Video Article

A Fully Automated Rodent Conditioning Protocol for Sensorimotor Integration and Cognitive Control Experiments

Ali Mohebi[1], Karim G. Oweiss[1,2,3]

[1]Electrical and Computer Engineering, Michigan State University

[2]Neuroscience Program, Michigan State University

[3]Cognitive Science Program, Michigan State University

Correspondence to: Karim G. Oweiss at koweiss@msu.edu

URL: http://www.jove.com/video/51128
DOI: doi:10.3791/51128

Keywords: Behavior, Issue 86, operant conditioning, cognitive function, sensorimotor integration, decision making, Neurophysiology

Date Published: 4/15/2014

Citation: Mohebi, A., Oweiss, K.G. A Fully Automated Rodent Conditioning Protocol for Sensorimotor Integration and Cognitive Control Experiments. *J. Vis. Exp.* (86), e51128, doi:10.3791/51128 (2014).

Abstract

Rodents have been traditionally used as a standard animal model in laboratory experiments involving a myriad of sensory, cognitive, and motor tasks. Higher cognitive functions that require precise control over sensorimotor responses such as decision-making and attentional modulation, however, are typically assessed in nonhuman primates. Despite the richness of primate behavior that allows multiple variants of these functions to be studied, the rodent model remains an attractive, cost-effective alternative to primate models. Furthermore, the ability to fully automate operant conditioning in rodents adds unique advantages over the labor intensive training of nonhuman primates while studying a broad range of these complex functions.

Here, we introduce a protocol for operantly conditioning rats on performing working memory tasks. During critical epochs of the task, the protocol ensures that the animal's overt movement is minimized by requiring the animal to 'fixate' until a Go cue is delivered, akin to nonhuman primate experimental design. A simple two alternative forced choice task is implemented to demonstrate the performance. We discuss the application of this paradigm to other tasks.

Video Link

The video component of this article can be found at http://www.jove.com/video/51128/

Introduction

Studying the relation between neurophysiology and behavior is the ultimate goal in systems neuroscience. Historically, there has been a tradeoff between animal model choice and behavioral repertoire[1-5]. While simple organisms like sea slugs[6] or squids[7] have been used extensively to study properties of single ion channels, neurons and simple neural circuits, higher order species are needed to study more complex functions such as spatial navigation, decision making[8-11] and cognitive control[12-14]. Despite being a standard animal model for human like behavior, use of nonhuman primates prompts cost and ethical considerations that precludes their use across a wide range of experiments in a single laboratory setting[15-18]. Simpler animal models such as rodents are generally preferred[19], provided they have similar neural substrates underlying the behaviors of interest.

There's ample evidence suggesting that rodents share similar cortical and subcortical structures as those found in primates[20-22]. Rodents are also known to integrate information across multiple sensory modalities to guide their action[23-25], for example, by coordinating whisking and sniffing during exploratory behavior[26] or by integrating auditory and visual/olfactory events[25,27].

Here we describe a framework for operant conditioning of rodents used to test cognitive tasks[28-32]. In this framework, subjects are required to fixate inside a nosepoke hole and maintain their snout inside the hole until the presentation of a go cue. The behavioral task is a five-hole nosepoke design that is conventionally used for 5-choice serial reaction time task studies. During the delay period, a range of instruction cues is presented to guide the subject to perform an action. This framework can easily be modified to suit a wide range of experiments in which training the subject to minimize its overt movement over a brief interval is needed. This permits studying the extent to which spiking activity of individual neurons is affected by specific cues during this interval. The protocol can minimize the training time and can reduce across-subject learning variability. A schematic flowchart of the task is shown in **Figure 1**.

Protocol

All procedures involving animals were approved by the Michigan State University Institutional Animal Care and Use Committee (IACUC).

1. Experimental Setup

1. Use an operant conditioning box which consists of a five-hole nosepoke wall on one side and a food delivery trough on the opposite side.
    1. The center nosepoke hole is considered as a "fixation" hole and the four other holes (two on each side of the fixation hole) are considered motor target holes. Each hole is equipped with a tri-color LED and an infrared beam emitter-detector system that detects when the animal enters and retracts from the fixation hole.
    2. Use a programmable tone generator to generate single frequency tones with millisecond precision and connect it to a speaker mounted inside the operant box. Control the tone generator and nosepokes through the behavioral tracking system using the appropriate software. Use a hardware and software system that provides millisecond timescale monitoring of behavioral events and control of cues and responses.

