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Rodent Research

Wistar rats do not show preference for either of two commonly used nutritionally sound food rewards in a T-maze

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ABSTRACT

Food rewards are commonly used as positive reinforcement in rodent behavioral experiments. Bioserv dustless precision pellets and Noyes formula P precision pellets are both used for this purpose in behavioral experiments in multiple laboratories, as they are nutritionally consistent with standard laboratory diets. Because of the nutritional value, they are superior to other positive food rewards such as chocolate. Whether male Wistar rats prefer either of these pellets was tested with a T-maze choice test, because Noyes formula P precision pellets could no longer easily be purchased in Europe. Rats did not show preference for either Bioserv dustless precision pellets or Noyes formula P precision pellets. Concluding, both pellet types can be used reliably as positive reinforcement in behavioral experiments. We advise against repeating of experiments replacing one of these pellet types with the other, to reduce the number of experimental animals needed.

Introduction

Food rewards are commonly used as positive reinforcement in rodent behavioral experiments. Rats and mice will quickly adapt their behavior for rewards such as chocolate (Naqshbandi et al., 2007), cheese (Evenden and Ko, 2007), chocolate chip cookies (Evenden and Ko, 2007), almond (van der Kooij et al., 2010), and cereals (Cohen and Gotthard, 2011). These types of food rewards have the disadvantage that animals can become nutritionally deficient, particularly as animals are usually on a moderately restricted diet to motivate them to perform the behavioral tests. Therefore, several companies have developed food reward precision pellets for use in automated feeders. These precision pellets are nutritionally consistent with (sweetened) standard laboratory diets (Baum, 1991), fulfilling species-specific nutrient requirements. Experimenters using commercial precision pellets thus do not need to be particularly concerned about possible adverse impact of the food rewards on the nutritional health of their animals.

Three main psychological components of reward can be distinguished: 1) the stimulus-action-consequence relationship; 2) the hedonic consequence of reward consumption; and 3) the motivation to learn and act (Berridge and Robinson, 2003). The type of reward used in behavioral experiments influences the second and third component, and thereby indirectly on learning the first. Thus, if food reward is used as a tool to assess behavior and cognition, it is important that the type of reward is kept constant within and between experiments.

Commercially available precision pellets have been used in behavioral tests comprising maze tasks (Denk et al., 2005), large arena tasks, (Salvetti et al., 2014) and operant tasks (Leenaars et al., 2013). Two types of food rewards for rats that have been used regularly are Bioserv dustless precision pellets (45 mg; BioServ, Frenchtown, USA) and Noyes formula P precision pellets (45 mg; Research Diets, New Brunswick, USA). The composition of these pellets is provided in Table 1 in the methods section. The 45 mg pellets are the standard size for use in rat skinner box food

This work was performed at the Netherlands Institute for Neuroscience in 2006. We recently received a grant for publishing these negative results, from a funding scheme dedicated to encouraging responsible use of laboratory animals.

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Table 1
Composition of the pellets

	BioServ dustless precision pellet	Noyes formula P precision pellets
Ingredients	Dextrose, sucrose, casein, fiber, corn oil, corn syrup, choline bitartrate, mineral mix, vitamin mix, flowing agents.	Sucrose, casein, maltodextrin, corn starch, corn oil, minerals, silicon dioxide, vitamins, magnesium stearate, DL-methionine.
Protein	18,80%	17,90%
Fiber	4,60%	4,90%
Fat	5,00%	4,90%
Ash	4,40%	4,10%
Carbohydrates	61,50%	66,80%
Energy	3.68 kcal/g	3.48 kcal/g

dispensers. The Noyes formula P pellets were highly preferred over Noyes FP (sucrose-fruit punch) and Noyes AI (grain-based, comparable with laboratory chow) pellets (van der Plasse et al., 2007).

Our laboratory, performing behavioral experiments in male Wistar rats, had to transfer from Noyes formula P precision pellets to unflavored purified Bioserv dustless precision pellets as the formula P pellets were no longer available from our preferred supplier. As far as we know, most (but certainly not all) EU behavioral laboratories have transferred to Bioserv pellets. Noyes precision pellets are still available from TestDiet (www.testdiet.com; 5TUL, 5TUT, and 5TUM) and also used by US behavioral laboratories (Wadhera et al., 2017).

