

Novel Object Recognition in the Classroom: Establishment of an Online Video Resource for Use by Instructors in Courses on Animal Learning, Memory and Behavior

Matthew J. Anderson & Justin J. McGraw Saint Joseph's University

Novel object recognition procedures are widely used by animal learning and memory researchers. In order to foster a greater understanding of the methods and usage of such procedures by students in animal learning, memory, and behavior classes, we have created a freely available online video resource containing video samples of novel object recognition training and testing trials. This article describes this resource, and offers some suggestions for its use in the classroom.

Keywords: Novel object recognition, novel object preference, rats, videos

The novel object recognition (aka novel object preference) task capitalizes on the findings of Berlyne (1950), where it was found that rats prefer to explore objects that they have not previously encountered over objects that are familiar. In 1988, Ennaceur and Delacour promoted the use of novel object recognition as a one-trial test of rodent working-memory, not involving the use of primary reinforcement (e.g., food, shock), and comparable to similar procedures employed in human and non-human primate subjects (but see Ennaceur, 2010). Since that time, the use of such procedures has grown rapidly.

Some evidence of the recent growth in usage of the novel object recognition task can be seen in Figure 1. Yearly (2000-2011) date restricted PsycINFO searches for the exact phrases, "novel object recognition" OR "novel object preference" were conducted and plotted to provide some sense of the rapid growth of novel object recognition task usage (searches conducted on 8/8/11, and were restricted to the "animal" population group). The exact number of results generated by means of this particular literature search method are likely to be conservative estimates, and may or may not accurately reflect the precise number of studies that have actually utilized such tasks during these periods. Indeed, one can imagine possibly obtaining a greater number of results with less restrictive search rules. Despite this possibility, however, the results still depict a recent period of rapid growth, and the trend is clearly evident.

Novel object recognition procedures have been used to examine the effects of age (e.g., Anderson, Barnes, Briggs, Ashton, Joynes, & Riccio, 2004; Dellu, Mayo, Cherkaoui, Le Moal, & Simon, 1992), predatory threat (Morrow, Roth, & Elsworth, 2000), chronic stress (e.g., Beck & Luine, 1999), rat strain (e.g., Andrews, Jansen, Linders, Pricen, & Broekkamp, 1995), pregnancy (Paris & Frye, 2008), numerous psychoactive substances (e.g., Ennaceur, Cavoy, Costa, & Delacour, 1989), and a variety of other factors on object recognition. Moreover, through some minor modification to the basic procedures, similar tasks have also provided environmental measures memory for familiarization (e.g., Bevins, Koznarova, & Armiger, 2001), and memory for an object's spatial location (e.g., Dix and Aggleton, 1999; Beck & Luine, 2002)(for review see Anderson, 2006a, b). This popularity and widespread usage likely stems from the novel object recognition task's simplicity and ease of implementation, as well as the appetitive nature of exposure to novel stimuli (e.g., Bevins, Besheer, Palmatier, Pickett, & Eurek, 2002).

The typical procedures of the novel object recognition task begin with initial handling of the subjects and pre-exposure to the environment in which object training and testing exposures are to subsequently occur. This pre-exposure is implemented as environmental familiarization has

Number of PsycINFO Results

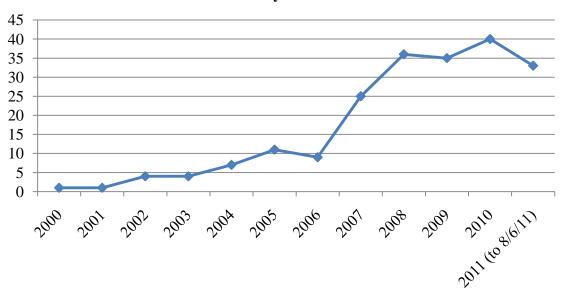


Figure 1. Depicted are the yearly date and animal population group restricted PsycINFO searches for the exact phrases, "novel object recognition" OR "novel object preference" (on 8/8/11 at approx. 11:00pm EST). The substantial recent growth in the use of novel object recognition tasks is evident.

