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SMARTER BAITS: THE EFFECTS OF STRESS ON BAIT AVERSION AND OPTIONS TO AVOID THE DEVELOPMENT OF BAIT AVERSIONS

Summary: In poisoning operations, sublethal consumption of the toxin, can produce bait aversion. This decreases the effect of the poisoning and may create problems due to the presence of uneaten toxin in the environment. The use of new bait additives may prevent aversion development. Here I report the effects of two bait additives, corticosterone and mifepristone, in altering bait aversion development in rats exposed to the widely used poison, monofluoroacetate (1080). Corticosterone is a glucocorticoid hormone, released in response to stress. Mifepristone (Ru 38486), inhibits the actions of this hormone. Imposed stress as well as administration of corticosterone, decreased consumption. Concurrent administration of mifepristone prevented these decreases. Mifepristone in low doses increased aversion in stressed, but not unstressed rats. At high doses, mifepristone both increased consumption and decreased aversion in all rats following exposure to 1080. Administration of corticosterone also produced dose-dependent effects on aversion. At low doses in unstressed rats corticosterone, alone, increased aversion, while at high doses in all rats it decreased aversion. Stress, and the hormonal outcome of this state, may thus contribute to aversion by influencing both consumption and aversion development.

Keywords: Toxins; aversion; sublethal effects.

Introduction

Sodium monofluoroacetate (1080) has been extensively used to poison vertebrate pests in New Zealand for several decades (Livingston, 1994; Eason *et al*, 1993). Aversion to re-baiting, following sub-lethal consumption of a bait-toxin, represents a well documented example of toxin limitation from an operational perspective (Hickling, 1994). Aversion to bait occurs when an animal consumes a sublethal dosage and associates the subsequent experienced ill-effects with the bait and/or toxin mix, thereafter avoiding that mix. Aversions to cyanide (Warbuton and Drew, 1994), 1080 (Hickling, 1994), and anticoagulants (Cook and Dean, 1996) have been documented.

Sublethal consumption can occur as a result of the animal being presented with a bait of poor quality or which has been degraded by environmental exposure. Bait that has broken into smaller pieces, and animals showing cautious feeding strategies (consuming only a small amount of any bait), can also contribute to the development of aversion.

Strategies to reduce this problem include: ensure that the bait-toxin does not degrade in the environment, that a lethal dose is correctly presented to the animal and, use dyes and other cues to mask the signals an animal uses in developing an aversion (Thomas, Henderson and Hickling, 1996; McDonald and Short, 1996).

Another strategy to avoid aversion is to include, within the bait, additives that disrupt the association between the bait-toxin and the ill-effects experienced. This association is a form of learning (Naheen and Khan, 1990) and can be disrupted chemically thus preventing aversion (Cook and Dean, 1996; Devine and Cook, 1998).

Stress in animals has also been shown to influence both food consumption and learning (Wood and Shors, 1998; Gardner, Rotherwell and Luheshi, 1998). This influence results from the effects of a hormone, corticosterone, released in response to stress. Both stress and corticosterone show an "inverted U" –type dose relationship to learning. Too little or too much corticosterone reduces learning ability (Wood and Shors, 1998; Xu, Anwyl and Rowan, 1997; Diamond *et al*, 1992). I tested the hypothesis that bait additives that influence stress responses could also influence bait consumption and aversion.

Methods

Experiment 1a

To determine if stress, (isolation or handling) affected food consumption and the development of bait aversion, I divided 80 male rats *(Rattus norvegicus)*, with live body mass of 300-400 g at 6 months of age, into two sets. Rats in set 1 were housed individually (singles) while rats in set 2 were housed in same-sex pairs (doublets). After 7 days on a diet of 40 g standard pellets (Diet 86, Sharpes Grains and Seeds Ltd, Lower Hutt, N.Z.) and 20 g of a highly preferred chocolate-based cereal (Cocopops ™, Kellogs, Australia; Dean, 1997) per animal, all rats were handled individually for 20 min at 6 h intervals for 1 day. Changes in food consumption, measured as g per kg live body mass per day, were analysed for evidence of feeding inhibition. Blood samples (Cook, 1997) were taken from each rat on day 1 and day 3 to provide a corticosterone baseline, and on day 9, 24 h after handling.