Before making the transfer, we needed to know if these rewards were equally motivating for Wistar rats. In literature, we only found one study testing motivation to press a lever on a variable interval (30 s)—fixed ratio (20 lever presses) schedule for both pellet types in four male Long-Evans rats (Baum, 1991). This underpowered study concluded that the motivation for both pellets is generally equivalent. The present study examines Wistar rat preference for either pellet type in a T-maze.

For this study, the term “preference” indicates a difference in motivation to acquire one reward over another. Preference between rewards can be measured with choice tests (Kirkden and Pajor, 2006, Habedank et al., 2018). In choice tests, animals are required to make repetitive discrete choices between alternative rewards. To result in valid outcomes, the rewards should not vary in more than one dimension, that is, the size and nutritional value should be equivalent. Alternatively, preference can be measured with operant tests (Kirkden and Pajor, 2006). However, these are complex and depend strongly on the animal’s motivation to perform the task (Cooper and Mason, 2001). The advantages of choice tests for unidimensional substitutes are that they allow animals to express their preference directly, which implies that they are relatively easy to interpret, and that they can be performed relatively quickly compared with other types of motivational tests (Kirkden and Pajor, 2006). Moreover, choice tests are relatively comparable with food preference testing in humans (Leenaars et al., 2016). A general disadvantage of this type of testing is that it is virtually impossible to distinguish an absence of preference from not being able to learn which alternative option is where. However, because of the intelligence of rats, it is likely that they do learn these types of tasks.

Other studies have successfully used the T-maze to test for rodent food preference (van der Plasse et al., 2007, Wadhera et al., 2017, Al’bertin, 2018, Correa et al., 2016, Capaldi et al., 1989, Buckley et al., 2011); for other types of preference testing (Hernandez-Lallement et al., 2014, Marquez et al., 2015, Karimi et al., 2017, Yohn et al., 2017, Mayeux-Portas et al., 2000); and for cognitive testing (van den Bos et al., 2012, Acevedo-Triana et al., 2017, Dławichowska and Lukaszewska, 1986, Marquis et al., 2008, Nocjar et al., 2007, Capaldi et al., 1992, Mendelson, 1966). The objective of this study was to determine the possible preference of male Wistar rats for Noyes formula P precision pellets versus Bioserv dustless precision pellets with a choice test in a T-maze.

Materials and methods

This study was carried out in accordance with Dutch legislation and European guidelines. The protocol was approved by the experimental animal committee of the Royal Netherlands Academy of Arts and Sciences (P_NIN2006-07).

We used 10 male Wistar rats (Harlan, Horst, the Netherlands; weight on arrival 250-300 g, estimated age 7-8 weeks), the most commonly used species and sex of rat in behavioral neuroscience. They were housed under reversed light (L:D 12:12; lights ON at 19:00) in groups of 5 in type-IV Makrolon (polycarbonate) cages (60 × 38 × 20 cm) with corresponding stainless steel wire covers containing the water bottle, and standard laboratory chow during the habituation phase. Before and between experimental sessions in the T-maze, rats were group-housed to prevent the stress induced by social isolation, that is, to increase their welfare. We did not expect the social interactions in the home cage to affect pellet preference. We provided standard sawdust for bedding and a shelter (25 cm of PVC tubing, cut through lengthwise to create a shelter 12 cm high) for cage enrichment. Rats 1-5 were housed together in one cage and rats 6-10 in a second cage. The cages were placed next to each other in the NIN animal house, in a room within conventional (i.e., non-SPF) facilities, with controlled temperature (20°C ± 2°C) and relative humidity (60% ± 20%). They were replaced weekly by clean cages, always on Fridays. When changing cages, one hand of sawdust from the old cage was transferred to the new cage to increase familiar odors.

