School for Skinks: Can Conditioned Taste Aversion Enable Blue-tongue Lizards (Tiliqua scincoides) to Avoid Toxic Cane Toads (Rhinella marina) as Prey?

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Introduction

Invasive species are affecting native biodiversity on a global scale (e.g. Vitousek et al. 1997; Mack et al. 2000; Clavero & García-Berthou 2005). Areas that have been isolated from other large landmasses through long periods of evolutionary time are at particular risk, because of the vast number of potential non-native immigrants. For example, Australia has been inundated with introduced species (Wilson et al. 1992; Burgman & Lindenmayer 1998). Many of these taxa have had significant ecological impacts and have contributed to high rates of extinction of native fauna (Burbidge & McKenzie 1989; Short 1998; Johnson 2006). We urgently need to find ways to reduce such impacts (e.g. Banks et al. 1998; Pimentel et al. 2000; McLeod 2004; Robley et al. 2004; Gren et al. 2007).

The invasion of cane toads (Rhinella marina) through Australia imperils native predators that are killed if they consume these toxic anurans. The magnitude of impact depends upon the predators’ capacity for aversion learning: toad impact is lower if predators can learn not to attack toads. In laboratory trials, we assessed whether bluetongue lizards (Tiliqua scincoides) – a species under severe threat from toads – are capable of learned taste aversion and whether we can facilitate that learning by exposing lizards to toad tissue combined with a nausea-inducing chemical (lithium chloride). Captive bluetongues rapidly learned to avoid the ‘unpalatable’ food. Taste aversion also developed (albeit less strongly) in response to meals of minced cane toad alone. Our data suggest that taste aversion learning may help bluetongue lizards survive the onslaught of cane toads, but that many encounters will be fatal because the toxin content of toads is so high relative to lizard tolerance of those toxins. Thus, baiting with nausea-inducing (but non-lethal) toad products might provide a feasible management option to reduce the impact of cane toad invasion on these native predators.

Abstract

The invasion of cane toads (Rhinella marina) through Australia imperils native predators that are killed if they consume these toxic anurans. The magnitude of impact depends upon the predators’ capacity for aversion learning: toad impact is lower if predators can learn not to attack toads. In laboratory trials, we assessed whether bluetongue lizards (Tiliqua scincoides) – a species under severe threat from toads – are capable of learned taste aversion and whether we can facilitate that learning by exposing lizards to toad tissue combined with a nausea-inducing chemical (lithium chloride). Captive bluetongues rapidly learned to avoid the ‘unpalatable’ food. Taste aversion also developed (albeit less strongly) in response to meals of minced cane toad alone. Our data suggest that taste aversion learning may help bluetongue lizards survive the onslaught of cane toads, but that many encounters will be fatal because the toxin content of toads is so high relative to lizard tolerance of those toxins. Thus, baiting with nausea-inducing (but non-lethal) toad products might provide a feasible management option to reduce the impact of cane toad invasion on these native predators.
crocodiles (Letnic et al. 2008), and marsupial quolls (Braithwaite & Griffiths 1994; Oakwood 2003, 2004; Woinarski et al. 2008; O’Donnell et al. 2010). That ecological impact has stimulated the expenditure of vast effort and resources (more than $16 000 000 of government funding between 2004 and 2010: Department of Environment and Conservation 2009) toward toad control, mostly by direct physical collection of toads. Given the rapid maturation and high fecundity of the target species, manual removal and trapping do not have any long-term effect on toad abundance (Taylor & Edwards 2005). As a result, the toad invasion front has spread at ever-increasing rates across tropical Australia (Urban et al. 2008).