been shown to increase subsequent exploration of objects (Sheldon, 1969; Besheer & Bevins, 2000). Subjects next receive some form of exposure to the initial training (aka sample) objects, during which they are thought to habituate to these stimuli (e.g., Ennaceur & Delacour, 1988). It is worth noting that some recent evidence has suggested that only a minimum amount of object exposure is necessary during the training phase, and that object interaction beyond this minimum amount does not necessarily result in greater preference for novelty at subsequent test (Gaskin, Tardif, Cole, Piterkin, Kayello, & Mumby, 2010). Following training, all subjects undergo novel object recognition testing in which they are allowed equal opportunity to explore the initial object and an object with which they have had no previous experience. Preferences to explore the various objects are noted, and a tendency to explore the novel object over the familiar sample is interpreted as evidence of memory for the training exposure (e.g., Ennaceur & Delacour, 1988; but see Gaskin et al., 2010).

Given the increased usage of the novel object recognition task by researchers, taking steps to allow for students in animal learning and memory or research methods courses to have some direct exposure to these methods seems vital. Thus, we decided to develop an online video resource containing sample novel object training and testing trials that would be freely available for use by

instructors. This resource may be of particular use in settings where actual live animal subjects are unavailable, or when instructors are seeking to reduce the number of live animals used in their classrooms. Moreover, instructors could integrate the use of these clips into discussions on habituation, memory, curiosity, and a variety of research methods topics. The video clips are available in several formats, and can be found on the following webpage: http://people.sju.edu/~mander06/NORVideos.htm. Alternatively, in the event of technical difficulties, M.J.A. can provide the clips to you on CD. Below we describe the origin of these videos, the methods employed when generating them, and the results that you can expect to obtain from them. We conclude with a brief discussion of several possible classroom activities that utilize the video clips.

A Disclaimer and Note on the Origin of the Videos

The rats appearing in the videos that we have chosen to make publically available constitute a subset of those subjects originally ran as part of a broader investigation that attempted to examine the relationship between lateral behavior and novelty preference. While this former research project was previously presented as a poster at the annual meeting of the Eastern Psychological Association (McGraw & Anderson, 2011), the results were inconclusive and not warranting publication on their

Table 1. Novel & Familiar Objects

Rat #	Training Objects	Novel Object at Test	Familiar Object at Test
1	Bottles	Duck	Bottle
2	Ducks	Bottle	Duck
3	Bottles	Duck	Bottle
4	Ducks	Bottle	Duck
5	Bottles	Duck	Bottle
6	Bottles	Duck	Bottle
7	Ducks	Bottle	Duck
8	Ducks	Bottle	Duck

own merit (and thus shall not be discussed further here). Moreover, the project itself encountered some technical difficulties and experimenter errors that resulted in the elimination of multiple rats. One should in no way attempt to use these videos for any legitimate research purpose. As a general preference for novelty was detected in this particular subset of subjects, however, they do lend themselves nicely to lab/classroom exercises and a meaningful discussion of the novel object recognition task.

Methods Employed in the Videos

All procedures had received approval from the Saint Joseph's University Institutional Animal Care and Use Committee prior to the start of the original research project. When running the study, adult female Long-Evans rats were obtained from our in-house breeding stock (originally derived from animals purchased from Taconic Farms Inc., Germantown, NY) and housed in pairs in standard plastic shoebox cages in a room lit on a reversed 14/10-h light/dark schedule, with all procedures taking place during the dark portion of their lighting schedule. Subjects were maintained on ad lib food and water throughout the experiments' entirety.

All subjects underwent handling for approximately three minutes before being transported from the main colony room to the lab room in which testing was to occur. Next, animals were individually placed in the center of a sealed wooden octagon-shaped open-field apparatus with approximately 40cm sides and 34cm tall walls for a period of three minutes. This was done in order to pre-expose the subjects to the empty training/testing apparatus and increase the likely of object interaction during subsequent phases of the study. This procedure was repeated for two additional consecutive days (i.e., three total days of handling followed by open-field exposure).

