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Rats anticipate damaged rungs on the elevated ladder: Applications for rodent models of Parkinson's disease

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The present study examined rats' ability to anticipate undetectable wider gaps between rungs produced when they stepped on and dislodged damaged rungs while they traversed a slightly inclined elevated ladder. Rats in the first of three experiments reduced running speeds when they encountered four evenly spaced damaged rungs either always placed on the first or second half of the ladder (the break-a-way (BW) phase) but quickly recovered to their baseline (BL) levels when damaged rungs were replaced with intact rungs (the recovery phase). Rats previously exposed to damaged rungs over the first half of the ladder increased their speeds above BL on its second "safer" half during the recovery phase, a delayed "relief-like" positive contrast effect. In Experiment 2, other rats decreased their speeds more as they approached a single damaged rung at a fixed location when it occurred before than after the mid-point of the ladder. Although they quickly recovered to BL speeds on the portion of the ladder after the damaged rung or replaced intact rung, they never showed any "relief-like"/escape effects. Rats also reduced their likelihood of dislodging the damaged rung with a fore paw over extended BW training. In the third experiment rats encountered a more easily dislodged damaged rung that was signaled by a closer intact rung on half the trials. Under these conditions rats displayed a more reliable positive contrast "relief-like" effect. We discussed how traditional associative and cognitive theories of aversive conditioning account for these findings and their relationship to normal changes in dopamine production and possible effects of reduced production from the substantia nigra pars compacta (SNpc) in the Basal ganglia in rodent models of Parkinson's disease.

Keywords: *Rattus norvegicus*; elevated ladder; skilled walking; anticipation; associative processes.

1. Introduction

In traversing difficult terrain, animals exhibit highly complex locomotor behavior to prevent injury from slips and falls from obstacles along the way. The elevated ladder

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walking task introduced in 1983 by Watson and McElligott, is a particularly good preparation for examining rats' performance as a function of terrain difficulty. In that task, rats are typically first trained to traverse a narrow elevated ladder to a larger "safer" flat platform baited with preferred food morsels and then are exposed to the ladder with more widely and unevenly separated rungs. Such post-training conditions disrupt rats' walking behavior as seen by hind leg slips through wider gaps between rungs and increased latencies to reach the solid platform. This task has been primarily used to show that rats and mice subjected to targeted cephalic or spinal cord injury exhibit greater and more persistent hind leg slippage (miss-steps) and less accurate or flexible forepaw rung grasping behavior than intact animals (Watson & McElligott, 1983; Soblosky *et al.*, 1996, 1997a, 1997b, 2001; Metz & Whishaw, 2002; Farr *et al.*, 2006; Jadaji & Metz, 2009; Whishaw *et al.*, 2010).

These studies are important as they have been showing the contribution of spinal and supra-spinal structures and pathways on animals' skilled walking performance, but is known about how they reflect higher cognitive processes. The operation of such processes by rats in a ladder rung walking task was recently demonstrated by Wallace *et al.* (2012). In that study, rats reduced their running speeds and increased leg slips when exposed to a ladder with unevenly spaced rungs after first learning to run on it with evenly spaced rungs. However, rats more quickly recovered to their baseline (BL) performance levels when exposed to a simpler than to a more complex repeated uneven rung placement pattern. Probe trials with novel gap patterns disrupted rats in both groups. According to Wallace *et al.* (2012), such differences in skilled locomotion reflected differences in rats' representations of spatial gap sequences.

Another reasonable implication from these findings is that fear of falling through unexpected gaps in the ladder motivated rats to somehow anticipate the locations of these gaps that appeared at fixed locations. Although rats might not have been able to visually detect these gaps in the darkened running room, Wallace *et al.* (2012) suggested that they could have detected variations in rung spacing through tactile sensations from their vibrissae and nose or body hair to prevent miss-grasps and miss-steps. This idea raises several questions about how rats might react if they could not tactually detect wider gaps between rungs at fixed locations. Would they remain more cautious while walking on the ladder because they are maintaining their fear of falling or because they are attempting to prevent any deleterious effects from miss-steps through wider gaps? In either case rats would be seen to anticipate where such gaps occur at fixed locations by reducing their walking speeds as they approach each gap. Will they become less disrupted by a wider gap that comes closer to the end of the ladder and its safer, baited flat platform as predicted by associative models of Pavlovian and instrumental conditioning Mazur (2013)? If rats increase their fear of falling from the elevated ladder as they approach a more dangerous section of it, might they also show some type of "relief-like" positive contrast when they leave it? That is, as they reduce their walking speeds as they approach an anticipated wider gap in the ladder they would greatly increase their speeds above BL when leaving it

and continue toward the end of the ladder. More traditional one-way escape/avoidance tasks in double runway (Lambert & Hammond, 1970; Miller & Woods, 1975; Candido *et al.*, 2002) and alternating lever pressing (Quirt & Cohen, 1974) preparations have shown such reliable “relief-like”, positive contrast effects. The main goal in this study was to determine whether these effects based on traditional associative learning theory will also be found in a ladder rung walking task by rats encountering undetectable gaps between its rungs. We asked whether rats would reduce their running speeds on a ladder as they approached any undetectable gap at a fixed location and increase their speeds above their normal (BL) walking rates when they leave such an unsafe section of the ladder. To accomplish this goal, we modified the ladder and the rats’ skilled walking task as follows.

To make such gaps undetectable to rats we replaced one or more intact rungs with damaged rungs at fixed locations on an elevated, slightly inclined ladder after rats had learned to traverse it consisting of only evenly spaced intact rungs. A rat could dislodge a damaged rung by stepping on it to create a wider gap on the ladder. As these otherwise undetectable damaged rungs occurred only within limited sections on the ladder, rats had a large area on the ladder before encountering any damaged rung and a large area after leaving any of them. To measure rats’ anticipation in approaching a damaged rung and its “relief” in leaving it, we installed a series of photo-beam sensors along the side walls of the ladder as shown in Fig. 1(A) to measure its running speed between each successive beam. We note that we slightly inclined this ladder 7.5° up to a rat’s baited holding cage to encourage it to keep moving forward along the ladder.

The present study consisted of three separate experiments, each containing different animals. In the first experiment, we arranged four broken rungs to occur at fixed locations over the first half of the ladder for some rats (1st Half group) or over the second half of the ladder for other rats (2nd Half group). In the second experiment, we only replaced a single intact rung with a damaged one at a fixed location either before or after the mid-point on the ladder so that all rats experienced a large area leading up to and away from it as they traversed the ladder. If stepping on a faulty rung elicits a fear reaction of falling through the ladder, rats would be expected to reduce their running speeds as they approach a series of or a single broken rung but should increase their speeds above previously established BL levels as they leave the last broken rung to exhibit an escape or “relief-like” reaction. If rats display changes in speeds suggestive of a more negative emotional affect, will they maintain these patterns on subsequent trials when the ladder no longer contains any damaged rungs? If earlier encounters with broken rungs produce Pavlovian conditioned emotional fear and “relief” reactions, rats should readily extinguish them during these extinction or “recovery” trials. However, if such changes in locomotion reflect instrumental avoidance/escape behavior, rats should resist extinguishing such responding according to traditional learning theories (Mazur, 2013).