Given the lack of success of attempts at toad removal, is there anything that can be done to reduce the ecological impact of this invasive species? One approach may be to manipulate the outcomes of encounters between toads and predators, by modifying predator behavior through conditioned taste aversion (CTA: Gustavson & Gustavson 1985; O’Donnell et al. 2010). CTA is an adaptive learning mechanism that enables animals to avoid food containing toxic compounds and occurs when ingestion of a novel food type is followed by digestive illness (Garcia et al. 1974). The animal associates the taste of that food with the illness and subsequently avoids consuming that food type (Garcia et al. 1955, 1966, 1974; Cowan et al. 2000). Even a single exposure can induce long-lasting CTA (Gustavson 1977; Gustavson & Gustavson 1985). Inducing such an aversion response might provide a humane (non-lethal) means of controlling wildlife damage to livestock and crops (e.g. Ziegler et al. 1982; Gustavson & Gustavson 1985; Gill et al. 2000; Baker et al. 2008). CTA also might be used to mitigate problems posed by invasive species. For example, O’Donnell et al. (2010) used a CTA approach to train captive marsupial predators (quolls, Dasyurus hallucatus) to avoid cane toads, thereby enhancing rates of quoll survival post-release, in a toad-infested landscape.

Could CTA also reduce the impacts of toad invasion on other vulnerable predator species? Apart from quolls, the other Australian taxa threatened by cane toads are reptiles (Shine 2010), and the feasibility of CTA with such animals remains unclear. Most CTA research has focused on mammals and birds (but see Burghardt et al. 1973; Paradis & Cabanac 2004). In this study, we examine the potential for the nausea-inducing chemical lithium chloride (LiCl) to condition a threatened predator (the bluetongue lizard, Tiliqua scincoides: Price-Rees et al. 2010) to avoid cane toads. Skinks have abundant taste buds in the oral epithelium and on the tongue, particularly on the tip (Schwenk 1985). Bluetongues frequently extrude their tongues to sample potential prey items (Cooper 2000) and did so in response to the food items offered in this study (S. J. Price-Rees, pers. obs.). Thus, they might be capable of acquiring taste aversion. In previous studies, LiCl has been used to create CTA in reptiles (Burghardt et al. 1973; Paradis & Cabanac 2004) as well as in mammals and birds (e.g. Ziegler et al. 1982; Gustavson & Gustavson 1985; Dimmik & Nicolaus 1990; Gill et al. 2000). Our study was also designed to clarify the ability of bluetongue lizards to develop taste aversion based on sublethal exposure to toad tissues alone and hence to evaluate the prospects for lizards to survive the toad invasion in the absence of any management (CTA-based) intervention.

Methods

Animals and Husbandry

Bluetongue lizards (genus Tiliqua) are widely distributed across Australia. Within the areas invaded by cane toads, the common bluetongues are Tiliqua s. scincoides (in eastern Australia) and T. s. intermedia (in northern Australia: see distribution maps in the study by Price-Rees et al. 2010). Both taxa are heavyset, short-limbed, large-bodied omnivorous generalist foragers, consuming a wide diversity of plant and animal material (Cogger 2000). Such dietary breadth should enhance the adaptive value of an ability to learn via CTA (Bernstein 1999).

All trials were conducted at the University of Sydney Tropical Ecology Research Facility, Middle Point, Northern Territory (NT: 12.58°S, 131.31°E). Lizards were obtained from commercial breeders (expt 1) or were wild-caught (expt 2; see later for details). Animals were maintained until they were feeding consistently in captivity. They were housed individually in plastic bins (700 × 340 × 400 mm) containing a sand substrate, a refuge shelter, a rock to assist with sloughing, and a water dish. The bins were kept in a shaded area under natural photoperiod and ambient temperature. Lizards were maintained on a diet of lizard chow (lean beef, dog chow, boiled egg, and vitamin supplements) and mixed fruit and vegetables, given twice a week. Water was provided ad libitum.

Novel Food

CTA works most effectively with novel food types, and the aversion effect is weakened by prior safe
exposure to that kind of food (Revusky & Bedarf 1967). We used minced cane toad legs to provide the novel prey type. Although cardioactive toxins (bufadienolides) are distributed through the toad’s body, most are concentrated in specific sites such as the parotoid glands and ovaries (Lever 2001). To minimize toxicity of the mince (to avoid mortality of predators), we removed the legs from dead (locally collected) toads, skinned them, soaked them in running water for 5 min, and then minced the meat in a domestic food processor.