To determine whether the effects of stress on food consumption could be reduced by corticosterone, I treated 10 – 10 rats, orally, from each set on day 11 with 1.0, 2.0 or 4.0 mg kg^{-1} mifepristone (Ru 38486, Sapphire Bioscience, Australia) a corticosteroid receptor antagonist. A group of 10 rats in each set were not treated with the corticosterone antagonist (controls). Rats continued to receive 40 g standard pellets, 20 g cocopops and water *ad libitum*. Blood samples were collected twice before (on days -3 and -1) and 1 day after treatment.

A sublethal dose of 1080 (1.0 mg kg^{-1} animal live body mass) was fed to each rat in cocopops on day 15. At the same time, 10 rats in each set received 1.0, 2.0 or 4.0 mg kg^{-1} mifepristone, with 10 rats receiving only the 1080 dose (controls). This procedure was designed to test the hypothesis that acquired aversions in response to sublethal doses of a toxin develop less strongly in unstressed animals. Three days after 1080 exposure (day 18) cocopops and pellets were offered to all rats. On the next day (day 19), 4 days after the poison exposure, cocopops containing 0.1 mg kg^{-1} 1080 as well as standard pellets were offered to all rats.

Experiment 1b

This experiment was conducted as above, with the following modifications. To validate further whether mifepristone had an effect on consumption, during isolation and handling stress, individually housed animals were fed, in addition to the pellets and cocopops, on days 1, 3, 5 and 8 (handling day) either 1.0 (10 animals), 2.0 (10 animals) or 4.0 mg kg^{-1} mifepristone alone (5 animals). To determine whether the effects of mifepristone were via competition with corticosterone 4.0 mg kg^{-1} mifepristone and 4.0 mg kg^{-1} corticosterone (Research Biochemicals International, Natick, USA) were fed to 5 animals. A further 10 animals received no drug treatments.

To determine if the additon of corticosterone would change aversion development, and if mifepristone could prevent this, all individually housed animals received 1080 at 1.0 mg kg^{-1} live body mass and corticosterone at 1.0 (10 animals), 2.0 (10 animals) and 4.0 mg kg^{-1} (5 animals) on day 15. Five of the animals received 4.0 mg kg^{-1} corticosterone and 4.0 mg kg^{-1} mifepristone.

The same experiment was performed on animals that had been housed in pairs (doublets) These were assumed to be less stressed than the animals kept in isolation. Doublet animals were fed corticosterone at 1.0 (10 animals), 2.0 (10 animals) or 4.0 mg kg^{-1} (5 animals), or received no drug treatments (10 animals) on days 1, 4 and 8 to test the effects of increasing corticosterone level on consumption. Five of the animals received 4.0 mg kg^{-1} corticosterone and 4.0 mg kg^{-1} mifepristone on the same days. On days 15 all animals received 1.0 mg kg^{-1} 1080. Corticosterone was also fed at 1.0 mg kg^{-1} (5 animals), 2.0 mg kg^{-1} (5 animals) and 4.0 mg kg^{-1} (5 animals) alone, or in combination with mifepristone at 1.0 mg kg^{-1} (5 animals), 2.0 mg kg^{-1} (5 animals) or 4.0 mg kg^{-1} (5 animals). Ten animals received no drug treatments.

To determine if isolation stress influenced consumption, housing conditions were reversed on day 19. Animals kept in pairs were isolated, and isolated ones were put in pairs. Food consumption was measured for the subsequent 5 days. Corticosterone titre in blood was measured daily in all animals.

Analysis

Analysis was conducted using a Sigmastat v 2.0 (Jandel Scientific Software) software package. Multivariate analysis was performed where data passed normality and equal variance testing; other procedures applied were repeat measure ANOVA and standard paired and unpaired t-tests. Non-parametric tests, (Mann-Whitney Rank Sums), were also performed where appropriate.

Results and Discussion

Animals in doublets consumed more (ANOVA $P<0.05$) per bodyweight than individual housed animals (Fig. 1), although the proportion of pellets to

cocopops consumed did not significantly differ**.** Administration of mifepristone, an antagonist of corticosterone, increased (ANOVA, $P<0.05$) consumption in the singles in a dose-dependent fashion (Table 1), but had little effect on doublets (Table 2). Administration of corticosterone to both the doublets and singles decreased (ANOVA, $P<0.05$) consumption in a dose-dependent fashion (Tables 1 and 2). This was prevented by concurrent administration of mifepristone.

