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Electric shock for aversion training of jumping spiders: Towards an arachnid model of avoidance learning

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ABSTRACT

Electric shock is used widely as an aversive stimulus in conditioning experiments, yet little attention has been given to its physiological effects and their consequences for bioassays. In the present study, we provide a detailed characterization of how electric shock affects the mobility and behaviour of *Servaea incana*, a jumping spider. We begin with four mobility assays and then narrow our focus to a single effective assay with which we assess performance and behaviour. Based on our findings, we suggest a voltage range that may be employed as an aversive stimulus while minimizing decrements in physical performance and other aspects of behaviour. Additionally, we outline a novel method for constructing electric shock platforms that overcome some of the constraints of traditional methods while being highly effective and easily modifiable to suit the study animal and experimental context. Finally, as a demonstration of the viability of our aversive stimulus in a passive avoidance conditioning task, we successfully train spiders to associate a dark compartment with electric shock. Future research using electric shock as an aversive stimulus with terrestrial invertebrates such as spiders and insects may benefit from the flexible and reliable methods outlined in the present study.

1. Introduction

Electric shock is used extensively as an aversive stimulus in conditioning experiments, with species-specific avoidance responses documented in taxa as varied as insects (Vergoz et al., 2007), crustaceans (Abramson et al., 1988), fish (Wodinsky et al., 1960; Xu et al., 2007), rodents (Kimble, 1955; Iwata and LeDoux, 1988), and primates (Barrett, 1977), including humans (Glotzbach et al., 2012). As a conditioning stimulus, electric shock offers a number of advantages, including ease of use, immediacy of onset and offset, and a precisely controlled area of effect. There is a need for careful consideration of physiological effects induced by electric shock, and the consequences of such physiological effects for bioassays. It is difficult to isolate the behavioural changes that are contingent on the chosen learning or memory paradigm in the absence of an understanding of the changes to physical state that result from aversive stimuli (Pritchett, 1968). For example, a lack of mobility in a spatial maze owing to physical effects of an aversive stimulus could result in a poor performance score, as animals would be less likely to reach a target than their more mobile counterparts. Through awareness of such collateral effects of conditioning stimuli it is possible to ameliorate risks through precautions or controls.

Invertebrates have long been used to study the behavioural, cellular and molecular basis of cognition, but in recent years the focus has been on developing a few key model systems, such as honeybees and *Drosophila* in depth, rather than sampling widely across taxa (Sattelle and Buckingham, 2006; Wolf and Heberlein, 2003). While much can be learned from delving deep into the workings of select model animals, a broader perspective remains integral to the central tenet of comparative cognition, drawing on assessments of how taxonomically disparate groups perform in analogous physiological or behavioural tasks (Eisenstein, 1997; Shettleworth, 2010).

Spiders have been underrepresented in the comparative literature, yet they have much to offer. Spiders inhabit nearly all terrestrial environments and exhibit extraordinary diversity. For example, spiders vary from a solitary lifestyle to living in dense and cooperative social groups, with predatory behaviour ranging from sit-and-wait strategies in webs to active pursuit as cursorial hunters (Wise, 1993; Foelix, 2011). Jumping spiders (Araneae, Salticidae) are particularly well suited for studies of cognition, with exceptionally acute vision in their large forward-facing 'primary' eyes and complex, visually mediated behaviour (Jackson and Cross, 2011). Most jumping spiders are cursorial hunters that use their

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extraordinary visual abilities to mediate navigation, hunting and communication. It is known that they can learn in a variety of contexts (Nakamura and Yamashita, 2000; Skow, 2005; Jakob et al., 2007; Leidtke et al., 2014), solve problems through trial and error (Jackson et al., 2001), perform challenging navigational detours (Tarsitano and Jackson, 1994) and behave flexibly in novel situations (Jackson and Wilcox, 2010). Despite a growing literature detailing impressive cognitive feats in spiders, much of this work has been conducted using methods that do not readily support comparisons with other taxa. For spiders in general and salticids in particular to enter the comparative framework, there is a need to adapt and apply the well-established standard tools of the comparative method.

There is a need for well-characterized aversive stimuli for use in conditioning experiments. In the present study, we provide a detailed characterization how electric shock, one of the most commonly used aversive stimuli, affects the mobility and behaviour of *Servaea incana*, a common Australian salticid species. We begin with four mobility assays and then narrow our focus to a single effective assay with which we assess performance after both short and long-term shock exposure. We also assess behaviour of spiders in the long-term exposure group. Based on these results, we suggest a voltage range that may be employed as an aversive stimulus while minimizing decrements in physical performance and other aspects of behaviour.