We conducted two experiments to assess the potential for CTA to induce bluetongues to avoid cane toads. Expt 1 (April–June 2009) gauged lizard responses to a known concentration of LiCl by injecting the chemical into lizards after they consumed toad-mince balls. Expt 2 (July–Sept. 2009) extended the work by presenting the toad-mince in a form better suited to field baiting, i.e. inside a sausage skin, and incorporating the LiCl within the mince. Sausage-style baits containing poison often have been deployed in the field to manage other invasive species (Marks et al. 2006; Hetherington et al. 2007).

Experiment 1

We obtained 30 lizards from commercial breeders: 20 T. s. scincoides from New South Wales and 10 T. s. intermedia from the Northern Territory. These lizards ranged in snout-vent-length (SVL) from 210 to 320 mm and in body mass from 220 to 569 g. Lizards were randomly allocated to treatment or control groups. Prior to experimental procedures, all lizards were deprived of food for 72 h. Lizards were then offered a 15-g ball of toad-mince at 13:00 h and monitored for the next 3 h. Then, any remaining food was removed and reweighed. Following the consumption of mince, treatment lizards were injected with LiCl (8 M, 0.5 mg/kg) and control lizards received a sausage without LiCl. Because lizards either consumed the sausage entirely or not at all, we scored consumption (after 3 h, as earlier) as a binary measure (yes or no).

Post-treatment testing of both control and treatment lizards was repeated for 7 wk. To compare sausage consumption rates of treatment vs. control lizards up until week 7 of the trial period, we carried out a logistic repeated-measures analysis using Proc Genmod v9.1 (SAS 2009b). The probability of eating a sausage was modeled using a logit link function over 7 weekly periods.

To determine whether the acquired aversion was associated with the skin of the sausage rather than its contents, all animals were offered a 10-g sausage containing lizard chow (the subjects’ regular diet) after the main experiments had finished (9 wk after their initial exposure to toad-mince sausage), under the same test conditions.

Video Analysis of Lizard Behavior

To determine whether the responses of bluetongue lizards to cane toads were modified by encounters with the toad baits, we videotaped the behaviors of all lizards (from both expts 1 and 2) when presented with live toads (5–10 g). To avoid predator mortality as a result of ingestion (Price-Rees et al. 2010), the toads were placed inside a mesh container that allowed visual and olfactory cues, but not direct contact. Prior to the CTA trials, lizards were presented with (1) an empty container, as a control to gauge response levels to the experimental situation and (2)
a live toad in a container, to gauge pre-trial response levels. Following the CTA trials, the lizards were presented with a toad in a container 2 and 10 wk post-trial to assess learning outcomes.

Trials were conducted between 11:00 and 17:00 h EDST during April to September 2009. Lizards were deprived of food at least 2 d prior to the trials. A video camera mounted on a tripod above the lizard’s usual enclosure recorded events as the enclosure lid was removed, and the container was placed in the furthest corner from the lizard’s current position. The lizard was then left undisturbed. The first 10 s of the video footage was discarded to account for initial disturbance, and each lizard was filmed for up to 60 min (as soon as the container was displaced by the lizard, the trial was terminated). From the video, we scored the time taken for the lizard to make contact with the container. These durations for treatment vs. control lizards were compared using a single-factor repeated-measures ANOVA. For expt 2, statistical analyses were conducted on pre-trial and 2-wk post-trial behavior responses. Statistical analyses were performed using JMP version 8.0 (SAS 2009a).

