Applied Animal Behaviour Science xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim

Research Paper

# Using mildly electrified grids to impose costs on resource access: A potential tool for assessing motivation in laboratory mice

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## ARTICLE INFO

*Keywords:*
Preference
Motivation
Mouse
Enrichment
Electric current

## ABSTRACT

Access to strongly preferred resources improves animals' welfare. Ethologists often assess strengths of preference by making resource-access costly, and observing animals' responses as costs increase (*e.g.* identifying the cost reached before animals cease to pay). Such costs typically comprise operant tasks (wherein the number of responses required is progressively increased), or aversive barriers such as variably weighted doors. However, operant training can be time-consuming, while pushing weighted doors can reflect non-motivational confounds like strength (and be prone to ceiling effects beyond which animals simply cannot move the weight). Here, for mice, we therefore validate a new technique for imposing costs that avoids these issues: crossing a mildly electrified grid. We used enriched cages to motivate 108 trio-housed C57BL/6, BALB/c and DBA/2 females to cross grids set at steadily increasing currents (0–0.6 mA, increased by 0.02mA–0.04 mA every 48 h). Both starting cages and enriched cages contained food, water, bedding and nesting material, so that mice never had to experience the current unless they chose to, and 98/108 mice traversed the grid from the outset. The hypothesis that stronger currents represent greater obstacles made several testable predictions that were all supported by our data. First, mice steadily ceased crossing as currents increased (only 16/98 still crossing at 0.6 mA, the maximum imposed). Second, videos of 13 cages showed that if mice did cross, they did so with longer latencies at higher currents ($F_{1,329} = 61.7$, $p < 0.0001$). Third, data from six focal DBAs in this subset revealed fewer crossings per 48 h as currents increased ($F_{1,47} = 16.0$, $p = 0.0001$), accompanied by correspondingly longer visits to the enriched cage ($F_{1,87} = 143.3$, $p < 0.0001$). Next, such effects were stable within individuals (*e.g.* the last and penultimate latencies to cross co-varied: $F_{1,329} = 61.7$, $p < 0.0001$), and different measures of motivation inter-correlated in a consistent manner (thus as crossings declined, median visit durations increased [$F_{1,89} = 66.9$, $p < 0.0001$] and latencies to cross tended to increase [$F_{1,89} = 1.78$, $p = 0.095$]; while median visit durations and latencies to cross also positively covaried [$F_{1,76} = 3.2$, $p = 0.04$]). Finally, the Maximum Prices Paid (MPPs) also differentiated between strains as expected from differences in nociception (BALB/cs and DBA/2s being more sensitive to shock than C57BL/6s; respective median MPPs of 0.1, 0.12, and 0.44 mA). Thus mice discriminate between varying intensities of electric current in a floor-grid, treating increasing currents as more aversive in an internally-consistent, graded manner. Mildly electrified grids can therefore be valid, useful tools for imposing access costs and thence measuring strengths of preference in laboratory mice.

## 1. Introduction

One of the main ways to assess animal motivation is by titrating it against a cost. For example, researchers may impose costs on access to a resource that are gradually increased over time, and then record effects such as point at which each subject becomes unwilling to pay the price (termed the 'breakpoint'). Assessing the Maximum Price Paid (henceforth MPP) – the peak price paid before the breakpoint – has long been used to address fundamental ethological questions (for instance about endocrine effects on female rats' motivations to reach potential mates; *e.g.* McDonald and Meyerson, 1973), as well as in research using laboratory animals to model disorders like addiction (to evaluate factors affecting how hard addicted animals will work to access drugs; *e.g.* Orio et al., 2010; Puhl et al., 2013; Stafford et al., 1998). Applied ethologists study motivation because it is important for animal welfare: allowing animals to perform highly motivated behaviours or to interact with highly-motivating resources is generally regarded as good for their well-being (*e.g.* Fraser and Nicol, 2011; Hughes and Duncan, 1988;

