SHORT COMMUNICATION

Vibration as an effective stimulus for aversive conditioning in jumping spiders

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Abstract.   Previous work has shown that jumping spiders are able to associate visual cues with shock. We tested the efficacy of vibration as an aversive stimulus. *Phidippus audax* (Hentz 1845) (Salticidae) were first allowed to choose between two video stimuli, a cricket and an oval. We then tethered spiders so they were oriented toward their preferred stimulus with their tarsi touching a platform, either vibrated by a motor (experimental group) or with the motor turned off (control group). Spiders were then given a second opportunity to choose between the stimuli. Experimental spiders were significantly less likely to choose the stimulus that they viewed during training compared to control spiders. Spiders stalked and ate prey soon after experiencing the training procedure, suggesting that vibration caused no lasting harm. In addition, freely moving spiders avoided a vibrating platform, supporting the assertion that the vibration itself is aversive.

Keywords: Associative learning, training, Salticidae

Aversive conditioning can be an effective protocol for investigating animal cognition. In aversive conditioning, a negative stimulus (also called an unconditioned stimulus), such as shock, is paired with a neutral stimulus such as an image or tone. After training, animals avoid the previously neutral stimulus (now called the conditioned stimulus). While vibration has been used in studies of invertebrate learning, it is more often used as a conditioned stimulus rather than an unconditioned stimulus. For example, antlion larvae (*Myrmeleon crudelis*) learned to associate a vibrational cue with the arrival of food (Guillette et al. 2009), earthworms (*Lumbricus terrestris*) learned that substrate vibration predicted the onset of a bright light (Ratner & Miller 1959; Watanabe et al. 2005), and honeybees (*Apis melifera*) learned that vibration predicted electric shock (Abramson 1986). Here we test vibration as an aversive, unconditioned stimulus for studies of spider learning.

We studied vibration for four reasons. First, animals often learn more efficiently about stimuli that have some biological relevance (Shettleworth 2010). Spiders sense air- and substrate-borne vibration using, respectively, trichobothria and slit sensilla in the tarsal cuticle (Foelix 2011). Spiders use vibration in mating displays (reviewed in Sivalinghem et al. 2010) and to detect the presence of predators and prey (reviewed in Foelix 2011). Second, vibration has been used effectively as an unconditioned stimulus in several studies of spider learning. In *Araneus diadematus* Clerck 1757 spiders learned to associate different frequencies with aversive or non-aversive prey (Bays 1962). Spiders also attend to vibration as part of a multimodal cue: vibration enhanced the ability of jumping spiders, *Habronattus dossenus* Griswold 1987, to learn a color discrimination task (VanderSal & Hebets 2007). Third, vibration can be consistently administered across animals and trials because it is easy for the experimenter to see when the spider is experiencing the stimulus. Finally, vibration chambers are inexpensive and easy to build. The apparatus described here required minimal assembly and cost under $40 USD.

We collected adult and penultimate *Phidippus audax* (Hentz 1845) from fields and structures in Hampshire County, Massachusetts, USA, during late summer and early fall of 2013 and spring of 2014. Spiders were housed in 18 × 13 × 10 cm high clear plastic cages with a green wooden dowel, a refuge tube and plastic foliage for enrichment (Carducci & Jakob 2000). Spiders were fed crickets

(*Acheta domesticus*) weekly and had constant access to water in cotton-stoppered test tubes. Spiders were starved for no less than four days before training and choice tests.

Our experimental design was to run initial choice tests to ascertain which of a pair of stimuli a naïve spider approached when given a choice, train the spider to associate vibration with its chosen stimulus, and finally give it a post-training choice test between the same two stimuli. The stimuli used were a silhouette of a cricket and a solid black oval (Fig. 1), which we knew from other work that *P. audax* could differentiate. We created stimuli in Adobe Illustrator CC and used ImageJ to adjust their sizes so that they were equal in area and approximately the same length and height. We turned the stimuli into videos with Adobe Flash for Macintosh, exported the videos as .mov files (30 frames per second), converted them to MPEG-4 for iPod in Apple iTunes, and presented them to the spiders on Apple iPods (generation 5; Apple Inc., Cupertino, CA). All trials were conducted in a room lit only by natural light filtered through a translucent blind; in pilot experiments, dim light improved spiders' attention to the videos.

