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Method Article

Low-cost conditioned place preference setup including video recording and analysis of behaviour

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A B S T R A C T

The conditioned place preference (CPP) paradigm is widely used in rodent research to test the rewarding and aversive properties of different stimuli. Despite its relative simplicity, commercially available CPP systems are often costly. Here we describe the construction of a CPP setup and a behavioral data analysis pipeline incorporating:

- a CPP box which can be built in a single day by using widely available and affordable materials.
- an open source computer system for data acquisition (based on Raspberry Pi).
- a freely available behavioural analysis software.

The behavioural analysis allows for measurement of both locomotor activity and time spent in a zone of interest. Including all components, our setup costs ~1/10 of the cost of the least expensive commercially available CPP boxes alone (not including data acquisition and analysis). We validated the setup by showing that a 4 mg/kg dose of amphetamine increases locomotor activity acutely in adolescent male mice and induces conditioned preference for the drug-paired compartment in the CPP test.

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Specifications table

Subject Area:	Psychology
More specific subject area:	Rodent models of reinforcement
Method name:	Conditioned place preference
Name and reference of original method:	*n/a*
Resource availability:	1. https://github.com/local-vision/Pi-Vision 2. https://www.realvnc.com/en/connect/download/viewer/ 3. https://sourceforge.net/projects/mp4video1click/ 4. https://sourceforge.net/projects/toxtrac/

Method

The conditioned place preference (CPP) paradigm is a widely used tool for assessing the reinforcing or aversive properties of drugs and other stimuli. In this paradigm an experimental animal is exposed repeatedly to a stimulus in a given context until a stimulus-context association is established through classical conditioning. As a result of the conditioning, if the stimulus in question has reinforcing (or aversive) properties, the stimulus-paired environment is preferred (or avoided, respectively) relative to a control environment and/or a baseline measurement of preference before conditioning [1,3,8,9].

The CPP apparatus consists of at least two compartments with distinct contextual cues and a recording system, which can track the amount of time an experimental animal spends in each compartment. Despite their relative simplicity, commercially available CPP setups are often costly and beyond the reach of low-budget projects. Moreover, data gathering and analysis systems used to track animals (e.g. video recording and analysis) may entail further expenses. Here we present a low-cost CPP setup incorporating a CPP box, an open-source video recording system, and freely available animal tracking software (grand total: ~300CAD/box). The principal components of our CPP setup are represented in Fig. 1.

First, using polycarbonate cement and sheets, we constructed a three-compartment CPP box. The dimensions of the box and the sheets used to construct it are provided in the supplementary

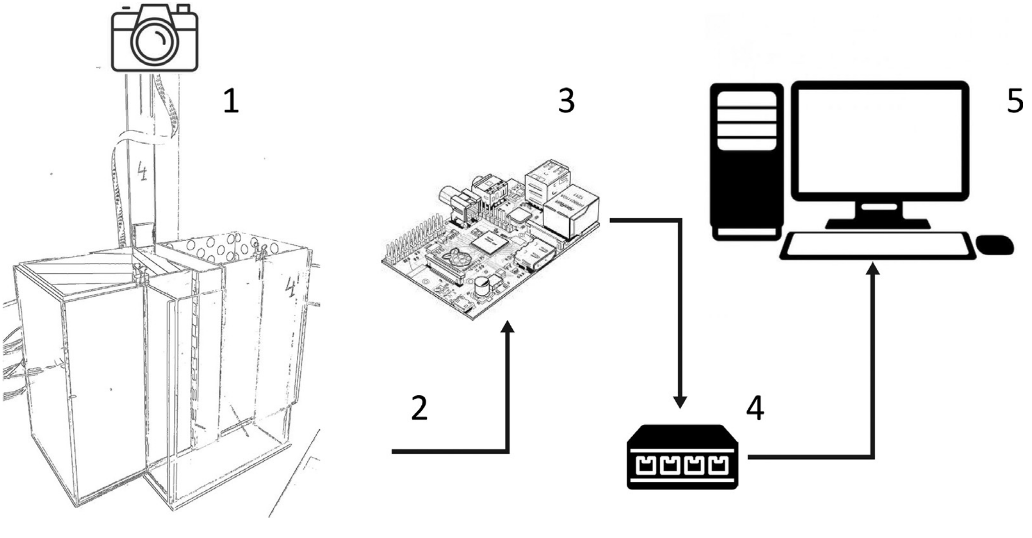

Fig. 1. Schematic representation of the experimental setup. A Raspberry Pi camera positioned above the CPP box (1) is connected, via flat cable (2), to a Raspberry Pi (3) which is in turn connected to an ethernet switch (4) via an ethernet cable. The switch connects to a desktop PC (5) via ethernet cable as well. The PC controls the Raspberry Pi(s) through freely available virtual network computing (VNC) software. The type of switch and PC determine how many boxes can be run simultaneously.

