

RESEARCH ARTICLE

The behavioural effects of predator-induced stress responses in the cricket (*Gryllus texensis*): the upside of the stress response

Shelley A. Adamo*, Ilya Kovalko and Brianna Mosher

Department of Psychology and Neuroscience, Dalhousie University, Halifax, NS, Canada B3H 4R2

*Author for correspondence (sadam@dal.ca)

SUMMARY

Predator-induced stress responses are thought to reduce an animal's risk of being eaten. Therefore, these stress responses should enhance anti-predator behaviour. We found that individual insects (the cricket *Gryllus texensis*) show reliable behavioural responses (i.e. behavioural types) in a plus-shaped maze. An individual's behaviour in the plus maze remained consistent for at least 1/2 of its adult life. However, after exposure to a model predator, both male and female crickets showed a reduced period of immobility and an increased amount of time spent under shelter compared with controls. These changes could be mimicked by injections of the insect stress neurohormone octopamine. These behavioural changes probably aid crickets in evading predators. Exposure to a model predator increased the ability of crickets to escape a live predator (a bearded dragon, *Pogona vitticeps*). An injection of octopamine had the same effect, showing that stress hormones can reduce predation. Using crickets to study the fitness consequences of predator-induced stress responses will help integrate ecological and biomedical concepts of 'stress'.

Key words: stress hormone, fight-or-flight, acute stress, animal temperament, animal personality, Orthoptera, Gryllidae.

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INTRODUCTION

Predators kill. Animals that avoid being eaten will have an advantage, provided that the fitness costs of that advantage do not outweigh the fitness benefits. Sensitivity to such fitness costs probably explains the evolution of stress response systems in animals. By using a response that can be turned on rapidly and transiently, animals can maximize the benefits of anti-predator adaptations, while minimizing their costs (Orr et al., 2010). This perspective suggests that understanding the costs and benefits of predator-induced stress responses will be important for interpreting ecological and biomedical data on them.

Data from vertebrates largely support the perspective that predator-induced stress responses have both costs and benefits. Acute predator exposure reliably activates stress responses (Apfelbach et al., 2005). This activation induces a coordinated set of physiological changes that is mostly consistent with the hypothesis that these changes prepare the body for extreme action (Sapolsky et al., 2000). Concomitant behavioural changes are thought to increase the ability of animals to escape predators (Apfelbach et al., 2005). Finally, studies have shown that the use of the stress response entails costs that are likely to lower fitness; for example, the stress response induces a reduction in sexual behaviour (Sapolsky et al., 2000). Nevertheless, it has proven challenging to provide a detailed account of the costs and benefits of predator-induced stress responses. For example, although predator-induced changes in behaviour are widely assumed to help animals evade predators, this has not been well demonstrated (e.g. Hendrie et al., 1996), and contradictory data exist (e.g. Maher et al., 2013).

Recent data suggest that predator-induced stress responses are more complicated than previously believed. The link between different components of the vertebrate stress response system [e.g. sympathetic nervous system and hypothalamic–pituitary–adrenal

(HPA) axis] and behaviour remains somewhat murky (Johnstone et al., 2012). In some species, stress responses and behaviour have been decoupled (Johnstone et al., 2012). Glucocorticoids, traditionally considered key stress-inducing hormones in vertebrates, may be stress-recovery hormones in many species (Sapolsky et al., 2000; Johnstone et al., 2012; Clinchy et al., 2013). For example, in tadpoles, acute exposure to a predator decreases corticosterone levels for the first 4 h; corticosterone levels increase above baseline only after a delay of several hours (Maher et al., 2013). Therefore, levels of this stress hormone increase too late to help tadpoles evade a predator. If corticosterone levels are artificially increased, tadpoles have reduced survival against predators (Maher et al., 2013). The rapidly activating sympathetic nervous system is more likely to be involved in immediate responses to predators (Wingfield, 2003). Unfortunately, the costs and benefits of activating the sympathetic nervous system have been little studied (Dickens and Romero, 2009; Breuner et al., 2013). This lack of information is surprising; much of the complexity regarding the costs and benefits of stress responses involves long-term effects (Sapolsky et al., 2000). Although predator-induced stress responses can also have long-lasting effects (Slos et al., 2009; Clinchy et al., 2013) that will complicate calculation of its cost, it should be possible to demonstrate the magnitude of the immediate benefit.