        Note: The amplitude of both tone and noise cues should be kept around 60 ± 3 dB SPL.

2. Early Habituation

1. Restrict the subject's food intake gradually to ~5 g per 100 g of the subject's normal weight (*e.g.* over the course of 3 days). The subject should maintain 85-90% of their *ad libitum* weight.
2. Habituate the subject to handling by the experimenter and familiarize the subject with the apparatus from the first day of starting the food deprivation protocol. Start handling the animal and place it in the operant conditioning box while providing food pellets in the pellet trough to encourage the subject to explore the cage and get familiar with the reward delivery location.

3. Subject Training

1. **General notes**
    1. The task suggested here needs precise coordination between perception of an auditory cue, minimizing movements during the delay period and movement execution.
    2. Gradually train the subject step-by-step to prepare them for the final desired behavior.
    3. Make sure that at the end of each step, the subject maintains >75% behavioral performance for at least three consecutive sessions before progressing to the next stage.
    4. Once the final stage is reached, keep the subject on the protocol for a week to ensure the performance is maintained at the desirable level.

2. **Start:** Familiarize the subject with the nosepoke holes, food delivery port and the association between the flashing holes and reward.
    1. Select one out of the four targets on a random schedule.
    2. Play the Go cue (a white auditory noise) and keep the LED inside the hole flashing (0.3 sec pulse duration).
    3. Set the software to reward the subject upon visits to the hole.
    4. Time-out the trial after 30 sec if the hole is not visited and start a new trial.
    5. Do not reward any visits to the incorrect holes.

3. **Target Selection:** Punish erroneous visits to the nonselected holes.
    1. Upon visits to incorrect holes, terminate the trial followed by 5 sec of black-out.

        Note: During a black-out epoch, the fixation hole LED is turned off in the cage. This means that the subject cannot initiate a trial and needs to wait until the fixation hole LED starts flashing.
    2. Select a new hole and start a new trial.

4. **Nosepoke:** Train the subject to poke inside the fixation hole to start a trial.
    1. Flash a yellow LED inside the fixation hole.
    2. Upon visiting the fixation hole immediately play the Go cue and start a new trial.
    3. Penalize incorrect visits by 5 sec of black-out.

5. **Delay:** Teach the subject to maintain their nose inside the fixation hole for a set period of time (delay period) that is increased gradually as training progresses.
    1. Wait for the subject to visit the fixation hole.
    2. Terminate the trial if the subject retracts within 500 msec. Otherwise, play the Go cue.
    3. Penalize premature retractions by a black-out period for 7 sec.
    4. Reward the correct visits by delivering a food pellet.

6. **Two Cues (with Light):** Increase and randomize the delay period length and introduce the auditory instruction cue.
    1. Increase the length of the delay period to an average of 1.5 sec.
    2. Choose a random delay period length at each trial based on a uniform density between 1.3-1.8 sec.
    3. Introduce the instruction cue as a single frequency auditory tone pulsed in triplets, with a pulse duration of 150 msec and interpulse interval of 100 msec.
        1. Play the instruction cue immediately after the subject enters the fixation hole.
        2. Assign two instruction cues to each of the targets.
        3. Only use one cue associated for each target at this stage.

   4. Let the subject use both auditory and visual cues to select the target hole.

7. **Two Cues (without Light):** Train the subject to only use auditory cues.
   1. Turn off the flashing LEDs inside the target holes so that the subject would only use auditory instruction cues.

8. **Four Cues:** Introduce the two other cues to the sequence of randomly presented instruction cues and repeat sections 3.5.3-3.6.1.

4. Behavioral Data Analysis

1. **Success Rate:** Define success rate as the percentage of correct visits to the targets divided by the total number of trials.
   1. Measure the success rate at each stage of training.

2. **Error types:**
   1. Premature retraction: measure the percentage of trials timed-out due to early retractions from the fixation hole.
   2. Comission error: Calculate the percentage of failed trials when the subject visits an uninstructed target
   3. Omission Error: Calculate the percentage of errors when the subject does not visit any of the targets after trial initiation.

3. **Measured variables:**
   1. Reaction Time (RT): For each trial, measure the delay between the onset of the Go cue and the subject retracting from the fixation hole.
   2. Time to Target (TT): Measure the duration between the subject retraction from the fixation hole and entering the target hole.