The experiments were performed in 10 male Wistar rats, using a within-subject crossover design described below and in Table 2. As

Table 2
Overview of the experimental procedure

Day	Test	# Pellets per arm per trial	# Trials	Trial type
1 (Monday)	Habituation	4	3	Open
2 (Tuesday)	Habituation	2	10	Open
3 (Wednesday)	Side preference	2	12	Choice
4 (Thursday)	Pellet preference 1, Even-numbered rats get Noyes pellets on the left; odd-numbered rats get Noyes on the right	2	2 + 17	Open + Choice
5 (Friday)	Pellet preference 2, Even-numbered rats get Noyes pellets on the right; odd-numbered rats get Noyes on the left	2	2 + 17	Open + Choice

Rats were always tested from 1 to 10 sequentially. Rats 1-5 were housed together in cage 1 and rats 6-10 in cage 2. On open trials, doors stay open. On choice trials, the door closes after a rat enters an arm. On day 4 and 5, rats started with 2 open trials, exploring both arms and eating all pellets, to learn which pellet was on which side (disregarded from all analyses).
The number of trials per day and the number of pellets per trial is based on experience within our laboratory. With experience, rats move faster through the maze, allowing for larger numbers of trials per day.

this was a pilot experiment, for which power analyses were not required at the time, no *a priori* power analysis was performed; the sample size was based on preceding comparably designed experiments at the Netherlands Institute for Neurosciences (Amsterdam, mainly unpublished). The comparison of three different Noyes pellet types mentioned in the introduction was performed in 8 male Wistar rats (van der Plasse et al., 2007). Other types of T-maze testing in our institute used a sample size of n = 5–8 rats per experimental group (Sanchez-Santed et al., 1997). Our sample size is also comparable with that used in more recent T-maze experiments from other groups (Al'bertin, 2018, Fatahi et al., 2018, Yohn et al., 2017). We do strongly recommend power analyses to be performed *a priori* for any future experiment.

Rats were monitored daily for welfare; they were observed in the home cage for any visible indicators of lack of wellbeing (e.g., piloerection, poor grooming, lethargy, discharge from nose or ears). Rats were left undisturbed for one week after arrival for acclimatization. They were habituated to daily handling for another week before starting experiments.

Food restriction to 16 g/rat/day as described before (van der Plasse et al., 2007) was started three days before first behavioral testing. This amount of food was generally used in our laboratory when performing behavioral experiments, to keep the rats motivated to perform tasks for food reward, while maintaining them at or over 90% of their free-feeding weight (e.g., van der Plasse et al., 2007). On the test days, the 16g of chow is supplemented with the pellets (Table 1) eaten on the maze (12-38 pellets, refer to Table 2). We did not adapt the amount of chow provided for this experiment, as only small numbers of pellets can be earned in testing (12-38 pellets of 45 mg each, i.e., 0.54–1.71 g if all trials are completed). The maintenance of 90% of the free-feeding weight was confirmed by daily weighing on experimental days. When rats were on food restriction, chow was provided toward the end of the dark phase, at least one hour after testing, to avoid learning to anticipate food during the test. Plain tap water was provided in the home cage from standard laboratory rat water bottles with metal drippers. Water was unrestricted throughout the experiment, except during the actual testing when rats were taken out of the home cage.

The protocol comprised habituation to the T-maze for two subsequent days. This was followed by control testing for possible side preference and testing for possible pellet preference on the three subsequent days. We implemented the side preference testing and used a crossover design for pellet preference testing as we were concerned about potential side preferences obscuring a pellet preference effect. While we recommend this strategy for preference testing, when using a T-maze for memory tests, the reward in each arm should usually be kept consistent.

Rats were always tested in order from 1 to 10 sequentially, during their dark phase, when they are naturally active. An overview of the experimental protocol, including the number of trials per day, is provided in Table 2.

T-maze and habituation

The T-maze had been made by our mechanics workshop. It consists of three equal arms of 34 cm long, 9.5 cm wide, and 19.5 cm high with one start-arm at a 90° angle of the lateral arms. Both lateral arms could be closed with remotely controlled doors. The end of each lateral arm contained a metal cup for pellets. The test room was located on the same floor as the rats were housed. It was lit with four 120-Watt spots resulting in roughly equal light intensity throughout the T-maze. The T-maze was cleaned with 70% ethanol after each trial to prevent odor transfer. All testing was performed during the dark phase.

In the first two days of the experiment, rats habituated to the T-maze. Each rat was rewarded with both pellet types in the maze: Bioserv dustless precision pellets (45 mg; BioServ, Frenchtown, USA) and Noyes formula P precision pellets (45 mg; Research Diets, New Brunswick, USA) mixed on both sides. The composition of Bioserv dustless precision pellets and Noyes formula P precision pellet types is shown in Table 1. The doors were not used during habituation. Pellet types were mixed during the habituation and the side preference test, to prevent the establishment of side preferences before the start of pellet preference testing, as this could result in confounding and bias.