Both pre- and post-training choice tests were run in a V-maze made of foam core (Fig. 2A). The end of each maze arm had a slot to accommodate an iPod. A hole cut through the bottom of the floor between the two arms allowed the spider to be inserted into the arena via a syringe. We coated the inside walls of the arena with Vaseline petroleum jelly (Unilever, Rotterdam, Netherlands) to keep spiders from escaping.

To begin a choice test, we placed a spider into an open-ended 30 ml syringe covered with opaque tape and plugged the syringe with a cotton ball wrapped in plastic wrap. We inserted the syringe into the bottom of the V-maze. We placed a V-shaped divider of clear acetate between the arms of the maze to prevent the spider from moving immediately into the maze upon release. After a 5-min rest, we removed the syringe plug and slowly depressed the plunger until it was flush with the floor of the maze. After the spider had clearly oriented to both stimuli (turning its body so that the anterior eyes were directed at the stimulus), the divider was removed and the spider was allowed to make a choice. A choice was defined as the spider walking 10 cm into an arm of the arena. If the spider did not orient to both stimuli at least once in 10 min before the divider was lifted or if it did not make a choice within 20 min after the divider
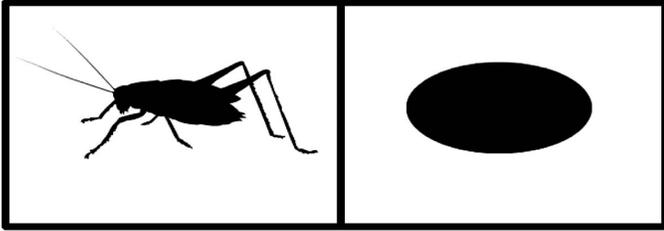
Figure 1.—The two stimuli used in the experiment.

was lifted, the trial was stopped and the spider was retested later that same day.

Spiders were trained in an alley constructed of foam core (15 cm × 9.5 cm × 11 cm high) (Fig. 2C, D). At one end of the alley, a slot held an iPod for playback of the training video. The distance between the spider and the iPod in the training arena was the same as the distance between the insertion point and the iPod in the choice testing arena. The opposite end of the alley had a 6.5 × 3.4 cm foam-core platform, separate from the floor of the arena and glued to the top of a 196 Hz 3V motor (Cermag Motor, Aristo-Craft, USA). An Arduino Uno (Smart Projects, Italy) was programmed to turn on the motor at specific intervals. We measured the vibration of the platform with an ADXL 335 3-axis accelerometer (Analog Devices, Norwood, MA, USA) driven by an Arduino Uno board. Raw data from the accelerometer was captured using CoolTerm (freeware.the-meier-s.org) at a sampling rate of 500 Hz and analyzed using the Data Analysis Tool Box in Excel (Microsoft Coporation, Redmond, WA, USA) and the Sinusoidal Motion Calculator (Vibration Calculator) from Advanced Mechanical Engineering Solutions (www.amesweb.info). The frequency spectrum for the motor showed a dominant peak at 196 Hz and a smaller peak at 190 Hz. The platform moved in all three axes of motion, with a peak displacement of 4.37 mm, 7.15 mm and 4.13 mm and a peak velocity of 68.67 mm/s, 112.38 mm/s and 64.87 mm/s along the x, y and z axes respectively.

For training with vibration, we tethered spiders in order to keep them oriented to a video of their preferred stimulus at a standard distance from the screen. We tethered each spider by waxing the tip of a microbrush (EasyinSmile Dental, Staten Island, NY) to the spider's cephalothorax as a "hat" (Fig. 2B). To attach the microbrush, we placed the spider in a 30 ml syringe with its tip cut off and a foam-padded plunger. We stretched a piece of Parafilm over the syringe opening and raised the plunger until the spider's cephalothorax and abdomen were firmly pressed against the Parafilm. We made a hole in the Parafilm to expose the cephalothorax while leaving the legs and abdomen immobilized. Using a heated wax carving tool typically used for the preparation of dental implants (GadgetWorkz, Orange County, CA), we melted several ml of a 1:1 beeswax/rosin mixture and dipped the microbrush into it. We then used a fine heated wax-carving tip to melt the mixture and attach the brush to the spider's cephalothorax while avoiding its eyes. We affixed hats at least 1 h before training.

