used a behavioural assay based on the performance of locusts in an arena (Roessingh et al. 1993; Simpson et al. 1999; Cullen et al. 2012). This arena consists of a rectangular space 41 cm long $\times$ 31 cm wide $\times$ 10 cm high with two clear perforated partitions at each end, behind each of which is a chamber 8 cm long $\times$ 31 cm wide $\times$ 10 cm high (Fig. 4). A ‘stimulus group’ of 30 long-term gregarious locusts is placed in one of these chambers so that a locust in the main arena can see and smell this group and is able to approach or avoid it (the other chamber is left empty). An experimental locust is introduced into the arena via a hole in the centre of its floor and detailed observations of its behaviour – both continuous measures of its location, velocity and trajectory as well as discrete events such as head, antennal movements and leg movements – are made for the next 500s.

Observations are made of the performance of 100 locusts that are known to be highly solitary and of 100 locusts that are known to be highly gregarious to generate a data set of expected behaviour for each phase in this arena. From this basic data set we can develop a multidimensional model of solitary and

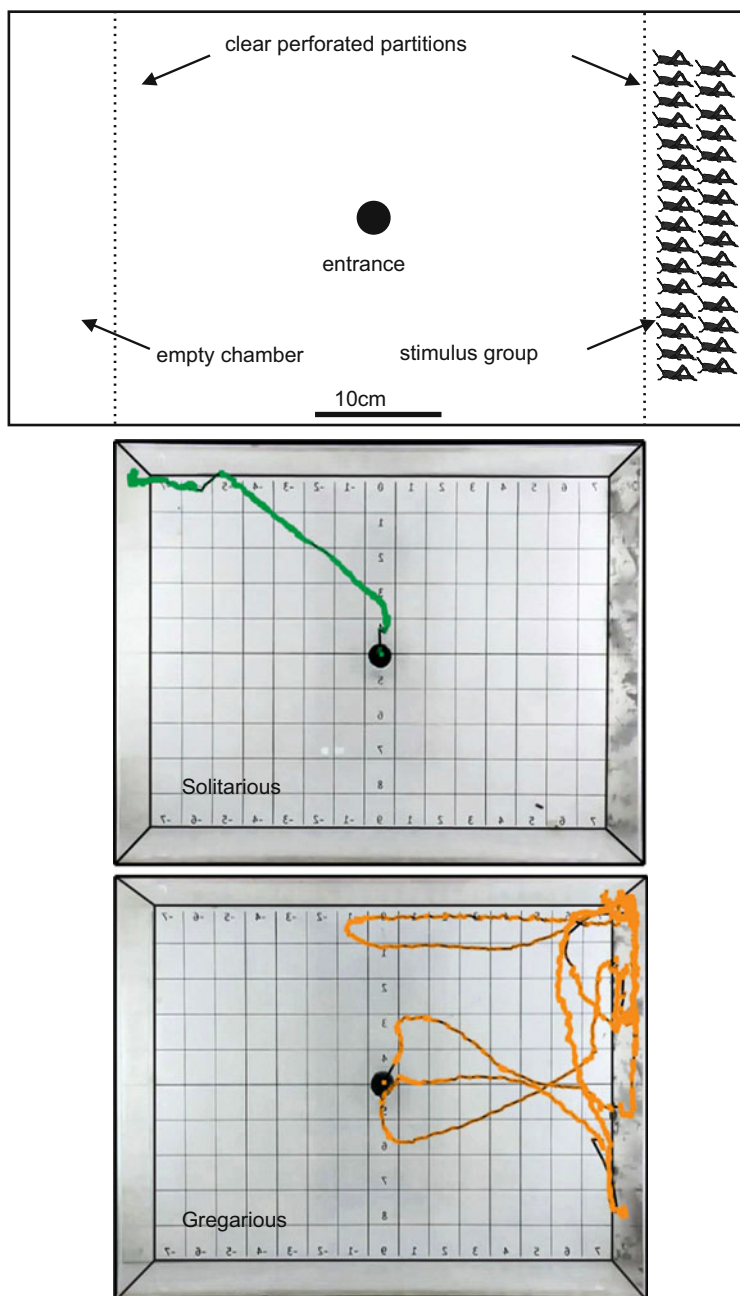


Fig. 4 The design of the behavioural arena (*top*) and representative trajectories of a solitary (*middle*) and gregarious (*bottom*) locust over the course of a 500-s observation period