A comparative approach using invertebrates may provide important complementary information about the costs and benefits of predator-induced stress responses. Invertebrates face many of the same evolutionary pressures as vertebrates (e.g. predation, competition for food and mates), but have simpler physiological systems. They have robust stress response systems that in some ways parallel those in vertebrates (Roeder, 2005; Adamo, 2008; Adamo, 2012). For example, in insects, the stress response begins with the release of a biogenic amine, octopamine (Orchard et al., 1993).

Octopamine is the chemical cousin of noradrenaline (norepinephrine), one of the key catecholamines used by the sympathetic nervous system (Purves et al., 2012). In fact, the octopaminergic system appears to be the functional equivalent of the vertebrate sympathetic nervous system (Roeder, 1999; Roeder, 2005). The noradrenergic and octopaminergic systems are thought to have evolved from the same ancestral pathway (Evans and Maqueira, 2005; Caveney et al., 2006). Predator exposure activates the stress response and increases neurohormonal levels of octopamine (Adamo and Baker, 2011). Elevated levels of octopamine produce a number of physiological changes that enhance physical performance, similar to the effects of vertebrate stress hormones (Roeder, 1999; Roeder, 2005). Octopamine modulates anti-predator behaviour in beetles (*Tribolium castaneum*) (Nishi et al., 2010) and an orb-weaving spider (Jones et al., 2011). The behavioural effects of predator-induced stress can also be monitored in insects. As in vertebrates (Sih et al., 2004; Bell, 2007), insects display behavioural responses that are consistent within individuals [e.g. crickets (Hedrick and Kortet, 2012; Niemelä et al., 2012)]. These behavioural styles can be changed by predator exposure (Niemelä et al., 2012). Finally, some of the costs of activating the stress response in crickets have been studied (e.g. Adamo and Parsons, 2006). For example, an acute stress response results in a transient decline in disease resistance (Adamo and Parsons, 2006).

In this paper, we demonstrate that individual crickets, *Gryllus texensis* (Cade and Otte, 2000), show a stable and consistent set of behaviours (i.e. behavioural strategy or behavioural type) (Sih et al., 2004; Bell, 2007) using a modified plus-shaped maze (plus maze). We show that the presence of a model predator shifts a cricket's behavioural strategy and that octopamine is probably involved in mediating this shift. Finally, we test whether activating the stress response or artificially raising octopamine levels enhances escape from predators. Such functional tests are rarely done (Hendrie et al., 1996), but are important for estimating the relative advantage supplied by predator-induced stress responses.

MATERIALS AND METHODS

Animals

Crickets (long-winged *G. texensis*) were collected near Austin, Texas, and have been maintained as a laboratory colony for many generations with occasional additions of fresh animals from the field. Pellets of dry cat food and water were provided *ad libitum* during rearing. Crickets were reared at 25±2°C on a 12 h/12 h light/dark cycle. Crickets were used between 10 and 24 days after the moult to adulthood. At this age crickets are sexually mature (Cade and Wyatt, 1984; Solymar and Cade, 1990) and within their natural lifespan in the field (Murray and Cade, 1995).

Adult bearded dragons, *Pogona vitticeps* Ahl 1927, were captive bred and had been kept as pets. Bearded dragons were kept in 40 gallon (~182 l) terraria. The ambient temperature within their enclosure was kept at 29±2°C during the day and 23±2°C at night. A combination of fluorescent and incandescent bulbs provided visible light, UVB and UVA on a 12 h/12 h light/dark cycle. Exo-Terra Plantation Soil (Hagen Inc., Montreal, QC, Canada) was used as a substrate, which was spot-cleaned daily. An Exo-Terra Reptile Cave made from food-grade resin was provided for shelter. Water was provided in a large water dish *ad libitum*. Bearded dragons were fed crickets (*Acheta domesticus* dusted with calcium powder) every other day and given kale, dandelion greens or mustard greens 6–7 days a week.

All studies were approved by the Animal Care Committee of Dalhousie University (no. I11-025 for crickets, no. I12-102 for

bearded dragons) and are in accordance with the Canadian Council on Animal Care.

