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A fighter's comeback: Dopamine is necessary for recovery of aggression after social defeat in crickets

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ABSTRACT

Social defeat, i.e. losing an agonistic dispute with a conspecific, is followed by a period of suppressed aggressiveness in many animal species, and is generally regarded as a major stressor, which may play a role in psychiatric disorders such as depression and post-traumatic stress disorder. Despite numerous animal models, the mechanisms underlying loser depression and subsequent recovery are largely unknown. This study on crickets is the first to show that a neuromodulator, dopamine (DA), is necessary for recovery of aggression after social defeat. Crickets avoid any conspecific male just after defeat, but regain their aggressiveness over 3 h. This recovery was prohibited after depleting nervous stores of DA and octopamine (OA, the invertebrate analogue of noradrenaline) with α-methyl-tyrosine (AMT). Loser recovery was also prohibited by the insect DA-receptor (DAR) antagonist fluphenazine, but not the OA-receptor (OAR) blocker epinastine, or yohimbine, which blocks receptors for OA's precursor tyramine. Conversely, aggression was restored prematurely in both untreated and amine depleted losers given either chlordimeform (CDM), a tissue permeable OAR-agonist, or the DA-metabolite homovanillyl alcohol (HVA), a component of the honeybee queen mandibular pheromone. As in honeybees, HVA acts in crickets as a DAR-agonist since its aggression promoting effect on losers was selectively blocked by the DAR-antagonist, but not by the OAR-antagonist. Conversely, CDM's aggression promoting effect was selectively blocked by the OAR-antagonist, but not the DAR-antagonist. Hence, only DA is necessary for recovery of aggressiveness after social defeat, although OA can promote loser aggression independently to enable experience dependent adaptive responses.

Introduction

Intra-specific aggression is common throughout the animal kingdom and a major hallmark of this behavior is that its expression is influenced by a wide variety of experiences (Huntingford and Turner, 1987; Archer, 1988; Hsu et al., 2006, 2009). In mammals, for example, experiences as diverse as physical exertion (Raichlen et al., 2011), the possession of a resource (Fuxjager et al., 2010) and winning (Hsu et al., 2006, 2009) can each promote the aggressiveness of an individual, whereas losing leads to a pronounced reduction in the expression of aggressive behavior (Hsu et al., 2006, 2009). Investigations of the consequences of social defeat and its importance for understanding social behavior and psychiatric disorders such as post-traumatic stress disorder are receiving increasing amounts of attention (Huhman, 2006). Comparatively little, however, is known about the neuronal control mechanisms underlying the effects of experiences such as social defeat on aggressive behavior.

Our work on male crickets (*Gryllus bimaculatus* de Geer) has shown that the physical experiences such as flying (Stevenson et al., 2005), winning (Rillich and Stevenson, 2011) and possession of a shelter (Rillich et al., 2011) all lead to a state of enhanced aggression which is mediated by the biogenic amine octopamine (OA; reviews: Stevenson and Rillich, 2012; Stevenson and Schildberger, 2013; Simpson and Stevenson, 2014), the invertebrate analogue of noradrenaline (Pflueger and Stevenson, 2005). The mechanisms underlying depressed aggression and its subsequent recovery in crickets and indeed other animals are practically not known. Most behavioral theories agree that information gathered from agonistic signals exchanged during fighting is somehow assessed to time the decision to flee, and our work on crickets shows that agonistic signals act to suppress aggression of the opponent (Rillich et al., 2007; for review see Elwood and Arnott, 2012). Neuromodulators are likely to play key roles, for example serotonin and nitric oxide, which can suppress aggression in both mammals (Nelson and Trainor, 2007) and insects (Iwasaki et al., 2007; see also Edwards and Spitzer, 2006 on serotonin and social dominance in crustaceans).

In this paper we specifically address the possibility that OA and/or dopamine (DA), are involved in the recovery of aggression after social defeat. As in many animals, crickets that have just lost a fight behave

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submissively towards previous victors (Stevenson and Rillich, 2013) and even unfamiliar opponents (Rillich et al., 2007), but slowly regain their aggressiveness after 3 h of social isolation (Stevenson and Rillich, 2013), after which they will readily engage and fight any conspecific adult male. The involvement of OA in recovering from this so-called loser effect is suggested by the finding that losers rapidly regain their aggressiveness after treatment with the insecticide chlordimeform (CDM, Stevenson et al., 2005), a tissue permeable OA-receptor (OAR) agonist (Roeder, 1995) with low affinity for other amine receptors (Hiripi et al., 1999). Here we show that aggression is also restored in losers by treatment with homovanillyl alcohol (HVA), a catecholamine metabolite, that is structurally related to DA and acts as a DA-receptor (DAR) agonist in honeybees (Beggs and Mercer, 2009). Taken together our experiments provide evidence that DA and OA can independently promote aggression in defeated crickets, but while OA is sufficient, DA alone is necessary for the normal recovery of aggressiveness after social defeat. This adds firm support to recent findings in fruit flies (Alekseyenko et al., 2013) and ants (Szczuka et al., 2013) suggesting that DA may play some role in insect aggression, and provides general insights into how amines operate in controlling social behavior, perhaps even in mammals (cf. O'Connell and Hofmann, 2011).

Methods

Experimental animals

Mature, 2–3 week old, adult male Mediterranean field crickets, *G. bimaculatus* (de Geer) were taken from a breeding stock maintained under constant standard conditions at Leipzig University (22–24 °C, relative humidity 40–60%, 12 h:12 h light:dark regime daily feeding on bran and fresh vegetables) and kept isolated in individual glass jars for at least 24 h prior to all experiments. After this time all known effects of previous social interactions on the expression of aggressive behavior have abated (Stevenson and Rillich, 2013), and we refer to the animals as being "socially naive." All animal treatments complied with the Principles of Laboratory Animal Care and the German Law on the Protection of Animals (Deutsches Tierschutzgesetz).

