

# Short-term spatial memory responses in aged Japanese quail selected for divergent adrenocortical stress responsiveness<sup>1</sup>

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**ABSTRACT** Stress-induced glucocorticoids can dampen learning and spatial memory via neuronal damage to the hippocampus. Cognition losses can be transient (associated with acute stress episodes) or permanent as in aged individuals who show chronic glucocorticoid-induced accelerated brain aging and neurodegeneration (dementia). Thus, chronic versus acute stress effects on spatial memory responses of quail selected for reduced (low stress, LS) or exaggerated (high stress, HS) plasma corticosterone (B) response to brief restraint were assessed. Aged food-motivated male LS and HS quail were tested for 10 min in a feed-baited 8-arm radial arm maze (RAM) 1) at 255 d of age (quail who had experienced lifelong management stressors but who were otherwise never intentionally stressed; that is, chronically stressed birds), 2) on the next day post-acute stressor treatment (5 min of restraint), and 3) on the next day without treatment (acute stress recovery). The RAM tests used the win-shift procedure in which visited arms were not rebaited. Radial arm maze performance was measured by determination of

the total number of arm choices made, the number of correct entries made into baited arms out of the first 8 choices, the time required to make a choice, and the number of pellets eaten. Line effects ( $P < 0.001$  in all cases) were detected for total number of arm choices made (HS < LS), number of correct entries made into baited arms out of the first 8 choices (HS < LS), time required to make a choice (HS > LS), and number of pellets eaten (HS < LS). However, neither the effects of day of RAM testing nor its interaction with line further influenced these variables. Thus, although selection for divergent plasma B responsiveness to an acute stressor was found to be associated with severe impairment of spatial memory in aged male HS compared with LS quail, the observed spatial memory impairments (HS > LS) could not be further altered by acute stressor treatment. Line differences in cognition may reflect lifelong management-induced stress episodes that periodically produce higher plasma B responses in HS than LS quail, which underlie HS quail memory deficits, or other etiologies, or both.

**Key words:** Japanese quail, spatial memory, corticosterone, stress

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## INTRODUCTION

It is well known that certain regions of the hippocampus of both mammals and birds are involved in various aspects of learning and spatial memory and that the hippocampus can be damaged via the action of glucocorticoid (GC) hormones associated with stress situations that often result in transient (acute stress-induced) or permanent (chronic stress-induced) impaired cognition (Dachir et al., 1993; Levy et al., 1994; Endo et al., 1996; McEwen et al., 1997; McLay et al.,

1998; Stillman et al., 1998; Diamond et al., 1999; Rose, 2000; Kitaysky et al., 2003; Sandstrom and Hart, 2005; Montaron et al., 2006; Hodgson et al., 2007; He et al., 2008). Indeed, many researchers ascribe to the well-established GC cascade hypothesis of brain aging and neurodegeneration that suggests chronic high levels of corticosteroids or stressful events, or both, underlie accelerated age-dependent hippocampal neuron degeneration (Landfield et al., 1978; Sapolsky et al., 1986; Kerr et al., 1991; Landfield and Eldridge, 1991; McEwen et al., 1997; He et al., 2008). Furthermore, in most animals, including humans, the memory-impairing effects of acute stress or GC administration are generally believed to be reversible, whereas the effects of chronic stress and GC on memory dysfunction may or may not be reversible (Payne et al., 2004). It is also noteworthy that, in mountain chickadees, moderate long-term

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chronic elevation of corticosterone (**B**) via implants apparently does not alter hippocampal anatomy and neurogenesis and such treatment actually facilitates feeding and food-caching, a behavior that at least partially relies on spatial memory for successful cache retrieval (Pravosudov, 2003; Pravosudov and Omanska, 2005). Moreover, the effects of stress on learning and memory performance have not always been straightforward in mammalian species (i.e., a diversity of effects including facilitating, impairing, and no effects have been reported). This led Sandi and Pinelo-Nava (2007) in a recent review to develop multiple hypothetical integrative models that make use of experimental classifying factors such as the source of stress, stressor duration and intensity, stressor timing with regard to memory phase, and learning type to help explain how stress may be affecting memory function.