Approximately one minute following the final pre-exposure to the empty open-field, each

animal underwent initial object training. Training involved individually placing each subject in the octagon open-field apparatus with two identical objects for a period of three minutes. During training a camera (Sony model: DCR-TRV260) mounted above the apparatus collected video that was recorded and analyzed by a Dell PC computer running a motion analysis program (EzVideoDV Automated Tracking System, AccuScan Instruments Inc., 2006).

Approximately one minute after training, each animal was returned to the octagon for novel object recognition/preference testing. The rat was placed in the octagon for three minutes where one of the original objects and a novel object that it had not previously encountered was presented. Testing sessions were recorded/analyzed by computer in the exact fashion as training.

The objects serving as initial and novel objects, as well as the object locations employed were counterbalanced across subjects (although some of this may be lost in the available video clips as multiple rats were excluded due to errors/problems as described above). Table 1 contains a list of the objects serving as novel and familiar objects for each of the subjects. A wide variety of objects have been employed in investigations utilizing the novel object task. In the present study we used two sets of objects: a small plastic rubber duck and a 120ml baby bottle with a plastic cap (cf., Anderson, Jablonski, & Klimas, 2008). Each object was obtained in triplicate in order to allow for the presentation of two of the objects during training sessions and the third during test sessions, reducing the likelihood of reliance on olfactory cues. As has been described elsewhere (Anderson et al., 2008), each object was glued to a 7.62cm X 7.62cm, 0.635cm thick black Plexiglas block. A screw passed through each Plexiglas block, allowing for the block and object to be secured to the approximate center of one of four quadrants in the octagon open-field. Following each trial the octagon arena and objects were cleaned with mild soapy water.

Analysis of the Videos

The training and testing exposure videos of eight subjects are presented online in multiple formats and are freely accessible at the webpage mentioned above. Alternatively, in the event of technical difficulties, M.J.A. can provide the clips to you on CD. Object interaction has been operationally defined in numerous ways by those investigators implementing the novel object task. In analyzing the video clips we employed two different methods in order to present the reader with several of the

possibilities. First, the clips were hand-scored by one of the investigators (M.J.A.), who calculated (via the iLab: Timer HD application developed by Negative Ninth Studio {© 2011} ran on an Apple iPad {running software version 4.3.4}) the amount of time that an animal was in contact with or had its snout facing within approximately one inch of the object (cf. Anderson, Karash, Ashton, & Riccio, 2003). Second, object interaction, as defined by the animal entering an approximate 8cm circular zone around the object, was automatically scored by the EzVideo DV Automated Tracking System (cf. Anderson et al., 2008). Our previous research has suggested that the object interaction scores generated by the computer tracking time spent in the vicinity of an object in such a manner is significantly positively correlated with the more traditional hand-scoring methods (Anderson et al., 2008), thus validating its potential use as a measure of object interaction. Indeed, the current data also appear to support this, as the time spent exploring both objects at training (r(6)=.843,p=.009), both objects at test (r(6)=.960, p<.001), the familiar object at test (r(6)=.989, p<.001), and the novel object at test (r(6)=.937, p=.001) gathered via the hand scoring and computer zone tracking each significantly methods were positively correlated.