Chemicals were obtained from Sigma Chemical Co. (St Louis, MO, USA) unless otherwise noted.

Evidence for behavioural type in *G. texensis*

The plus maze was shaped like a plus sign and constructed of black acrylic. It consisted of four arms (L×W×H 14×8×6.5 cm each) and an open central area (L×W×H 8×8×6.5 cm). During the trial, two opposing arms were covered with heavy black bristle board, creating dark spaces underneath. The other two arms were left uncovered. Crickets prefer covered areas (Hedrick and Dill, 1993). Crickets were gently transferred from their home cage to the central, uncovered area. They remained under the transfer cup for 1 min. The cup was then removed and we measured the time the cricket remained motionless. We called this time period 'freezing'. Freezing is a stereotypic anti-detection response associated with predation avoidance across taxa (Stynoski and Noble, 2012) including crickets (Niemelä et al., 2012). We also measured the number of times each cricket entered an arm of the plus maze as an assessment of its tendency to explore its environment. We measured the amount of time each cricket spent locomoting, both inside and outside the covered arms, as a proxy for behavioural activity. We also measured the time it spent under the covered arms to assess its tendency to avoid open spaces. Total trial time was 10 min. Pilot studies demonstrated that longer trials (20, 30 or 60 min) did not yield a different pattern of results. Crickets ($N=12$ males, $N=11$ females) were tested four times in the apparatus over 10 days. The apparatus was cleaned with disinfectant between trials.

At least 1 day prior to the plus maze trial, sexually mature crickets (i.e. crickets that had mated) (Cade and Wyatt, 1984; Solymar and Cade, 1990) (S.A.A., personal observation) were removed from mixed-sex bins and placed into individual containers (L×W×H 17×15×9.5 cm) with food and water *ad libitum*. Crickets remained in their individual containers for the entire 10 days. Different people scored the crickets on different trial days. The data sheets were filed away after each trial, meaning that each person running the trial was unaware of the cricket's previous score.

Effects of mock predator exposure and octopamine on plus maze behaviour

Sexually mature crickets were isolated from the general colony and placed into individual containers (10.5 cm diameter×7 cm) with food and water *ad libitum*. Crickets were then assigned into one of five weight-matched groups: model predator exposed, sham exposed, octopamine injected, sham injected and control. We used the same procedure as in an earlier study (Adamo and Baker, 2011) to mimic predator exposure. This method reliably increases neurohormonal octopamine titres in this species (Adamo and Baker, 2011). Briefly, crickets were placed into a container (L×W×H 17×15×9.5 cm) with a loose opaque divider separating it into two halves. In one half of the container was the cricket, in the other half was a robotic hamster (ZhuZhu Pets, Cepia, St Louis, MO, USA). The robotic hamster made electronic noises and moved randomly about its half of the container, hitting the side as well as the divider. The robotic hamster never contacted the cricket. Arrhythmic vibrations, such as those produced by the robotic hamster, induce anti-predator behaviour in crickets (Dambach, 1989). The crickets tended to run continuously during the trial. Sham-exposed crickets were also placed into a similar container with a divider for 3 min; however, the robotic hamster remained inactive. Control crickets stayed in their individual tubs and were not disturbed before entering the plus maze. To

determine whether changes in behaviour caused by exposure to the model predator can be mimicked by octopamine, a fourth group of crickets received an injection of 2 nmol of octopamine dissolved in 2 μ l of water (see Fields and Woodring, 1991). Sham-injected crickets were injected with 2 μ l of water. All injections were made with a 10 μ l Hamilton syringe through the pronotal membrane. Crickets were placed in the plus maze 5 min after injection or after model predator exposure. Each cricket was used in only one trial.

Effects of mock predator exposure on predator evasion

Crickets were isolated, weighed and assigned into three groups. The groups were as described above: control (male $N=18$, female $N=21$), model predator exposed (male $N=19$, female $N=20$) and sham-exposed (male $N=18$, female $N=21$); 5 min after exposure, crickets were used in the live predator trial. A second set of trials was run with control ($N=19$ male, $N=20$ female), sham injected ($N=19$ male, $N=20$ female) and octopamine injected ($N=19$ male, $N=20$ female) crickets; 5 min after injection, crickets were used in the live predator trial.