The passive-avoidance paradigm is based on the animal learning to avoid an environment in which an aversive stimulus was previously delivered. The response that is punished is typically one that the animal normally performs. This means that the task is for the animal to learn to suppress a normal response when in a given context, and thus behave contrary to their innate preferences (Bammer, 1982). The passive avoidance paradigm is well established in insects (bees: Abramson, 1986; Agarwal and Guzman, 2011; cockroaches: Disterhoft et al., 1971; Disterhoft, 1972 and ants: Abramson, 1981), and has been used successfully to assess colour discrimination in spiders through colour-heat pairings (Nakamura and Yamashita, 2000). Here, we conduct an initial preference test to determine if spiders prefer the dark or light compartment of a two-sided arena, then train spiders to avoid their preferred compartment by pairing it with electric shock. Following a 20-min break we return spiders to the arena to see if avoidance behaviour persists in the absence of electric shock, and if spiders can retain at least short-term memory of this aversive event.

2. Methods and results

2.1. General Method

2.1.1. Animals

Equal numbers of adult male ($n = 95$) and female ($n = 95$) *S.incana* were collected from *Eucalyptus* trees in Sydney, Australia. Spiders were maintained in a controlled environment laboratory (24–26 °C; 65–75% relative humidity; 11:1:11:1 light:dusk:dark:dawn cycle) where they were individually housed in ventilated 1.125 L plastic cages (11 × 11 × 12 cm tall) containing a folded sheet of paper (2 × 3 cm) that was shaped as a 'tent' under which spiders could shelter and build nests. Spiders were fed weekly on an alternating diet of two house flies (*Musca domestica*) or two Queensland fruit flies (*Bactrocera tryoni*). All experiments were conducted 3–4 days following a feeding. Supplementary moisture was provided by lightly misting each cage with a spray bottle once each week. All experiments were conducted during daytime hours (8 am to 4 pm) under full-light (50:50 metal halide and halogen ceiling lights).

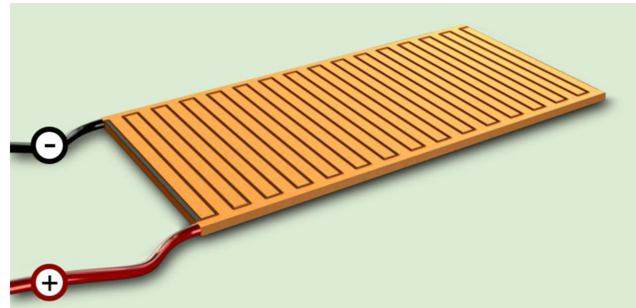

Fig. 1. Schematic of custom-designed electric shock platform, constructed as a printed circuit. Spiders receive a shock when a power source is activated and they bridge parallel opposite- charged bars of the platform.

Spiders were weighed to the nearest 0.1 mg (Shimadzu Model N595, electronic balance, Shimadzu Corporation, Kyoto, Japan) within 3 h following their final trial. In addition to weighing, each spider was photographed from above using a digital camera (ProgResC10) focused through a stereomicroscope (Olympus SZX12, Olympus Corporation, Tokyo, Japan). To minimize movement during photography, spiders were gently restrained on the flat surface of an inverted Petri dish under clear plastic film (Glad Products, Australia). Cephalothorax length and width were measured using the open source image-processing software ImageJ (v1.30, National Institutes of Health, Bethesda, MD, U.S.A.).

2.1.2. Shock chamber

Spiders were confined to a rectangular arena (75 mm wide × 150 mm long × 75 mm tall) constructed from white corrugated plastic board (Corflute®). The top of the arena was open to permit video recording, while the walls were lightly dusted with non-scented talcum powder to make the walls slippery and prevent spiders from escaping. The shock platform – on the floor of each enclosure – was a rectangular board (2 mm thick × 150 mm long × 75 mm wide) covered with a pattern of parallel copper bars alternately of positive and negative charge (Fig. 1). Previous studies have used adhesive copper tape (Skow, 2005), strips of aluminium (Bednarski et al., 2012), or wire (Agarwal et al., 2011) to achieve a similar design, but each has drawbacks owing to inconsistent voltage or susceptibility to damage. Here we adopt a novel technique that produces highly uniform voltage across the platform while eliminating risk of subjects failing to contact bars or becoming injured. To create a shock platform, a grid-like pattern (5 mm bars spaced 1 mm apart) was chemically etched onto the copper side of a blank printed circuit board backed with epoxy fibreglass laminate (Jaycar Electronics, Australia). The pattern was designed using Adobe Photoshop CS5.5 (Adobe Systems, San Jose, CA, U.S.A.) and printed onto toner transfer film (Press-n-Peel, Techniks Inc., New Jersey) using a standard laser printer (Hewlett-Packard 4250 LaserJet). The pattern was then transferred to a blank copper board using a hot iron, followed by chemical etching in a hot ammonium persulphate bath. Boards produced using this method are durable and can be cleaned with circuit board cleaner, alcohol and water without affecting their conductivity.

Alternating bars of the copper shock platform were wired to the positive and negative terminals of a 60 V (max 3amp) DC power supply (Sanke Electrical Co., Ltd., China) (see Fig. 1). When the power supply was active, shock was delivered to spiders each time they bridged the gap between parallel copper bars, completing the circuit. Due to the narrow gap between opposite-charged bars, failure to complete the circuit was very rare.