In addition to the different ways of defining object interaction, there are multiple ways to examine novel object preference. First, one can simply compare the time spent with the novel and familiar objects by each subject via a paired-samples t-test. This type of analysis yielded evidence of a significant novelty preference while employing the hand-scoring method (t(6)=3.246, p=.018) and a marginally significant novelty preference for the computer zonescoring method (t(6)=2.065, p=.085). Similarly, one can generate an absolute novelty preference score by subtracting the seconds spent with the familiar object from those spent with the novel object (novel time familiar time). Such a score could be compared to chance performance (a score of "0") via a one-sample t-test, and yields results similar to the paired-samples t-test analysis (hand scoring: t(6)=3.246, p=.018; computer/zone scoring: t(6)=2.065, p=.085). Alternatively, one can calculate a percent preference for novelty ({novel time / [novel time + familiar time]} X 100), which could be compared to chance performance (a score of "50") in a one-sample t-test. Such analyses also yield a significant novelty preference for the hand scoring (t(6)=3.957, p=.007) marginally significant novelty method. and preference for the computer/zone scoring (t(6)=2.178, p=.072) method. All analyses were performed with SPSS 16.0 for Mac, Release 16.0.2.

Tables 2 and 3 contain the scores of each subject on each of the various measures according to the hand scoring and computer/zone scoring methods, respectively, and ought to provide instructors with a general idea of what they can expect from the videos. It is worth noting that rat #3 was excluded from each of the novel object preference analyses just described above as she failed to explore either object at test. We included her videos so that students could get a sense for the behavioral variability and problems frequently encountered in this task, and how such difficulties are often addressed (e.g., exclusion of such rats from analyses). If instructors do not wish to discuss such issues in their classes they can simply choose to not have their students score the videos from that particular subject.

As with previous studies (Gaskin et al., 2010), time spent exploring the initial objects in the present video clips does not perfectly correlate with novelty preference at test. In other words, those that spend more time exploring the objects at training do not necessarily show a greater novelty preference. Pearson correlation analyses (again, performed without rat # 3) examining the relationship between the time spent exploring the objects at training and the various novelty preference measures each failed to achieve significance, although one was marginally significant and all were in the positive direction as would be expected. For the computer-zone scoring method, the total time spent exploring objects at training was not significantly related to the percent preference for novelty measure (r(5)=.361, p=.426), but displayed a marginally significant positive correlation with the absolute novelty measure (r(5)=.726, p=.065). For the hand-scoring method, the total time spent exploring objects at training was not significantly related to either the absolute (r(5)=.626, p=.132) or percent (r(5)=.372, p=.411)preference for novelty measures. The one marginally significant relationship and the general positive directions of the remaining correlations are consistent with the notion that the training experience does influence subsequent novelty reactions. relationships are far from perfect, however, the results are also consistent with the notion that only a minimum amount of sample object exposure is necessary to demonstrate a subsequent novel object preference, and that little is gained beyond that minimally required experience (Gaskin et al., 2010).

Table 2. Scores According to the Hand-Scoring Method.

Rat #	Both Objects at Training (sec)	Novel Object at Test (sec)	Familiar Object at Test (sec)	Both Objects at Test (sec)	Absolute Novelty Pref.	Percent Novelty Pref.
1	15.9	8.4	2.2	10.6	6.2	79.25
2	11.5	1.9	0	1.9	1.9	100.00
3*	19.3	0	0	0		
4	11	1	1.8	2.8	-0.8	35.71
5	27.2	18.5	3.1	21.6	15.4	85.65
6	20.2	6.6	0.3	6.9	6.3	95.65
7	10.1	11.2	1.4	12.6	9.8	88.89
8	24.2	6.2	0	6.2	6.2	100
Mean	17.43	6.73	1.10	7.83	6.43	85.59
SD	6.38	6.13	1.20	7.02	5.24	22.46

^{*}Note: Rat 3 was excluded from analyses of novelty preference reported in text

Table 3. Scores According to the Computer/Zone-Scoring Method

Rat #	Both Objects at Training (sec)	Novel Object at Test (sec)	Familiar Object at Test (sec)	Both Objects at Test (sec)	Absolute Novelty Pref.	Percent Novelty Pref.
1	15.1	4.7	5.06	9.76	-0.36	48.16
2	11.7	2.3	0	2.3	2.3	100
3*	46.71	0	0	0		
4	13.79	0.87	3.23	4.1	-2.36	21.22
5	40.17	32.17	7.29	39.46	24.88	81.53
6	25.6	7.03	0.63	7.66	6.4	91.78
7	5.03	12.47	3.79	16.26	8.68	76.69
8	34.3	10.16	0	10.16	10.16	100
Mean	24.05	8.71	2.50	11.21	7.10	74.20
SD	15.03	10.45	2.78	12.51	9.10	29.40

^{*}Note: Rat 3 was excluded from analyses of novelty preference reported in text.

