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Watching from a distance: A robotically controlled laser and real-time subject tracking software for the study of conditioned predator/prey-like interactions

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HIGHLIGHTS

- Robotic laser beam device to explore the distance-behavior relationship between predator and prey.
- Software for simultaneously controlling and detecting the laser beam and rodent.
- Worked calculations of the dynamic distance relationship between predator and prey.
- Rats will learn to case or avoid the laser beam depending on the reward-punishment contingency.
- Technical schematics and an overview of the software are presented.

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ABSTRACT

Background: The physical distance between predator and prey is a primary determinant of behavior, yet few paradigms exist to study this reliably in rodents.
New method: The utility of a robotically controlled laser for use in a predator–prey-like (PPL) paradigm was explored for use in rats. This involved the construction of a robotic two-dimensional gimbal to dynamically position a laser beam in a behavioral test chamber. Custom software was used to control the trajectory and final laser position in response to user input on a console. The software also detected the location of the laser beam and the rodent continuously so that the dynamics of the distance between them could be analyzed. When the animal or laser beam came within a fixed distance the animal would either be rewarded with electrical brain stimulation or shocked subcutaneously.
Results: Animals that received rewarding electrical brain stimulation could learn to chase the laser beam, while animals that received aversive subcutaneous shock learned to actively avoid the laser beam in the PPL paradigm. Mathematical computations are presented which describe the dynamic interaction of the laser and rodent.
Comparison with existing methods: The robotic laser offers a neutral stimulus to train rodents in an open field and is the first device to be versatile enough to assess distance between predator and prey in real time.
Conclusions: With ongoing behavioral testing this tool will permit the neurobiological investigation of predator/prey-like relationships in rodents, and may have future implications for prosthetic limb development through brain–machine interfaces.

1. Introduction

This paper examines the use of a platform for studying complex spatial components of behavioral responses in rats to dynamic stimuli associated with reward or punishment. Simply manipulating the distance between predator and prey may be sufficient to evoke very complex behavior (McNaughton and Corr, 2004;

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Blanchard et al., 1990, 2011; Griebel et al., 1996, 1995). Indeed, predator–prey interactions have not been a major focus of neuroscience, in part because of this complexity; yet the brain's ability to deal with these situations is requisite for an organism's survival.

Technical attempts have been used to model predatory–prey interactions in both humans and animals. For example, Mobbs et al. (2007) used a computer-based predator–prey game, whereby a human subject had to navigate an object through a two-dimensional maze while avoiding a predator object. If the subject were caught by the predator, he would receive a cutaneous electrical shock. A similar strategy has been attempted in which rodents directly interact with robots. Choi and Kim (2010) used a robot ("robogator") which would surge toward a rat that was foraging for food. Other investigators have used cutaneous shocks to train rats to avoid a robot (Telensky et al., 2011) or other rats (Telensky et al., 2009). Rat predatory behavior is less well described. Rats have been found to commit muricide (mouse killing) (Hsuchou et al., 2002; Karli, 1956) and have been used as a predator to study mouse defensive behaviors (Griebel et al., 1996, 1995). In addition rats also predate upon insects (e.g. cockroaches) but only few studies have been devoted to this behavior (Comoli et al., 2003; Sukikara et al., 2006).

A biological predator (e.g. a cat) or prey (e.g. cockroach) has good ecological validity; however their behavioral variability and the complexity of predator–prey interaction make for difficult experimental control and advanced interpretation. The use of a physically realistic, robotically controlled predator or prey is not only challenging to build, but the degree of realism may also produce varying reactions in rodents. Hence, a testing system employing an easily-positioned abstract stimulus (e.g. a laser beam) has utility for characterizing how behavioral reactions depend on the distance and trajectory of a threat or reward relative to an animal.

We describe an easy to construct, robotically controlled laser beam that can chase or be chased by a rat. Software is utilized to detect whether or not the animal is within range of the laser beam, at which point the animal will be given either an aversive subcutaneous shock or rewarding medial forebrain bundle (MFB) stimulation. This approach appears to be ideal for the study of neural correlates involved in reward seeking (predation-like behavior) and neural correlates involved in fear and avoidance (prey-like behavior) where multiple acquisition trials are necessary to correlate neuron activity with behavior.