The predators were three female bearded dragons, *P. vitticeps*. The first two dragons were used in an alternating fashion for the first 39 trials, and the third bearded dragon was used for the final 39 trials.

For each set of trials, one cricket from each of the three groups was placed beneath an overturned opaque cup (8 cm diameter) and moved to a plastic enclosure ($L \times W \times H$ 72 \times 37 \times 34 cm). The cups were placed in the enclosure along a line 11 cm from one of the short walls (37 cm) and running parallel to it. A shelter composed of cardboard and duct tape ($L \times W \times H$ 32 \times 4.5 \times 5 cm) ran along this short wall as well. The three cups were equidistant from each other and the long walls of the enclosure (i.e. cups were about 9 cm apart and 9 cm from the wall of the container). The starting position for each group was rotated with each trial. At the start of the trial, a bearded dragon was added to the enclosure, halfway between the two long walls of the container and 25 cm from the end of the container wall opposite the crickets. The three cups were then lifted simultaneously by means of a rod attached to all three cups. The bearded dragon was released from its start position as soon as the crickets were uncovered. The first cricket to enter the shelter and the first to be consumed by the bearded dragon were recorded. Individual crickets could be identified by unique traits such as antennal length and cuticular colour. Trials were videotaped. The live predator trials lasted less than 1 min. Captured crickets were killed instantly by the lizards. For each trial, only crickets of the same sex were used. Therefore, no between-sex comparisons were possible.

Statistics

Statistics were performed using IBM SPSS Statistics (v19) and GraphPad Prism (v5.0b, GraphPad Software, La Jolla, CA, USA). Behavioural data were not normally distributed, even after attempted transformation, with the exception of locomotion. Locomotion was normally distributed after log transformation. A principal components analysis (PCA) was performed on the behavioural measures (locomotion, time spent freezing, the number of arm entries and the time spent in the covered arms). This method of analysis is recommended for studies of behavioural styles (Carter et al., 2013). PCA performed for data reduction can be used on non-normal data (Jolliffe, 2002). Only the first component of the PCA had an eigenvalue above 1 (score=2.26) and it explained 56.5% of the total variance ($N=23$ crickets).

The validity of the PCA was shown by the measure of sampling adequacy (Kaiser–Meyer–Olkin=0.635) and a highly significant

Bartlett's test of sphericity ($P < 0.0001$). The resulting first PCA component was also non-normally distributed, even after attempted transformation. Non-parametric factorial analysis (equivalent to a two-way ANOVA) was performed according to Meddis (Meddis, 1984). Non-parametric tests for homogeneity of variances were conducted using the non-parametric Levene test (Nordstokke et al., 2011). The alpha criteria were adjusted when necessary to account for multiple tests on the same data set (Benjamini and Hochberg, 1995) (calculated using www.marum.de/Binaries/Binary745/BenjaminiHochberg.xlsx).

RESULTS

Evidence for behavioural type in *G. texensis*

The PCA analysis demonstrated that cricket behaviour in the plus maze spanned a continuum between two different strategies. A high score on the first PCA component described animals that spent little time immobile in the exposed central area but that spent much of their time locomoting in, and moving between, the covered arms. A low factor score denoted crickets that remained immobile (in the open) for part of the trial. These patterns are evident from the component loadings for each behaviour: entries, 0.850; locomotion, 0.449; time frozen, -0.807; time within the covered arms, 0.828.

Crickets scoring highly on the first PCA component were deemed to be 'shelter-seeker/explorers' and low scoring crickets were considered 'freezers'. These scores were consistent and repeatable within individuals (Kendall's coefficient of concordance $W=0.8875$, $P < 0.0001$; $N=23$ crickets, tested four times each over 10 days). There were no significant differences in component scores across trials (Fig. 1; Friedman's test=6.18, $P=0.10$), suggesting that it is stable across time. This was tested explicitly with a Dunn's multiple comparison *post hoc* test. There was no significant difference in scores between the first and the last (fourth) trial (difference in rank sum=1.0, $P > 0.05$). This result suggests that animals did not habituate to the plus maze. There were no sex differences (Mann–Whitney tests for the shelter-seeker/explorer score, time frozen and time under the covered arms, $P > 0.05$ for all tests), except that females were