For each trial, the rat was placed in the start arm and left in the maze to explore until it had visited both arms, ate all the pellets, and no longer showed increased locomotor activity or rearing (novelty response). No formal criteria were used to evaluate the novelty response. However, after exploring the maze and eating the pellets, rats mostly started washing or just stayed in one place. On day 1, four pellets were provided in each lateral arm during each trial, two of which were placed on the maze arm before the metal cups. From day 2 onward, this was reduced to two pellets per arm in the metal cups only, as rats generally explore the maze faster on the second exposure. The number of trials per day and the number of pellets per trial is based on experience within our laboratory. With experience, rats move faster through the maze, allowing for larger numbers of trials per day. During habituation, we provide 4 pellets per arm per trial. As of day 2, we only provide 2 pellets per arm trial.

Side preference

We examined if the rats had any spontaneous side preference before the onset of preference testing on the third day of the experiment. In the side preference test, the door closed after entering an arm in 12 sequential trials. The rat in the maze was still rewarded with mixed pellets, one of each type on each side. The number of choices for each lateral arm was registered to determine potential side preferences.

If rats did not choose an arm within two minutes after starting the trial, the trial was ended. Side preference for the right arm was expressed as a fraction of the total number of completed trials.

Pellet preference

Pellet preference was tested on day 4 and 5 of the experiment in a crossover design. Each day started with two open trials (no doors) in which rats could freely explore the maze and find 2 pellets of one type in each lateral arm. The open trials were excluded from all analyses. These two exposure trials were followed by 17 choice trials in which the door closed after the rat entered a lateral arm.

Noyes pellets were on the right side of the T-maze on day 4 (referred to as "order A") for five rats (rat 1, 3, 5, 7, and 9); they were on the left side for the other five (referred to as "order B"). The side on which each pellet type was provided was changed for each individual rat on day 5 compared with day 4, resulting in a fully crossed design. We used alternation for the order of receiving each pellet type on each side of the maze instead of randomization. This way, the order is perfectly counterbalanced. We preferred this perfect balance over chance-induced imbalances, which may occur with randomization of small numbers of animals. This could result in unwanted baseline differences between the groups. The combination of this alternating order and the crossover design should prevent potential side preferences from affecting the observed pellet preference.

If rats did not choose an arm within two minutes after starting the trial, the trial was ended. Pellet preference for Noyes pellets was expressed as a fraction of the total number of completed trials.

Statistical analysis

Data are presented as average values ± standard error of the mean (SEM), unless otherwise stated. Statistical analyses were performed with SPSS (Chicago, USA). Differences were considered significant at $P = 0.05$.

For side preference, all completed trials on day 3 were included. For pellet preference, all completed choice trials on day 4 and 5 were summed and included. Our outcome, preference, was expressed as a fraction of the number of completed trials to include all rats in the analyses. Analyzing fractions or percentages of choices is a common procedure for T-maze experiments (Al'bertin, 2018, Fatahi et al., 2018, Karimi et al., 2017, Wadhera et al., 2017).

The experimental unit was the single rat. Deviation from the normal distribution of the preference data expressed as a fraction of choices was tested with a Kolmogorov-Smirnov test. Fraction of choice data did not statistically deviate from the normal distribution ($D = 0.23$; $P = 0.13$ for side preference, $D = 0.15$; $P = 0.20$ for pellet preference, $N = 10$ for both). While the Kolmogorov-Smirnov test may not be too informative for a sample of $n = 10$, we did not have reason to expect deviation from the normal distribution (based on visual inspection of the data) and used parametric statistical tests for our primary analyses. Besides, we analyzed the separate sequential choices in sensitivity analyses.

Side preference and pellet preference were analyzed separately. The presence of a side preference was tested with a one-sample two-tailed T-test comparing the fraction of choices for the right arm with the expected average of 0.5, comparable with the approach used by, for example, Wadhera et al. (2017) and Marquez et al. (2015).