Results

Experiment 1

At their first exposure, all lizards (both treatment and control) consumed the entire 15-g ball of mince. Following treatment, all LiCl-injected lizards were ill (i.e. lethargic and unresponsive) and 10 of the 15 animals vomited within 24 h. Of the control (saline-injected) lizards, 10 showed no ill effects, but the other five vomited after consuming toad-mince. In the first week of post-treatment testing, both the treatment and control groups exhibited an abrupt decline in the amount of toad-mince consumed. In this week, treatment lizards ate less toad-mince than did control lizards (\( \bar{x} = 0.16 \pm 0.11 \) g SE vs. 6.47 \( \pm 1.71 \) g SE; \( F_{1,29} = 13.16, p = 0.001 \)). This difference persisted, but became less marked, over the next 7 wk (repeated-measures ANOVA: treatment effect \( F_{1,27} = 8.46, p = 0.007 \); time effect \( F_{7,21} = 47.73, p < 0.0001 \); time*treatment \( F_{7,21} = 4.30, p = 0.004 \); see Fig. 1a). Neither control nor treatment lizards returned to the pre-trial consumption rates by the end of the 7-wk study (Fig. 1a).

Experiment 2

Prior to the experimental treatment, 100% (seven of seven) of control lizards and 90% (9 of 10) of treatment lizards consumed the toad sausages presented. Of the nine treatment lizards that consumed sausages, five were lethargic and unresponsive thereafter, and four of them vomited within 24 h. The other five treatment animals displayed no obvious signs of illness after eating the sausage. Control lizards showed no illness and no vomiting. In the first week post-treatment, treatment lizards displayed an immediate decline in the consumption of novel

![Fig. 1: Consumption of a novel prey type (minced cane toad flesh) by captive bluetongue lizards (\( Tiliqua scincoides \)) before exposure to conditioned taste aversion (CTA) trials (week 0) and during the following 7 wk of post-treatment testing. The upper graph (a) shows results from expt 1, where control lizards were injected intramuscularly with saline after their first meal of cane-toad mince, whereas treatment lizards were injected intramuscularly with lithium chloride (LiCl) to induce CTA. The figure shows mean values (based on 15 lizards per treatment group) and associated standard errors. The lower graph (b) shows results from expt 2 and depicts the proportion of captive lizards that consumed a sausage of cane-toad mince before exposure to CTA trials (week 0) and during the following 7 wk of post-treatment testing. Control lizards were given sausages containing toad-mince only, whereas treatment lizards received sausages laced with a nausea-inducing chemical (LiCl) to induce CTA.](image-url)
biats. Only 20% of treatment lizards consumed the proffered sausages compared with 100% of the control lizards. The repeated-measures logistic analysis indicated a significant Group*Time interaction (Z = 2.16, 1 df, p < 0.031), as well as significant main effects of Group (treatment and control; Z = 4.74, 1 df, p < 0.0001) and Time (Z = 4.88, 1 df, p < 0.0001, Fig. 1b). Nine weeks after their initial exposure to toad-mince sausages, all but two of the lizards (one treatment and one control) consumed sausages containing lizard chow, suggesting that the acquired aversion was related to the content of the sausages rather than to their skins.

Video Analysis of Lizard Behavior – Experiment 1
Prior to the CTA trials, lizards contacted a container with a live cane toad significantly quicker than they contacted an empty container (F1, 28 = 4.72, p < 0.04) in the case of both control and treatment animals (F1, 28 = 0.29, p = 0.59). At later tests, however (2 wk post-treatment and 10 wk post-treatment), treatment (LiCl-injected) animals were significantly slower to approach and investigate the toad stimulus than were their control counterparts (repeated-measures ANOVA: F1, 27 = 4.31, p < 0.05; Fig. 2). This difference between treatment and control lizards increased from an average of 4.5 min pre-treatment, to 14.2 min after 2 wk, and 20 min after 10 wk (Fig. 2). Latencies to approach the toad stimulus were greater after 2 wk than at zero or 10 wk (F2, 26 = 7.42, p < 0.003) in both groups (interaction trial*group F2, 26 = 0.95, p = 0.40).