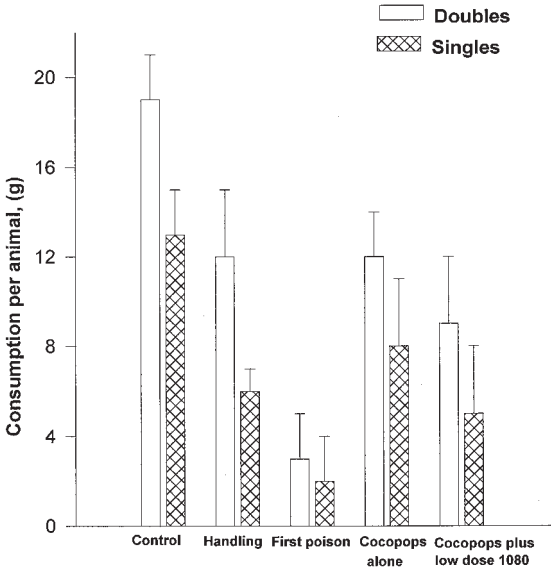

Figure 1: *Cocopop consumption for non-drug treated animals, either singles or doublets, under different conditions. Baseline represents consumption during periods in which the food alone was provided. Handling stress represents consumption when stress was imposed by handling the animals. Measurements are shown for the first exposure to cocopops containing 1080 at a dose of 1.0 mg kg^{-1} and subsequent exposure to cocopops alone and then subsequently cocopops associated with 0.1 mg kg^{-1} 1080. Measures represent the group mean and standard deviation over a 24 h period.*

Handling stress also had a marked effect on food consumption in the rats, significantly reducing (ANOVA, $P<0.01$) the daily food consumption in all groups. Administering corticosterone produced a greater reduction while administering mifepristone increased the consumption in these handled animals. Mean (± S.D.) blood corticosterone levels increased ($P<0.01$) in singles (19 ± 2 mg 100 ml $^{-1}$) as compared to doublets (3 ± 3 mg 100 ml $^{-1}$), and in all groups were slightly elevated on the day after handling (28 ± 6 mg 100 ml $^{-1}$ for singles, and 11 ± 4 mg 100 ml $^{-1}$ for doublets). Stress is known to influence food consumption (Gardner *et al.*, 1998), probably through the elevated corticosterone levels that accompany stress. Isolation (or alternatively over-crowding) can impose stress (Cook, 1996; Jacobson and Cook, 1998; Cook, 1998) onto an animal, and it would appear from the above results that this was the case for the rats. Separating doublets into singles, not only significantly (ANOVA, $P<0.01$) increased their corticosterone levels, but also reduced their food consumption (ANOVA, $P<0.01$). Doubling up singles had an opposite effect (ANOVA, $P<0.01$, Fig. 2).

These results suggested that features of an animal's social and stress environment could markedly affect its consumption of a bait. This, in turn, could reduce or promote sublethal consumption and subsequent development of aversion.

Stress has also been shown to influence learning (Wood and Shors, 1998) and an "inverted U"- type relationship exists between dose of corticosterone and learning performance (Xu, Anwyl and Rowan, 1997; Diamond *et al*, 1992). To test if stress also has an effect on learning, I compared the development of aversion in single vs. doublet animals following sublethal exposure to bait containing 1080 (Fig. 1). I also administered either corticosterone, the corticosterone antagonist mifepristone, again at different dosages, or a combination of both, in conjunction with exposure to bait containing 1080 (Tables 1 and 2). Animals, that received no drug treatments, showed an initial reduction (ANOVA, $P<0.01$) in eating both cocopops (poisoned) and

Table 1: *Consumption of cocopops (g kg^{-1} live mass, mean ± s.d.) in single housed animals with different treatments.*

Control	Mifepristone			Corticosterone		Mifepristone + corticosterone*	
	1.0 mg kg^{-1}	2.0 mg kg^{-1}	4.0 mg kg^{-1}	2.0 mg kg^{-1}	4.0 mgkg^{-1}		
Control	13±2	17±4	18±3	19±2	10±2	4±3	14±4
Handling	6±1	9±2	11±3	12±2	3±3	2±2	10±2
Day 15 poison	2±2	2 ±2	4±3	7±3	1±2	2±3	5±2
Day 18 cocopops	10±3	4±3	2±3	14± 4	4±2	12±3	11±4
Day 19 repeat poison	5±3	2±1	1±3	11± 3	2±2	13±4	8±3