Although corticosteroid-induced depression of central nervous system-mediated cognitive responses is the most frequently observed outcome in many different mammalian and avian species, because chronic **B** treatment enhances spatial memory independent of hippocampal effects in wild birds (Pravosudov, 2005), further study in this area in avians is warranted. The Japanese quail lines of Satterlee and Johnson (1988) that have been selected for either a reduced (low stress, **LS**) or exaggerated (high stress, **HS**) plasma **B** response to brief mechanical restraint provide a useful model for such study. In addition, these lines permit the study of adrenal cortical influences on cognition using a domestic bird species that is fed ad libitum, does not store food, and is largely unreliant on spatial memory to eat.

We have so far shown that selection in the **LS** quail line has been accompanied by several intuitively desirable physiological and behavioral changes. For example, **LS** quail show reduced adrenocortical responsiveness after exposure to many different nonspecific systemic stressors, less cortical bone porosity, less underlying fearfulness, increased sociality, and less developmental instability than do quail of the **HS** line (Satterlee and Johnson, 1985; Satterlee and Roberts, 1990; Jones et al., 1992a,b, 1994, 1999, 2000, 2002; Satterlee et al., 1993, 2000; Jones and Satterlee, 1996; Marin and Satterlee, 2004; Satterlee and Marin, 2006; Davis et al., 2008; Kembro et al., 2008). Compared with quail of the **HS** line, **LS** quail also exhibit less pronounced reductions in **BW** after exposure to multiple sequential stressors (Satterlee and Johnson, 1985) and accelerated puberty and enhanced reproductive performance in both males (Satterlee et al., 2002, 2006, 2007; Marin and Satterlee, 2004; Satterlee and Marin, 2004) and females (Marin et al., 2002; Satterlee et al., 2008). However, although much is known about social, fear, and sexual behavior in quail stress response line birds studied at various ages, learning and memory behaviors have yet to be studied in young or old **LS** and **HS** birds. Because **HS** quail comparatively exhibit exaggerated plasma **B** responses to many different stressors (their

nonspecific responsiveness nature; see above), then, theoretically, in aged birds, the cumulative effects of lifelong unavoidable stress exposures should provide for a comparatively repeatedly elevated plasma **B** situation in **HS** quail akin to the chronic exogenous **B** or intentional stress exposure treatments that have been used by many researchers in the past (see above). Potential unavoidable lifelong stressors would include events and management practices associated with hatching (e.g., egg pipping, chick emergence, and the pulling and handling of hatched chicks; Ramade and Bayle, 1980; Scott et al., 1981; Tanabe et al., 1983; Freeman and Manning, 1984), leg banding and housing into brooder environments, cold brooding temperatures (Scott and Washburn, 1985, 1986), the installation of wing bands, debeaking procedures (Davis et al., 2004), general bird capture and contact with humans with (Gildersleeve et al., 1988; Satterlee and Johnson, 1988; Jones et al., 1992b, 1994) and without (Jones et al., 1994) brief handling or short- or long-term restraint, and adult bird activities [e.g., animal-caretaker interactions associated with feed replenishments and routine weighing (Jones et al., 2005), cage cleaning-sanitization procedures, peck order establishment and reestablishment when birds are mixed, and mating stress (Sefton and Siegel, 1975; Cunningham and Siegel, 1978)]. Moreover, we know that in adults of an early generation of the quail stress lines, the average absolute and relative (mg/100 g of **BW**) adrenal gland weights of **HS** quail have been found to be 14 to 16% greater than those of random-bred quail (Carsia et al., 1988). This finding can be interpreted to mean that the adrenal glands of **HS** birds reach a greater mass due to the constant exaggerated adrenocortical stress responsiveness of adult **HS** quail to day-to-day perturbations experienced during their lifetime. Thus, herein, we hypothesize that line differences in spatial memory ( $\text{HS} < \text{LS}$ ) should exist in aged quail due to their contrasting genomic predispositions of stress responsiveness ( $\text{HS} > \text{LS}$ ). In addition to determining whether untreated (unintentionally stressed) quail of the **LS** and **HS** lines show differences in spatial memory, it would be worthwhile to determine whether, and if so, in which direction, line differences in cognition exist when an intentional acute stressor is given and if and comparatively how **LS** and **HS** quail exhibit spatial memory recovery from an acute stressor.