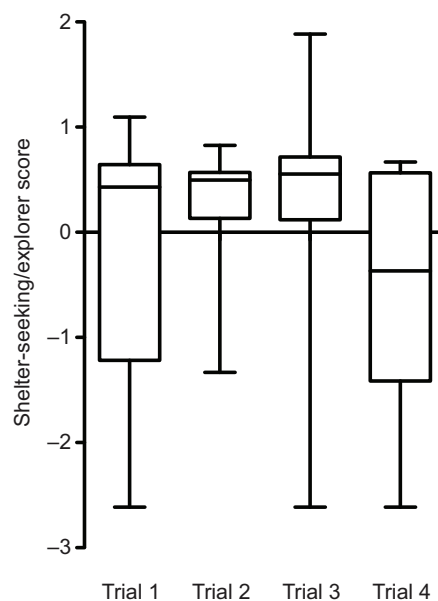


Fig. 1. Change in 'shelter-seeking/explorer' score over the four trials. There is no significant trend with time (see Results). The central line represents the median and the lower and upper bars represent 1st and 3rd quartiles. The error bars denote the range.

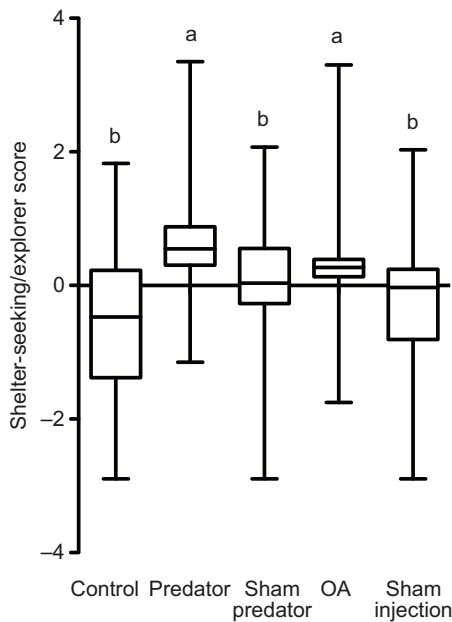


Fig. 2. Effect of model predator exposure or octopamine injection on shelter-seeking/explorer score. Both mock predator exposure and octopamine (OA) injection increase shelter-seeking/explorer score. The central line represents the median and the lower and upper bars represent 1st and 3rd quartiles. The error bars denote the range. Bars with different letters above them are significantly different from one another.

somewhat more active than males (log locomotion data, $F_{1,21}=6.34$, $P=0.02$); however, this result is only marginally significant after correction for multiple tests [after Bonferroni correction, alpha criterion (0.05 level)=0.0125 (Benjamini and Hochberg, 1995), no significant results]. There was no correlation between the ‘shelter-seeker/explorer’ score (mean over four trials) and weight (Spearman’s $r=-0.13$, $P=0.64$, $N=23$).

Effects of predator exposure and octopamine on plus maze behaviour

A PCA was performed separately on these data, extracting a single component with an eigenvalue of 2.0 explaining 49% of the variance. This component showed the same relationship to the four behavioural variables as described in the section above. It correlated positively with a strategy of multiple entries, greater locomotion and time spent under the covered arms, but correlated negatively with time spent frozen in the central area (component loadings: entries, 0.682; locomotion, 0.622; time frozen, -0.747; time in covered arms, 0.744).

Across the five treatment groups [model predator exposed (male $N=17$, female $N=17$), sham exposed (male $N=17$, female $N=17$), octopamine injected (male $N=16$, female $N=17$), sham injected (male $N=16$, female $N=16$) and control (male $N=17$, female $N=17$)], there were no significant differences due to sex for any of the behavioural measures (Mann–Whitney tests, all $P>0.3$). Therefore, male and female data were pooled for each treatment group and analysed using a Kruskal–Wallis test followed by a Dunn’s multiple comparison *post hoc* test where appropriate. There were significant differences in shelter-seeking/explorer scores across the five groups (Fig. 2, Kruskal–Wallis, $KW=40.7$, $P<0.0001$). Dunn’s multiple comparisons showed that both predator-exposed and octopamine-injected crickets had higher scores than did controls ($P<0.01$). To examine the changes in more detail, we studied the individual