(a) Rat walking up ladder during Baseline (b) Rat approaching and dislodging rung



Fig. 1. (a) Photograph of a rat walking up an inclined elevated ladder leading to its baited holding cage during a BL session. No photographs or videos were taken during the first of three experiments in this study. See text for further details concerning the apparatus and actual room lighting during an experiment. (b) Frames from a digital video recording in the second experiment of a rat about to step on a damaged rung with its right forepaw and after it had dislodged it and replaced that paw back on to the intact rung located before the damaged now broken rung.

Animals in the second experiment were exposed to considerably more sessions than animals in the first experiment but only ever experienced a single damaged rung on the ladder. By presenting only one such rung in that experiment we were able to easily record each rat's paw placements on a specific target rung; that is, the damaged rung in the break-a-way (BW) phase of that experiment. Therefore, we could determine whether rats changed their paw placement patterns on this rung during that longer BW phase from paw placement patterns seen in their prior BL and subsequent recovery trials. In the third experiment, we also presented rats with a single damaged rung whose location varied within a small defined mid-section of the ladder. We asked whether placing an extra intact rung 2.5 cm closer to a damaged rung on some trials would enable rats to more easily detect the otherwise moveable damaged rung and change its "approach to" and "leaving from" speeds and paw placement patterns from those they display without a warning signal rung. That is, would rats learn to step over a "signaled" damaged rung? An example of frames from a video camera that recorded each rat's stepping behavior on the target rung in these last two experiments is shown in the B panels of Fig. 1.

2. Materials and Methods

2.1. *Subjects*

Three separate batches of male Long-Evans hooded rats were purchased from Charles River Breeding Farms, St. Constant, Quebec for this study. The first batch consisted of 25 animals that served in the first experiment. Each of the second and third batches consisted of six animals and served in second and third experiments, respectively. All rats were approximately 90 days old and 300 g at the beginning of their respective experiment and were housed in group cages (3 per cage) in our colony room when not otherwise engaged during an experimental session. The colony room remained on a reversed 12 h dark/light cycle with lights going off at 800 and coming on at 2000. Rats began their various daily experimental activities within 2 h of the beginning of their dark cycle. These rats also served as subjects in two other experiments, an object recognition task and a serial reaction time task, before 1200 of each day and were run in the current experiment at 1500. At the end of their session in this experiment each rat received its end-of-day food rations of 20–25 g rodent chow in individual holding chambers for two hours before being replaced back into their colony room group cages. Water was continuously available in their individual holding and group cages. These conditions maintained rats at approximately 90% of their free-feeding weights.

2.2. *Apparatus*

The apparatus consisted of a 198 cm long by 30.4 cm wide ladder with 9.5 cm high vertical aluminum side walls set 12.7 cm apart from each other as shown in Fig. 1. A 5.5-cm long metal plate occurred at each end of the ladder. The open 187-cm long area between these plates contained thirty-two 17.5 cm long \times 0.64 cm dia. stainless steel rungs evenly separated from each other by 5.5 cm. The ladder was inclined 7.5° with its starting point 75 cm and its end point 197 cm above the floor of the running room. A small table, at the higher end of the ladder, contained the rat's individual holding cage baited with a thin slice of apple. A total of 18 pairs of infra-red photo-beam sensors, each 5.4 cm above the rungs, were evenly positioned 11 cm apart along the side walls to create 17 segments, each containing three rungs. These sensors detected when a rat intercepted each successive beam as it traveled up the ladder from which its running speed (cm/s) was calculated over each segment by a program developed with LabView software (National Instruments, USA) and automatically stored in an Excel file in a desktop PC computer. Four shorter 12.5 cm long stainless steel rungs were used to fabricate the damaged rungs by inserting an iron set screw into each of their ends to allow a 2.54 cm long \times 0.64 cm dia. rare earth rod magnet to be attracted to them. Each broken rung could remain suspended on the ladder until a rat stepped on it to create a wider 11 cm gap between intact rungs before and after each broken rung. We determined that the minimum force a rat required to separate (dislodge) a damaged rung from its end magnets was 144 g in the first and second experiments. In the third experiment, we reduced this force to

33 g by resetting the damaged rung's set screws further in from their flush end positions. Along with this modification to the damaged rung, we also renovated intact, unbreakable rungs by permanently attaching the same type of earth magnet rods to make them visually identical to the damaged rung. The only illumination in the running room aside from the computer monitor on a laboratory bench at the end wall came from indirect lighting of two 40-W incandescent lamps on a side wall. A commercially available (JVC) digital camcorder was located beneath the ladder to record rats' paw placements on the single target rung during the second and third experiments.

2.3. Procedure

Rats were given a five-day pre-training phase to acclimate them to handling and food maintenance schedule procedures and to shape them to run up the ladder for a slice of apple in their individual holding cages. Following this pre-training phase, rats in the first experiment were run on the ladder for 21 daily sessions divided into three phases consisting of a 9-session BL training phase on the intact ladder, followed by a three-session "break-a-way" phase with four widely- and evenly distributed damaged rungs that broke away when stepped on, and finally a nine-day recovery or extinction phase on the "repaired" ladder with intact rungs that replaced the damaged ones. During the BW phase, 13 randomly selected animals received the 4 broken rungs at fixed locations, one each within segments 3, 5, 7, and 9 over the first half of the ladder (the 1st Half group) while the other randomly selected 12 rats received these broken rungs at fixed locations, one each within segments 11, 13, 15, 17 over the second half of the ladder (the 2nd Half group).

The six rats in the second experiment received two runs of 27 sessions following initial pre-training. Each run was divided into three equal 9-session BL, BW, and recovery phases. Half the animals in the first run were exposed to a damaged rung at a fixed location in segment 8 and the other half to it at a fixed location in segment 12 during the BW phase. Following the last recovery phase in that run, each rat received a second run of 27 sessions similarly evenly divided into these three phases but with the damaged rung at the other location in its BW phase. Thus, this experiment was a within-subjects temporally counterbalanced design for varying the damaged rung's two locations.

In the final experiment, six other experimentally naïve rats received a 6-session BL training phase and a 12-session BW phase following their initial pre-training phase. The BL phase consisted of three two-session blocks that alternated between segments with a target intact rung signaled by an added closer rung and segments without this added rung. Each of these two types of trials occurred in segments 9, 10, and 11 in an ascending order. We limited the number of BL sessions in this experiment to prevent rats from habituating (ignoring) changes in rung spacing configuration in those segments that would eventually contain a damaged rung. The BW phase consisted of four three-session blocks, with the damaged target rung randomly changed over the

three mid-section segments. The two odd numbered BW blocks of sessions contained a signaled faulty rung while the two even numbered BW blocks contained the non-signalized faulty rung. One of the three segments that contained the damaged rung was randomly varied over sessions without repetition within each block of BW trials. We note that unlike the previous two experiments, we did not fix the position of the target rung over trials thus making the extra signal rung a better predictive signal than the more variable mid-location of the damaged rung. We also omitted a recovery phase in this experiment for reasons later explained.

Throughout the study, the experimenter sprayed the ladder with lemon scented disinfectant between animals and ran rats in a different order each day.