gregarious behaviour. We use binary logistic regression to analyse the data, since it is able to condense several discrete behavioural variables into a single metric, P_{greg} , which is the probability that a locust belongs to the gregarious model population on the basis of its performance. This model is typically able to correctly categorise >90 % of locusts as either solitary or gregarious. A P_{greg} of 0 means that the locust's behaviour is indistinguishable from the solitarily reared model population, whereas a P_{greg} of 1 indicates fully gregarious behaviour. The statistical model can then be applied to locusts of unknown phase state to determine their P_{greg} on the basis of their behaviour when introduced into the arena. Transitional states between the two extremes can be quantified so that a direct link can be made between changes in the central nervous system (CNS) and behaviour, which, in turn, gives us a way to assess quantitatively the effects of pharmacological or other treatments on the propensity of locusts to gregarize or solitarize.

Gregarious and solitary locusts differ in many aspects of behaviour; the arena only reveals some of these and the logistic regression model uses four different measures that span the gamut of these behavioural differences. Two of the behaviours are related to general activity, walking speed and proportion of time spent totally inactive. Gregarious locusts walk at approximately twice the speed of solitary locusts and spend much less of their time motionless (60 %) compared to solitary locusts, which are motionless for about 95 % of the time (Fig. 4; compare the path-lengths of the solitary and gregarious locusts). Another key measurement in the model is the time spent adjacent to the stimulus group of locusts at one end of the arena. Solitary locusts avoid this end of the arena and typically move to the opposite end, whereas two-thirds of gregarious locusts will spend at least some time in the arena in close proximity to the other locusts (approximately 20 % of the total time in the arena on average; compare the paths of the solitary and gregarious locusts in Fig. 4). The final behaviour in the model is the frequency of grooming, with gregarious locusts much more likely to groom than solitary locusts.

The final value of P_{greg} obtained after observation is derived from a weighted sum of all of these behaviours. Changes in any one of these behavioural variables are insufficient to cause a major change in P_{greg} ; only a concerted change in all four of them will cause a pronounced shift towards either end of the solitary-gregarious axis.

Changes in CNS Neurochemistry During Phase Change

Our own research has largely concentrated on the Desert Locust, *Schistocerca gregaria*. Our first attempt to understand the neurochemical mechanisms driving phase change was to perform a large-scale analysis of changing neurochemistry during the entire process, from solitary to gregarious and back again (Rogers et al. 2004). The amounts of 13 different neurotransmitters and/or neuromodulators in different parts of the CNS were measured using high-performance liquid

chromatography (HPLC). We measured these neurochemicals in both long-term solitary and long-term gregarious locusts, as well as in locusts undergoing phase transition in both directions. Eight of these substances were more abundant in both the brain and the thoracic ganglia of long-term solitary locusts than in long-term gregarious ones, and three were less abundant, with only two showing no significant difference (Fig. 5a). Isolating larval gregarious locusts led to rapid changes in seven neurochemicals within 24 h, equal to or in most cases exceeding the differences seen between long-term solitary and gregarious locusts. Over subsequent generations of isolation, the long-term differences between phases fully established themselves. During the process of gregarization, crowding larval 3rd generation solitary locusts led to rapid changes in six neurochemicals within the first 24 h, by which time gregarious behaviour was already being fully expressed and presenting many possible targets for further analysis. We also looked, however, at the critical 4 h window during which gregarization is first established. Here the result was clear: only serotonin (5-hydroxytryptamine, 5HT) showed a dramatic increase (Fig. 5b). This increase was confined to the thoracic ganglia and could be induced by either the mechanosensory or the sight + smell gregarizing pathway (Anstey et al. 2009). After crowding solitary nymphs for the duration of the entire 5th larval instar, the amounts of all chemicals except octopamine were similar to those of long-term gregarious locusts. These measurements show that the expression of many neuroactive substances in the CNS changes and presumably underpins the behavioural and physiological changes that are the hallmarks of phase differences.