Video Analysis of Lizard Behavior – Experiment 2
Overall response patterns were similar to exp 1 but not statistically significant. Mean latency to approach the container for treatment lizards increased from 15.0 min (±6.59 min) pre-trial to 26.0 (±7.9 min) 2 wk later. In contrast, control lizards showed little change in this respect (14.1 ± 8.0 vs. 15.2 ± 7.6 min). Nonetheless, analysis revealed no statistically significant effects on bluetongue responses (group effect: F1, 5 = 0.37, p = 0.54; time effect: F1, 15 = 1.23, p = 0.28; time*group: F1, 15 = 0.84, p = 0.37). At 10 wk post-trial, the mean latency to approach the container for both control and treatment lizards remained longer than for the pre-treatment trials (± 30.5 and 24.9 min).

Discussion
Both expts 1 and 2 (using toad-mince as the novel food type) showed that tiliquine skinks are capable of learning taste aversion. These results are consistent with the findings of Paradis & Cabanac (2004) that also demonstrated a marked decline in intake of novel foods by basilisk lizards when the food was paired with a nausea-inducing drug (LiCl). In our study, the lizards responded to post-feeding administration of LiCl by becoming less likely to consume that prey type in subsequent trials. Interestingly, however, in exp 1, one-third of the control lizards (i.e. that had been given toad-mince without the drug) also showed symptoms of nausea, and this group also became less likely to consume that prey type even though no nausea-inducing chemical had been added. Clearly, toad-mince alone was capable of inducing taste aversion, albeit less strongly than occurred if LiCl was used. Based on latency to approach a toad in the videotaped trials, the lizards’ aversion responses appear to have been generalized from toad-mince to live cane toads. Our second experiment (using toad-mince sausages as the food type) gave less clear-cut results, which may reflect the means by which the LiCl was administered. When administered orally via the toad sausages, only 50% of the treatment lizards exhibited ill effects compared with 100% when LiCl was injected.
The lizards’ behavioral responses to a live toad were more variable in this experiment also. Some lizards displayed an increased latency to approach a toad, while others showed no change in behavior. Nonetheless, broad patterns of response were similar between the two experiments, including a significant tendency for both treatment and control lizards to exhibit increasing aversion to toad-mince sausages (but not other sausages) through time. In combination, our results suggest that bluetongue lizards can acquire taste aversion to cane toad flesh per se and that the use of nausea-inducing chemicals can accelerate that training process.

If bluetongue lizards can learn not to eat cane toads, why has the invasion of this toxic anuran been accompanied by lizard mortality (Sanson 2010) and a dramatic reduction in abundance (Price-Rees et al. 2010). In other predator taxa, a capacity for aversion learning has prevented population-level declines following toad invasion (Shine 2010). The answer may lie in the high toxin content of toads, combined with the sensitivity of bluetongues to this toxin. Tiliquine skinks sometimes attack large prey items, especially slow-moving animals such as cane toads (Greer 1989; Shea 1998). A large toad contains enough toxin to kill Australian predators much larger than bluetongue lizards (e.g. Varanus panoptes – Smith & Phillips 2006; Doody et al. 2009; Crocodylus johnstoni – Letnic et al. 2008). The cane toad invasion front comprises large individuals, with little breeding (and thus few small toads) (Brown et al. 2006). Hence, the first toads likely to be encountered by foraging bluetongue lizards will be animals large enough to kill the predator rather than induce taste aversion learning (Shine 2010). Our trials used toad tissue that had been selected and processed to remove most toxins (i.e. skinned, soaked, from the legs rather than from toxin-rich body parts), so that the amounts of toxin were a tiny fraction of those present in an adult toad. Despite these precautions, the lizards in our trials frequently exhibited signs of nausea and almost universally developed taste aversion. Ingestion of the full toxin complement of a live toad, especially a large specimen, would be likely to cause rapid death rather than behavioral change.