2.4. *Data analysis*

Running speeds. Although each rat produced a running speed (cm/s) over each of the ladder's 17 segments during each experimental session, we eliminated its speeds from the 1st segment because a rat was already part way in that segment when it began running up the ladder. We also reduced the considerable amount of speed data generated by each rat over the remaining 16 segments over all sessions to produce more easily analyzable data as follows. First we calculated the average of each segment's speed over each block of three sessions for each rat. This recalculation produced 16 averaged segment speeds in each of the three blocks of BL trials, for the one block of BW trials in the first experiment and the three blocks in the second experiment, and for each of the three blocks of trials in the recovery phase in both experiments. We further decreased each rat's dataset in each experiment by eliminating its averaged segment speeds on its first two blocks of BL trials. We used only a rat's averaged BL segment speeds from that phase's third block for comparisons to the averaged data from its respective segments in the BW and recovery phases. We observed that rats displayed their highest and most stable asymptotic speeds on the third block of BL trials that averaged from around 20 cm/s on the 2nd segment and remained around 40 cm/s over each of the remaining 15 segments of the ladder. Finally, to further reduce the amount of data to a more manageable level, we recalibrated each rat's averaged segment speeds from each BW and recovery block of trials as speed difference scores from those of respective segments in its 3rd block of the BL phase. Thus, each rat's final data consisted of 16 speed difference scores in one or three blocks in the BW phase in the first and second experiment, respectively and of 16 speed difference scores in three recovery blocks in each experiment. Consequently a minus or plus speed difference score in any segment in these two phases indicated a speed below or above of that segment's BL speed, respectively. Although we analyzed speed difference scores from a total of four blocks of trials over the last two phases in the first experiment, for ease of explication we designate the fourth block of trials only as that of the BW phase and the 5th, 6th, and 7th block of trials as those from the 1st, 2nd and 3rd block of the recovery phase. In the second experiment, the speed difference scores from the last

six blocks of trials are similarly designated as the 1st, 2nd, and 3rd BW blocks followed by the 1st, 2nd, and 3rd recovery blocks.

We conducted statistical analyses of the speed difference scores in the first two experiments as follows. For the first experiment, we carried out two separate sets of statistical analyses on rats' speed difference scores. The first consisted of a series of one-way ANOVAs within each group over each half of the ladder (eight segments) for each block. These analyses along with post hoc one-sample *t*-tests for each segment and pair-wise (LSD) comparisons between segments provided information about which BW and recovery phase segment's speed difference score significantly departed from its BL phase's segment speed (set at 0) and how these difference scores varied over successive segments. The second set consisted of two-way ANOVAs and appropriate post hoc comparisons that compared groups within each of their safer and unsafe ladder areas.

For the second experiment, we used only those speed difference score data from nine segments containing the target rung that was located within that section's 5th segment. Thus, when a damaged or replaced intact rung occurred in the 8th or 12th segment, we examined speed difference scores from the 4th to the 12th segment or from the 8th to the 16th segment, respectively. We note that our main comparisons concerned speed difference scores based on target rung locations (pooled over runs) within the BW and recovery phases. As in the first experiment, we first conducted a one-way ANOVA within each block at each broken rung location to determine whether each BW or recovery phase segment's difference speed score significantly departed from its respective BL speed set at 0. Then we conducted a two-way (2 target rung locations \times 9 segments) ANOVA (repeated measures on both factors) for each block to determine the statistical reliability of any apparent differences in these patterns as a function of target rung location. We conducted supplementary statistical tests for comparisons among BW phase blocks at each target rung location when warranted. Although we also carried out the same type of statistical analyses to compare runs (pooled over target rung location), we confine our description of results from the analysis concerning the effect of target rung location for reasons given later.

In the third experiment, each segment's speed for each rat was averaged over each of the three signaled and non-signaled sessions in each BL and BW phase (pooled over segment location). As in the second experiment, we used only the averaged segment speeds from those nine segments that included the middle segment containing an intact or damaged target rung. Thus, when the target rung occurred in segment 9, or 10, or 11, we examined averaged segment speeds over segments 5 through 13, or over segments 6 through 14, or over segments 7 through 15, respectively. For ease of explication we designate each of these nine segments as the 1st through 9th segment with its 5th segment containing a signaled or non-signaled target rung. Unlike the first two experiments we could not further recalibrate BW segment speeds into difference scores because the pattern of BL speeds differed as a function as type of rung configuration as we later describe. Therefore, we directly compared rats' BW segment speeds with their respective BL segment speeds within

each target rung configuration by two-way and one-way within-Ss ANOVAs and appropriate post hoc LSD and t -test comparisons.

Paw placement patterns. From our examination of each rat's video recordings, we determined how often it touched the "target" rung with one of its fore paws and hind paws within each phase in each run in the last two experiments and also how often it touched the closer signal rung in the third experiment. From these data we also calculated the proportion of times rats dislodged the damaged rung with either one of their fore or hind paws during each block of BW sessions in the last two experiments and the proportion times they touched the added, intact signal rung in the third experiment. We analyzed these data by appropriate two-way ANOVAs to determine the effects of target rung location and runs over phases and within each BW phase in the second experiment and by a one-way ANOVA to determine the effects of signaling the target rung.

Any effect from a statistical analysis was considered significant at $p < 0.05$.

3. Results and Discussion

We describe and discuss the results from each experiment separately.

3.1. *Experiment 1: Effects of location of the "unsafe" and "safer" half of the ladder*

Figure 2 shows the speed difference scores on each segment within the unsafe half (boxed in by broken borders) and the safe half of the ladder for each group. We first report results from each group's currently or previously "unsafe" half during its respective BW and recovery blocks of trials and then similarly report results from each group's "safer" half of the ladder.

As seen in the boxed-in unsafe areas of Fig. 2 and confirmed by one-way ANOVAs, each group produced consistently significant below-BL speeds during its BW phase, 1st Half group: $F_{1,12} = 55.43$, $p < 0.001$; 2nd Half group: $F_{1,11} = 91.41$, $p < 0.001$. Each group's below-BL speed difference score was higher in the first segment of that area than in each of the remaining seven segments to produce a significant effect for segments, 1st Half group: $F_{7,84} = 3.19$, $p = 0.005$; 2nd Half group: $F_{7,77} = 6.67$, $p < 0.001$. The two-way ANOVA failed to uncover a significant difference between groups. During the recovery phase, each group continued to produce below BL speeds on some segments that generated an overall significant effect, 1st Half group: $F_{1,12} = 13.19$, $p = 0.003$; 2nd Half group: $F_{1,11} = 14.07$, $p = 0.003$. However, the 1st Half group increased its speeds up to BL (i.e., they reduced minus speed difference scores) over the last two segments in first block of recovery trials to generate a significant effect for segments, $F_{7,84} = 4.61$, $p < 0.001$. Although the 2nd Half group slightly but significantly increased its segment speeds in the first block of recovery trials from those in the previous block of the BW trials as revealed by paired-samples t -tests at each segment, $t_{s11} \geq 2.00$, $p_s \leq 0.035$, it continued to maintain significant below BL speeds (i.e., minus speed difference scores). These different patterns of