The Key Role of Serotonin in Initiating Gregarization

The rapid transient increase in serotonin which accompanied the initial acquisition of gregarious behaviour prompted us to examine its role more closely. In our first experiments, we determined how close a correspondence there was between serotonin titres and the extent of behavioural change (Anstey et al. 2009). We crowded solitary locusts for 0, 1, or 2 h to generate the entire gamut of behaviour from solitary to gregarious and measured the amount of serotonin in the thoracic ganglia of each locust; there was a significant positive correlation between the amount of serotonin and the degree of gregarious behaviour (Fig. 6a). Locusts that behaved the most gregariously ($P_{greg} > 0.8$) had approximately three times more serotonin than the most solitarily behaving locusts ($P_{greg} < 0.2$). Furthermore, the amount of serotonin corresponded solely to the degree of gregarization, not the duration of crowding *per se*. A similar relationship was seen in locusts gregarized through the mechanosensory and through the sight + smell pathway.

This strong correlation between the degree of gregarization and serotonin titre in the thoracic ganglia suggested a causal link. To demonstrate causality, we performed the following experiments. First, blocking the action of serotonin prevented behavioural gregarization. Locusts injected with a mixture of two

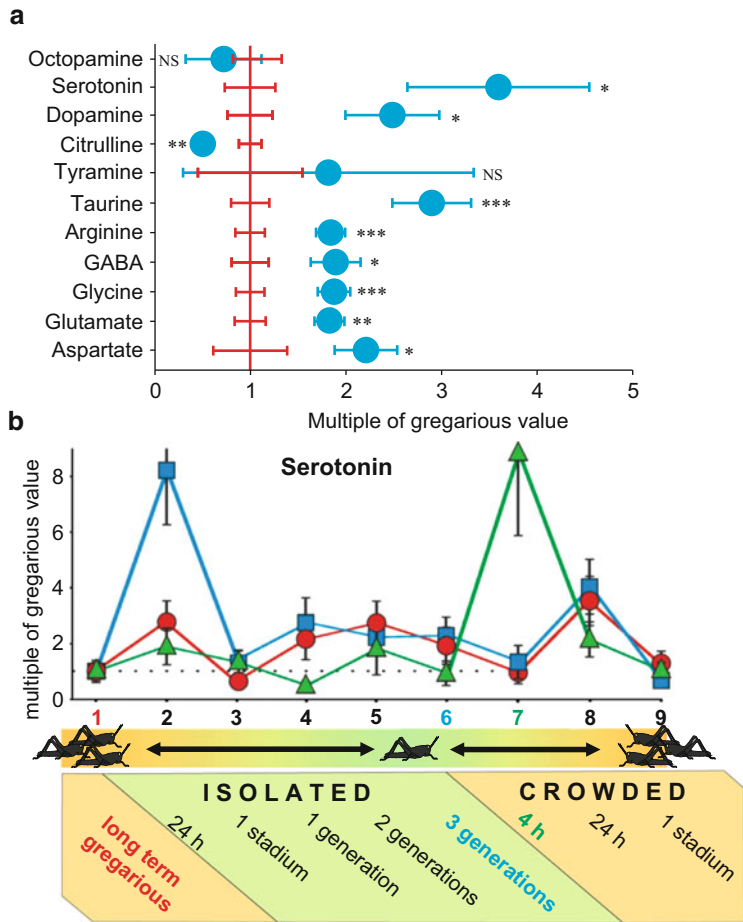
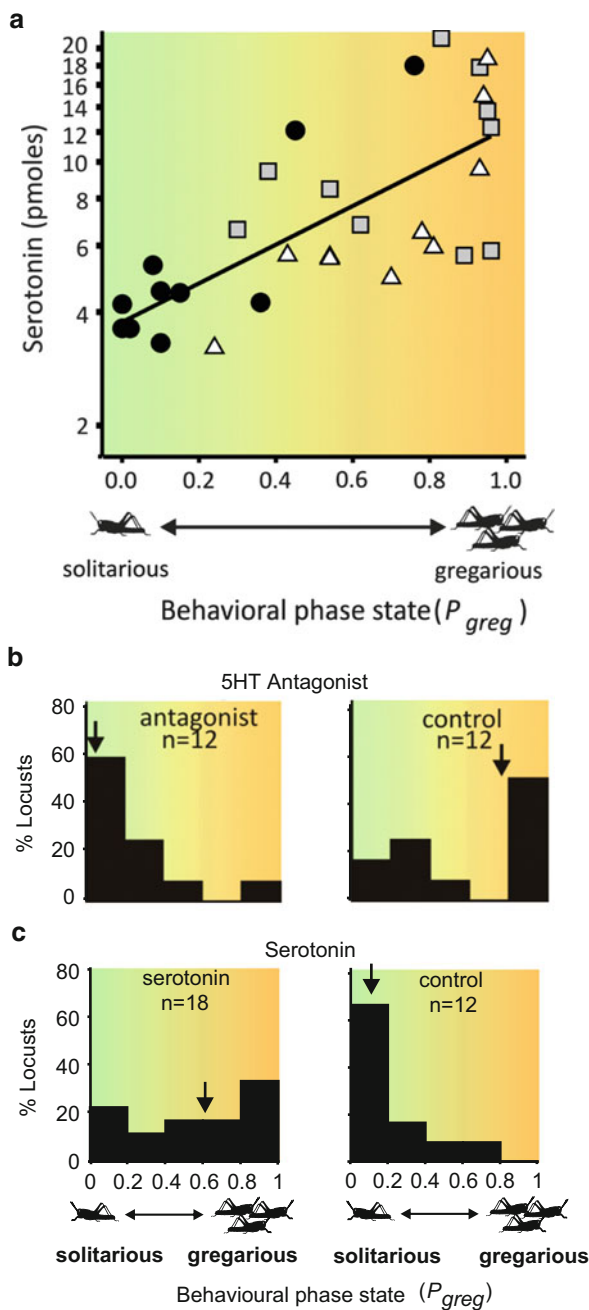


