Reinforced Variability Enhances Object Exploration in Shy and Bold Rats

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Abstract

In an open-field test, the Long-Evans (LE) strain of rats was identified as "bold" and the PVG strain as "shy." Some members of each strain then experienced 14 sessions of a common enrichment procedure, namely exposure to a series of novel objects (Exposed). Others in each strain were explicitly reinforced with food pellets for variable interactions with the same objects (Reinforced). Both experience and strain influenced object interactions. In particular, Reinforced rats interacted more variably with the objects – contacting, probing, pushing and so forth – than did the Exposed; and LEs interacted more variably than PVGs. Foraging proficiency in the same rats was then studied in a transfer-of-training test. Food pellets were hidden among never-before experienced objects and the rats were permitted to explore freely. Reinforced rats discovered and consumed more pellets than Exposed; and LEs discovered and consumed more than PVGs. Thus a bold genetic strain and reinforcement of variability independently contributed to successful foraging behavior.

Keywords: Reinforced variability; Shy - bold; Foraging; Novel objects; Enrichment; Open field

Highlights:

* Reinforcement increases variable responses to novel objects
* Bold rats respond more variably to novel objects than shy rats
* Reinforcement of variability potentiates enrichment experience
* Reinforcement of variability improves foraging proficiency

1. Introduction

Rats will often approach and explore novel objects, a reaction common to many species [1]. However, not all rats explore equally, and strains of rats, as well as individuals within a strain, differ [2,3]. This, together with other related findings, has led researchers to characterize some animals as 'bold,' or those who readily approach and explore spaces and objects, and others as 'shy,' or those who tend to be avoidant [4,5]. In the present study, rats from bold and shy strains are explicitly rewarded for variable interactions with novel objects. Of particular interest is whether reinforcing response variability will cause shy animals to approximate behaviors that characterize bold.

1.1. Three questions

We asked three questions: First, what are the effects of variability reinforcement on rats' explorations of novel objects? Second, does variability reinforcement influence strain differences? Third, does a history with variability reinforcement affect foraging-type discoveries in new contexts, i.e., a question of transfer of training? The background for each of these questions will be discussed.

1.2 Reinforcement of variability

Operant response variability is controlled by reinforcers contingent upon the variability [6]. Such control is analogous to the effects of reinforcers on response rates, latencies, forces, and topographies and has been demonstrated for many types of responses and across many different species, including lever-press sequences in rats, key-peck interresponse times in pigeons, saccade eye movements in adult humans, block building in children, and verbal replies in individuals with autism (see [7] for a review). Two studies are most relevant to the present research. First, Pryor, Haag & O'Reilly [8] rewarded porpoises for variable body movements, including flips, turns, jumps and the like. With continued training, the porpoises came to generate responses that had never before been observed in any porpoise. The second study was an unpublished undergraduate senior thesis by Arnesen [9] in which one group of rats was rewarded (with food pellets) for variable responses to novel objects and a second group received pellets independently of their behaviors. The groups differed significantly in a later test of their ability to discover hidden pellets (see Section 6).

1.3 Shy-bold strain effects

Shy strains of rats (also referred to as fearful or neophobic) are less likely than bold (fearless or neophilic) to explore a novel space or objects and more likely to show signs of anxiety, e.g., low levels of interactions, thigmotaxis, and frequent defecations [10]. We compared two strains, the PVG strain that has

been characterized as shy and Long-Evans (LE) as bold. We know of no direct comparison between the PVG and LE strains, but studies have compared each to a common third species, e.g., Sprague- Dawley (SD). For example, Schmitt & Hiemke [11] placed two novel objects in the center of an open field and permitted PVGs and SDs to explore during a 10 min test. The PVGs were less likely to enter the center of the field, less likely to approach the novel objects, less likely to move actively, and defecated more. In another experiment, when PVGs and SDs were exposed to a cat in an adjoining chamber, the PVGs showed greater fear – they were more likely to freeze in place – than the SDs [12]. LE's, on the other hand explored more than SDs and approached novel objects more [13]. LEs were also more likely to approach and manipulate a newly experienced lever than SDs [13]. Importantly, the PVGs' hesitancy to explore was not due to physical incapacity: Biesiadecki [14] found that PVGs outperformed a number of other strains on measures of physical dexterity and strength [15]. The present experiment asked whether extrinsic reinforcement of variable object-directed responses would affect shy PVGs and bold LEs differently.

1.4 Hidden Food Test (HFT)

After some rats had been reinforced for responding variably to objects, Arnesen [9] tested all rats in a room containing 25 never-before experienced objects, with food pellets hidden within or under each. The HFT combines aspects of laboratory-based foraging procedures, e.g., radial-arm mazes where rats must vary their choices of location in order to obtain a food reinforcer, with food-puzzle type procedures common with caged primates, where an animal must figure out how to gain access to a hidden or difficult-to-obtain food reward. Arnesen [9] found that the rats that had been reinforced for variable responses spent more time exploring new objects and discovered more food pellets than the control rats. Because the test was given in a novel context and the objects differed from those used during training, the HFT provides an index of generalization or transfer of training.

