



A novel apparatus/protocol designed for optogenetic manipulation and recording of individual neurons during a motivation and working memory task in the rodent

Samuel D Dolzani¹, Shinya Nakamura¹ & Donald C. Cooper^{1*}

Innovative molecular tools allow neuroscientists to study neural circuitry associated with specific behaviors. Consequently, behavioral methods must be developed to interface with these new molecular tools in order for neuroscientists to identify the causal elements underlying behavior and decision-making processes. Here we present an apparatus and protocol for a novel Go/No-Go behavioral paradigm to study the brain attention and motivation/reward circuitry in awake, head-restrained rodents. This experimental setup allows: (1) Painless and stable restraint of the head and body; (2) Rapid acquisition to simple or complex operant tasks; (3) Repeated electrophysiological single and multiple unit recordings during ongoing behavior; (4) Pharmacological and viral manipulation of various brain regions via targeted guide cannula, and; (5) Optogenetic cell-type specific activation and silencing with simultaneous electrophysiological recording. In addition to the experimental advantages, the head-restraint system is relatively inexpensive and training parameters can be easily modulated to the specifications of the experimenter. The system runs on custom LabView software. In summary, our novel apparatus and protocol allows researchers to study and manipulate components of behavior, such as motivation, impulsivity, and reward-related working memory during an ongoing operant behavioral task without interference from non task-related behaviors. For more information on the custom apparatus, software or to collaborate please visit www.neuro-cloud.net/nature-precedings/dolzani.

Here we describe a new rodent head-fixation apparatus and contextual Go/No-Go discrimination task that allows direct manipulation and recording of individual neuronal activity during ongoing behavior. The novel Go/No-Go task utilizes head-restrained, behaving rodents discriminating between two different frequencies of tones that correspond to the “Go” and “No-Go” components of the paradigm. The task begins with a presented auditory Go or No-Go cue that requires the rat to acknowledge with a lever press response. A delay period is inserted until a white noise (WN) cue is presented. During this WN cue the rat must respond with a lever press for the Go condition or omit responding for the No-Go condition. This head-fixed protocol is advantageous because it allows the rat to initiate only on trials where the discriminative initiation tone was attended to. This allows a more accurate assessment of correct and incorrect responses after the delay period, thus providing a more reliable measure of working memory. After learning has plateaued according to criteria (>75% correct responding) it is possible to then manipulate desired brain regions pharmacologically and/or optogenetically with simultaneous *in vivo* electrophysiological recording. By using a head-restrained anesthetic-free condition, one can rule out any pharmacological artifacts that may confound electrophysiological recordings in anesthetized animals. Depending on the task complexity training subjects to an appropriate criterion of >75% correct responding can take between days and several weeks. Once criterion is achieved, behavioral components, such as motivation, impulsivity, and behavioral inhibition can be measured and studied in the

context of specific brain regions that may be causally related involved in the behaviors.

RESULTS

The time needed for acquisition of each component of the behavioral task varied between subjects and therefore some subjects reached criteria and progressed through the training at a faster rate than others. 60-90 minute daily training sessions are

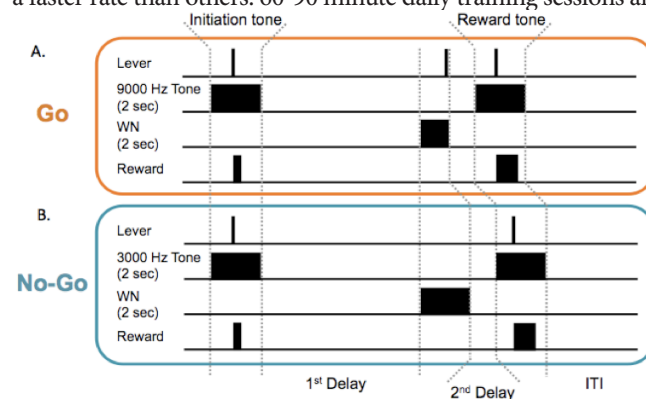


Fig 1. The subject is required to press the lever and receive a small sucrose reward during the initiation tone to start the trial. During the period of white noise (WN), pressing the lever (Go) or withholding from pressing the lever (No-Go) is required depending on the tone. The reward tone is delivered if the rat responds correctly during WN.