During training, a spider was suspended by the end of its hat from an alligator clip attached to an adjustable XY microscope stage, which allowed us to easily orient the spider to the video. The spider was lowered onto the vibration platform until all of its tarsi made contact. We ensured that the cephalothorax and abdomen were clear of the platform because in pilot studies we had found that vibrating these disoriented the spider.

After the tethered spider was oriented to the video screen, it was given a three-minute rest with a blank screen followed by 10 training bouts (modified from Skow 2007). In each bout, the preferred stimulus appeared on the video screen and the spider was given 5 seconds of vibration every 10 sec for 30 sec. Each training bout was followed by a one-minute break during which the iPod displayed a
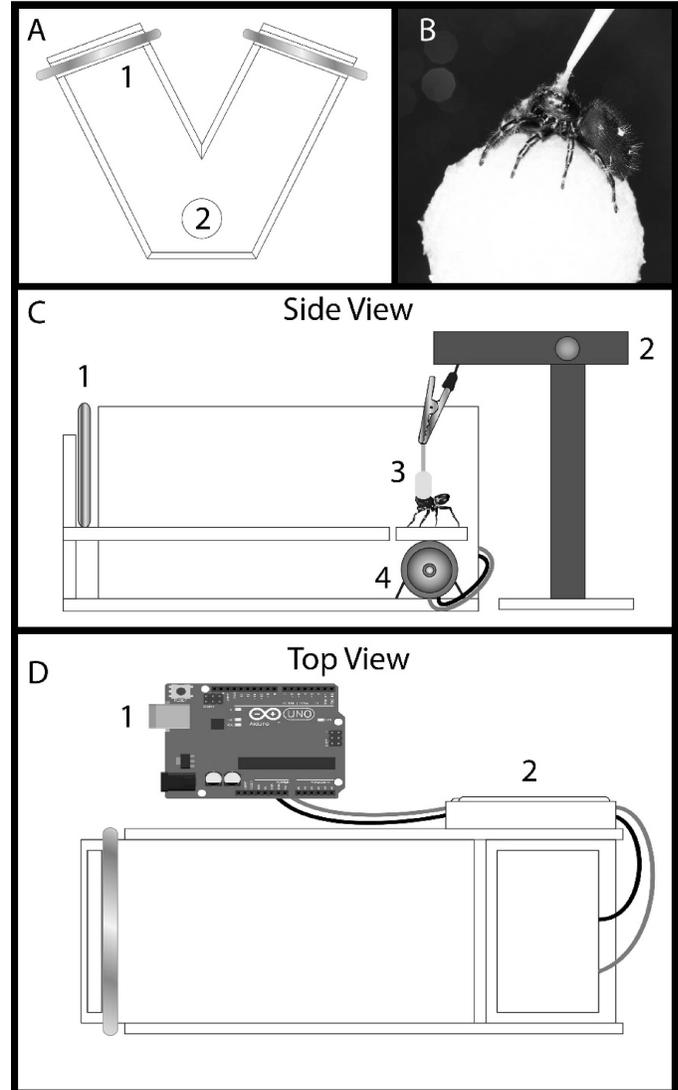

Figure 2.—A. Top view of the choice test arena. An iPod (1) was placed at the end of each arm, 12 cm from the center of the arena. A hole (2) was cut into the center of the arena to allow spiders to be introduced via a syringe with the end cut off. B. A spider tethered by a hat. C. Side view of the training arena. An iPod (1) was placed in an open slot at the far end of the arena opposite the spider. The spider was suspended over the vibration platform using a modified X/Y microscope stage (2) by a waxed hat (3). The vibration platform was glued to a small, 196 Hz motor (4), which provided the vibration. D. Top view of the training arena showing the placement of the Arduino Uno (1) and the battery case (2).

black screen. After the tenth training bout, we used forceps to gently pry off the spider's hat and repeated the choice test with the two stimuli randomly assigned to different arms of the choice arena. Control spiders underwent the same procedure except that the motor was disconnected from the vibration platform during training. If a spider did not make a choice after 20 min, the trial was ended and the spider was retrained and tested at least 24 h later. The vibration platform, syringe, plug and arena surfaces were cleaned with alcohol between trials.