2. Experiment 1: Open Field

2.1 Introduction

The open field test assesses rodent emotional and exploratory responses [10] and we used it to compare LEs and PVGs. As is common, the behavior of an individual rat in an unfamiliar open space was observed for 10 min. The three main dependent variables were time spent in the center of the field, frequency of rearing, and time spent moving overall. Animals that choose to spend time in the center of the open field, rear on their hind legs to visually explore the space, and move frequently are generally described as bold, a characterization supported by additional tests, such as in the elevated-plus maze [11]. Because we initially placed each rat in the center of the open field, we additionally recorded durations of initial freezing responses.

2.2 Methods

2.2.1. Animals

Fourteen Long-Evans (LE) and 14 PVG rats, all females, were obtained from Harlan Sprague-Dawley at three to four weeks of age. Each strain was comprised of seven littermate sister pairs from different litters. The sisters were housed together (one sister pair per cage) with tail markings distinguishing the two individuals, maintained on a 12-12 light/dark schedule with free access to food and water, and handled daily. When approximately nine weeks old, they were tested in the open-field.

2.2.2. Materials

The open field consisted of a wooden box, 69 x 91 cm, with 20 cm high walls and Plexiglas tops. The floor was covered with grey linoleum on which a 15 cm square grid pattern was marked in gray. Two identical open-field chambers were located side-by-side, although completely separated from one another, to permit video recording with a single camera.

2.2.3. Procedure

Each rat was placed in the center of an open-field chamber with sisters in adjoining chambers and permitted to remain for 10 min. We recorded times spent in the center and periphery; frequency of rearing responses; and time spent moving, assessed by crossings of the grid pattern. The "center" was defined as the four squares centrally located, with the 12 surrounding squares representing periphery (see Figure 2). Chamber floors were cleaned with a 70% alcohol/30% water solution after each rat was removed to eliminate odor cues with approximately 5 min elapsing between tests to permit the chambers to dry.

3. Results and Discussion

Upon being placed in the center of the open field, all rats froze and remained unmoving with all 4 paws contained within the center 4 squares. LEs froze for an average of 11.4 s (SD=10.9) and PVGs for an average of 17.2 s (SD=8.0), a difference that did not reach statistical significance ($t[26]=1.626$, ns). In the remainder of the session, LEs spent more time in the center than did the PVGs, $t[26]=-2.331$, $p=0.028$ (Figure 1A), but unlike the initial freezing responses, later time spent in the center represented voluntary movement. The number of rearing responses was higher for the LEs than PVGs, $t[26]=-3.55$ $p=0.001$ (Figure 1B). The two strains did not differ significantly, however, in time spent moving $t[26]=-1.061$, ns. (Figure 1C). (Levene's test for Equality of Variance indicated that in all three cases, the variances did not differ significantly. Additionally, Welch's t test resulted in essentially the same significance levels).

Therefore, on two common measures of the shy-bold axis –choosing the central part of the open field and rearing – LEs were assessed as relatively bold and PVGs as shy. The absence of difference in movement is consistent with studies reporting that that PVGs outperformed a number of other strains on measures of physical dexterity (Johnson & Mitchel, 2003, Biesiadecki, 1999). These results, which provide the first direct comparison of LEs and PVGs, are consistent with the characterization of LEs as bold and PVGs as shy.

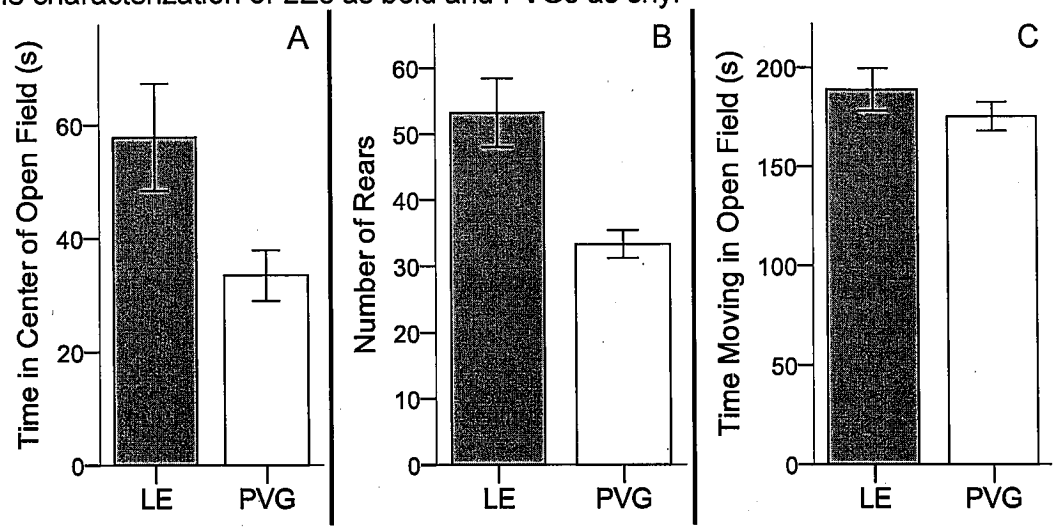

Fig 1.