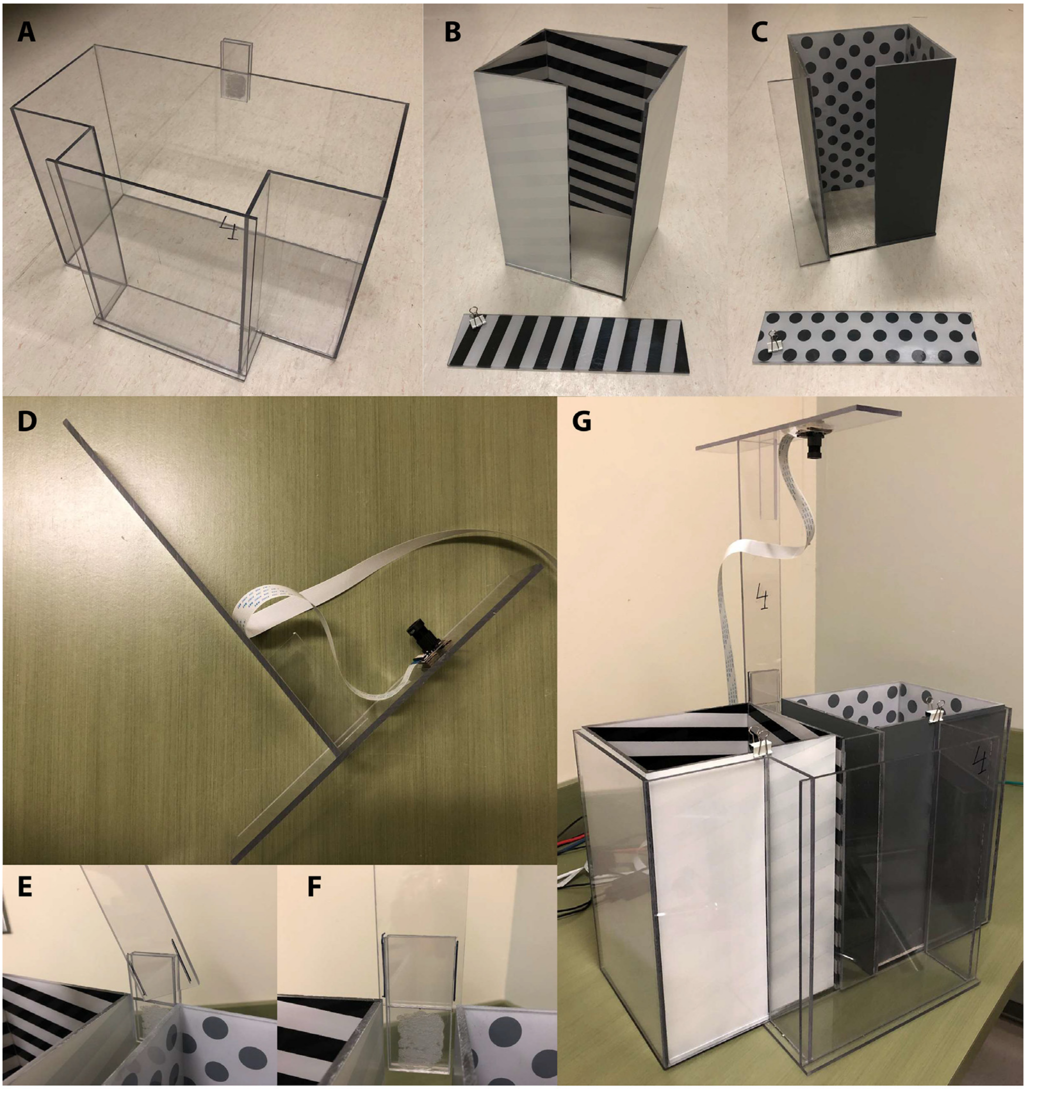

Fig. 2. Illustrative images of the CPP box. The main body of the box (A) consists of a single large compartment which accommodates two smaller compartments with distinct contextual cues and removable doors (B,C). A removable arm is holding the camera for recording of behaviour (D). The arm slots in between two polycarbonate pieces attached to the back of the main body (E,F). During conditioning, the patterned compartments are closed off (G), but remain opened during the CPP test phase.