In a separate test, we confirmed that spiders treated vibration as aversive by testing them in an arena (8.2 cm × 5.2 cm × 11 cm high) with half its floor comprised of the vibrating platform used in training

and the other half comprised of a stationary platform. The sides of the arena were covered in a thin layer of Vaseline to prevent escape. We tested two groups of spiders. For the vibration group ($n = 15$), a spider was placed in the arena, with the motor on the vibrating side turned off, and allowed to explore freely. After the spider had explored both sides of the arena floor and returned to the vibrating platform, we turned on the motor. We then recorded the amount of time the spider spent on the vibrating platform during a two min interval. The control group ($n = 15$) was treated identically but the vibrating platform was not turned on. We compared the amount of time each group spent on the vibrating platform with a Mann-Whitney U test.

While we saw no evidence during the learning experiment that spiders behaved abnormally after training, we also tested whether spiders stalked and fed on prey after being exposed to vibration. We followed the same hatting and training procedure described above except that we omitted the initial choice trial and showed the cricket video to all test spiders during training. After training, we removed the hats from the spiders, returned them to their cages, and allowed them to rest for 15 minutes. A single live cricket was then placed in each cage. We recorded the spiders' latency to attack and eat the prey.

Training with vibration was effective. Spiders appeared to be disturbed by the vibration of the platform and moved their legs rapidly in response (see Video 1, online at http://www.bioone.org/doi/suppl/10.1636/S14-49). Spiders exposed to vibration were significantly more likely than control spiders to choose the stimulus they did not see during training (Fisher exact probability test, $P < 0.04$). After vibration training, 7 of 30 spiders chose the stimulus that they saw during the training procedure, in contrast to 16 of 30 control spiders. In a separate test, freely moving spiders spent less time on a vibrating platform than did a control group where the vibration was turned off (Mann-Whitney U Test, $z = -3.69$, $P < 0.0002$). However, vibration did not appear to cause long-lasting harm, as all 15 spiders presented with live crickets after vibration training captured and ate the crickets within 15 min, and 13 of these did so within two minutes.

Two additional explanations for the training data should be considered. First, we cannot exclude the possibility that the tethering procedure itself was aversive and could cause spiders to avoid the stimulus they viewed during training; in fact, we view this as likely. However, the addition of vibration did significantly affect post-training decisions. Second, it is possible that vibration itself is not aversive to spiders, but that tethering with the wax hat is the aversive stimulus and that vibration only serves to increase the spiders' attention to it, similar to the role of vibration in VanderSal & Hebets (2007). However, freely moving spiders avoided a vibrating platform, supporting the hypothesis that vibration in itself is aversive.

Training with a 196 Hz vibration did not appear to cause long-lasting harm. However, we did not test other speeds. Our artificial vibration was in the range of seismic signals produced by other jumping spiders. For example, *Habronattus dossenus* has a mating display with three seismic components with fundamental frequencies of 5.7–65 Hz and peak frequencies of 260–1203 Hz (Elias 2003). It is possible that faster vibrations would be harmful.