CTA relies on specific neural mechanisms (Kiefer 1985). Because the cranial nerves carrying taste, gastric and visceral information converge in the same area within the brain stem (Nachman et al. 1977; Bernstein 1999), a digestive illness is more likely to be associated with taste than with other cues such as scent, which is not processed in this region (Garcia & Hankins 1977; Garcia & Rusiniak 1980). Accordingly, taste may be important in the induction of CTA in bluetongue skinks. Vomeronasal function in lizards has been studied extensively (e.g. Burghardt 1970; Cooper & Alberts 1990; Graves & Halpern 1990; Cooper 1994, 1997; Martínez-Marcos et al. 2001), but the role of taste in mediating behavior has received less attention (Burghardt 1970; Simon 1983; Schwenk 1985). Further work could usefully explore the role of taste in discriminating among prey types and hence in the development of aversion to specific food types.

What can we conclude about the feasibility of using CTA to modify feeding responses of free-ranging bluetongue lizards? Our study is only the first step in exploring this idea, but the results are broadly encouraging. First, wild-caught bluetongues readily consumed sausages containing minced cane toad tissue. Second, the experience of consuming toad tissue induced nausea in some lizards, even without the use of drugs. Third, consumption of a non-lethal meal of toad tissue resulted in a decreased propensity to eat more of this novel food type, and an increased latency to approach live toads. Fourth, LiCl facilitated the taste aversion learning process.

Lithium chloride is not the only drug that could be used to induce CTA (Riley & Tuck 1985) but seems well suited to this role. More specifically, Nicolaus et al. (1989) identified the following prerequisites for a CTA compound to be successful under field-based conditions: (1) it should induce a strong, enduring CTA after single oral dose, (2) the effective illness-inducing dose should not cause mortality or have ongoing detrimental effects in either target or non-target species, (3) the compound must be physically stable within the bait, so it will not deteriorate under field conditions, and (4) at the effective dose, it should be undetectable when incorporated within the baits. Below, we evaluate the evidence for these issues:

1. In our study, LiCl produced a strong aversion to toad-mince after a single dose. The aversion persisted for at least 2 mo.
2. LiCl is considered relatively safe for animals and the environment (Burns 1983).
3. LiCl is stable under a wide range of conditions (Burns 1983).
4. The only evidence concerning detectability is that the videotaped trials revealed a greater aversion (i.e. reluctance to approach) toward live toads in treatment lizards than control lizards. We would not have expected such a shift in behavior unless the aversion created by the LiCl was focused on toad
cues rather than LiCl cues. Previous studies where detectability of LiCl has been a problem were conducted on large mammals, which were given a dose of 7–45 g of LiCl per animal (Burns 1980), much greater than the dose used in the present study (0.13–0.31 mg per animal). Nonetheless, it is clear that toad-mince alone can induce CTA, rendering the effects of LiCl difficult to distinguish from a background level of aversion development. If detectability of LiCl proves to be a problem, the drug can be encapsulated within the bait such that it is not exposed to the animal’s oral mucosa (Rusiniak et al. 1976; Hetherington et al. 2007).

Before CTA-based methods could be deployed in the field proactively (in advance of the toad invasion front) to reduce toad impact on predators, we need more information on issues such as optimal places and times for bait deployment, and the identity and fate of predators that consume baits. Critically, field deployment must not cause collateral damage to other native species, and it would be vital to evaluate the possibility of inadvertent negative consequences (e.g. if some native species prove highly sensitive to either toad-mince or LiCl, or if individual predators consume so many baits that the total toxin content reaches dangerous levels). The example of the cane toad itself shows how easily a well-intentioned attempt at manipulating natural systems can create major problems. Fortunately, the risks posed by CTA methods are likely to be minimal, in that any field deployment would be in areas about to receive vast numbers of invading cane toads anyway. Thus, ill effects from occasional overdoses of toad toxin are likely to be trivial compared with the effects of the full-scale toad invasion about to arrive. If CTA methods can maintain viable predator populations for a few years after the initial toad invasion, the increasing availability of newly recruited (small, relatively non-toxic) toads may provide an opportunity for the progeny of those predators to learn taste aversion themselves, without the need for additional management intervention (Shine 2010). Although our results are preliminary, they suggest that additional research would be worthwhile into the potential effectiveness of CTA approaches at reducing invasive species impact.

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