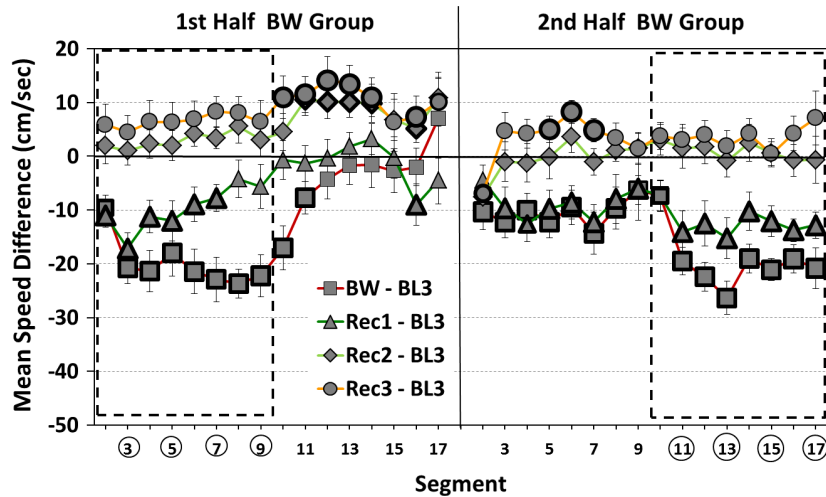


Fig. 2. Mean speed difference at each segment between the BW block and the third block of BL trials (BW-BL3) and between each of the three blocks of recovery trials and the third block of BL trials (Rec1-BL3; Rec2-BL3; Rec3-BL3) for each group in the first experiment. The areas boxed in with a dashed border contain data from unsafe half of the ladder containing a damaged rung at a fixed location within each of the four circled segments. The remaining areas represent the safer half of the ladder within each group. Enlarged bold bordered data markers designate segment speeds significantly different ($p < 0.05$) from their respective third block of BL trials' (BL3) segment speeds set at 0 (horizontal line). Vertical error bars are \pm SEM.

recovery performance between groups generated a significant group by segments interaction, $F_{7,161} = 2.44$, $p = 0.021$. All rats similarly increased their speeds to BL levels (i.e., reduced their minus speed scores) over their 2nd and 3rd recovery blocks. Thus, rats were more similarly disrupted by dislodging damaged rungs that occurred on either half of the ladder but somewhat more different in their recovery performance patterns.

The difference between groups during the first recovery block may be attributed to rats in the 2nd Half group anticipating only one hedonically positive event as they ran up the previously unsafe area of the ladder; that is, to the baited holding chamber, while rats in the 1st Half group anticipated two hedonically positive events; that is, a long safer half of the ladder leading up to the baited holding chamber. These differences between groups disappeared by the 2nd recovery block suggesting that each group had learned that its previously unsafe area no longer contained damaged rungs.

As seen in the unboxed safer areas of Fig. 2, groups greatly differed as they ran over their respective segments. In the BW phase block, the 1st Half group increased its speeds (i.e., reduced its minus speed difference scores) up to BL levels by the third segment (segment 12) of its safer half and maintained these levels over all but the next to last segment in the first block of recovery trials. A significant effect for segments, $F_{7,84} = 6.28$, $p < 0.001$, and post hoc one-sample t -tests confirmed these observations. The 2nd Half group displayed similar significant below-BL speed

difference scores over segments in both the BW and the first block of the recovery trials, $F_{S_{1,11}} = 17.14; 10.13$, $ps = 0.002; 0.009$. A significant segments effect, however, occurred during the first recovery block, $F_{7,77} = 2.26$, $p < 0.038$, due to slight but significant changes in speed difference scores between segments 3 and 7 and between segments 7 and 8. Significant groups by segments interactions within each phase, $F_{S_{7,161}} = 3.16; 3.63$, $ps < 0.01$, supported these observed differences between groups.

Traditional associative learning theories (Mazur, 2013) can account for these group differences as follows. The first or “safer” half of the ladder for the 2nd Half group would be expected to elicit a negative conditioned emotional state by occurring before the “unsafe” half to partially suppress rats’ forward movement. The safer half for the 1st Half group would not be expected to elicit any sort of suppressive conditioned emotional response as it occurred after the unsafe half. Rather it should have elicited positive conditioned emotional “relief” or escape from the unsafe side effect to promote more rapid recovery of speeds to BL as was uncovered in the first recovery block. Indeed, this difference between groups became more apparent over the second and third blocks of recovery trials. As seen in Fig. 2 and supported by a significant main effect, the 1st Half group developed above-BL speeds on all but a single segment within each of the last two recovery blocks, $F_{S_{1,12}} = 9.20; 8.42$, $ps = 0.01; 0.013$. The 2nd Half group, however, developed segment speeds not significantly different from BL during the second block of recovery trials but did significantly exceed BL on three segments in its third block recovery block to generate an overall significant segments effect, $F_{7,77} = 3.96$, $p = 0.001$. A significant groups effect in the 2nd block of the recovery phase, $F_{1,23} = 5.90$, $p = 0.023$, and a significant groups by segments interaction the 3rd recovery block, $F_{7,161} = 2.90$, $p = 0.007$, further confirmed these differences.

A positive contrast effect by the 1st Half group did not occur until the second block of recovery trials by which time any disruptive effects within the first, previously unsafe half of the ladder had disappeared. Perhaps rats had not forgotten which half of the ladder had previously been “unsafe” or “safe” while traversing it during the recovery phase. Retrieval of such memories, while not sufficient to reduce (suppress) forward movement below BL within a previously unsafe half, may have been sufficient to produce delayed “relief” reactions in the safer half for the 1st Half group. This idea is consistent with a cognitive theory of aversive conditioning (Seligman & Johnston, 1973) and the idea that extinction training does not eliminate memories of previously acquired associations (Bouton & Bolles, 1985).

We note that traditional associative learning theory (Mazur, 2013) can account for the difference between groups on the first block of the recovery trials in an unsafe area. However, according to such a theory, rats should have shown less disruption on the unsafe area nearer the end of the ladder and its baited holding chamber. Perhaps exposing rats to four evenly spaced damaged rungs obscured any such location effect. It is also possible that exposing rats to so many faulty rungs within the first half of the ladder also obscured any positive contrast effects on the safer half of the ladder. This possible obscuring factor would have been eliminated in the second experiment

where rats received only a single damaged rung over many more BW sessions. Thus, we examined the performance by rats in the BW and recovery phases of the second experiment to determine whether they would be disrupted more when they encountered the damaged rung further way from the end of the ladder and whether they would be more likely to reliably develop a positive contrast speed effect after dislodging the faulty rung.

We also noticed that rats did not always dislodge every damaged rung in their unsafe area in this experiment. Although we neglected to record when and which damaged rung they failed to dislodge, we note that every rat failed to dislodge at least one damaged rung. This effect led us to ask whether such “failures” reflected a deliberate attempt by rats to avoid dislodging rungs by exerting less pressure on them in the unsafe area or merely reflected rats’ inadvertent skipping over some rungs that by chance were damaged. We attempted to answer this question in the next experiment by recording paw placements on a single damaged rung over more BW sessions.