* Both 4.0 mg kg^{-1}

Table 2: *Consumption of cocopops (g kg^{-1} live mass, mean ±s.d.) in doublet housed animals with different treatments.*

Control	Mifepristone			Corticosterone		Mifepristone + corticosterone*	
	1.0 mg kg^{-1}	2.0 mg kg^{-1}	4.0 mg kg^{-1}	2.0 mg kg^{-1}	4.0 mg kg^{-1}		
Control	19±2	18±4	19±1	19±2	10±2	8±3	16±4
Handling	12±3	15±2	18±3	18±2	9±3	3±2	13±2
Day 15 poison	6±2	7±2	9±3	11±3	3±2	5±3	8±2
Day 18 cocopops	16±3	15±3	16±3	17±4	6±	13±3	11±4
Day 19 repeat poison	9±3	8±1	12±3	14±2	3±2	12±4	8±3

*Both at concentrations of 4.0 mg kg^{-1}

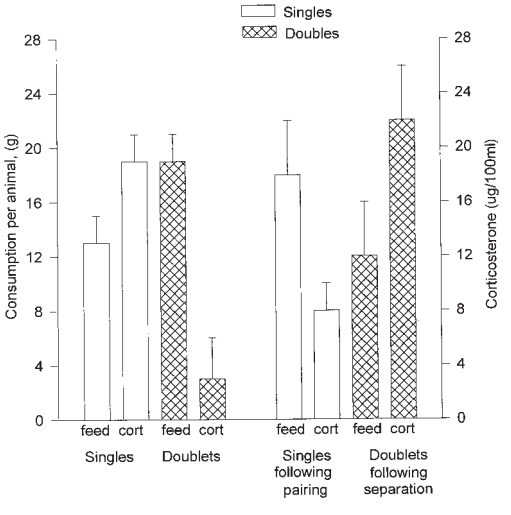

Figure 2: *The influence of single housing as compared to doublet housing of rats on both basal corticosterone and feed consumption. Data is also shown when rats previously housed as singles were subsequently housed as doublets and vice-versa . This is presented for the switched groups 5 d after the switch. Measures represent the group mean and standard deviation over a 24 h period. Feed = consumption of cocopops. Cort = concentration of corticosterone.*

pellets (non-poisoned) on the first day of poison exposure. More cocopops (unpoisoned) were eaten on day 18 than on the "poisoning day" (day 15), but this amount was still less than on pre-poisoning days. On day 19 when cocopops were again re-poisoned, albeit with a much lower dose of 1080, consumption was reduced, slightly more in singles than in doublets. In the doublets, however, it was not possible to measure individual food consumption and this may have biased the results.

In the singles that received mifepristone similar trends were seen at low doses, except that aversion to favoured food was greater (ANOVA, P<0.05) on all days compared to non-treated animals. At the highest dose of mifepristone, little aversion to the favoured food was seen except on the first poison exposure when pellet consumption was also reduced. Administration of corticosterone at a low dose slightly increased aversion. This disappeared at the highest dose of corticosterone. These effects of corticosterone could be prevented by concurrent administration of mifepristone. In the doublets, administration of corticosterone at low doses produced an increase in aversion, while the highest dose reduced aversion. Concurrent administration of mifepristone again prevented these effects. Mifepristone alone at high doses decreased aversion in doublets.

These results suggest that both stress, and the level of corticosterone, influenced the development of aversion. In some cases animal ate the same amount or more suggesting that the effects of stress on aversion could be dissociated from the effects on consumption. The results suggest that too little or too much corticosterone action inhibits the development of aversion, whereas a high level of corticosterone inhibits consumption (low level does not). The use of corticosterone or mifepristone, offers practical approaches to prevent bait aversion. We have to know, however, the existing state of stress in the target group. If we are able to assess this, then they may offer an adjuvant for preventing aversion and sublethal consumption. It is worth noting, however, that irrespective of stress state, mifepristone at the highest dose reduced aversion and promoted consumption. Administering mifepristone may be a useful tool for preventing bait aversion. This approach may offer a development strategy for "smarter" bait products particularly when combined with other additives that appear to decrease aversion (Devine and Cook, 1998) and products that may increase consumption such as neuropeptide Y (Gardner *et al*., 1998). These approaches may reduce the total amount of 1080 currently needed to meet control targets and mitigate risks associated with the use of 1080.

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