Thus, the present experiments were designed to test the above 3 hypotheses. To achieve baseline results, markedly aged male **LS** and **HS** breeder quail were tested for differences in their spatial memory performance in finding food rewards in a radial arm maze (**RAM**). The same birds were then retested in the **RAM** the following day immediately after being briefly restrained in a crush cage that served as an intentional acute stressor. A third sequential day of **RAM** testing was then used to determine if the **LS** and **HS** quail had returned to baseline spatial memory levels the day after the acute stress.

## MATERIALS AND METHODS

### *Birds and Husbandry*

Offspring from generation 41 of the LS and HS lines were studied. Satterlee and Johnson (1988) have described the genetics that underlie the first 12 generations of pedigree selection and the more recent genetic history of the lines, up to generation 34, is discussed in detail elsewhere (Satterlee et al., 2000, 2006; Marin and Satterlee, 2004). Although line differences in levels of plasma B were not measured in the present study, recent studies (J. F. Cockrem, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, New Zealand, and D. G. Satterlee, unpublished data) in the stress lines attest to the maintenance of divergent adrenocortical responsiveness (plasma B, HS > LS) to a variety of nonspecific systemic stressors (e.g., mechanical restraint, handling, and exposure to a novel object). Indeed, Satterlee et al. (2007) have recently offered explanations as to why the gene(s) that control the adrenocortical responsiveness trait in these lines have become fixed.

### *Test Procedures*

Aged (243 d old) adult quail of the LS and HS lines were housed in groups of 12 families per line, each family made up of 3 females and 2 males within a cage of an Alternative Cage Designs 4-tier cage battery (Alternative Design Manufacturing and Supply Inc., Siloam Springs, AR). Individual cage dimensions were 50.8 × 15.2 × 26.7 cm, length × width × height, respectively. Before any treatments, all birds were being maintained on a breeder mash ration (21% CP; 2,750 kcal of ME/kg) with feed and water supplied ad libitum. The daily photoperiod was 14L:10D (approximately 280 lx during the lighted portion of the day); lights-on was at 0600 h and lights-off was at 2000 h daily.

At 243 d of age, all birds were switched to a pelleted game bird ration (20% CP; Purina Game Bird Breeder Layena Product 5438, St. Louis, MO) with feed and water continued ad libitum. At 247 d of age, the females were removed from all cages allowing the males (eventual behavioral test subjects; see below) to reside in their home cages. At 248 d of age (after 5 d of acclimation to the pelleted diet), 20 adult males (2 males/cage) from each of 10 randomly selected families per line (20 LS + 20 HS quail) were fasted between 0600 h to 0800 h to stimulate their food drive. Then, beginning at 0800 h and in an alternating line sequence, 4-bird same-line groups of LS or HS males were captured from their home cages, transported about 10 m to a separate room, and placed in the center hub area of a rat RAM (8-arm RAM, Stoelting Co., Wood Dale, IL) for training (10 min/group). The Stoelting maze used had a central hub octagonal space area that measured 24.1 cm from side to side of each of the 4 opposing walls and each of the 8 arms that radiated off the hub was

50.8 cm long × 11.4 cm wide. Lighting and temperature conditions in the RAM test room were similar to those present in the room wherein the quail were home-caged. Feed pellets were scattered about the RAM to facilitate exploration of the maze and to train the birds to food rewards associated with successful explorations. Group training occurred throughout the day until all 40 males were trained. When training was complete, all birds were refed in their home cages. The above group training procedures were repeated daily for the next 3 d for a total of 4 d of group RAM training. From 252 to 254 d of age, the males of both lines (again in an alternating line sequence) were further trained daily (for a total of 3 more days) in the 8-arm RAM using the same feed withdrawal and refeeding schedules (fasting between 0600 to 0800 h daily and refeeding once all trainings were complete) and training times (10 min) as used in the previous 4 d of group RAM trainings, except that the birds were trained as individuals with 1 feed pellet as a bait at the end of each RAM arm.