Representative Results

The suggested framework enables training the subject on a range of cognitive tasks. Here we implemented an instructed delay task designed to investigate the mechanisms of goal-directed actions in the rodent prefrontal cortex. **Figure 1** shows a flowchart of the experimental design.

To ensure that the subject understands the task requirement at every step, performance measures should be continuously assessed. **Figure 2** shows an example performance of one subject across multiple sessions. Once the subject acquired the task, it was implanted with a 32 channel microelectrode array in the prelimbic area (corresponding to the medial prefrontal cortex). Multiunit activity and local field potentials (LFPs) were recorded. Single neuron spike trains were isolated using standard spike sorting techniques[33] and events associated with different epochs of the task were marked. **Figures 3** and **4** show some sample results of selective multiple single unit modulations during critical epochs of the task.

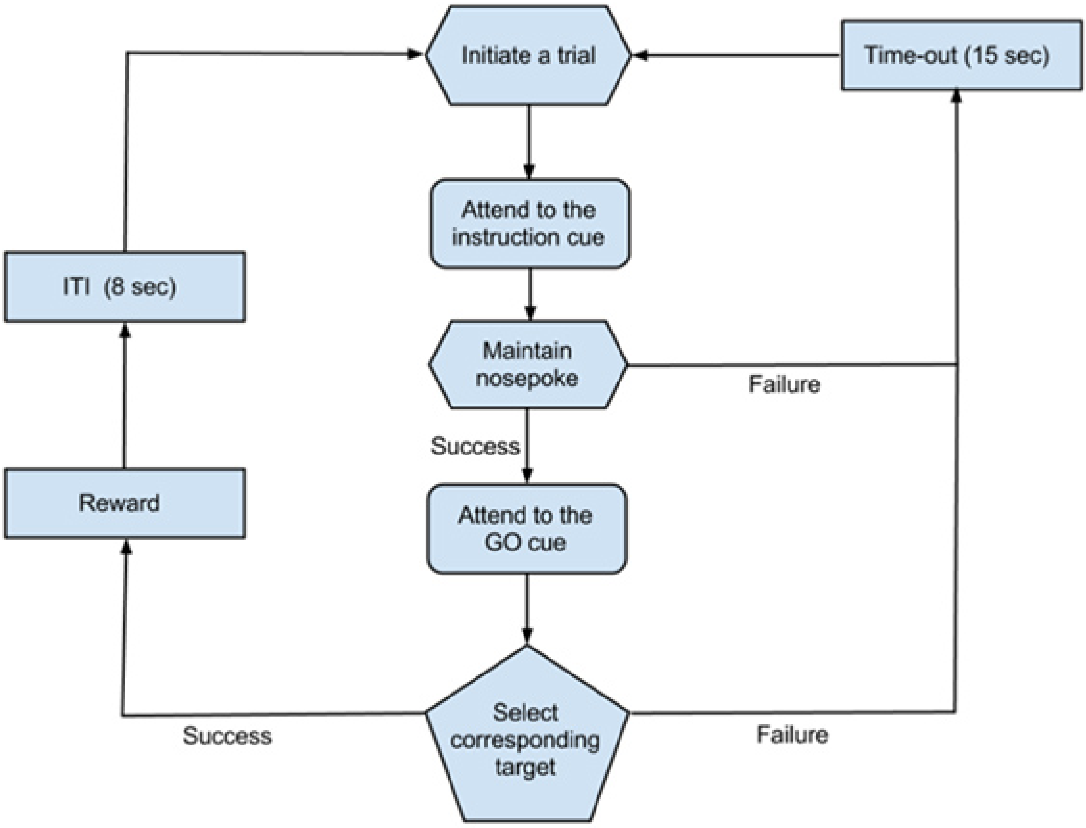

Figure 1. Flowchart of a sample trial showing the sequence of actions and events during a trial. The subject self-initiates a trial by poking the nose inside the fixation hole. Briefly after the nosepoke, an instruction cue (a single frequency tone) is played followed by a delay period. The subject is required to maintain the nose inside the fixation hole until the presentation of the Go cue. Any premature retraction will cause the trial to be aborted and the subject is penalized by a time-out. After a delay period of random length, a Go Cue (auditory white noise) is presented and the subject is free to move towards the instructed target. Successful trials are rewarded by a 45 mg food pellet while failed trials are timed out for 15 sec. Click here to view larger image.