2. Materials and methods

2.1. Animals

Five Male Fischer–Brown Norway (FBN) rats (average weight = 393 ± 7.78 SEM, 6–8 months old) were bred in-house and used for reward conditioning while 5 male Long–Evans (LE) rat (average weight = 592 ± 36.04 SEM, 8–12 months old) were used for testing avoidance conditioning. Rats were housed in an animal colony on a regular light cycle (12/12 h) and behavioral tests were conducted during the animals' natural wake time. All procedures were conducted in accordance with the Canadian Council for Animal Care and approved by the University of Lethbridge Animal Welfare Committee.

2.2. Surgery

For predator–prey-like experiments involving avoidance conditioning, Long Evans rats were anesthetized with isoflurane (1.5–2% by volume in oxygen at a flow rate of 1.5 L/min). A midline incision was made in the skull, and the skin and periosteum were blunt dissected to expose the skull. Bilateral subcutaneous electrodes were placed at the dorsal aspect of the neck between the trapezius muscles and the skin, and then sewn in place with 4.0 silk. A small connector assembly (ASSEMBLY-8-NT, NeuroTek-IT Inc., ON, Canada M6H3J9) was connected to the electrodes and cemented to the rat's skull. Dental cement was packed around the connector for adherence to the skull.

For predator–prey-like experiments involving reward conditioning, FB rats was anesthetized and the skull was exposed as (as above) Two bipolar twisted, teflon-coated, stainless steel (Medwire, 316SS-3T, coated diameter 0.0045 in.) electrodes were targeted, through a craniotomy, to the right MFB (4.0AP, 1.5ML, 8.2DV and 2.5AP, 1.8ML, 8.5DV). Preliminary confirmation of electrode positions were conducted by passing small amounts of current (similar to reward currents below) into the MFB during surgery. It was observed that the respiratory rate would increase under these conditions, which appeared to have predictive value over the quality of reward training (data not presented).

Electrodes were connected to a head assembly (ASSEMBLY-8-NT, NeuroTek-IT) which was attached to the skull (as above). All animals were given seven days to recover before the behavioral procedure started.

2.3. Behavioral training

2.3.1. Avoidance conditioning

LE rats were connected to a cable via the head assembly attached to the skull, which was in turn connected through a torque sensing commutator (CMTR-18-NT, NeuroTek NeuroTek-IT Inc., ON, Canada M6H3J9) to a stimulus isolation unit for delivery of current to the subcutaneous electrodes. On the day of experiment, rats were placed in the center of a square chamber (2 × 2 × 2 ft). In some cases the chamber floor and walls were lined with black flocking paper (#40, Edmund Optics, Inc., Barrington, NJ 08007-1380, U.S.A) to reduce reflections of the laser beam. A small piece of colored tape or plastic was placed on the connector assembly to track the animal's position using an over-head webcam and software (described below). The walls of the chamber were dark blue or black to reduce reflections of the laser beam. After 5–15 min in the chamber, a low wattage (Sothiclights Electronics CO, 532mw green, 457558883) laser beam approached within 87 mm (1.7 mm/pixel × 50 pixel) (automatically determined with the software) of the rat at a speed of ∼251 mm/s. Baseline consisted of approach and retraction of the laser beam to within 50 pixel of the animal's position every ∼60 s for an hour without use of aversive shock stimulus. On the days to follow training included shock stimuli delivered subcutaneously, when the laser approached within 50 pixel, to associate the approach of the laser with aversive stimuli. The laser was retracted by 1–2 ft following stimulation. The animal was given an inter-trial interval of 60 s after which the laser would then be advanced toward the animal. If the animal learned to avoid the advancing laser beam, no shock was given. Approach of the laser beam occurred with an inter-trial interval of 30–90 s (Kamin et al., 1963; Stuchlik et al., 2004) for up to an hour. During the inter-trial interval, the laser beam was retracted to the center of the arena until the next trial. This training was repeated for four days after baseline.

2.3.2. Reward conditioning

FBN rats were first connected to a cable via the head assembly attached to the skull, which was in turn connected through a torque sensing commutator (CMTR-18-NT, NeuroTek-IT Inc., ON, Canada M6H3J9) to a stimulus isolation unit for delivery of current to the MFB electrodes. An elastic (Coban) belt was attached around the mid region of the rat. A red piece of plastic was connected to the belt so that the rats position could be detected using the webcam and software (described below). The rat was placed in the center of