materials. It consists of one large casing (Fig. 2A) which holds two smaller chambers with distinct contextual cues for conditioning. During pairing sessions, the two chambers can be closed off using barriers secured by small binder clips (Fig. 2, B & C). The outer casing is shaped so that it forms a connection between the conditioning chambers, effectively creating another compartment (Fig. 2G). This third compartment is used as a starting point during the unforced choice CPP test [4]. Finally, a removable polycarbonate arm supporting a camera (dimensions given in supplementary materials) is positioned above the box for recording of animal behaviour (Fig. 2 D, E & F). We used grey, black, and white spray paint to make the patterns on the outside of the conditioning chambers (masking tape and circular price tags were used as stencils). The floor of each conditioning chamber was covered with either the embossed or smooth side of a beige mat to provide distinct tactile cues and a suitable background for animal tracking. Each box can be constructed in one day by a single person.

We used a wide-angle Raspberry Pi camera for data collection. The Raspberry Pi (RPi) is an open-source, small-scale personal computer running a Linux-based operational system. It controls the camera via a freely available software (Pi-Vision, Suppl. [1]) and stores the recorded videos on an SD card in a .h264 file format. We equipped each box with its own camera and RPi, the latter equipped with a protective casing and powered by micro-USB connected to a wall socket adapter. Each RPi was connected to an ethernet switch which in turn connected to a main desktop computer (PC) controlling all RPis (Fig. 1). We used freely available virtual network computing (VNC) software to access RPis remotely from the main PC (RealVNC®, Suppl. [2]). Every time the RPis are plugged into a power socket, they can be accessed and controlled via a remote desktop created by the VNC. The VNC allows for file transfer via ethernet so that the videos initially stored on the RPis after recording can be easily transferred to the main PC for conversion and analysis. We used a freely available software for conversion to .mp4 file format (Mp4 Video 1 Click, Suppl. [3]) which is required by the video analysis software. Our video setup allowed for recording at up to 1080p, but we limited ourselves to lower resolutions to save disk space and to speed up data analysis (Suppl. videos 1&2).

Animal tracking was done using ToxTrac, also a freely available software [6,7]. ToxTrac has a user-friendly interface and provides an in-depth behavioural analysis including time spent in zones of interest, movement speed and distance travelled, as well as detailed description of animal positioning. More extensive description of the software is available at the developer's website (Suppl. [4]). Exemplary images of the ToxTrac analysis are presented in Fig. 3A. Fig. 3B shows a random frame form a recording of a CPP test session.

Validation

We validated our setup using a biased CPP design. Adolescent male mice (PD 21 at start of experiment, $n = 16$) were first exposed to the CPP boxes, in drug-free conditions, for 20 min and given the opportunity to explore all three chambers freely. We recorded these sessions to establish if mice had a baseline preference for one of the conditioning chambers. Half of the mice ($n = 8$) were then assigned to an experimental group and treated with 4 mg/kg amphetamine in the morning and saline in the afternoon, every other day, for a total of 10 days (5 injections of amphetamine and saline). We chose an amphetamine dose and treatment regimen which we have shown to produce changes in the development of the mesocorticolimbic dopamine system [2,5]. For the experimental group amphetamine injections were paired with the context which was preferred less at baseline. The other half of the mice were assigned to a control group ($n = 8$) and given two injections of saline per day, one in each of the two sides of the CPP chamber. Twenty-four hours after the last injection, all mice were once again given the opportunity to explore all three chambers of the CPP box freely, in drug-free conditions. We recorded mice during treatment sessions to measure locomotor behaviour and also during the CPP test session to assess chamber preference.

As expected, during drug-pairing sessions, amphetamine-treated mice had increased locomotor behaviour relative to saline controls, main effect of substance: $F(1, 14) = 154.8$, $p < .001$ (Fig. 3C). Locomotion scores increased across days, $F(4, 56) = 11.80$, $p < .001$, but this effect was observed only in the amphetamine group: saline-treated animals had similar locomotion scores over the 5 pairing sessions, day x treatment interaction $F(4, 56) = 12.36$, $p < .001$. Finally, amphetamine-treated animals showed a significant preference for the context paired with the drug injections when contrasted against their saline counterparts, $t(14) = 3.08$, $p = .008$. We defined preference as an increase in time spent in the drug-paired chamber from pre- to post-conditioning.