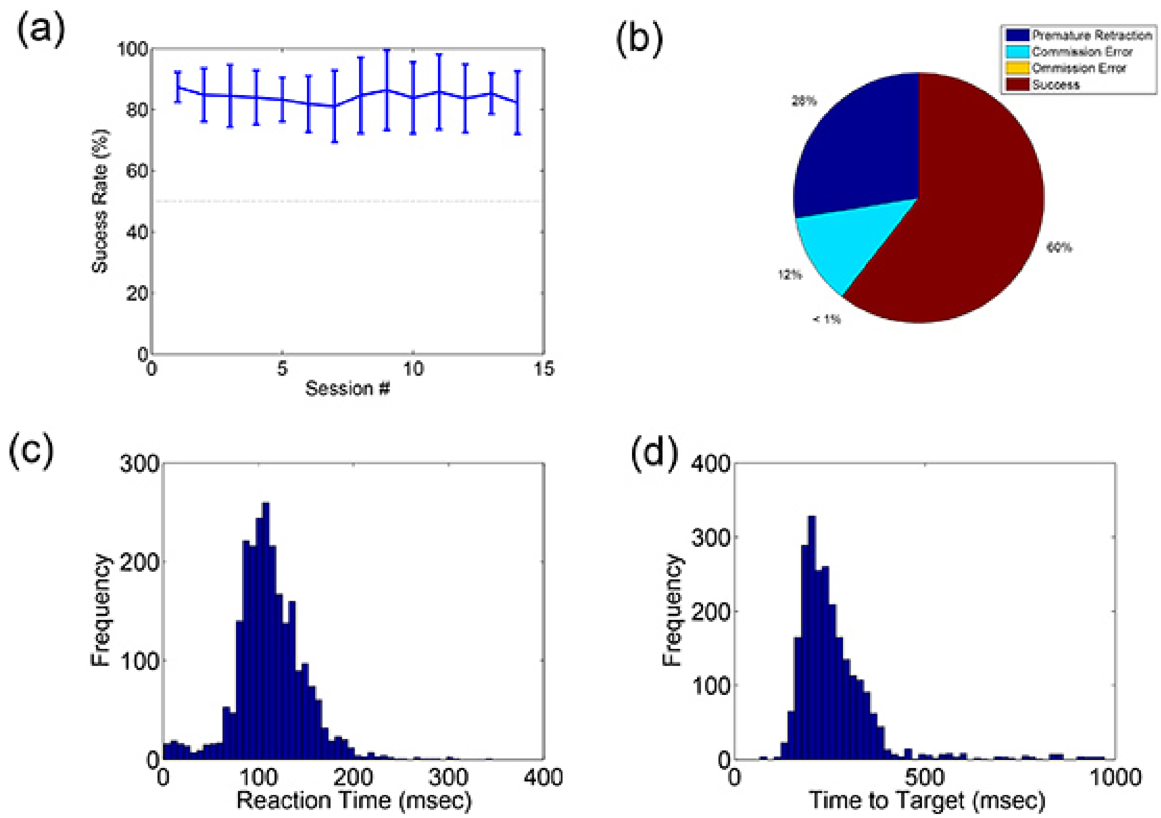

Figure 2. Behavioral performance scores measured across multiple sessions. (**a**) Success rate is defined as the ratio of the number of successful trials to total number trials in every session. Results are shown for a fully trained subject across 14 recording sessions. (**b**) Distribution of error types. Premature retraction occurs with early retraction before the Go cue. Commission error is defined as visiting any target other than the one that was instructed and the omission error occurs when the subject does not reach for any target within 5 sec from the Go Cue. (**c**) A histogram of reaction time - the period between the onset of the Go cue and the subject's breaking out the fixation hole beam - showing the distribution of the reaction time across different trials. (**d**) A histogram of time to target - the period between breaking out of the fixation hole and breaking in the target hole - showing the distribution of the time to target across different trials. Click here to view larger image.