It is recognized that fasting ad libitum-fed quail, when applied for a sufficiently long enough period, could be stressful. Therefore, the LS and HS quail in terms of plasma B responsiveness may have perceived feed withdrawal differently. But, unfortunately, we do not know if this occurred in the present study because the only study of the effects of fasting on plasma B response that we have done in LS and HS quail used the much harsher circumstances of both feed and water withdrawal applied by cooping overnight for a very long period of time 12 to 20 h (Jones et al., 1992b). Although this study showed plasma B responses in HS quail to be greater than those found in LS birds, it was impossible therein to separate the potential effects of bird capture and handling for transport to the coops, the novelty of the non-home cage environment (the newness of the coop environment per se), test subject isolation distress from familiar conspecifics left behind in the home cages, the establishment of new social interactions-hierarchies when mixed with strangers in the coops, and stocking density (crowding) changes from the main intended treatment stressor component of a simple feed and water fast. Furthermore, it deserves note that, unlike in Jones et al. (1992b), in the present RAM study, only feed (water being continued) was taken away and for much shorter periods of time, 2 h before testing of the first birds tested each day and for slightly longer periods of time, but never exceeding 6 to 8 h on a given test day, for birds tested throughout the day. Also, it needs to be recognized that at least some feed restriction was needed presently to increase food drive during RAM testing; otherwise, the use of ad libitum-fed birds at testing would have likely been met with much less or no motivation by the test subjects to consume the feed pellet rewards when traveling down the arms of the RAM. Pre-RAM food or water, or both, restriction protocols similar to the protocol we presently used are well documented in the literature for RAM studies (Stillman et al., 1998).

The day after the third and final day of individual bird RAM training (i.e., beginning at 255 d of age), all of the males were individually tested in the RAM for 10 min (once again in an alternating line sequence) for their spatial memory behaviors in accordance with the win-shift procedure in which visited arms of the RAM were not rebaited. This test day was labeled as a baseline test day because the birds were captured from their home cages and directly transported to the behavioral test room for RAM testing without delay. However, the reader is reminded that although the baseline test day was used to detect whether spatial memory line differences existed in the LS and HS quail without the use of any intentional stressors, it nevertheless was considered to be reflective of the effects of the lifelong unavoidable management stressors. A test bird was considered to have made a choice of having entered an arm of the RAM once it moved from the central area of the RAM and to a point where more than half of its body was located halfway down the length of a maze arm. In most cases that this occurred, the test subject was observed to have eaten the feed pellet bait. Reentries into previously visited arms using the same arm travel distance criteria were scored as errors. Each test trial was terminated when all 8 arms of the RAM were visited, when 16 entries were made, or at the end of 600 s (total test session time), whichever came first.

The day after baseline test day testing (at 256 d of age), all of the males were individually tested a second time in the RAM for 10 min using the procedures described above for baseline test day except that individuals were restrained for 5 min in a crush cage immediately after being captured from their home cages and before RAM testing. This test day was designated acute stress day and it was used to assess whether an acute stressor right before RAM testing alters RAM performance when compared with the baseline behavioral responses that were observed on the previous day, the baseline test day. We believe that 5 min of restraint in a crush cage represents an appreciably greater stressor than just bird capture and handling for transfer to the RAM based on several previous studies. For example, using LS and HS quail, Jones et al. (1994) showed that capture and 5 min of restraint before blood sampling, capture and return to the home cage for 5 min before recapture and bleeding, and no human disturbance treatments were found to yield plasma B responses of the following ranked order (highest to lowest): capture and 5 min of restraint before blood sampling > capture and return to the home cage for 5 min before recapture and bleeding > no human disturbance. In addition, Satterlee and Marin (2006) found that control quail of the LS line that were simply captured and transported to an open field showed a higher mean ambulation rate, less freezing behavior, and increased numbers of floor sectors entered than did HS controls and LS and HS quail stressed by 5 min of immobilization before testing. Finally, in rats, a precedent study that demonstrates short-term memory reduction after brief nor-

mothermic restraint treatment using a RAM has been done (Stillman et al., 1998). Indeed, this study, which prompted our present work and from which we modeled our present treatments, showed that 15 min of normothermic restraint treatment decreased the number of correct entries into baited RAM arms out of the first 8 choices made (**NUMCOR8**) and the total number of RAM arm choices (**TOTCHOICES**) responses while increasing the time required to make a choice (**TIME/CHOICE**) response in RAM-tested rats. We measured the same 3 behavioral variables presently in making our control (unrestrained) versus 5-min restraint stressed and LS versus HS quail comparisons on the acute stress day.