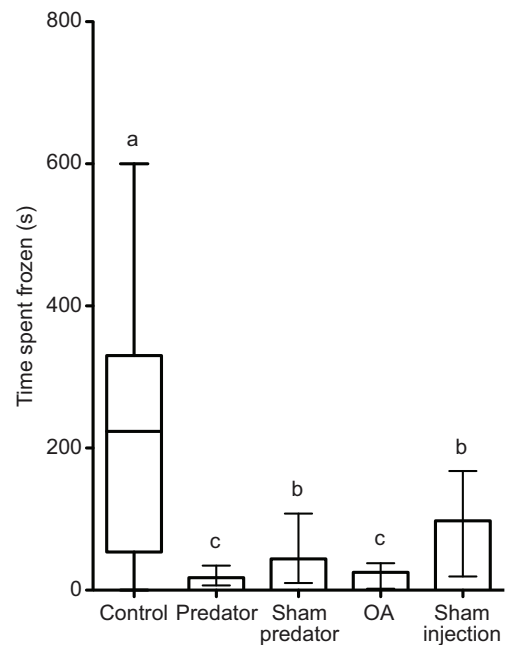


Fig. 3. Time spent frozen after exposure to a mock predator or octopamine (OA) injection. The central line represents the median and the lower and upper bars represent 1st and 3rd quartiles. The error bars denote the range. Bars with different letters above them are significantly different from one another.

behaviours, except for number of arm entries because that value tended to be low in all groups. Time spent frozen was significantly different across groups (Fig. 3, Kruskal–Wallis, $KW=39.2$, $P<0.0001$). Model predator-exposed and octopamine-injected crickets both spent less time frozen than did control, sham-injected or sham-exposed crickets (Dunn’s rank sum multiple comparison test, $P<0.05$). Sham-injected and sham-exposed crickets were frozen for a significantly shorter time than controls (Dunn’s rank sum multiple comparison test, $P<0.05$).

The median time spent within the covered arms was also significantly different among groups (Fig. 4, Kruskal–Wallis, $KW=53.8$, $P<0.0001$). The time spent within the covered arms was not significantly different between the model predator-exposed and octopamine-injected crickets. Model predator-exposed and octopamine-injected crickets both spent more time within the covered arms than did sham-injected, sham-exposed or control crickets (Fig. 4, Dunn’s rank sum multiple comparison test, $P<0.01$).

The median time spent locomoting was not significantly different across groups (Kruskal–Wallis, $KW=5.63$, $P=0.229$).

Variability of the shelter-seeker/explorer score was less in the predator-exposed or octopamine-injected groups than in controls (non-parametric Levene’s test, $F_{4,162}=4.7$, $P=0.001$; Fig. 2).

Effects of predator exposure on predator evasion

Model predator-exposed crickets ($N=22/39$) were significantly more likely to be the first individuals to reach shelter first than were either the control ($N=8/39$) or sham-exposed ($N=9/39$) groups (chi-squared test, $\chi^2=14.08$, $P=0.005$). Similarly, octopamine-injected crickets ($N=19/39$) were significantly more likely to be the first individuals to reach shelter first than were either the control ($N=7/39$) or sham-injected ($N=13/39$) groups (chi-squared test, $\chi^2=8.3$, $P=0.02$).

Model predator-exposed crickets ($N=19/39$) were significantly more likely to survive exposure to a predator than either the control