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http://dx.doi.org/10.1016/j.applanim.2017.09.013
Received 18 April 2017; Received in revised form 14 September 2017; Accepted 17 September 2017

Please cite this article as: Walker, M., Applied Animal Behaviour Science (2017), http://dx.doi.org/10.1016/j.applanim.2017.09.013

Kirkden and Pajor, 2006; Mason and Bateson, in press). The costs typically used by applied ethologists fall into two main categories: operant tasks and aversive barriers. In operant paradigms, animals are trained to perform a novel response such as lever or key pressing in order to gain access to a resource (*e.g.* Cooper and Mason, 2001). The number of responses needed to gain access is progressively increased until the animal stops responding, this MPP being used to infer how valuable a resource is (*e.g.* Bokkers et al., 2004; Hovland et al., 2006). Alternatively, barrier methods such as weighted doors (*e.g.* Cooper and Mason, 2000; Olsson and Keeling, 2002; Petherick and Rutter, 1990; Seaman et al., 2008; Tilly et al., 2010), traversable water baths (*e.g.* of varying depths (Sherwin and Nicol, 1995) or lengths (Sherwin and Nicol, 1996)), and narrow gaps (*e.g.* Bubier, 1996; Cooper and Appleby, 1996) are used to titrate resource acquisition against an aversive experience. Again, researchers progressively alter this obstacle so that it becomes increasingly aversive or costly, and again the last level successfully crossed, the MPP, is used to indicate the motivation of the animal to access the resource.

These different techniques for imposing a cost on resource access each have potential scientific and practical pros and cons. For example, relying on operant tasks requires not only time to train animals, but that the tasks must be sufficiently biologically relevant to ensure that failures to 'pay' are not just failures to learn (*e.g.* hens cannot learn to peck a key to gain access to litter, though they can learn this operant task for food; Dawkins and Beardsley, 1986). Furthermore, if researchers instead using aversive barriers, results can potentially be subject to confounds because influenced by non-motivational factors. For example, larger animals may be physically unable to fit through narrow gaps despite being still motivated to access the resource; weighted doors may likewise be prone to ceiling effects beyond which the animal simply cannot push them, and stronger animals are also more likely to be able to push heavier doors, quite independent of their motivations to access the resource. Finally, some barrier tasks are not sufficiently graded in how costly they are, decreasing their abilities to reveal small differences in motivation (cf. *e.g.* Sherwin and Nicol 1996's differentially long water barriers, which were treated by mice as costly if present, but not as more costly if they were long rather than short).

One possible technique not yet tried by applied ethologists could avoid these issues: using electric current to impose a cost on accessing resources. Electric current is a universal punishment (Braud et al., 1969; Miller et al., 1962; Seligman and Maier, 1967), and animals typically show a graded behavioural response, in terms of increasing avoidance behaviour, to increasing levels of current (Ramabadran and Bansinath, 1986). Furthermore, electrified grids have been successfully used in some early fundamental research into motivational processes (*e.g.* McDonald and Meyerson, 1973). We therefore hypothesized that imposing different degrees of electric current *via* mildly electrified grids could be validly used as a graded cost to accurately measure motivations (here, to access environmental enrichment) in laboratory mice. This hypothesis makes several testable predictions. First, if increasing electric currents are perceived as increasingly costly, mice should react to higher currents by crossing less frequently and staying with the resource for longer. These predictions are based on many findings that as a cost to access a resource increases in magnitude, the frequency with which subjects pay the cost decreases, resulting in fewer visits, but with subjects spending longer interacting with that resource (or ingesting more if it is consumable) at each visit. Such responses have been well-documented across diverse species, resources and cost-types (*e.g.* Collier et al., 2002, 1990; Collier and Hirsch, 1971; Collier and Johnson, 1990; Cooper and Mason, 2000; Seaman et al., 2008; Stafford et al., 1998). Second, if increasing currents are perceived as increasingly costly, mice should also show longer latencies to cross them. This prediction is based upon much evidence that the more aversive a stimulus is, the longer are animals' latencies to interact with it (*e.g.* rats' latencies to eat increase when food is laced with bitter quinine: (Thompson et al., 2016); and the latencies of mice to cross deep [more aversive; Cameron and Perdue, 2005] *vs.* shallow water [Sherwin and Nicol, 1995]). Third, known strain differences in electric shock nociception (*e.g.* Kazdoba et al., 2007) should predict strain differences in response to increasing current, with the more nociceptive strains (*e.g.* BALB/c and DBA/2) showing greater changes in behaviour, and lower MPPs as the current is increased, than less nociceptive strains (*e.g.* C57BL/6s). Finally, assuming that motivations to use enrichments vary between individuals in a stable manner (cf. *e.g.* Walker and Mason, 2011), and assuming that the different indices of cost perception should co-vary, then the behavioural effects of increasing current should be stable and consistent within individuals while varying between individuals, such that long latencies to cross to access enrichment at one current predict long latencies at another, and furthermore, longer latencies should co-occur with longer visits and fewer traverses, longer visits to the enrichment, and lower MPPs to access it should all inter-correlate.