To determine the extent of performance recovery from the acute immobilization stressor, the following day (at 257 d of age; acute stress recovery day), all of the males were individually tested a third and final time in the RAM for 10 min using procedures identical to those described above for the baseline test day. Although we do not have direct RAM testing stressor behavioral or physiological evidence to support the use of a 1-d interval as being a sufficient enough amount of time to allow for acute stress recovery, the literature teaches that plasma B elevations in response to all sorts of short-term stressors (even more severe ones than were used herein) are widely known to be minute-to-minute or, at best, hour-to-hour situations. For example, Hull et al. (2007) have shown that control quail handled by repetitively picking them up, inverting them, and putting them down for 15 min showed transient plasma B elevations that had dissipated by 60 min. This suggests that it is highly unlikely that plasma B levels remained elevated above basal levels a full 24 h in response to the milder short-term stressor (10 min of RAM treatment) used herein. Furthermore, the precedent rat study of Stillman et al. (1998) mentioned above used even more severe stressors (15 min of restraint coupled with cold water immersion) in conducting their RAM studies in which the same treatment application timeline (training day 7 = baseline, stress day testing 24 h later, and post-stress day recovery 24 h later) was applied using the same variables as we studied.

During all 3 d of RAM testing, quail behavior in the RAM was videorecorded using an HP Elite Autofocus Webcam camera (Hewlett-Packard Co., Palo Alto, CA) that hung approximately 2 m above the RAM.

### **Variables Measured**

Upon replay of the video recordings, the following measures were recorded: **TOTCHOICES**, the time to complete a session, and **NUMCOR8**. The **TIME/CHOICE** was calculated as the total time for completion of the session divided by the total number of entries. The reader is reminded that for the determination of **TOTCHOICES**, **NUMCOR8**, and **TIME/CHOICE**, a test trial was terminated when all 8 arms of the RAM were visited, when 16 entries were made, or at the end

of 600 s (test session ceiling time), whichever came first. These 3 variables and test constraints were the same as those used by Stillman et al. (1998) in studying spatial memory responses in rats under similar experimental conditions. In addition, the number of feed pellets (**PELLETS**) eaten by a test subject (0 to 8) at the end of a full 10 min spent in the RAM was presently recorded.

## Statistical Analyses

The dependent variables examined were TOTCHOICES, NUMCOR8, TIME/CHOICE, and PELLETS. Each of these variables was statistically analyzed by a repeated measures 2-way ANOVA using stress line (LS vs. HS) as the between-subjects factor and day of test (baseline test day vs. acute stress day vs. acute stress recovery day) as the within-subject factor. Post hoc mean comparisons were performed using Duncan's multiple range test.