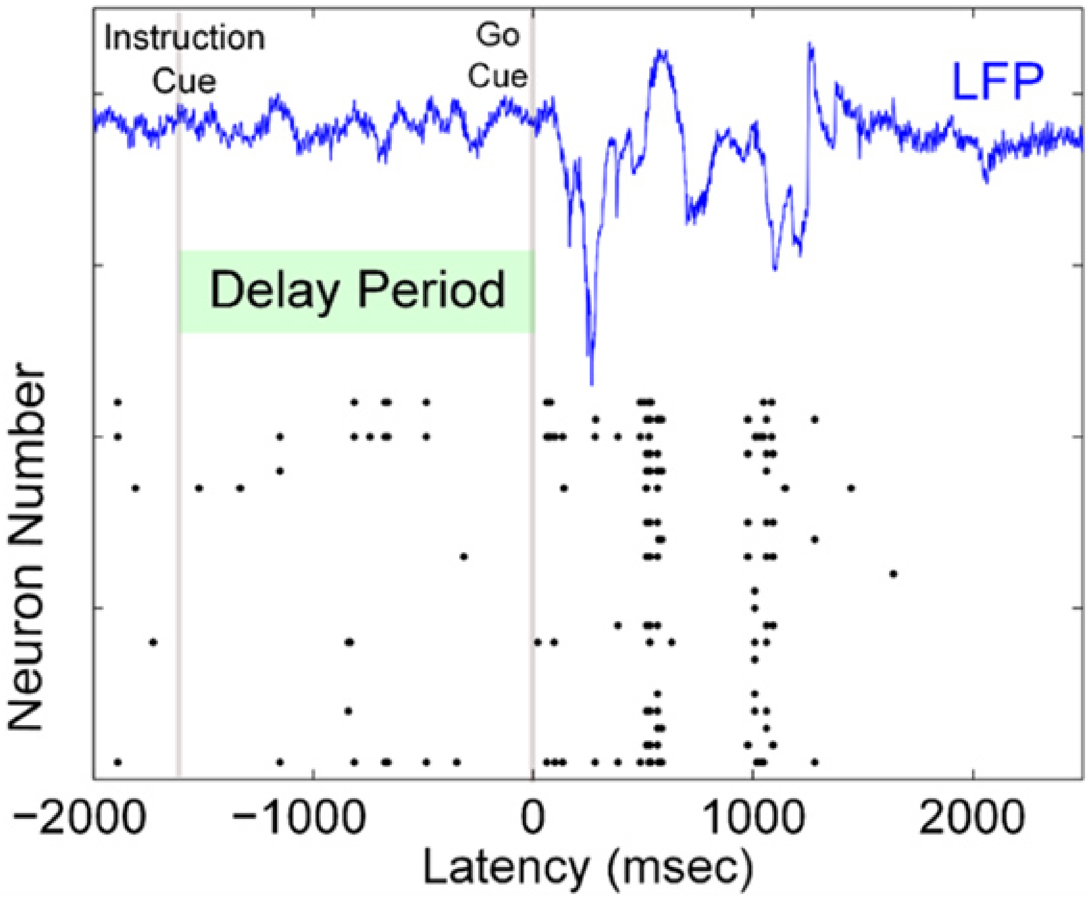

Figure 3. Neurophysiology data from a sample trial. After the subject mastered the task and maintained a high performance level for at least a week, it was implanted with a 32 channel microelectrode array in the prelimbic area of medial Prefrontal cortex (mPFC) and multiple single unit activity was recorded along with local field potentials. A sample trace of LFP variation along with a raster plot of 22 simultaneously recorded units (each row is a unit and each dot represents one spike) are shown. Markers for behavioral events are also plotted on top of the traces. These traces show high prediction power of motor intention after the Go cue (Analysis not shown here). Click here to view larger image.

Sensory Cue	Spatial Target Location
1 KHz	Right
2 KHz	Right
4 KHz	Left
8 KHz	Left

Table 1. Instruction cue assignment. The table shows the corresponding motor target assigned to each instruction cue.

Sensory Cue	**Spatial Target Location**
1 KHz	Right
2 KHz	Right
4 KHz	Left
8 KHz	Left

Table 2. Training time table. The table shows the length of training session spent for each subject (2 training session/day) for adult female Sprague-Dawley rats (3-4 months old).