Our experiment demonstrates that vibration provides an alternative to another proven aversive stimulus, shock (e.g., Skow 2007; Bednarski et al. 2012). Shock has several drawbacks that vibration does not share. It is important to consistently apply the negative stimulus in order to ensure that all animals have the same opportunity for learning, but shock can be inconsistent. In a typical operant chamber, the animal receives an electric shock when it completes an open electrical circuit by touching two adjacent metal strips, rods, or parts of a grid. Larger animals such as rats or mice are in constant contact with both parts of the circuit, but we have noticed that spiders can learn to position their legs between even close strips and thus avoid shock. In addition, the amount of shock a spider receives varies depending on which part of its body completes the circuit. The amperage of the current passing through the animal depends on the electrical resistance of the animal's tissues. The resistance of chitin, which forms the spider's exoskeleton, increases with its thickness (Rao & Mehrotra 1997). Thus, the amount of shock a spider receives depends on whether the circuit is completed by the thick chitin of tarsal claws or by the thin chitin of the abdomen. In pilot data, we measured the current the spiders received in a shock chamber and found that it varied as they moved around. In contrast, vibration is visible so it easy to confirm that spiders are all receiving the same training.

Given the increasing interest in spider learning and cognition (reviewed in Jakob et al. 2011), vibration as a negative stimulus should be a useful addition to our tool kits. This training procedure could easily be modified in order to accommodate other experimental designs. While our trial used a tethered spider suspended over the vibration platform, the chamber could be modified to accommodate a free-running spider or could have a shuttlebox design.

LITERATURE CITED

Abramson, C.I. 1986. Aversive conditioning in honeybees (*Apis mellifera*). Journal of Comparative Psychology 100:108–116.

Bays, S.M. 1962. Study of training possibilities of *Araneus diadematus* Cl. Experientia 18:423.

Bednarski, J.V., P. Taylor & E.M. Jakob. 2012. Optical cues used in predation by jumping spiders, *Phidippus audax* (Araneae, Salticidae). Animal Behaviour 84:1221–1227.

Carducci, J.P. & E.M. Jakob. 2000. Rearing environment affects behaviour of jumping spiders. Animal Behaviour 59:39–46.

Elias, D.O. 2003. Seismic signals in a courting male jumping spider (Araneae: Salticidae). Journal of Experimental Biology 206:4029–4039.

Foelix, R.F. 2011. Biology of Spiders, 3rd edition. Oxford University Press, Oxford.

Guillette, L.M., K.L. Hollis & A. Markarian. 2009. Learning in a sedentary insect predator: Antlions (Neuroptera: Myrmeleontidae) anticipate a long wait. Behavioural Processes 80:224–232.

Jakob, E.M., S.M. Long & C.D. Skow. 2011. Plasticity, learning and cognition. Pp. 307–347. *In* Spider Behaviour: Flexibility and Versatility. (M.E. Herberstein, ed.). Cambridge University Press, Cambridge.

Rao, P.A. & K.N. Mehrotra. 1997. Influence of diflubenzuron on chitin and its consequential effect on electrical resistance of the cuticle of *Schistocerca gregaria* Forskal. Journal of Entomological Research 21:253–257.

Ratner, S.C. & K.R. Miller. 1959. Classical conditioning in earthworms, *Lumbricus terrestris*. Journal of Comparative and Physiological Psychology 52:102–105.

Shettleworth, S.J. 2010. Cognition, Evolution and Behavior, 2nd ed. Oxford University Press, Oxford.

Sivalinghem, S., M.M. Kasumovic, A.C. Mason, M.C.B. Andrade & D.O. Elias. 2010. Vibratory communication in the jumping spider *Phidippus clarus*: polyandry, male courtship signals, and mating success. Behavioral Ecology 21:1308–1314.

Skow, C.D. 2007. Jumping spiders and aposematic prey: the role of contextual cues during avoidance learning. Doctoral dissertation,

University of Massachusetts Amherst, Amherst. Online at http://scholarworks.umass.edu/dissertations/AAI3254952

VanderSal, N.D. & E.A. Hebets. 2007. Cross-modal effects on learning: a seismic stimulus improves color discrimination learning in a jumping spider. Journal of Experimental Biology 210:3689–3695.

Watanabe, H., T. Takaya, T. Shimoi, H. Ogawa, Y. Kitamura & K. Oka. 2005. Influence of mRNA and protein synthesis inhibitors on the long-term memory acquisition of classically conditioned earthworms. Neurobiology of Learning and Memory 83:151–157.