## RESULTS

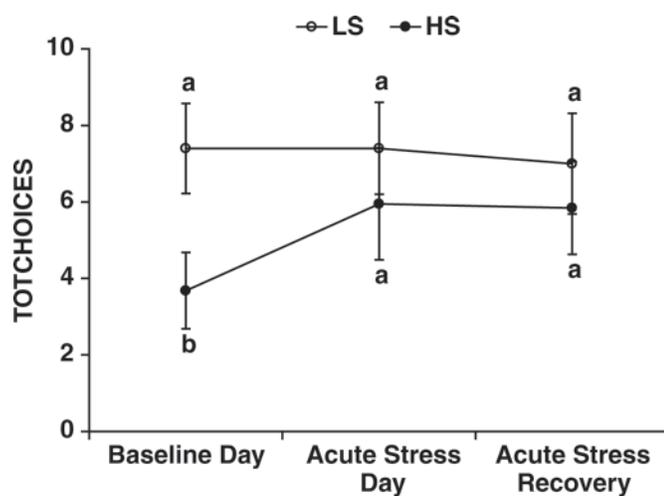
Significant effects ( $P < 0.001$  in all cases) were detected for mean ( $\pm$ SE) line differences in TOTCHOICES (HS,  $5.2 \pm 0.7 < LS, 7.3 \pm 0.7$ ), NUMCOR8 (HS,  $2.9 \pm 0.4 < LS, 4.8 \pm 0.4$ ), TIME/CHOICE (HS,  $281.0 \pm 33.9$  s  $> LS, 181.9 \pm 32.4$  s), and PELLETS (HS,  $2.5 \pm 0.4 < LS, 4.4 \pm 0.5$ ). Test day and its interaction with line did not affect any of these variables. Post hoc tests of the line  $\times$  day treatment means resulted in the statistical differences depicted by the letters contained in Figure 1 (TOTCHOICES), Figure 2 (NUMCOR8), Figure 3 (TIME/CHOICE), and Figure 4 (PELLETS). The following outcomes on the initial day (baseline test

day) of RAM testing are noteworthy: 1) HS quail made half as many RAM arm choices (TOTCHOICES), took approximately twice as long to make a choice (TIME/CHOICE), and made half as many correct choices in the RAM (NUMCOR8) than did LS quail and 2) at the end of a full 10 min of testing, on average, LS quail ate 2.4-fold more of the food pellets that were placed at the ends of the baited 8 RAM arms than did HS quail.

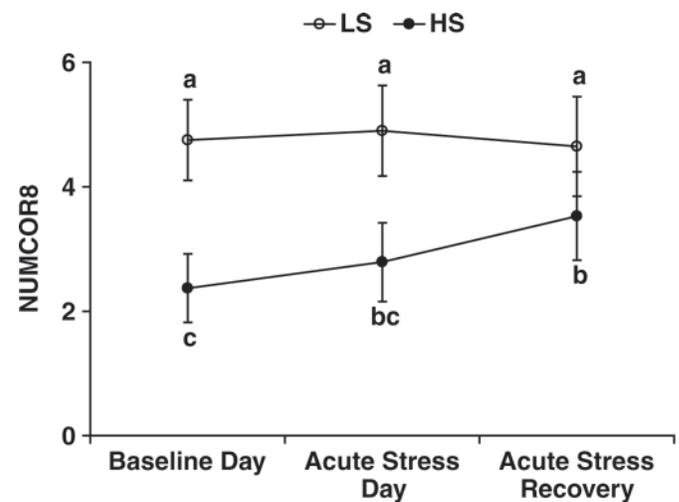
## DISCUSSION

### Baseline Test Day

Overall, short-term spatial memory, as measured by multiple behavioral parameters in a RAM, was impaired in aged male HS compared with LS quail. The line differences detected were particularly evident on the first day of RAM testing (d 1, baseline test day) wherein the birds were not intentionally stressed beyond their lifelong experiences. Although we did not determine whether hippocampal neurogenesis has been affected differentially in the LS and HS quail, it is tempting to speculate that our d 1 findings support the GC cascade hypothesis of brain aging and neurodegeneration that suggests chronic administration of corticosteroids or stressful events, or both, underlie accelerated age-dependent hippocampal neuron degeneration (Landfield et al., 1978; Sapolsky et al., 1986; Kerr et al., 1991; Landfield and Eldridge, 1991; McEwen et al., 1997; He et al., 2008), which, in turn, is associated with impaired cognition (Dachir et al., 1993; Levy et al., 1994; Endo et al., 1996; McEwen et al., 1997; McLay et al., 1998; Kitaysky et al., 2003; Sandstrom and Hart, 2005; Montaron et al., 2006; He et al., 2008). It deserves noting



**Figure 1.** Mean ( $\pm$ SE; vertical bars) total number of choices (TOTCHOICES) made by low-stress (LS) and high-stress (HS) quail to enter arms of a baited 8-arm radial arm maze on d 1 (baseline day), 2 (acute stress day), and 3 (acute stress recovery day) of testing. Note: a test trial was terminated when all 8 arms of the maze were visited, when 16 entries were made, or at the end of 600 s (test session ceiling time), whichever came first. Means with different letters are significantly different at  $P < 0.05$ .