3.2. *Experiment 2: The effects of the location of a single damaged rung*

Running speed difference scores. Initial statistical analyses of segment speeds on the third block of the BL phase as a function of either target rung location (pooled over runs) or of runs (pooled over target rung locations) revealed only a significant effect for runs resulting from higher BL segment speeds in the second than first run (43 ± 5 cm/s vs. 35 ± 3 cm · s), $F_{1,5} = 7.65$, $p = 0.040$. Only a negligible non-significant difference in BL speeds between segments containing the target rung in segment 8 and in segment 12 (40 ± 3 vs. 39 ± 6 cm/s) was found. In view of the upward drift in BL speeds over runs, we confine our description and discussion of results based on target rung location where speed difference scores were derived from equivalent BL speeds around each target rung location. We further note that changes in speeds over successive segments leading up to and away from the segment containing the target rung were similar under either type of analysis.

Figure 3 shows the speed difference scores on the nine segments speeds over the three BW (a) and three recovery blocks (b) when a damaged rung occurred on segment 8 (left panels) or on segment 12 (right panels). As seen Fig. 3(a), rats reduced their speeds below BL on the segment just after the one containing the target rung in either location and then increased them to levels near BL over the remaining segments in each block. These observations were confirmed by a significant segments effect when the damaged rung occurred in segment 8, $F_{S_{8,40}} = 2.55$; 3.04; 4.90, $ps \leq 0.024$, or in segment 12, $F_{S_{8,40}} = 4.06$; 5.34; 6.04, $ps \leq 0.001$. However, rats displayed more consistent and greater declines in speeds below BL on segments that occurred up to and including the one following the damaged rung in segment 8 than in segment 12. This difference was most striking in the third block of BW trials. That is, rats produced a significant overall below-BL speed in each of the BW blocks when the damaged rung occurred in segment 8, $F_{S_{1,5}} = 12.71$; 13.45; 38.21, $ps \leq 0.016$, but only a significant overall below-BL speed in the second BW block when it occurred in

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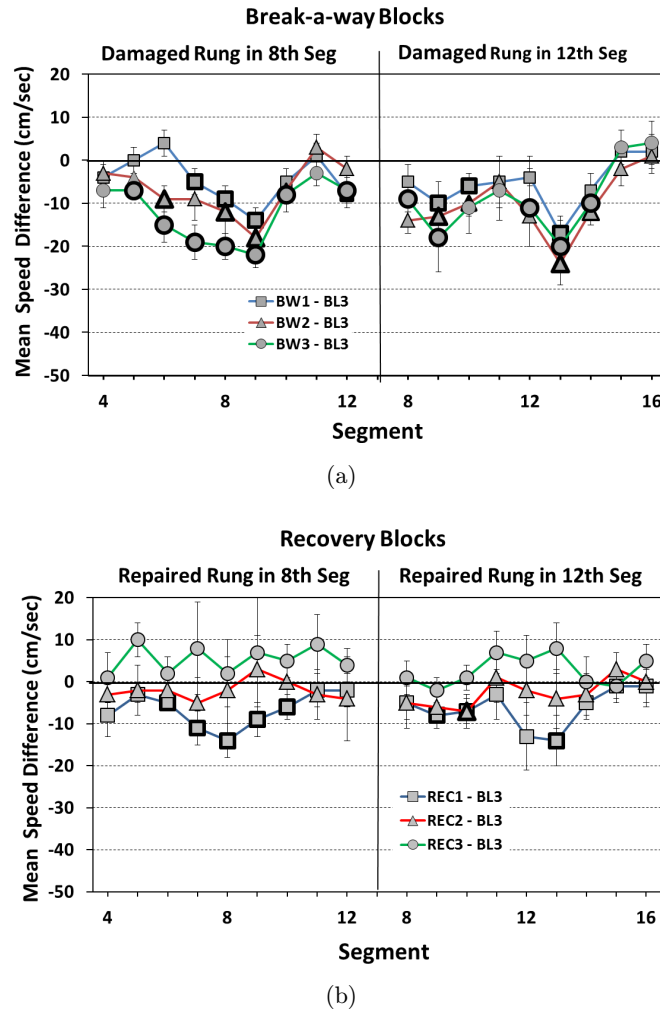


Fig. 3. (a) Mean speed difference at each segment between each BW block and the third block of BL trials when a damaged rung occurred in the 8th segment (left panel) or in the 12th segment (right panel). (b) Mean speed difference at each segment between each recovery (Rec) block and the third block of BL trials when a damaged rung occurred in the 8th segment (left panel) or in the 12th segment (right panel). Enlarged bold bordered data markers designate segment speeds significantly different ($p < 0.05$) from their respective third block of BL trials' (BL3) segment speeds set at 0 (horizontal line). Vertical error bars are \pm SEM.

segment 12, $F_{s_{1,5}} = 10.36$, $p = 0.024$. The more obvious differences between damaged rung location over segments during the third block of the BW phase was further confirmed by a significant interaction between damaged rung segment location and segments within those trials, $F_{8,40} = 3.41$, $p = 0.004$. Comparisons between blocks in the BW trials for each damaged rung location also produced a significant blocks effect when the broken rung only occurred in segment 8, $F_{2,10} = 8.43$, $p = 0.007$. Pair-wise comparisons from this effect revealed that rats developed an overall significantly greater below-BL speed in their third block (-12 ± 1.9 cm/s) than in either their

second (-7 ± 1.9 cm/s) or first block (-5 ± 1.5 cm/s). We note that rats did not increase their segment speeds above BL on any segments that followed that containing the damaged rung. Thus, rats showed no relief-like, positive contrast effects during the BW trials.

These results fail to show that rats habituated or otherwise adapted to several exposures to a single damaged rung. Rather, they more consistently reduced their running speeds as they approached and dislodged a rung located closer to (in segment 8) than further from (in segment 12) the start of the ladder. Consequently rats' anticipation of the baited chamber by their arrival at segment 12 appeared to counteract anticipation of its faulty rung.

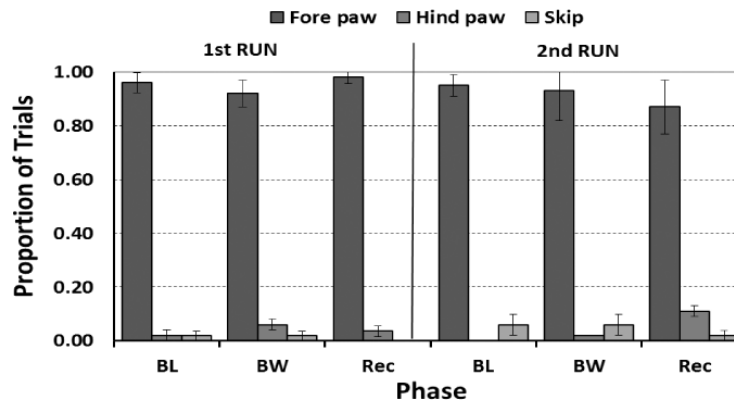
One finding requiring some further explanation is that rats actually reduced their speeds more in the segment following the one containing the broken rung. Our inspection of rats' video clips, however, reveal that, as rats stepped on a damaged rung with their fore paws, their heads were already extended across the next segment's photo beam. Therefore, the apparent slower speeds in the latter segment were likely an artifact of the animals pausing while still within the former as it was dislodging the damaged rung.