4. Experiment 2: Reinforcement of Variable Responses to Novel Objects

4.1. Introduction

Pairs of LE and PVG sisters from Experiment 1 were randomly divided into two groups, with one sister rat reinforced with food pellets for variable interactions with novel objects (Reinforced group) and the other sister provided access to the same objects for the same duration, and given the same number of food pellets in the chamber at the beginning of each session (Exposed group). The overall design was a 2 x 2 comparison of how strain (LE vs. PVG) and experience (Reinforced vs. Exposed) affect object interactions.

4.2. Methods

4.2.1. Animals

Rats and housing were the same as in Experiment 1 but now the rats were maintained under a 22- to 23-h food deprivation regimen with food freely available for one hour per day, this period following experimental sessions when those were provided.

4.2.2. Materials

Experimental chambers were the same as in Experiment 1, i.e., the open-field boxes. A Gerbrands feeder providing 45-mg food pellets was attached to the front wall of each chamber.

Fig 2

4.2.3. Habituation to apparatus

Five 10-min habituation sessions, one per day for each rat, followed completion of Experiment 1. During each of the first four of these sessions, 20 45-mg Noyes formula "A/I" food pellets were placed in a small bowl located under the pellet-dispenser. The chamber was otherwise empty. During these sessions, some rats did not consume the pellets and formula P pellets were substituted during the fifth session. These were readily consumed by all rats and used in all remaining sessions. Sister rats were simultaneously run in the side-by-side, identical chambers throughout this experiment.

4.2.4. Training

The training phase consisted of 14 10-min sessions, one per day, during which each rat experienced 10 different objects. A single object was present during each session. Table 1 describes the objects in the order experienced by all rats. The first object was presented for 3 sessions, the second and third objects were presented for 2 sessions each, and the remaining 7 were presented for one session each. One member of each sister pair was randomly assigned to the Reinforced condition, the other to the Exposed condition, and these assignments were maintained throughout the experiment. At the start of each session, the sisters were placed in the side-by-side chambers with identical

objects in the center of each (Figure 2). The rats were placed midway between the object and the pellet-dispenser front wall, facing the object.

4.2.4.1 Reinforcement of Variable Responses (Reinforced)

Seven LEs and seven PVGs were reinforced with food pellets for variable responses directed at the target object. The rats were initially reinforced for orienting toward, visually inspecting, approaching and sniffing the object, then contacting it with front paws or nose. A mirror was set at an angle against the laboratory wall that allowed the first author to view the rats (without hovering above them) and to dispense pellets in real time contingent upon their responses. As the 10-min session proceeded, reinforcement was made contingent upon more active engagement with the object, such as pushing it, rearing and leaning on it, biting or chewing it, and entering or probing hollow parts of the object. Throughout this training, a variability contingency was in effect such that a maximum of 3 repetitions could be reinforced across a ~2-min moving window. As with all shaping procedures, each animal experienced a training sequence tailored to the individual. With increasing time spent in a session, the variability requirement led to increasingly varied interactions with the objects, such as climbing on or crawling under the object, flipping it over, or moving it across the chamber. The most commonly reinforced responses for each of the 10 objects are shown in the right-hand column of Table 1.

Table 1
Description of objects used in Experiment 2 and common responses.

Object	Description	Examples of Reinforced Responses
Object 1	black rubber cork	Contact with nose/paws, bite or gnaw on edge, push/roll with nose, push/roll with paws, carry in mouth across box.
Object 2	lacrosse ball	Contact with nose/paws, roll ball away from body, roll ball towards body, rear on top of ball.
Object 3	tinker toy	Contact or roll with nose/paws, knock over.
Object 4	open-sided cylinder with lid	Contact or roll with nose/paws, move through space between lids, rear/climb on top.
Object 5	pvc pipe corner	Contact pipe, enter pipe, stand/rear on top of pipe.
Object 6	pellet bottle with holes	Contact outside with nose/paws, stick head through holes into bottle, enter bottle, exit bottle backwards, turn around inside bottle and exit forwards, enter and exit using different pathways through holes.
Object 7	ladder (jungle gym)	Contact ladder with paws, climb up ladder, back down ladder, jump off tower, spin

		tower in arena.
Object 8	cube with twine	Contact cube with paws, drag through arena, kick/toss cube airborne.
Object 9	metal screen tube with natural corks	Contact tube/corks with nose/paws, jump over tube, drag object through arena with mouth/paws.
Object 10	bowl with 8 screws	Contact screws, remove screw from bowl, move bowl, get inside of bowl.