We performed one sensitivity analysis (an analysis that was designed after the results from the primary analysis were available) for side preference: a binomial regression of all the choices on 12 individual trials per rat overall with an intercept-only model. This generalized linear mixed model (GLMM) included rat number as the subject variable, choice as the dependent variable (right arm as the response, left arm as the reference), and individual trials as repeated measures.

In the absence of an overall side preference, the presence of pellet preference was tested with a one-sample two-tailed T-test comparing the fraction of choices for Noyes formula P precision pellets with the expected average of 0.5. We performed two sensitivity analyses for pellet preference. The first is a two-tailed T-test comparable with the main analysis excluding the two rats that omitted trials. Our second sensitivity analysis was a binomial regression of all the choices on 2×17 individual trials per rat overall with an intercept-only model, comparable with the GLMM (Noyes as the response, Bioserv as the reference) for side preference.

With logistic regression, an intercept of 0 (the logit of 0.5) reflects a fraction of choices equal to 0.5. We report the output on the logit scale as provided by SPSS.

Results

Side preference

One rat (rat 6) missed one trial, otherwise all trials were completed. Of 12 trials, rats chose the right arm of the maze 1-10 times (range). All rats entered both arms at least once. The average choice percentage for right versus left was 58.1 (± 7.4%). Side preference was not statistically different from chance ($T_{(9)} = 1.1$; $P = 0.30$). The sensitivity analysis for the choices on the individual trials showed similar results; the intercept was 0.32 (95% confidence interval [CI] −0.25−0.89; $P = 0.27$).

The number of rats choosing the left arm in the consecutive trials is provided in Supplement Table S1. Choices made by individual rats are provided in Supplement Table S2.

As the pellets were offered on both sides of the maze for each rat on the subsequent test days, side preference was not taken into account for further analyses.

Pellet preference

All rats completed all open trials (no doors) on both days of pellet preference testing. One rat (rat 6) did not complete 11 trials on day 4 of the experiment; all other rats completed all day 4 trials. Two rats did not complete all trials on day 5: rat 5 missed 6 trials; rat 6 missed 10 trials. Of 34 choice trials (over 2 days), rats chose Noyes over Bioserv pellets 7-20 times (range). All rats chose Noyes pellets at least 7 times and Bioserv pellets at least 6 times. Average choice percentage for Noyes formula P precision pellets versus Bioserv dustless precision pellets was 48.0% (± 2.2%). Pellet preference was not statistically different from chance ($T_{(9)} = -0.91$; $P = 0.39$). Our post hoc power to determine a 10% difference in the fraction of choices for Noyes pellets from the theoretical 50% with an $\alpha = 0.05$ was 0.98 (determined with GPower 3.1 for a two-tailed T-test [one sample case; difference from constant], based on the observed standard deviation).

The first sensitivity analysis excluding rat 5 and 6 (the two rats not completing all trials) showed similar results (average choice percentage for Noyes: 47% (± 2.7%); $T_{(7)} = -0.96$; $P = 0.37$). The second sensitivity analysis for the choices on the individual trials also showed similar results; the intercept was −0.096 (95% CI −0.269−0.077; $P = 0.28$).

Choices made by individual rats are provided in Supplement Tables S3 and S4.

Discussion

Here, we show that male Wistar rats do not have a statistical preference for either Bioserv dustless precision pellets or Noyes formula P precision pellets when both of them are provided in a T-maze choice test on two subsequent days. This is the first study directly comparing these reward pellet types in a T-maze; there is no sign of a difference in preference.

The only other study that we are aware of that directly compared these two commonly used pellet types is the study by Baum (1991). While his $n = 4$ study is most likely underpowered, he concluded that overall the Noyes and Bioserv pellets were preferred equivalently by male hooded Long-Evans rats, using variable interval operant tests. The present study found no overall preference for Noyes or Bioserv pellets in male Wistar rats in a T-maze, with a power of 0.98 for a 10% difference. Our sensitivity analyses are consistent with our main analyses. While the number of rats tested may be considered low, which could result in increased chances of a type-II error, we are confident that there is no difference in preference between Noyes and Bioserv pellets because of the high (post hoc determined) power, the consistency of the planned and sensitivity analyses, and the absence of clear pellet preferences in individual rats (Supplementary Tables 1 and 3).