Figure 3(b) shows the mean segment speed difference scores for each block of recovery trials. As seen in this graph and supported by a significant segments effect at each target rung location, $F_{S_{8,40}} = 2.28; 3.35$, $p_s = 0.041; 0.036$, rats maintained their below-BL speeds on segments leading up to and on the one previously containing a faulty rung during their first block of recovery trials. Thus, rats had not merely reacted to dislodging the damaged rung in segment 12 but were anticipating such an event in the previous BW trials. Although rats continued to show more consistent and overall below-BL speeds over segments when the target rung occurred in segment 8 than in segment 12, this location effect just missed significance, $F_{1,5} = 6.48$, $p = 0.052$. Post hoc one-sample t -tests, however, revealed that rats produced significant below-BL speeds over more segments when the target rung was within segment 8 than within segment 12. Rats recovered as quickly (by their 2nd recovery phase block) to their BL performance as had rats in Experiment 1, but failed to show any above-BL speeds over segments following either segment containing the target rung. Consequently, unlike rats in Experiment 1, they did not evidence any delayed "relief-like" effects during either run's recovery phase.

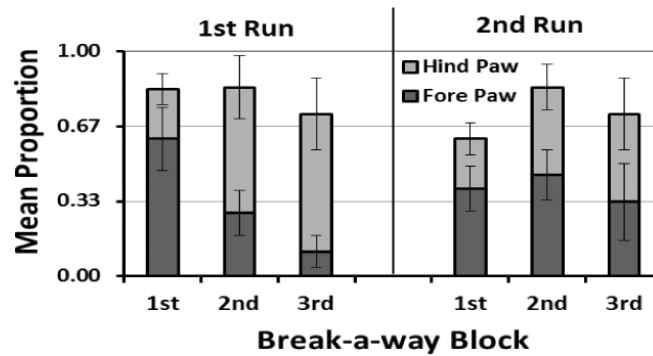
Paw placement patterns on the target rung. Figure 4(a) shows the proportion of trials that rats touched the faulty or the replaced intact (target) rung with either a fore paw or hind paw or stepped over it in each run's respective BW and recovery phase. Examination of these data did not reveal any significant change in rats' likelihood of touching it with one of their fore paws either as a function of the target rung location or the experiment's run. As seen in Fig. 4(a), rats overwhelmingly touched the target rung with one of their fore paws and seldom if ever only touched it with one of their hind paws or failed to touch it with either by hopping over it. Therefore, animals did not change their paw placement patterns during either run's BW phase. However, four rats did not always dislodge the broken rung on every

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(a)



(b)

Fig. 4. (a) Proportion of trials during each phase of each run in the second experiment that rats touched the target rung with one of their fore paws, with only one of their hind paws, or did not touch it with either of these paws (Skip). (b) Proportion of trials within each BW block that rats dislodged the damaged rung with a fore paw or a hind paw during the first run (left panel) and during the second run (right panel). Vertical error bars are \pm SEM.

session in either run's BW phase. Of the two rats that dislodged the broken rung on every session in the first run, only one continued to do so in its second run. The proportion of sessions that rats dislodged the rung during their first and second runs averaged $80 \pm 8\%$ and $74 \pm 8\%$, respectively and of those sessions, they did so with their fore paws on only $41 \pm 8\%$ or $51 \pm 15\%$ in their first and second runs, respectively.

Figure 4(b) shows the pattern of paw placements causing the damaged rung to be dislodged during each run's BW block of trials. As seen in the left panel in Fig. 4(b), rats primarily dislodged the damaged rung with one of their fore paws on its first run's initial block of BW trials but substantially decreased doing so over the next two blocks while they appeared to increase dislodging it with one of their hind paws. This observation was confirmed by a significant blocks effect for fore paw data, $F_{2,10} = 6.12$, $p = 0.018$, that LSD pair-wise comparisons found was caused by a significant difference between the third and first block of trials, $p = 0.001$. The

corresponding opposite change for using a hind paw to dislodge the target rung missed significance, $F_{2,10} = 3.34$, $p = 0.077$, but LSD pair-wise comparisons uncovered significant one tail differences between the 1st and 2nd, or 3rd blocks, $ps = 0.02$; 0.04 . These changes were not replicated in the second run as shown in the right panel of this figure but rats often touched the target rung with one of their fore paws without dislodging it until they stepped on it with a hind paw.

Results in the first run of this experiment might suggest that rats deliberately learned to reduce their fore paw pressure on pre-target and target rungs in attempting to avoid dislodging the latter with that paw. Perhaps rats could detect which rung was damaged when they lightly touched it with a fore paw without breaking it free from its magnets. It is also plausible that supposed changes in fore paw pressure only reflected a more automatic, classically conditioned anticipation of this aversive event. Failure to replicate these results in the second run, though, suggests that rats might not have found the reintroduction of a damaged rung as surprising or aversive. Moreover, the fact that rats increased their BL speeds on the second run, also suggests that they had reduced fear of falling through the ladder consisting of evenly but widely spaced rungs. Direct measurement of rats' paw touch pressures on rungs in future research with our preparation would be required to test these speculations. We note that findings from gait biomechanics research (Cham & Redfern, 2002) reveal that humans adapt to a potentially slippery surface on an inclined ramp by reducing their joint movements to decrease their force in walking. Such changes might correspond to those observed in changes in rats' fore paw reaching and grasping behavior on rungs in the elevated ladder in earlier studies (Soblosky *et al.*, 1997a, 1997b).

One obvious way rats could have avoided dislodging a broken rung would have been by stepping over it with their hind paws once they had detected it with their fore paws. We did not observe any such skipping or hopping behavior in these rats, however. Perhaps rats were unable to use this response strategy because rungs were already either too widely spaced, or too difficult to distinguish from a faulty rung or too strongly held by its end magnets. This reasoning prompted us to run the final experiment in this study as previously described to control for these possible obscuring factors.

3.3. *Experiment 3: Effects of signaling a more easily dislodged target rung with a closer intact rung*

Running speeds. As already noted we did not calculate speed difference scores for the BW phase as in the previous two experiments because as seen in Fig. 5 rats developed a markedly different BL speed pattern when exposed to the added closer (signal) rung in that phase. That is, only when the relative 5th segment contained the added intact rung (see black triangles in left panel) did rats reduce their running speeds as confirmed by a significant segments effect in a one-way ANOVA, $F_{8,40} = 3.31$, $p = 0.005$. Pair, wise (LSD) comparisons further revealed that after significantly decreasing