We have described the construction of a low-cost CPP apparatus, and the use of an open source recording system with freely available analysis software which amounts to a complete pipeline for CPP behavioural data gathering and analysis. We hope that this method will provide an affordable alternative to more costly commercially available products, with comparable functionality (Table 1).

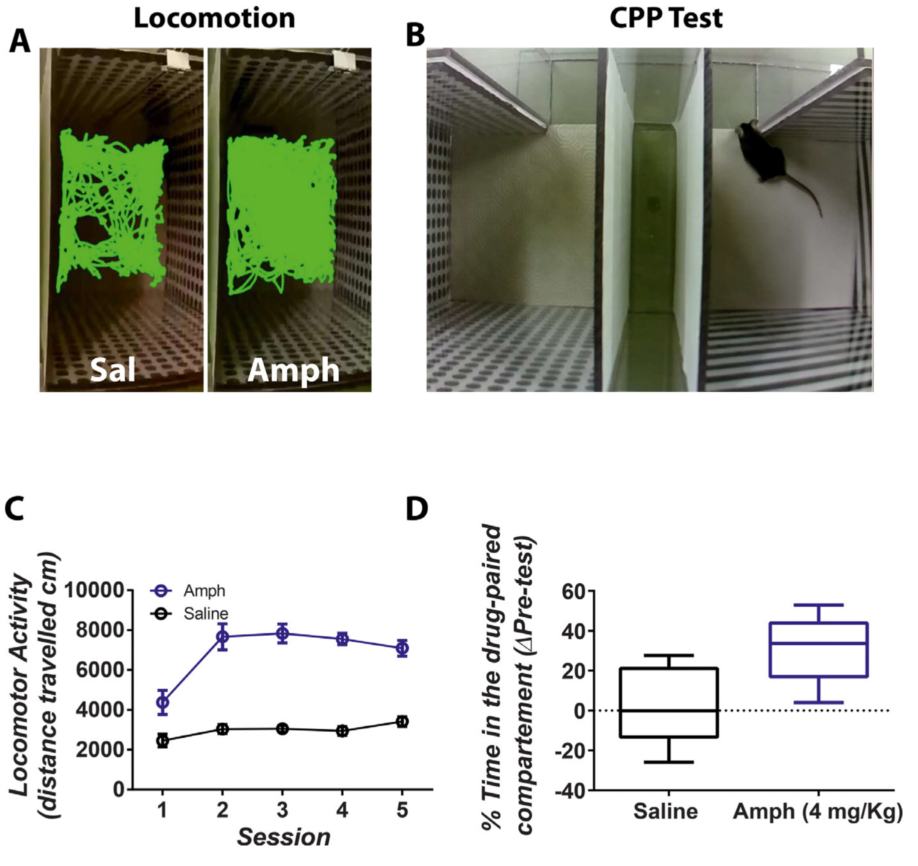

Fig. 3. Locomotion and preference for the drug-paired compartment following amphetamine or saline treatment. **A.** Example images of animal tracing during saline or drug pairings with a compartment of the CPP box. **B.** A random video frame from a recording during the CPP test phase. **C.** Locomotion during pairing sessions. **D.** Preference score for drug or saline-paired compartment expressed as a difference form pre-test preference.

Table 1
List of materials used and their source.

Item	Quantity	Source
Polycarbonate sheets	n/a	Johnstonplastics.com
Polycarbonate glue	1 tube	Amazon.com
Raspberry Pi 3.0	1/box	Amazon.com
Raspberry Pi case	1/box	Amazon.com
Raspberry Pi power cable	1/box	Amazon.com
Ethernet switch	1 total	Amazon.com
Raspberry Pi camera	1/box	Amazon.com
Flat cable	1/box	Amazon.com
Ethernet cable(s)	1/box (+ 1)	Amazon.com
Spray paint	n/a	Local art supplies shop
Binder clips	2/box	Amazon.com
Screws	4/box (optional)	Local hardware store
Tape	n/a	Amazon.com
Mats	n/a	Local dollar store

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Declaration of Competing Interests

The Authors confirm that there are no conflicts of interest.

Supplementary materials

1. https://github.com/local-vision/Pi-Vision
2. https://www.realvnc.com/en/connect/download/viewer/
3. https://sourceforge.net/projects/mp4video1click/
4. https://sourceforge.net/projects/toxtrac/

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2020.100899.

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