Protocol	A24	A25	A26	A28	A29	Average
Start	4	2	4	4	4	3.6
TargetSelection	3	5	5	4	4	4.2
Nosepoke	8	7	9	5	2	6.2
Delay	8	8	5	4	3	5.6
Two Cues (with Light)	5	4	5	5	2	4.2
Two Cues (without Light)	10	7	9	11	17	10.8
Four Cues	13	12	14	18	11	13.6
Total	**51**	**45**	**51**	**51**	**43**	**48.2**

Discussion

Rats have been widely used in neuroscience research for over a century. Since Thorndike's introduction of the concept of the law of effect in cats[34], operant conditioning has been the standard approach to test different aspects of animal behavior. Many neuroscience experiments involving decision making and motor preparation include a delay period between the instruction cues and the action interval. It is desirable to minimize movements during these delay periods to reduce any confounds to the neural data being acquired. While conventional maze navigation experiments in rodents capitalize on rodents' great capacity to forage for food, they are limited by the movements that the animal execute and therefore cannot be used to test more complex questions such as decision making and motor planning. While maze tasks are easy to implement as subjects learn to navigate rapidly, overt behavior is unrestricted during every phase of the task (*e.g.* the central arm of a T-maze).

Here we described a flexible framework inspired by visual attention studies in rodents. The representative results we provided demonstrate that animals can learn the task, even when multiple sensory cues are associated with a single motor target. This design was selected to test the capacity of the working memory used to guide motor behavior. The most critical step within the protocol is to train the subject to maintain their nose inside the fixation hole for the entire duration of the delay period.

Because frontal areas are reciprocally connected to many cortical and subcortical areas, precise timing of the behavioral events and synchronizing the timing of those events to the acquired neural data can alleviate the risk of potential confounds. Computer-automated registration of behavioral events (such as nosepoke or cue trigger) can occur with millisecond precision. Video tracking of subject movement can also be performed and the data can be synchronized with behavioral events to provide precise correlation between neural activity and behavior.

More complex cognitive abilities of rodents can be studied using this paradigm. For example, we have used it to implement a rodent version of the delayed match-to-sample task with an auditory sensory modality rather than spatial navigation. The subject was cued with a sample auditory cue followed by a matching cue and had to decide on target locations based on the matching decision.

Troubleshooting:

The implementation of the experimental design is very straightforward using a computer software and subjects should be able to master the task over approximately 25-30 training sessions. Deviations from this schedule might be due to lack of motivation, or confusion that may be caused by:

1. Inaccurate auditory tone frequency: The design is highly dependent on the pitch of the instructed cue. The experimenter should check both the frequency of the audio output and the amplitude of the tone.
2. Food delivery: Often when the subject is not motivated to perform the task, the food delivery system should be checked for any possible defect that may have disengaged the reward delivery system.

To summarize, technological advances in recording and stimulation of large ensembles have enabled measuring and interrogating the neural circuitry underlying action preparation and execution with millisecond precision. Rodents are among the best candidates across different animal species to be used for such research given their ability to perform cognitive tasks and the availability of techniques tailored to rodents. The protocol described in this article may help to design experiments to answer specific questions about the cognitive aspects of action preparation and execution.

Disclosures

The authors declare no competing financial interests.

Acknowledgements

This work was supported by the NINDS grant #NS054148.