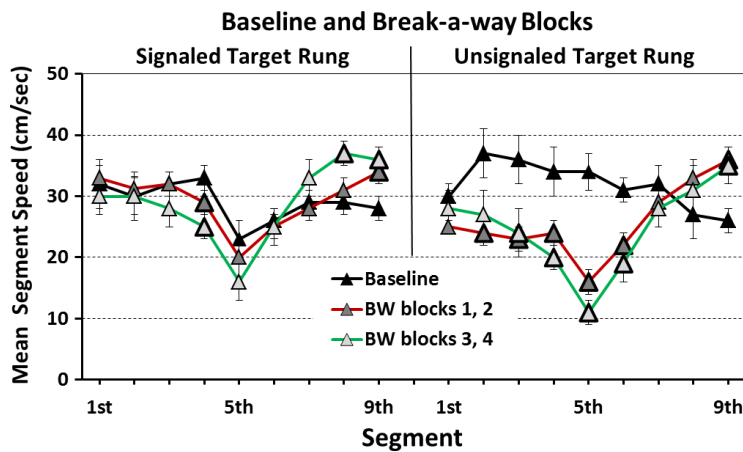


Fig. 5. Mean segment speeds during BL and BW blocks in the third experiment when the 5th segment contained an extra “signal” rung before the target rung (left panel) and when no extra “signal” rung was present (right panel). Enlarged bold bordered data markers represent segment speeds significantly different ($p < 0.05$) from their respective BL segment speeds. The added intact signal rung before the target rung occurred on the 1st and 3rd blocks of BW trials (left panel) and was absent on the 2nd and 4th blocks of BW trials. Vertical error bars are \pm SEM.

their BL speeds on a 5th segment, rats significantly increased them back to those of a 4th segment over the next three segments. In the absence of the added rung during BL trials (see black trials function in right panel) rats did not appear or significantly change their speeds over segments.

Comparisons between each BW block of trials and BL trials with the added closer signaling rung (left panel) revealed that rats reduced their speeds on the relative 5th segment to similar levels and similarly increased them back to their 4th segment BL speeds by their 7th segment. Rats developed slightly lower and higher BW speeds than BL speeds on the 4th and on the 8th and 9th segments, respectively. These observed differences were more prominent for comparisons within the last than first block of BW trials as confirmed by a significant blocks by segments interaction, $F_{8,40} = 6.51$, $p < 0.001$, and by paired-samples t -tests that uncovered significant differences from BL levels on 4th, 8th, and 9th segments. Despite failing to obtain a similar significant interaction for comparisons within the first block of the BW trials, paired samples t -tests also revealed significant differences at the 4th and 9th segments.

Comparisons between each BW block of trials and baseline trials without an added signal rung before the target rung (right panel) found that rats similarly reduced their running speeds on their BW blocks below baseline on segments leading up to and on their 5th segment but increased their speeds to BL on the 7th segment and above it by the 9th segment. These observations were supported by a significant blocks by segments interactions in each of the non-signaled BW block, $F_{8,40} = 7.31$; 9.41 , $ps < 0.001$, and by specific significant paired-samples t -tests.

Although adding an extra rung in a mid-section segment reduced rats' BL running speeds, it did not affect their BW performance compared to that on blocks without an

added closer “signal” rung. By the end of the BW phase, rats displayed similar changes in running speeds on their 4th, 8th and 9th segments under either target rung signaling condition. A supplementary statistical analysis directly comparing performance in the BW phase between the two target rung conditions failed to uncover any significant differences over the last two blocks of trials. Unlike findings from Experiment 2, rats displayed reliable, above BL speeds over the last one or two segments under either target rung condition and thus exhibited more immediate positive contrast “relief-like” effects.

Paw placement patterns. Inspection of each rat’s video clips as it moved across its 5th segment revealed that only one animal failed to dislodge the damaged rung on all 12 BW sessions. That animal did not touch the broken rung with either one of its fore or hind paws on its first BW trial but touched the “signal” rung with both. However, it dislodged the damaged rung on the remaining 11 BW trials with one of its fore paws even though it also touched the signal rung with fore and hind paws on two of the five sessions. Of the other five rats that dislodged the damaged rung on every BW session, four did so always with a fore paw and one did so only once with one of its hind paws during the last block of BW trials that did not contain any added signal rung. These observations clearly show that the presence of an added “signal” rung before a faulty rung did not reduce rats’ likelihood of dislodging the latter with one of their fore paws. Clearly reducing the strength of the broken rung’s end magnets also insured that rats would dislodge it when they touched it with one of their fore paws.

Figure 6 shows rats’ probability stepping on the extra “signal” rung on BL and BW trials. As is evident in this figure rats did not increase their likelihood of stepping on or grasping the signal rung with either a fore paw or a hind paw when the rung after it had been replaced by a damaged rung. Analysis of these data by a 2(paws) \times 3 (blocks) within-Ss ANOVA did not reveal any substantial or significant change in rats’ probability of stepping on the extra rung on BW phase blocks from BL levels. Although rats appeared to step on the extra signal rung more with their fore than hind paws in each block, this difference was not significant, $F_{1,5} = 3.18$, $p = 0.135$.

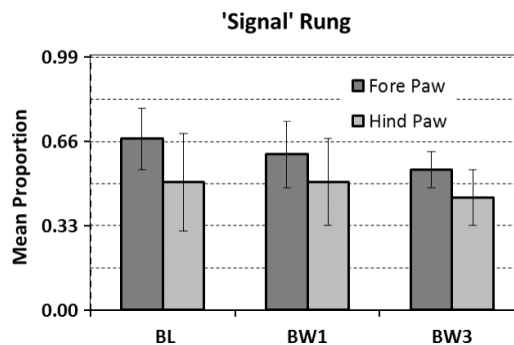


Fig. 6. Proportion of trials in each BL and BW block of the third experiment that rats touched the “signal” rung with their fore or hind paws. Vertical error bars are \pm SEM.

Findings in this final experiment show that introducing an extra closer rung before the faulty rung did not substantially affect rats' BW performance. Specifically rats did not use this rung to avoid dislodging the faulty rung by stepping over it. Either the number of sessions with this extra rung was insufficient for them to learn that it signaled a damaged rung, or the distance required to reach over the latter rung to the next spaced intact rung was still too great to support a skipping/hopping gait strategy. However, unlike rats in the previous experiments, rats in this experiment clearly showed a positive-contrast effect in their running speeds after emerging from the segment with the damaged rung. This apparent "relief-like"/escape reaction suggests that they found dislodging a damaged rung more aversive than rats in the previous experiment either because the location of the broken rung was not fixed within the same segment or was more easily dislodged or both.

4. General Discussion

The major goal in this study was to examine the associative nature of rats' skilled walking performance on the elevated ladder. We asked whether rats would display persistent cautious locomotion when they encountered faulty rungs at fixed locations and would continue such disrupted behavior when re-exposed to the repaired ladder. In the first of the three experiments, rats reduced their running speeds over the area containing or leading up to several damaged rungs. In the second experiment, other rats reduced their speeds as they approached a single faulty rung at a fixed location without decreasing this effect over several trials. More importantly they decreased their likelihood of dislodging it with a fore paw during BW trials in their first run and were about as likely to dislodge it with their hind paws as with their fore paws in the second run in that experiment. In either experiment, rats increased their running speeds up to BL levels as they moved away from the broken rung area of the ladder and also recovered to their BL patterns over three sessions when subsequently run on the "repaired" ladder. In neither experiment did rats display an escape/"relief-like" reaction by increasing speeds above BL during the BW phase but showed a delayed "relief-like" effect during the recovery phase in the first experiment.