Evaluation of aggression

Aggressive behavior was evaluated in dyadic contests (cf. Stevenson et al., 2005) between pairs of previously isolated adult male crickets that were matched for body mass (<5% mass difference). The opponents were placed at opposite ends of a small, Perspex-glass rectangular fighting arena (l·w·h: 16 × 9 × 7 cm) having a sand-covered floor and divided halfway along its length by an opaque sliding door. On removing the door the animals generally interact with each other within seconds. Their interactions follow an escalating sequence of stereotyped motor performances (Stevenson et al., 2000, 2005), which do not differ significantly from fights that occur in the field as part of their normal behavioral repertoire (Alexander, 1961). The intensity of aggressiveness was scored on a scale of 0–6 (Hofmann and Stevenson, 2000; Stevenson et al., 2000) denoting the level to which a fight escalates before the winner is established by the retreat of one contestant: Level 0: mutual avoidance without aggression. Level 1: one cricket attacks, the other retreats. Level 2: antennal fencing, whereby the contestants face each other and lash each other's antennae. Level 3: one contestant spreads its mandibles in a threat display. Level 4: mandible spreading by both crickets. Level 5: mandible engagement, whereby the two opponents interlock their mandibles. Level 6: grappling, an all-out fight involving repeated mandible engagements with biting, and body flipping. A contest may be concluded at any of the levels when one opponent retreats, leaving the established winner, which becomes hyper-aggressive and generally generates the characteristic rival song and body jerking movements (Rillich and Stevenson, 2011). The losers normally actively avoid contact to any conspecific male for 1–3 h (Stevenson and Rillich, 2013). Fight duration, from first contact until conclusion, was measured to the nearest second with a stopwatch. Very occasionally, the animals appeared to lose contact with each other so that fighting paused for a brief period before resuming when contact was regained. As in our previous studies, we chose to deduct the duration of these pauses in the few cases they occur in order to give a more representative measure of the actual time spent fighting.

Pharmacological treatments

Various pharmacological treatments were performed to evaluate the role of amines in loser recovery. To minimize variations due to random differences in daily performances, we took the precaution of evaluating single pairs of crickets from control and test groups in parallel and accumulated data from multiple experimental sessions (three groups per session, test sequence changed at each). The numbers of cricket pairs for each test group is given in tables and figures.

Unless stated otherwise, all drugs were obtained from Sigma Aldrich (Deisenhofen, Germany). Their effects were tested by injecting 10 µl solutions into the hemocoel via the pronotal shield using a micro-syringe (Hamiliton®, Bonaduz, Switzerland). The most effective concentrations that induced noticeable changes in aggressive behavior, but without having any obvious detrimental effect on general motility were determined in pilot investigations.

Hydrochloride salts of native OA and DA were found to have no obvious effect on aggression at concentrations as high as 20 mM, which is due we suspect to the permeability barrier of the ganglion sheath (cf. Schofield et al., 1984). Contrasting this, the tissue permeable OAR-agonist chlordimeform hydrochloride (CDM; cf. Roeder, 1995) and the DAR-agonist homovanillyl alcohol (HVA: Beggs and Mercer, 2009) were both effective at 1 mM in saline solution containing 1% DMSO (dimethylsulfoxide; saline components in mmol L^{-1}: NaCl 140, KCl 10, $CaCl_2$ 7, $NaHCO_3$ 8, $MgCl_2$ 1, N-trismethyl-2-aminoethanesulfonic acid 5, D-trehalose dihydrate, pH 7.4). The following amine receptor antagonists were dissolved in the same solvent (saline, 1% DMSO) and applied at the same concentration (10 mM): epinastine hydrochloride, a selective OAR-blocker (Roeder et al., 1998), fluphenazine dihydrochloride a D1/D2 dopamine receptor (DAR) blocker in insects (Degen et al., 2000) and yohimbine hydrochloride an insect tyramine receptor (TAR) blocker (Roeder, 2005). Previous experiments have shown that aminergic drugs injected into the hemocoel require about 30 min to become effective, and that their effects last for up to 4 h (Stevenson et al., 2005; Rillich et al., 2011; Rillich and Stevenson, 2011).

The roles of the amines OA, DA and TA in loser recovery were also evaluated by applying the competitive synthesis inhibitor α-methyl-tyrosine (AMT). We have previously shown that a comparatively high dosage is required in order to effectively deplete OA and DA from the cricket central nervous system as determined by immunocytochemistry (Stevenson et al., 2000) and we followed the same protocol in this study. Briefly, each animal received two successive injections of 1.5 mg AMT in 20 µl saline administered at 48 h intervals, and evaluated aggressive behavior 48 h after the last injection. Controls received two successive injections of saline only. Since AMT inhibits the conversion of tyrosine to OA's precursor TA, this amine is probably also depleted in addition to OA and DA by this treatment.

Experimental procedure

All experiments were performed during daylight hours, avoiding times when aggression tends to be depressed (just after midday and on generally dreary days; cf. Dixon and Cade, 1986; Stevenson et al., 2000). Fights were first staged in an *initial fight* to establish clear winners and losers and the hierarchical relationship verified by re-matching the same opponents 1 min later to assure that the designated loser retreated immediately from the designated winner. To chart the time course of loser recovery, the same opponents were re-matched 15, 30, 60 or