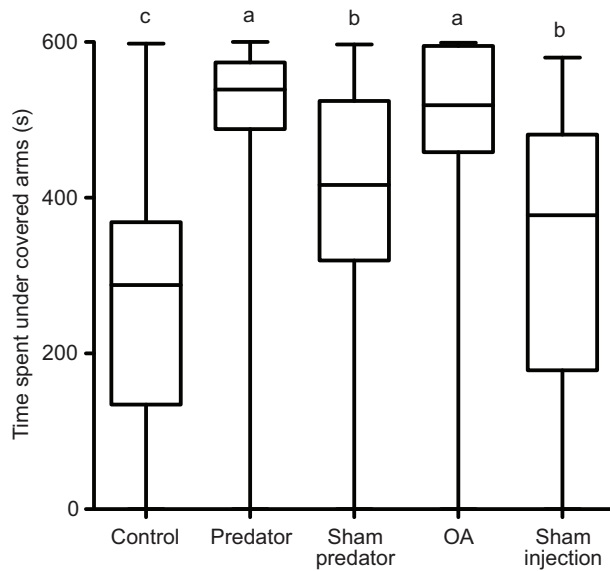


Fig. 4. Time spent under the covered arms after exposure to a mock predator or octopamine (OA) injection. The central line represents the median and the lower and upper bars represent 1st and 3rd quartiles. The error bars denote the range. Bars with different letters above them are significantly different from one another.

($N=9/39$) or sham-exposed ($N=11/39$) crickets (Fig. 5, chi-squared test, $\chi^2=6.4$, $P=0.05$). Similarly, octopamine-injected crickets ($N=19/39$) were significantly more likely to survive exposure to the predator than either the control ($N=8/39$) or sham-injected ($N=12/39$) crickets (Fig. 5, chi-squared test, $\chi^2=7.4$, $P=0.025$).

DISCUSSION

Individual *G. texensis* crickets exhibited distinct behavioural types in the plus maze that were consistent for at least half of their adult lifespan. However, these strategies were not immutable; they were sensitive to the presence of a predator (Fig. 2). Exposure to a mock predator shifted crickets away from using freezing as an anti-predator strategy and increased the time they spent under cover (Figs 2–4). Rodents show a similar pattern of behaviour in the elevated plus maze (Apfelbach et al., 2005; Eilam et al., 2012; Hacquemand et al., 2013). Predator exposure also reduces freezing in a related species of cricket tested using a different protocol (Niemelä et al., 2012). These results are consistent with current thinking about defence strategies. Defensive behaviours are thought to exist within a hierarchy (Hanlon and Messenger, 1996). Freezing occurs during low threat events, but if a predator continues to advance, animals flee (Hanlon and Messenger, 1996). Cricket defensive behaviour fits this framework; crickets freeze when the predation risk increases from low to moderate, probably to avoid detection by nearby predators. However, if an attack is imminent, crickets flee. Our study, and those of others (e.g. Apfelbach et al., 2005; Niemelä et al., 2012), shows that the threat level needed to shift animals from freezing to fleeing can be lowered by previous exposure to a predator. This shift is probably adaptive because being attacked by a predator in the immediate past is likely to be a good predictor that another attack is imminent if predator cues re-occur. Being able to alter anti-predator behaviour depending on the environment is likely to provide important fitness benefits (Storm and Lima, 2010).

Our results also provide support for the concept that the stress response is a continuum (e.g. Hacquemand et al., 2013), as might

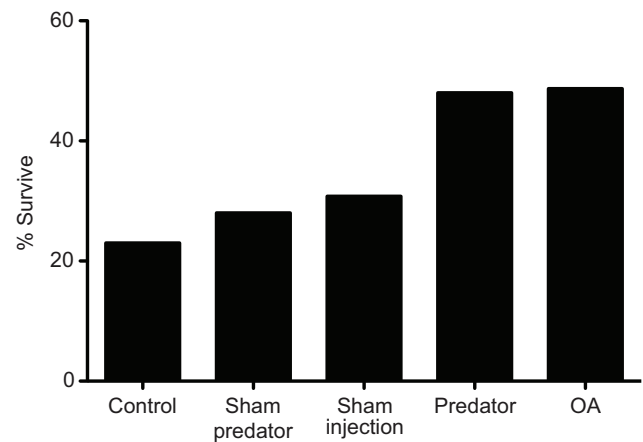


Fig. 5. Percentage of crickets that survive the encounter with the predator. Sample size is $N=39$ /group except for the control bar. This bar represents the average of the two control groups (from the first and second trials, $N=78$). Mock predator-exposed and octopamine (OA)-injected crickets were significantly less likely to be eaten than controls.

be expected if it is involved in determining the appropriate level of defensive behaviour. Our sham controls sometimes had behavioural scores that were intermediate between the unhandled controls and the predator-exposed or octopamine-injected crickets (e.g. Fig. 2). Similarly, handling stress in birds induces a smaller stress response than a predator attack (Pakkala et al., 2013). These results suggest that the stress response is not an all-or-none event, but that it can have graded effects depending on the severity of the threat.