ideal for minimizing restraint stress while maintaining the ability to train the animal. Animals that achieved >1000 lever-presses/session demonstrated learning of the association between lever-pressing and reward-delivery and were allowed to proceed to the next step of training (See detailed methods at www.neuro-

1. Department of Psychology and Neuroscience, Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80303 USA

* Correspondence should be sent to D.Cooper@colorado.edu

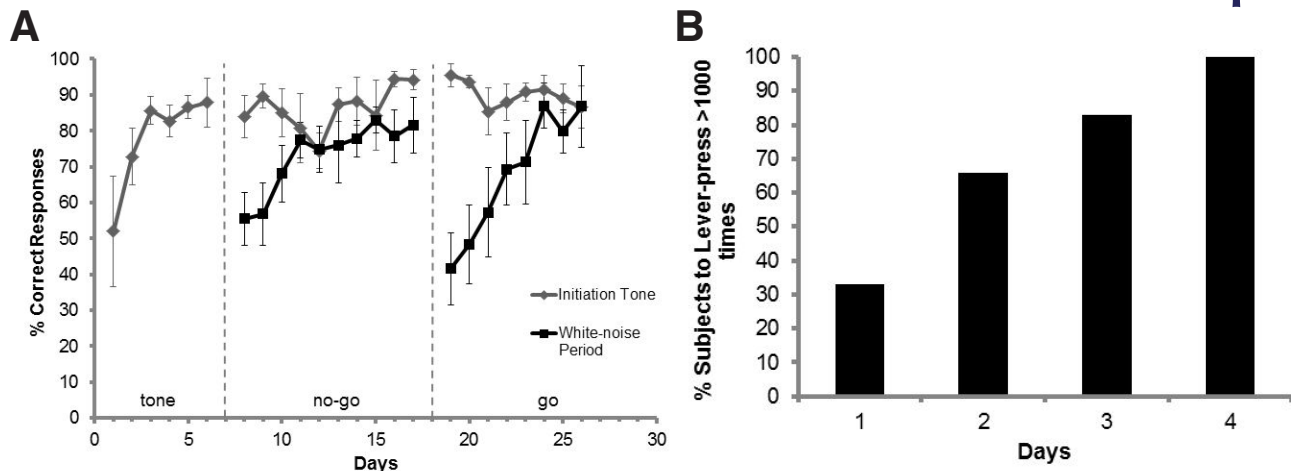


Fig 2. A. Six subjects completed the “lever” training condition in which sucrose delivery was contingent on lever-pressing. After successfully pressing the lever >1000 times/training session, rats proceeded to the next stage of training. **B.** Subjects learned the association between tone presentation and sucrose delivery, followed by the “No-Go” and “Go” constituents of the Go/No-go task (n=6).

cloud.net/nature-precedings/dolzani) (Fig. 2A). Subjects were then trained for 6-11 days to press the lever for sucrose in the presence of a 3kHz or 9kHz tone (Fig. 2B). Next, subjects were trained to perform the “No-Go” constituent of the task over the course of 5-18 days, until correct responding exceeded 75% for at least two consecutive days (Fig. 2B). Subjects then learned the “Go” component of training over the course of 4-19 days, depending on their accuracy (Fig. 2B).

DISCUSSION

The development of an operant tone discrimination task, such as the novel Go/No-Go contextual tone discrimination procedure presented, provides a behavioral model to closely examine the brain motivation/reward circuitry in order to understand decision making at the systems and cellular level. The preliminary data presented confirms validity of this construct. Our progressive training procedure ensures that subjects learn an operant response to achieve positive reinforcement, however the amount of time needed to train to criteria varies between subjects. Once subjects are trained to discriminate between a Go and No-Go tone the experimenter may choose to implement a random Go/No-Go sequence, whereby the animal must modify their behavior according to the type of trial that is presented. The random Go/No-Go task is useful for studying working memory/attention and the role of specific brain regions. Pharmacological, electrophysiological, and optogenetic techniques can be employed to facilitate these studies using this newly developed paradigm.

METHODS

All procedures were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee of the University of Colorado at Boulder. See www.neuro-cloud.net/nature-precedings/dolzani for detailed methods.

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AUTHOR CONTRIBUTIONS

SDD and SN performed the experiments. SN and DCC designed the experiments. SDD, SN and DCC wrote the manuscript.

PROGRESS AND COLLABORATIONS

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