4.2.4.2. Exposure to Objects (Exposed)

During the same sessions that a Reinforced rat received pellets contingent upon its variable interactions, its sister in the Exposed group had access to the same objects in the adjoining chamber for the same time periods. For the Exposed rats, food pellets were placed in the bowl at the front of the chamber prior to each session, with the number of pellets equal to that received by the sister rat during the previous session. (During the first session of this phase, Exposed rats received 30 pellets.) Thus, although the Exposed rats were not explicitly reinforced for interacting with the objects, they had free access for periods equal to that of their Reinforced sister and consumed the same number of food pellets.

In overview, each rat (14 LE's and 14 PVG's) experienced ten different objects. One sister in each pair was reinforced for variable interactions while the other sister was permitted to interact with identical objects for the same periods but without the explicit requirement to vary. All sessions were recorded on videotape for later analysis. Before each session, the floors and objects were cleaned with ethanol/water solution to minimize odor cues.

5. Results and Discussion

The first of two main measures was Object Interaction Time (OIT), or time in contact with the object. The definition of "contact" was limited to the mouth, face area and front paws (so that accidental contacts were excluded, e.g., with the side or rear of the rat's body). Analyses of OIT were based on the last six objects experienced, each for a single session. Figure 3A shows that the Reinforced groups, both LEs and PVGs, spent more than twice as much time in contact with the objects than did their paired Exposed sisters, but that PVGs and LEs did not differ. Analysis of variance showed a significant effect of training ($F[1, 24]=208.0$, $p<0.0001$), but not strain ($F[1,24]=0.248$, ns) or interaction $F[1,24]=0.09$, ns. Thus both shy and bold rats were readily reinforced for interacting with novel objects.

A different pattern of results emerged with respect to variability of the object-directed interactions, as shown by the Object Interaction Variability (OIV) measure. It was based on the relative *in*frequency – or rareness of occurrence --

of the types of responses that the rats made to the objects: the less common a given rat's response, compared to all 28 rats in the experiment (including its own responses), the higher the score. Object Interaction Variability was calculated as follows: For each of the objects separately, the first author reviewed the video records of all 28 rats and defined the types or classes of responses that had been made. For example, in the presence of the PVC pipe, the main response classes observed (across all rats) were contact with the pipe, entry into the open-ended pipe, travel through the pipe, and climbing on the pipe. The *number of rats* (plus 1) that did *not* emit a particular response class became the "diversity score" for that particular class. Thus a rare response (one that only 3 rats emitted) was assigned a higher diversity score (a score [28 – 3+1]=26) than one that 20 rats had emitted (yielding a score of 9). These diversity scores were then assigned to each rat. Note that a particular score could be assigned only upon the first occurrence of a given response. For example, if rat #7 emitted response "C", and C had a diversity score of 11, then 11 would be added to that rat's total. The same rat's score would not change if C were again emitted. Thus, the sum of the diversity scores provided an index of the types, or classes of responses by a rat to an object – with rare classes receiving higher scores than commonly emitted ones. Number of repetitions by any one animal did not influence its OIV. We intended to base OIV on the last 6 objects experienced, as was done for OIT, but before the analyses could be completed, videotaped data from two sessions were accidentally destroyed (sessions 6 and 10) and therefore responses to objects 5, 7, 8, and 9 were used to calculate OIV.