After mock predator exposure or octopamine injection, not only did the crickets' anti-predator behavioural strategy shift but also variability in behavioural style in the plus maze declined compared with controls (e.g. Fig. 2). These results suggest that under normal conditions, a number of behavioural strategies may be adaptive, but in the presence of predators, optimal solutions quickly converge on a much smaller subset of strategies. Such shifts in anti-predator behaviour can be long lasting and, in crickets, can be transmitted transgenerationally (Storm and Lima, 2010).

Injection of octopamine led to a reduction in the use of immobility (i.e. freezing) as an anti-predator behaviour (Fig. 2). Similarly, raising octopamine levels decreased the use of immobility as an anti-predator behaviour in both an orb-weaving spider (Jones et al., 2011) and the beetle *T. castaneum* (Nishi et al., 2010). These results suggest that octopamine may be involved in altering the threshold for different defensive behaviours in many arthropods. However, the lack of effect of octopamine on locomotion seems puzzling, given its postulated role in mediating general arousal (Roeder, 1999). Nevertheless, octopamine also had no effect on general locomotion in the orb-weaving spider (Jones et al., 2011). We hypothesize that one of the effects of elevated octopamine is that it shifts anti-predator behaviour towards defensive behaviours used for more serious threats. In our plus maze study, crickets injected with octopamine may be more inclined to flee into hiding than controls, but once in a dark, safe place, they will be less likely to move out of it. Therefore, in our plus maze, with its covered dark areas, octopamine-injected crickets may be less likely to express elevated locomotion.

Model predator-induced changes in behaviour increased the ability of crickets to evade an actual predator (Fig. 5). Exposure to model predators is known to activate the stress response in this species (Adamo and Baker, 2011). Moreover, an injection of the

stress neurohormone octopamine also enhanced anti-predator behaviour (Fig. 5). These results suggest that a predator-induced stress response assists crickets in evading a predator. Therefore, one important benefit of stress hormones in insects appears to be to promote survival during a predator attack. This paper supplies a rare example of the magnitude of the survival benefit due to a predator-induced stress response.

Previous work has demonstrated some of the costs of activating the stress response in crickets. For example, flight-or-fight behaviours induce a large increase in metabolic rate (e.g. Hack, 1997), leading to the consumption of scarce resources. Octopamine promotes the consumption of these resources by inducing the release of lipid from fat stores (Fields and Woodring, 1991). This mobilization requires energetic and molecular resources (Nation, 2008). Such metabolic costs are not trivial for small animals. For females, the amount of lipid mobilized during a stress response (Adamo et al., 2008) is equivalent to 1.4% of the resources in an egg (see Shoemaker and Adamo, 2007). When resources are scarce, additional demands decrease reproduction in this species (Adamo and Lovett, 2011). Acute activation of the stress response also induces the reconfiguration of physiological networks that results in a reduction in function for a number of physiological systems (e.g. immunity) (Adamo et al., 2008; Adamo, 2009; Adamo, 2010). The full costs of these shifts remain unknown.

This paper shows that crickets are useful models for studying the costs and benefits of predator-induced stress responses. Although studies in vertebrates (e.g. Sapolsky et al., 2000; Wingfield, 2003; Øverli et al., 2007; Romero et al., 2009; Oswald et al., 2012; Romero, 2012) continue to increase our understanding of these stress responses, a complementary examination of them in invertebrates can add an important perspective. Crickets, with their short generation time, lack of parental care and straightforward stress response systems are tractable models for addressing questions regarding the fitness consequences of predator-induced stress responses. Such information will be important in integrating ecological and biomedical concepts of stress.

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AUTHOR CONTRIBUTIONS

B.M. helped design the live predator trials, ran some of the behavioural trials and critically read the manuscript. I.K. helped design the plus maze trials, ran some of the behavioural trials and critically read the manuscript. S.A.A. proposed the original concept, designed the overall trial, analyzed the data, wrote the paper and helped run some of the behavioural trials.

COMPETING INTERESTS

No competing interests declared.

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