Fig. 5 Changes in brain chemistry accompanying phase change. **(a)** Differences in the amount of 11 neurochemicals in the CNS of adult long-term solitary (blue) and gregarious (red) locusts. Amounts are expressed as multiples of the mean gregarious quantity, with error bars representing the SEM. Significances are indicated by asterisks: * $P<0.05$; ** $P<0.01$; *** $P<0.001$. **(b)** The trajectory of change in the amount of serotonin (5-hydroxytryptamine, 5HT) in different parts of the CNS during the entire phase-change process, starting with the isolation of long-term gregarious on the left for increasing periods before the commencement of crowding of third-generation isolated locusts. Data are mean $\pm$ SEM; green are the thoracic ganglia; red is the central region of the brain and blue the optic lobes (Data are from Rogers et al. (2004))

serotonin receptor antagonists failed to gregarize in response to either mechanosensory or sight + smell stimuli, in contrast to saline-injected controls, which gregarized normally (Fig. 6b). Second, we interfered with serotonin synthesis by using α -methyltryptophan (AMTP), a competitive antagonist of tryptophan hydroxylase. AMTP-injected locusts failed to gregarize after having their hind femora stroked for 2 h, in strong contrast to control animals. Third, we showed

Fig. 6 The key role of serotonin in behavioural gregarization of *Schistocerca gregaria*. **(a)** There was a strong correlation between the extent to which locusts became gregarious after 0 h (circles), 1 h (triangles) or 2 h (squares) crowding and the amount of serotonin expressed in the thoracic CNS. **(b)** Blocking the action of serotonin through the use of 5HT receptor antagonists prevented locusts from becoming gregarious after 2 h of mechanosensory stimulation to a hind femur, whereas locusts treated with a saline control gregarized normally. **(c)** Topically applying serotonin directly to the thoracic ganglia induced a shift towards gregarious behaviour, even though the first time the experimental locusts had ever been exposed to another locust was when they were tested in the arena. Data are histograms of locusts falling into different ranges of P_{greg} , an aggregate measure of solitarious-gregarious behaviour (Data are from Anstey et al. (2009))