Rats were less disrupted in approaching a faulty rung closer to than farther away from the end of the ladder in the second experiment. This difference was attributed to a heightened anticipation of a positive hedonic event as they approached the reward at the end of the ladder that counteracted any disruptive effects from encountering the faulty rung. The question remains, however, whether rats' decline in running speeds reflects classical (Pavlovian) conditioned anticipatory fear of falling through the ladder or an instrumental adaptation to avoid or limit the effects of dislodging a faulty rung with their forepaws. That they easily extinguished their cautious approach to the location of that rung might seem to favor the former over latter explanation. However, rats were unable to avoid stepping on and dislodging it with one of their hind paws when they did not do so with one of their fore paws. Thus, rats had ample opportunity to quickly learn that any deliberative reduction in forepaw

pressure was unnecessary during recovery sessions and thus could have easily learned that stepping on that rung would violate a learned expectancy that it would collapse. Therefore, according to a cognitive theory of instrumental avoidance learning (Seligman & Johnston, 1973) animals can easily extinguish an acquired avoidance response when they are forced to test invalid expectancies that otherwise might maintain that behavior. According to this notion, if rats in the third experiment would have learned to avoid stepping on a signaled faulty rung, they should have become more resistant to extinction of any avoidance/escape tendencies in a recovery phase. We decided not to run such a recovery phase because we were unable to train rats not to step on a signaled faulty rung in that experiment. Rats in that experiment, however, did exhibit a consistent positive contrast “relief-like” or escape effect during the BW phase presumably because the faulty rung had been made easier to dislodge with their fore paws and its location on the ladder was not fixed.

From a methodological aspect, we consider the examination of rats’ locomotion on the elevated ladder easier if not more suitable than on flat surfaces for assessing their higher cognitive processes. We base this idea from research of kinematic and electromyographic analysis of gait behavior revealing greater engagement of supra-spinal and cortical structures while rats are walking on an elevated ladder than on a runway or treadmill (Bolton *et al.*, 2006; Canu & Garnier, 2009). Proprioceptive and kinesthetic information from rats’ more variable forelimb placement and rung grasping behavior on the elevated ladder provides more precise information to supra-spinal and cortical structures to help guide their hind limbs (Bolton *et al.*, 2006). Although information for appropriate limb placement during unimpeded walking on a flat surface of a treadmill need only go to spinal cord structures (Guillot *et al.*, 2008), it may also have to go to supra-spinal structures during interrupted walking. Earlier research of kinematic and electromyography analysis of hind limb placement by cats on the treadmill (Gorassini *et al.*, 1994; Hiebert *et al.*, 1994, 1995) reveals that proprioceptive information from hind limb extensor and flexor muscles’ Golgi tendon organs to supra-spinal structures must occur to insure appropriate hind limb movement and placement in the absence of ground support to it. Unlike rats in the present study, cats in those studies were prevented from obtaining any information with their fore limbs about the presence of a hole on the treadmill. In our research when rats obtained information about a wider gap between rungs produced by a fore paw they seldom if ever appeared to slip through this gap with a hind limb before stepping onto the next intact rung in the second experiment. They might have slipped through such a wider gap with one of their hind limbs, however, if they could only dislodge the damaged rung with one of those paws. We could not measure how far they did so or how quickly they stepped onto the next intact rung in our current preparation. Obviously further modifications to our ladder would be needed to obtain such important kinematic information in any future research.

As previously noted, the skilled walking task on the elevated ladder was originally proposed as more suitable than other tasks of locomotion to assess movement deficits in rodent models of progressive neuro-degenerative diseases because animals could

not use compensatory behaviors to gradually overcome movement/balance problems (Metz & Whishaw, 2002; Farr *et al.*, 2006). In previous research from our laboratories on rodent models of Parkinson's disease, we only measured balanced walking behavior on the rotarod in rats (Somayajulu-Nițu *et al.*, 2009) or on the narrow beam in rats (Muthukumaran *et al.*, 2014) or mice (Muthukumaran *et al.*, 2014; Sikorska *et al.*, 2014). In those studies, only short-term movement deficits were assessed following exposure to certain neurotoxins; e.g., the herbicide paraquat or MPTP, that target dopaminergic neurons in the substantia nigra pars compacta (SNpc) of the basal ganglia. Of particular importance were findings that either prophylactic or therapeutic treatment with possible neuro-protective compounds ameliorated such neurotoxic effects over a few weeks. However, future pre-clinical research requires an examination both of long-term changes in locomotor behavior as a function of progressive neuro-degeneration in untreated animals and of neuro-protection in animals given prophylactic or therapeutic interventions. Under such long-term testing conditions, we suggest the elevated ladder skilled walking task would be more appropriate than the beam or rotarod walking. Of particular interest to us is the fact that rats' performance on our modified skilled walking task reflects higher cognitive and emotional processes that have been shown to deteriorate and change in humans afflicted with neuro-degenerative movement disorders such as Parkinson's disease (DeFina *et al.*, 2013; de Lau *et al.*, 2005; Wu *et al.*, 2012). Therefore, our modified task could also be used to examine similar cognitive and emotional changes in rodent models of this or other experimentally induced neuro-degenerative conditions. Specifically we would want to determine if rats become less able to adjust to changes in the location of a faulty rung and find such events more aversive as higher sensorimotor cerebral areas become more dissociated from those of the basal ganglia.

We plan to use our modified elevated ladder skilled walking task on further research with rodent models of Parkinson's disease that will examine the effects of neuro-degeneration in SNpc and reduction of its production of dopamine. Therefore, we close our discussion by focusing on the application of this behavioral task for testing predictions based on current knowledge of SNpc's influence on motor behavior. These proposed predictions are based on a recent review on the function and neuro-physiology and anatomy of the midbrain basal ganglia (Rothwell, 2011). According to this review, it is generally conceded that basal ganglia do not directly affect an animal's movements because they do not directly receive or send information from or to sensory and motor outputs. Rather they indirectly influence such behavior through connections to forebrain areas, specifically the cerebral cortex and thalamus. The SNpc provides direct output of dopamine to two different types of striatum receptors, D₁ and D₂. Upon the occasion of an unexpected highly salient event such as, in our case, stepping on and dislodging a rung on an elevated ladder, SNpc should produce a burst of firing and increases the release of dopamine. Such increases of dopamine should affect D₁ receptors to facilitate anticipatory changes in a rat's approach to and perhaps escape from the dislodged rung. With successive presentations of this rung collapsing event, associations to maintain appropriate

movement changes should become more reliably instantiated within striatal and cortical circuits to allow SNpc to return its normal homeostatic slower tonic firing activity. When an expected faulty rung is absent, the SNpc would be expected to decrease its tonic firing which would reduce dopamine release that affects D₂ receptors. Over successive presentations of the absence of this previously expected event, SNpc neurons will again return of their tonic firing activity accompanied by extinction of previous anticipatory changes in the animal's locomotion. With progressive decrease of dopaminergic neurons in rodent models of Parkinson's disease, rats in our preparation would be expected to exhibit slower acquisition of anticipatory behavior to a post-morbid introduction of unexpected faulty rungs and slower rates of extinction of any pre- or post-morbid acquired anticipation of them.

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