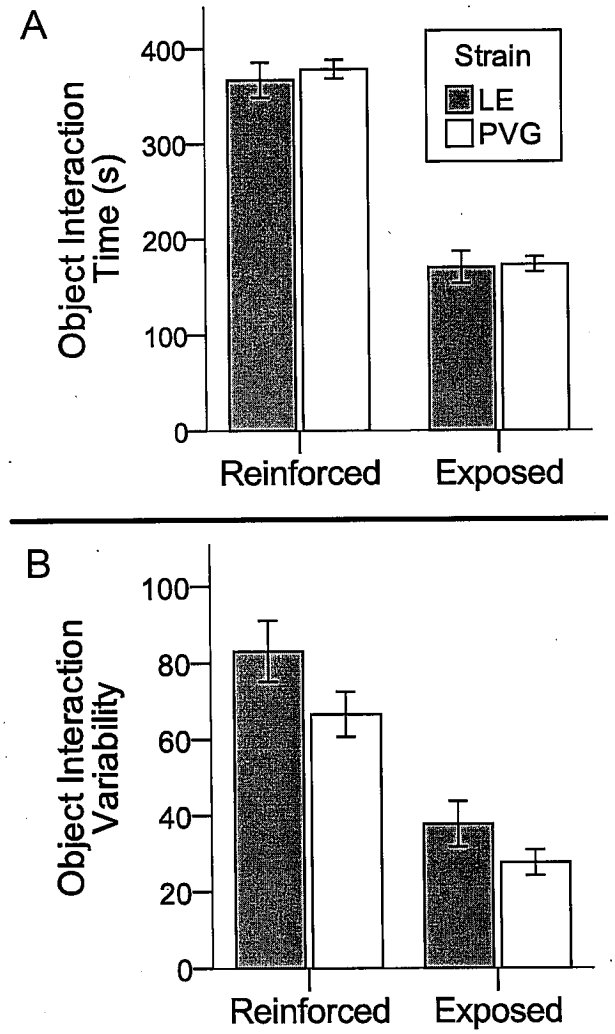

Fig 3

Figure 3B shows Object Interaction Variability averaged across objects 5, 7, 8, and 9. Both training experiences (Reinforced versus Exposed) and genetic strain (LE versus PVG) influenced OIV, with LEs responding more variably (i.e., their responses tended to be unusual ones) than PVGs and Reinforced rats responding more variably than Exposed. ANOVAs support these observations: training, $F[1,24]=48.712$, $p<0.0001$, and strain, $F[1,24]=4,868$, $p=0.037$. As indicated by the figure, the interaction between experience and strain was not statistically significant $F[1,24]=0.28$, ns.

The OIV differences cannot be attributed to number of pellets consumed. In the Reinforced condition, PVGs received an average of 46 pellets per session across the final six objects and the LEs an average of 43.5 pellets; $t[12]=0.773$, $p = 0.454$, ns; and, of course, because of the experimental design, Reinforced and Exposed rats received identical numbers of reinforcers. Note that the OIV measure was based on global rarity of responses whereas the reinforcement-of-

variations contingency was based on non-repetitions in real time, or recency. We conclude that both genetic strain and reinforcement contingencies influenced the variability of rats' explorations of novel objects.

6. Experiment 3: Hidden Food Test

6.1. Introduction

When foraging for food or water, animals often interact with never-before-experienced objects. Experiment 3 gave the same rats described in Section 4.2.1 the opportunity to explore novel objects with the possibility of uncovering hidden food pellets. The main questions were whether the rats' previous experience in the Reinforced versus Exposed conditions in Experiment 2 would yield different levels of success under this transfer-of-training, or generalization test; whether shy versus bold rats would succeed at different rates; and whether strain and experience would interact. In a procedure referred to as the Hidden Food Test (HFT), 25 never-before-experienced objects were located on the floor of a small room with food pellets hidden within or under each of the objects. The hungry rats were free to explore (or not), with the possibility of discovering and consuming the hidden pellets.

6.2. Methods

6.2.1. Animals

The same 14 LEs and 14 PVGs from Experiment 2 were used and we also added 8 LEs and 8 PVGs, obtained from Harlan Sprague-Dawley, to serve as controls that had no previous experience with novel objects. These new rats were handled each day for 30 days, and were 13 weeks old at the beginning of this experiment. They were fed Noyes pellets in their home cages before the experimental test and will be referred to as Handled controls.

6.2.2. Materials

The HFT was given in a 2.4 x 1.5 meters laboratory room, with a yellow composition floor and bright overhead fluorescent lighting. The rats had never before experienced the room. The room was empty except for 25 objects that were placed on the floor, these being different from any used during Experiment 2 and different from one another, organized in an approximate 5 x 5 grid, leaving a minimum of 15 cm empty around the periphery. Hidden in or under each object were one to three 45-mg Noyes formula P food pellets. The objects, starting in the bottom right corner and moving to the upper left were: plastic sand mill; metal pipe; paper ball; pencil box; step ladder; vegetable steamer; toy mini-truck; cardboard box; plastic cup; tinker-toy; tires; toy dump truck; upside-down coconut shell; small plastic ball; plastic container; jewelry box; infant ring toy; brush; can; blocks; toy Ferris wheel; metal pail; PVC pipe; plunger; toy

school bus. In some cases, the pellets could easily be seen (by the experimenters, and presumably by the rats), but in others, they were hidden within or under the objects. No attempt was made to mask pellet odor. In some cases, to gain access to the hidden pellets, manipulation of the object was required, e.g., the drawer of the jewelry box had to be pulled open to obtain the pellets contained within.

6.2.3. Procedure

Each rat, approximately 22 h food deprived, was gently placed on the floor of the room and remained there for 15 min during which time a video camera recorded the rat's activities for later analysis. At the end of the session, the number of food pellets consumed was recorded. As in Experiments 1 and 2, the floor was cleaned with 70% alcohol, 30% water solution after each rat was removed and objects placed in the same configuration and pellets replaced before another rat was tested.