that serotonin or serotonin receptor agonists induced behavioural gregarization in the absence of stimuli associated with other locusts. Serotonin in saline or a saline control was topically applied to the exposed thoracic ganglia for 2 h. Serotonin-treated locusts behaved significantly more gregariously in the arena, even though they had never previously been exposed to other locusts; control-treated locusts remained highly solitary (Fig. 6c). Fourth, locusts injected with a mixture of two serotonin receptor agonists, 1 mM α -methylserotonin and 1 mM 5-carboxamidotryptamine, showed a significant shift toward gregarious behaviour compared to saline-injected controls.

Behavioural Phase Change in the Migratory Locust, *Locusta migratoria*

The migratory locust represents perhaps an independent evolution of the full phase-change phenotype. The time course and mechanism of phase change show similarities and differences with that of *Schistocerca*. As in *Schistocerca*, the behavioural transitions from solitary to gregarious and vice versa have different time courses, but in *Locusta* behavioural gregarization is slower than solitarization and is not fully achieved even after 64 h of crowding (Guo et al. 2011). Several olfaction-related genes are strongly regulated and display opposite expression trends in response to changes in population density during both gregarization and solitarization. These include several chemosensory protein (CSP) genes, which code for proteins that are secreted into the lymph of insect chemosensory organs where they are thought to assist in ligand binding (Jacquin-Joly et al. 2001; Ban et al. 2003), but they are also more widely expressed in insect tissue and may have a role in development (Kitabayashi et al. 1998). The other gene of interest is a Takeout (TO) gene, which is also expressed in insect chemosensory organs and has a role in modulating taste sensitivity according to nutritional status (Meunier et al. 2007) and in a circadian manner (Sarav-Blat et al. 2000). The CSP gene LmigCSP3 and the TO gene LmigTO1 in locusts are implicated in the behavioural change between avoidance of and attraction to conspecifics by regulating peripheral odour sensitivity (Guo et al. 2011).

A second and contemporaneous report by the same group (Ma et al. 2011) identified *pale*, *henna* and *vat1*, genes involved in dopamine biosynthesis, synaptic release and cuticular melanisation, as critical targets related to behavioural phase changes. Moreover, injection of dopamine or a dopamine agonist initiated gregarious behaviour as well as serotonin agonists, suggesting that the mechanism mediating the initial shift to gregarious behaviour in *Locusta*, whilst showing some parallels with that to *Schistocerca*, has some differences in the signalling pathway.

Monoamines and Their Role in Social Behaviour

The demonstration that serotonin and dopamine play key roles in the initiation of gregarious behaviour in locusts is consistent with the important roles these monoamine neurotransmitters play in regulating social interaction throughout the animal kingdom. Serotonin, for example, modulates the propensity to engage in intra-specific aggression and establish social status in dominance hierarchies (Miczek et al. 2007; de Boer et al. 2009; Kravitz 2000; Dierick and Greenspan 2006; Yeh et al. 1996; Neumeister et al. 2010). Animals subjected to socially mediated stress often manifest long-term changes in the amount of serotonin in the CNS together with profound changes in perception, cognitive function and behaviour (Fuchs and Flügge 2003), and in humans these are associated with affective disorders such as anxiety and depression (Ansorge et al. 2007).

Dopamine is involved in mediating the brain's response to rewarding situations in both vertebrates and invertebrates (Dayan and Balleine 2002; Perry and Barron 2013) and mediates animals' susceptibility or resilience to social stress (Krishnan et al. 2007; Chaudhury et al. 2013). Dopamine signalling facilitates pair-bond formation in mammals (Young and Wang 2004; Young et al. 2011). In eusocial insects such as bees, alterations in dopamine titre are associated with reproductive dominance (Harris and Woodring 1995; Sasaki et al. 2009) and the changing tasks that workers perform (Schulz and Robinson 1999; Wagener-Hulme et al. 1999; McQuillan et al. 2012).