Of note, Baum (1991) suggested that under certain circumstances, differences between these pellet types may occur because 1) the pellets can clearly be distinguished by sight, smell, and taste; 2) the BioServ pellets soften more than the Noyes pellets during use; and 3) three of his four rats possibly showed a minimal preference for BioServ pellets. In Baum's study, high humidity softened the pellets, and they were occasionally crushed in the dispenser. In our experiment, we used pellets straight from the airtight containers provided by the suppliers, in a humidity-controlled

laboratory. The pellets can be distinguished by smell and taste, but rats did not show a clear preference for one over the other. Because humidity may affect the preference and the rewarding value of pellets, for future studies, we recommend reporting of the type of food storage and the frequency of replacement in home cages and automated dispensers.

We are unaware of studies comparing rodent preference for commercially available pellet types on a T-maze or otherwise, other than the already discussed studies by Baum (1991) and van der Plasse et al. (2007). Of note, searching for preference tests using the T-maze paradigm in PubMed is challenging, as no Medical Subject Heading is available for the T-maze paradigm. Searching for "maze learning" results in many irrelevant hits, mainly for the Morris water maze, the elevated plus maze, and the radial maze. In Embase, the Emtree term "T-maze test" combined with the Emtree term "food preference" only resulted in 2 hits (09 April 2018) (Al'bertin, 2018, Correa et al., 2016). In PsycInfo, the combined psychological index terms "T mazes" and "Food Preferences" only resulted in one hit (December 2018) (Buckley et al., 2011). Because of these challenges in searching, our discussion of the literature on food preference testing using T-mazes may be incomplete.

Strengths of our design are first, the high power, second, the a priori confirmation that on average rats did not show side preference before pellet preference testing, and third, the fully crossed design counterbalancing for possible confounders. Our sensitivity analyses were consistent with the main analyses. The GLMM modeling approach used in two of them has an advantage over the T-test for a fraction; all available individual trial data are used in the analyses. Besides, for larger studies, this type of approach allows for including additional predictors.

A weakness in our design is the lack of blinding of the experimenter who handled the rats and operated the doors. While we did not have a priori assumptions on rat preference for either type of pellet, unconscious bias could have affected the results. We do not find a preference for either pellet type. Another weakness in our data is the number of omissions; rat 5 and 6 did not complete all trials. As these rats did complete the first trials of each session, indicating that they did understand the task and were willing to start, this is most likely due to a lack of motivation to work for food. Excluding these data from the analyses did not alter our results.

A restriction to the external validity of these data arises from using male rats only; we cannot extrapolate to female rats with certainty. As common in the neurosciences, at the time, our laboratory exclusively used male animals. Unfortunately, restricting to male animals still seems common practice in recent T-maze experiments (Al'bertin, 2018, Correa et al., 2016, Fatahi et al., 2018, Karimi et al., 2017, Wadhera et al., 2017, Yohn et al., 2017). Many scientists argue that using male animals only is preferable because they lack an estrous cycle and are therefore less variable in their physiology and behavior. For mice, this can no longer be defended, as a meta-analysis of 293 studies shows that variability in behavioral, morphological, physiological, and molecular traits was not significantly greater in females than in males (Prendergast et al., 2014). For future experiments, we highly recommend a mixture of both sexes.

No difference in preference was observed between Noyes formula P precision pellets and Bioserv dustless precision pellets in male rats from two stocks as shown in two separate studies from two unrelated groups. For studies in male rats, data from experiments using either pellet type can be pooled for analyses if the study has used a well-balanced design. While changing from one type to the other cannot be recommended within subjects because unfamiliarity may affect the results, changing pellet type between subjects (between experiments or within long-running experiments) should not affect study outcomes.

While these findings remain to be confirmed for female rats and for other species, to the authors, careful extrapolation of the findings seems reasonable. As long as a counterbalanced experimental design has been used, repeating measurements or even entire experiments when changing from one of these pellet types to the other is thus not necessary. Whenever possible, repetition of experiments should be avoided to reduce both the number of experimental animals used and the waste of research resources.

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The data set generated and analyzed during the present study is fully provided in this article and the corresponding supplement.

Conflict of Interest

The authors declare that they do not have any competing interests.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jveb.2019.01.007.

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