7. Results

Object Interaction Time in the present experiment was defined as the sum of the times spent in contact with 23 of the 25 objects. Excluded from the calculation were two objects, #5 (Ladder) and #25 (school bus): the rats tended to hide under these without moving and with little exploratory activity. Figure 4A shows that the rats who had been reinforced for variable responses in Experiment 2, the Reinforced group, spent more time exploring objects than did the Exposed rats who, in turn, spent more time exploring than did the Handled controls. As in Experiment 2, strain exerted little influence on OIT. These observations were supported by an ANOVA, with a significant effect of training, $F[2,38]=20.124$, $p<0.0001$, but not of strain, $F[1,38]=0.087$, ns, nor interaction $F[2,38]=0.443$, ns. LSD post-hoc tests confirmed that each training condition differed significantly from the other two (p's<.01). Although the effect was not statistically significant, the largest strain difference was seen in the experimentally naïve Handled animals, with the LEs spending more time (108 s on average) exploring the objects than the PVGs, (65 s on average) ($t[14]=0.984$, ns).

Both experience and strain influenced the number of discovered and consumed pellets (Figure 4B). Reinforced rats consumed more pellets than the Exposed, who, in turn, consumed more than the Handled controls; and in each of these three training conditions, LEs consumed more pellets than PVGs. ANOVA supported these observations with significant effects of both training, $F[2,38]=21.270$, $p<.0001$, and strain, $F[1,38]=6.043$, $p=.019$ but not a significant interaction $F[2,38]=0.264$, ns. LSD post-hoc tests confirmed that each training condition differed significantly from the other two (p's<.02). Thus, in a novel-to-the-rats foraging-type situation, rats who had previously been reinforced for

variable responses to novel objects now uncovered and consumed more hidden food pellets than rats who had equal opportunity to interact with the same objects; a bold strain of rats was more adept at this task than a shy strain; and these two contributors to foraging proficiency did not interact.

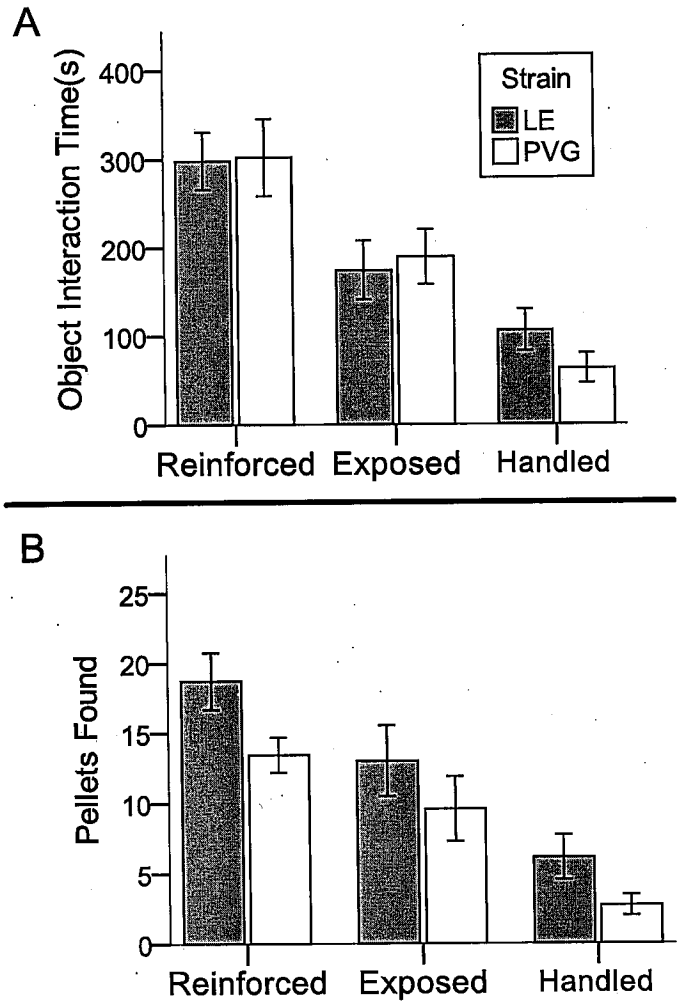

Fig 4

8. Discussion

The variability of operant responses can have many causes, including uncontrolled events in the environment, experimental errors, neurological insult, drugs, motivational variables, withdrawal of reinforcement, and others. In addition, particular levels of variability can be reinforced, from repetitive responding to highly unpredictable. For example, if a sequence of left and right responses is required for reinforcement, that sequence comes to be emitted

repetitively and responses are easily predicted. In that case, reinforcement is contingent on low variability. Repetition lies at one end of the variability continuum, and if reinforcement depends upon responses approximating a random model, then responding approaches, or in some cases equals, that model [16]. Intermediate levels of variability can be reinforced as well [7]. More generally, variability can be described as an operant, much like response force and speed: discriminative cues control when and where an animal varies; levels change as a function of the particular schedules of reinforcement; and choices to vary or repeat depend upon relative frequencies of reinforcement [6]. Operant variability has been confirmed by studies involving many different species and types of responses, including response sequences in people, pigeons and rats; interresponse times in pigeons; swimming movements, flips and turns in porpoises; block constructions in children; saccadic eye movements in people; and variable vocal outputs in song birds and walruses, to name a few (for review, see [7]).