The Extension and Maintenance of Gregarious Behaviour

Serotonin is essential for the initiation of gregarious behaviour in *Schistocerca gregaria*, but it cannot be responsible for the consolidation and maintenance of the gregarious phase. The initial increase in serotonin that drives gregarization decays to baseline levels within 24 h. Furthermore, locusts that have spent their entire lives in the gregarious phase have half the amount of serotonin in their CNS compared with long-term solitary locusts. Gregarious behaviour, which leads to the constant presence of reinforcing stimuli from other locusts, maintains the gregarious phenotype but, with time, gregarious behaviour becomes more robust and is retained even after periods of isolation from other locusts. Newly gregarious locusts that have been crowded for just 24 h lose their gregarious behaviour after only 4 h of isolation (Roessingh and Simpson 1994), but locusts that have been gregarious for many generations only start to become behaviourally solitary after days of isolation. How, then, is the transient spike in serotonin translated into a longer-lasting modification of behaviour and ultimately more extensive phenotypic change?

We investigated the role of two protein kinases in the consolidation of gregarious behaviour (Ott et al. 2012): the *foraging* gene product, a cGMP-dependent

protein kinase (PKG) implicated in switching between alternative group-related behaviours in several animal species; and cAMP-dependent protein kinase (PKA), an effector protein with a pre-eminent role in different forms of learning. Injecting the PKA inhibitor KT5720 prior to crowding prevented locusts from becoming gregarious, whereas injecting KT5823, an inhibitor of PKG, did not. Neither drug altered the behaviour of long-term gregarious locusts. RNA interference (RNAi) against *foraging* strongly reduced its expression in the CNS but this did not prevent gregarization upon crowding. By contrast, solitary locusts with an RNAi-induced reduction in the PKA catalytic subunit C1 expression behaved less gregariously after crowding. Furthermore, RNAi against the PKA regulatory R1 subunit, which inhibits catalytic activity by PKA, promoted more extensive gregarization following a brief period of crowding. These results suggest that PKA, acting in concert with serotonin, which has a highly evolutionarily conserved role in mediating neuronal plasticity under many conditions and in many species (Kandel 2001), has been co-opted into effecting the wide-ranging transformation from solitary to gregarious behaviour.

In addition to causing rapid but short-term changes in neuronal function through phosphorylation of existing proteins, the PKA effector pathway mediates changes in gene expression (Müller and Carew 1998). Several recent studies have highlighted the extensive differences in gene expression between the solitary and gregarious phases of both *Schistocerca gregaria* (Badisco et al. 2011a) and *Locusta migratoria* (Kang et al. 2004; Ma et al. 2006; Guo et al. 2011). In a recent study, using microarrays developed from an Expressed Sequence Tag (EST) database of *Schistocerca gregaria* central nervous tissue, 214 differentially expressed genes were detected between long-term solitary and gregarious locusts, of which only 40 % have a putatively known function (Badisco et al. 2011b). These included genes encoding proteins that are associated with CNS development and modelling, sensory perception, stress response and resistance, and fundamental cellular processes. Genes for heat shock proteins and proteins which confer protection from infection were up-regulated in gregarious locusts, which may allow them to respond to acute physiological challenges. By contrast, the longer-lived solitary locusts appeared to be more strongly protected from the slowly accumulating effects of ageing by an up-regulation of genes related to anti-oxidant systems, detoxification and anabolic renewal. Gregarious locusts also had a greater abundance of transcripts for proteins involved in sensory processing and in nervous system development and plasticity. An earlier study in *Locusta* identified 532 differentially expressed genes between phases (Kang et al. 2004). These studies probably only provide a flavour of the true scale of differential gene expression that accompanies phase change, and progress continues to be hampered by the lack of a full genome sequence for any species of locust.

Clearly much remains to be done before we have a full conceptual understanding of how social environment, neural circuitry and gene expression interact to produce the profound behavioural transformations associated with phase change in locusts. These analyses hold the promise of providing us with general and profound insights

into the interplay between the environment and mechanisms of phenotypic plasticity and, in particular, the neurobiology of social interaction.

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