References

1. Goldstein, E. B. *Cognitive psychology: Connecting mind, research, and everyday experience*. Wadsworth Publishing Company (2008).
2. Kandel, E. R., Schwartz, J. H., Jessell, T. M., & *et al. Principles of neural science*. 4th edition. McGraw-Hill New York (2000).
3. Cisek, P., & Kalaska, J. F. Neural mechanisms for interacting with a world full of action choices. *Ann. Rev. Neurosci.* **33,** 269-298 (2010).
4. Kalat, J. W. *Biological psychology*. Wadsworth Publishing Company (2012).
5. Banich, M. T., & Compton, R. J. *Cognitive neuroscience*. Wadsworth Publishing Company (2010).
6. Carew, T. J., Pinsker, H. M., & Kandel, E. R. Long-term habituation of a defensive withdrawal reflex in aplysia. *Science.* **175,** 451-454 (1972).
7. Hodgkin, A. L., & Huxley, A. F. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **117,** 500 (1952).
8. Romo, R., & Salinas, E. Flutter discrimination: neural codes, perception, memory and decision making. *Nat. Rev. Neurosci.* **4,** 203-218 (2003).
9. Romo, R., & de Lafuente, V. Conversion of sensory signals into perceptual decisions. *Prog. Neurobiol.* doi:10.1016/j.pneurobio.2012.03.007 (2012).
10. Shadlen, M. N., Britten, K. H., Newsome, W. T., & Movshon, J. A. A computational analysis of the relationship between neuronal and behavioral responses to visual motion. *J. Neurosci.* **16,** 1486-1510 (1996).
11. Beck, J. M. *et al.* Probabilistic Population Codes for Bayesian Decision Making. *Neuron.* **60,** 1142-1152 (2008).
12. Goldman-Rakic, P. S. Circuitry of primate prefrontal cortex and regulation of behavior by representational memory. *Compr. Physiol.* (1987).
13. Miller, E. K., Erickson, C. a & Desimone, R. Neural mechanisms of visual working memory in prefrontal cortex of the macaque. *J. Neurosci.* **16,** 5154-5167 (1996).
14. Fuster, J. M., Alexander, G. E., & *et al*. Neuron activity related to short-term memory. *Science.* **173,** 652-654 (1971).
15. Fetz, E. E., & Baker, M. A. *Operantly Conditioned Patterns of Activity and Correlated Responses Cells and Contralateral Muscles*. (1973).
16. Carmena, J. M. *et al.* Learning to control a brain--machine interface for reaching and grasping by primates. *PLoS Biol.* **1,** e42 (2003).
17. Georgopoulos, A. P., Schwartz, A. B., & Kettner, R. E. Neuronal population coding of movement direction. *Science.* **233,** 1416-1419 (1986).
18. Donoghue, J. P., Sanes, J. N., Hatsopoulos, N. G., & Gaál, G. Neural discharge and local field potential oscillations in primate motor cortex during voluntary movements. *J. Neurophysiol.* **79,** 159-173 (1998).
19. Abbott, A. Laboratory animals: the Renaissance rat. *Nature.* **428,** 464-466 (2004).
20. Fuster, J. *The prefrontal cortex*. Academic Press (2008).
21. Britten, K. H., Shadlen, M. N., Newsome, W. T., & Movshon, J. A. The analysis of visual motion: a comparison of neuronal and psychophysical performance. *J. Neurosci.* **12,** 4745-4765 (1992).
22. Abbott, A. Neuroscience: The rat pack. *Nature.* **465,** 282-283 (2010).
23. Uchida, N., & Mainen, Z. F. Speed and accuracy of olfactory discrimination in the rat. *Nat. Neurosci.* **6,** 1224-1229 (2003).
24. Jaramillo, S., & Zador, A. M. The auditory cortex mediates the perceptual effects of acoustic temporal expectation. *Nat. Neurosci.* **14,** 246-251 (2010).
25. Cohen, L., Rothschild, G., & Mizrahi, A. Multisensory integration of natural odors and sounds in the auditory cortex. *Neuron.* **72,** 357-369 (2011).
26. Deschênes, M., Moore, J., & Kleinfeld, D. Sniffing and whisking in rodents. *Curr. Opin. Neurobiol.* **22,** 243-250 (2012).
27. Raposo, D., Sheppard, J. P., Schrater, P. R., & Churchland, A. K. Multisensory decision-making in rats and humans. *J. Neurosci.* **32,** 3726-3735 (2012).
28. Bari, A., Dalley, J. W., & Robbins, T. W. The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. *Nat. Protoc.* **3,** 759-767 (2008).
29. Brasted, P. J., Dunnett, S. B., & Robbins, T. W. Unilateral lesions of the medial agranular cortex impair responding on a lateralised reaction time task. *Behav. Brain Res.* **111,** 139-151 (2000).
30. Gage, G. J., Stoetzner, C. R., Wiltschko, A. B., & Berke, J. D. Selective activation of striatal fast-spiking interneurons during choice execution. *Neuron.* **67,** 466-479 (2010).
31. Erlich, J. C., Bialek, M., & Brody, C. D. A cortical substrate for memory-guided orienting in the rat. *Neuron.* **72,** 330-343 (2011).
32. Mohebi, A., & Oweiss, K. G. Neural ensemble correlates of working memory in the rat medial prefrontal cortex. *41 st Ann. Meet. Soc. Neurosci.* (2011).
33. Oweiss, K. G. *Statistical signal processing for neuroscience and neurotechnology*. Academic Press (2010).
34. Thorndike, E. L. Animal intelligence: An experimental study of the associative processes in animals. *Psychol. Monographs: Gen. Appl.* **2,** i-109 (1898).