In the present experiment we reinforced (with food pellets) variable responses by rats to novel objects. The variability-reinforced rats were compared to different rats that were exposed to the same objects and received the same number of food pellets, but without the reinforcement-of-variability contingency. Within each of these conditions, two strains were compared: LEs characterized as bold (with a relatively high natural tendency to explore novel objects) and PVGs as shy (with less of a tendency to explore). Thus the design crossed experience (Reinforced versus Exposed) with genetic strain (LE versus PVG). A second major focus of the present work was whether training-to-vary would generalize to a new situation and facilitate foraging success. The results were these:

(i) Reinforced rats responded more variably to the novel training objects than did the Exposed rats, and this was true across both shy and bold strains.

(ii) Bold rats varied more than shy and this was true across training conditions. In brief, both experience and strain independently influenced levels of response variations.

(iii) In similar fashion, both experience and strain influenced later performances in a foraging-type environment where food pellets were hidden within and under 25 objects (these different from the training objects). The rats that had previously been reinforced for object variations discovered and consumed more food pellets than the Exposed controls; and bold LEs discovered and consumed more pellets than shy PVGs. A third experimentally naïve Handled group (comprised of both LEs and PVGs) demonstrated the lowest levels of exploration and discovered fewest pellets.

Two comparisons are worth special note. The first is that variability reinforcement caused shy PVG responses to approach those of the Exposed LEs. In fact, more time was spent interacting with objects, and the variability of those interactions was higher in the Reinforced PVGs than the Exposed LEs (Experiment 2). When tested in Experiment 3 in the Hidden Food Test, the Reinforced PVGs again spent more time exploring the objects than the Exposed LEs and found equal numbers of hidden pellets. Simply stated, reinforcement of

variable interactions overcame the naturally shy exploratory tendencies of PVGs, at least when compared to Exposed animals. Genetics contributed as well in that variability and exploratory successes were higher in LE than PVG.

Second, training-to-vary with one set of objects enhanced successful explorations in a different environment and with different objects. Rats who had previously been reinforced for variations discovered significantly more pellets in this foraging-type food-puzzle test environment than did the Exposed rats and the effects were large. For example, the Reinforced rats discovered almost fives times more pellets than experimentally-naïve Handled animals. Variability training significantly increased foraging success.

Generalization of variability training has been observed in some (but not all) other situations. For example, Lee, McComas and Jawor [17] reinforced variations in verbal responses by individuals diagnosed with autism and found generalization to different contexts and questioners in some of the participants. However, when Napolitano, Smith, Zarcone, Goodkin & McAdam [18] reinforced variability of block constructions in the same population, generalization to different block materials was generally not observed. Similarly, Goetz & Baer [19] found only meager generalization when normal pre-schoolers were reinforced for building novel block constructions. The successful generalization of training seen in the current study may partly be due to the relatively large number of preliminary training experiences – training with ten different objects (see, also, Stokes et al. [20]).

Response variability may be naturally reinforced in outside-of-laboratory environments. For example, variable search strategies are observed when animals forage [21, 22] and discovery of food may serve to maintain both search behaviors and the variability of such behaviors. Laboratory models are consistent with variable search in that choices are stochastic to a large extent when outcomes are uncertain [23]. Another example is seen in songbirds where male song variability is influenced by the presence and responses of female conspecifics [24]. A third example comes from the animal play literature, both non-human and human. Levels of variability by one individual are modulated as a function of the responses of playmates [25]. However, not all animals vary equally, and not all environments engender high levels of behavioral variations. Explicit application of reinforcement-of-variability contingencies may be especially useful in environments where animals or people are constrained, such as in laboratories, zoos, prisons, or classrooms; and for animals or people who experience difficulty in varying their responses – whether due to genetics, disease, or prior experiences.

Reinforcement of variable interactions may also be relevant to the enrichment experiences that are the focus of much research. Since the groundbreaking work of Rosenzweig and co-workers, behavioral, cognitive, and physiological effects of enrichment have been well documented [26]. When studied in rat models, enrichment often involves a combination of social housing (as opposed to isolate housing) and presence of multiple objects (as opposed to an empty cage). The present research suggests that "enrichment" effects may be potentiated by explicit reinforcement of variable interactions.