Ethical note: We recognized the potential ethical concerns of using electric current (cf *e.g.* Sherin and Nicol, 1995), and took these very seriously. This experiment was designed to allow mice to cross an electric grid *entirely voluntarily*. They did so in order to access an enriched cage, but both the starting cage and the enriched cage contained food, water, and nesting material, meaning that the mice *never* had to cross the electric grid if they found it too aversive. We also selected our starting and maximum currents carefully (see Methods). For reference, our maximum current was set at 0.6 mA, while humans generally report currents < 3 mA as mildly sensational and currents > 3 mA as painful (Lee, 1971). This work was approved by the University of Guelph Animal Care Committee (protocol number 2430).

## 2. Methods

### 2.1. Animals and home cage housing

One hundred and eight unrelated weanling female mice were purchased from Charles River Laboratories (Quebec, Canada) and housed in mixed-strain trios at approximately 3 weeks of age: one BALB/c, DBA/2, and C57BL/6 per cage (as previously validated as statistically powerful, humane and not influential on strain-typical phenotype; Walker et al., 2016). These mice were raised in 36 large rat cages (21H × 47L × 25Wcm; Allentown Inc., USA) that had corncob bedding (Lab Supply, USA), Shepherd Enviro-dri© nesting material (6–8 g; USA), a UDEL® polysulfone plastic mouse house shelter, and a home-made steel mesh elevated platform (5H × 40L × 4Wcm long, covered in duct tape) added to permit ready access to the water bottles. Thirteen cages contained a working stainless steel mesh 5" upright wheel (Ware Manufacturing Inc., USA); 12 cages, a working plastic mouse igloo & 'fast-trac' wheel combo (Bio Serv®, USA); and 11 cages, a 'locked' wheel that could be climbed on but not used for running. Furthermore, eight of the cages received additional enrichment items: a small paper cup (Dixie®, USA), a Nestlet™ (Ancare, USA), tissues (Kleenex®, USA), a cloth hammock (a roughly 12 × 12 cm piece of a sock attached to the cage lid *via* cable ties), occasional Cheerios® (General Mills, USA), and an autoclaved pinecone (approximately 7–10 cm tall). Table 1 details how many cages were configured with each set of items. These diverse enrichments were part of another experiment (Walker, 2016): we were not testing hypotheses about the animals' relative motivations to access specific items. All mice were specific pathogen free and given *ad libitum* food (Harlan® Teklad Global Diet [14% protein]) and water. The room

**Table 1**
Number of cages broken down by the type of running wheel in the cage and whether or not the additional listed environmental enrichment (EE) items were provided.

| | Plastic Wheel | Metal Wheel | Locked Wheel |
|---|---|---|---|
| Additional EE Present | 3 | 3 | 3 |
| Additional EE Absent | 9 | 10 | 9 |