Possible Classroom Exercises

Simple Behavioral Recording and Analysis

Given the popularity of the novel object recognition task, there is great value in simply having individual students score and analyze the videos using the hand scoring method and those analyses described above. Instructors could also have their students describe and track other rodent behaviors of their choosing (e.g., grooming, locomotion, turning, etc.), and may even wish to note any changes in such behaviors over the course of the training and testing sessions.

Operational Definitions Activities

Instructors could have different groups of students generate different operational definitions of object exploration, have the various groups score the videos according to their chosen definition and compare/contrast the results. Do the different measures correlate with one another? Similarly, one

may ask if there is a high degree of inter-rater reliability on the various measures.

Inter-rater Reliability

When utilizing the technique of hand scoring object interaction times, researchers often employ multiple raters that are blind to the experimental conditions. The scores of the multiple raters are typically averaged in order to reduce the influence of individual subjectivity and bias, and to provide more accurate measures of the variables in When utilizing such a technique, question. researchers will also typically assess the inter-rater reliability of the raters themselves. Instructors could divide students into pairs, and have each member of the pair individually score the videos and calculate the time spent exploring both objects at training, both objects at test, the familiar object at test, and the novel object at test, as well as the percent preference for the novel object for each rat. Students could then be asked to assess the degree of inter-rater reliability of their pair by performing Pearson correlations (or

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some other appropriate statistic) on the sets of scores generated for each of the principle measures.

Experimenter Bias

While I have provided in Table 1 (as well as on the webpage containing the video clips) a key that informs readers of which objects served as novel and familiar for each of the subjects, instructors could choose to withhold such information from their students. Indeed, if instructors wish to reduce the likelihood of bias in their students' scoring of the various clips, they may wish to have them score the testing clips before the training ones. This would prevent the students from knowing which object was the novel and which was the familiar for any given subject. Alternatively, it may be interesting to have half of the students score the training clips before the testing ones, while the other half scores the clips in the reverse order. This would allow for an examination of the possible effects of experimenter bias and expectancies on the scoring, and could lead to some interesting class discussion on the topic.

The lead author of this report has recently employed the videos in an even demonstration of expectancy effects. instructing students on the workings and typical findings of the novel object recognition task, students were asked to score only the test session videos (omitting rat # 3). Half of the students were provided the key informing them which objects were novel and which were familiar prior to hand-scoring the clips for object interaction. The remaining students scored the clips blindly and were given the key only after they had scored the videos. Each student then calculated a percent preference for novelty score for each rat, and averaged these scores for the entire sample (again, omitting #3). The overall average percent preference for novelty score of those students that were given the key prior to scoring the clips ended up being several percentage points higher than that of those that had scored the clips blindly, thus allowing for the discussion of possible expectancy effects. The only problems encountered when running this activity were technical in nature. Students were required to view the clips on their own laptops, and many encountered problems relating to missing plug-ins and/or otherwise outdated versions of internet browsers and/or video players.

Author Note

We sincerely hope that instructors find this online video resource of use in their classes. Instructors should feel free to use this resource as

they see fit to achieve their instructional/classroom goals (again, the clips are not appropriate for research purposes {as explained above}). We would be very interested in learning about how you utilize these videos in your own classes, and intend to post any additional classroom activities submitted by readers to the video resource webpage itself. If you would like us to share your classroom activity in this manner, please email a description of the activity to Dr. Matthew J. Anderson at mander06@sju.edu.

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