8.1. Caveats

Reinforcement of variable response interactions necessarily involves reinforcement of object contact. We did not use a contact-only control-group in Experiment 2 because (a) reinforcement of every contact would have produced an inordinately high frequency of reinforcement (and much higher than in the Vary group) and (b) the alternative – namely yoking frequencies of contact reinforcement to the Vary rats – would have introduced intermittent reinforcement which, as indicated above, elicits variability. We therefore chose a control in which the rats were exposed to novel objects – as in many enrichment studies – but without the contingent food reinforcement.

Our second procedural decision was to provide the Exposed animals in Experiment 2 with food pellets in the experimental context, so as to match those received by the Vary rats, but to do so as a batch of pellets at the beginning of each session rather than intermittently. This decision was based on our previous observations that non-contingently presented intermittent pellets resulted in the rats standing in front of the pellet dispenser for extended periods. In the present case, Exposed rats readily consumed all of the pellets at the beginning of each session, leaving ample time to explore and interact with the objects.

Our results must therefore be interpreted in light of procedural decisions, and future research can attempt to assess the extent to which reinforcement of contacts, *per se*, contributed to the Vary responses and the influence of reinforcer intermittency. Additionally, in this case as well as many other reinforcement-of-variability demonstrations [7], associated consequences, e.g., motivational, activity levels, motor outputs, may be responsible at least in part for some positive effects. In operational terms, however, reinforcing variable interactions significantly increased response variability both under the conditions of reinforcement and in a later transfer-of-training problem-solving situation.

8.2. Conclusion

We conclude that reinforcement of variability provides a functional way to increase variable object interactions and to improve foraging proficiency. The positive effects of reinforced variability were observed in both naturally shy (or hesitant to explore) animals and naturally bold (likely to explore).

Figure Legends

Fig 1. Comparison of Long-Evans rats (LE), shown by gray bars, and PVG rats, shown by open bars, in the open field test. (A) Average time (s) spent in the central zone of the open field. (B) Average number of rearing responses. (C) Average seconds spent moving. Error bars show standard errors.

Fig 2. The experimental chamber and one of the novel objects in Experiment 2. The side-by-side chambers were identical, as were the objects in each chamber. One rat in one chamber was explicitly reinforced (food pellets) for variable interactions with the object; the sister rat in the other chamber was permitted equal time to explore the object, but without the reinforcement of variations.

Fig 3. (A) Average Object Interaction Time (s) with novel objects in rats that were reinforced for varying their interactions (Reinforced) and others (Exposed) that were simply permitted to spend equal time interacting (but without explicit reinforcement of variations). (B) Object Interaction Variability, an index of the frequency of rare responses, in the same animals. Filled bars represent Long-Evans bold rats and open bars represent PVG shy rats. Error bars represent standard errors.

Fig 4. Performances during the Hidden Food Test in which rats were permitted to forage among 25 novel objects on the floor of a laboratory room (A) Object Interaction Time (s), or the total time during the 15 min session that rats spent interacting with the objects. (B) Number of food pellets discovered and consumed. Reinforced rats had previously been rewarded for variable interactions with novel objects; Exposed rats had spent equal time in the presence of the same novel objects; and Handled control rats had simply been handled. Filled bars represent bold Long-Evans rats and open bars shy PVGs. Standard errors are indicated.

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Table 1
Description of each object used in Experiment 2 and a list of common responses.

Object	Description	Examples of Reinforced Responses
Object 1	black rubber cork	Touch with paw(s), bite or naw on edge, push/roll with nose, push/roll with paws, carry in mouth across box.
Object 2	lacrosse ball	Contact with nose/paws, roll ball away from body, roll ball towards body, rear on top of ball.
Object 3	tinker toy	Contact or roll with nose/paws, knock over.
Object 4	open-sided cylinder with lid	Contact or roll with nose/paws, move through space between lids, rear/climb on top.
Object 5	pvc pipe corner	Contact pipe, enter pipe, stand/rear on top of pipe.
Object 6	pellet bottle with holes	Contact outside with nose/paws, stick head through holes into bottle, enter bottle, exit bottle backwards, turn around inside bottle and exit forwards, enter and exit using different pathways through holes.
Object 7	ladder (jungle gym)	Contact ladder with paws, climb up ladder, back down ladder, jump off tower, spin tower in arena.
Object 8	cube with twine	Contact cube with paws, drag through arena, kick/toss cube airborne.
Object 9	metal screen tube with natural corks	Contact tube/corks with nose/paws, jump over tube, drag object through arena with mouth/paws.
Object 10	bowl with 8 screws	Contact screws, remove screw from bowl, move bowl, get inside of bowl.