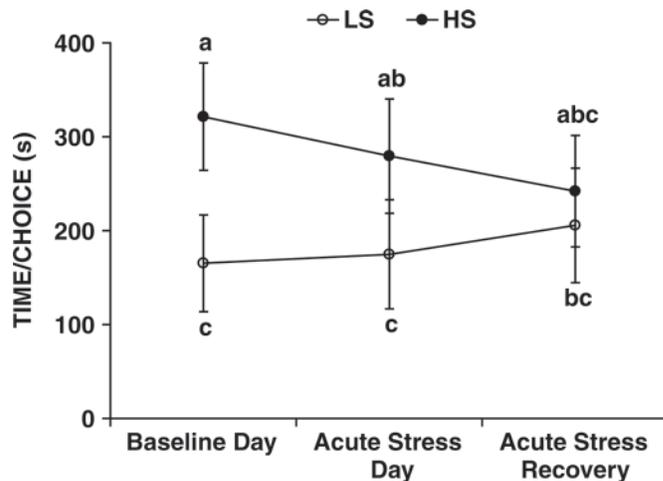


**Figure 2.** Mean ( $\pm$ SE; vertical bars) number of correct entries into baited arms of an 8-arm radial arm maze out of the first 8 choices made (NUMCOR8) by low-stress (LS) and high-stress (HS) quail on d 1 (baseline day), 2 (acute stress day), and 3 (acute stress recovery day) of testing. Note: a test trial was terminated when all 8 arms of the maze were visited, when 16 entries were made, or at the end of 600 s (test session ceiling time), whichever came first. Means with different letters are significantly different at  $P < 0.05$ .

that our quail maintained for 255 d under the present intense confinement-rearing conditions should have experienced a multitude of unavoidable daily management stressors associated with the hatching, brooding, and breeding phases of their lives (see Introduction and Materials and Methods for details of potential stressors). We submit, therefore, that one possibility to explain our findings is that the cumulative effects of such lifelong periodic stress episodes could have, in many instances, produced higher plasma B responses in HS than LS quail. Over the life of the quail, habitually exaggerated adrenocortical stress responses in HS birds may have been sufficient enough to cause the short-term memory deficits observed in them.

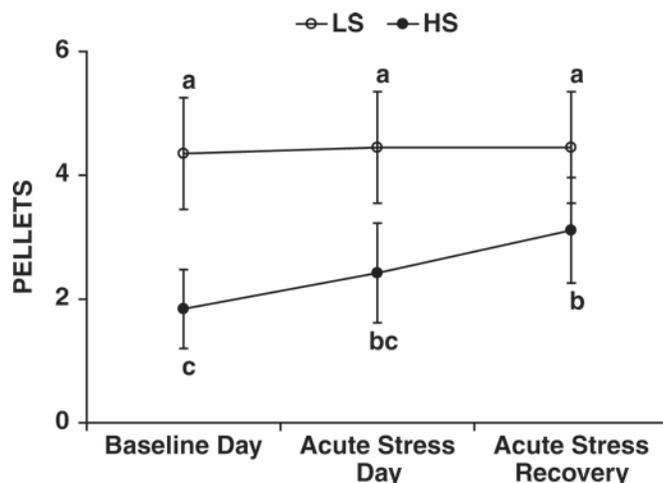
It is important to note that several other possibilities exist for explaining the line differences in the short-term memory outcomes described above for the baseline test day. Presently, plasma B responses in LS and HS quail to the proposed lifelong management stressors, posthandling for transfer to the RAM apparatus, and following restraint in a crush cage were not assessed. In addition, we collected no data on RAM performance in young HS and LS quail or on the speed with which unstressed birds of both lines learned the RAM task during training. Thus, in lieu of not knowing the influences of these variables, in addition to our proposal that lifelong exposure to management stressors impairs short-term spatial memory to a greater degree in HS quail, the observed line differences in RAM testing might simply reflect 1) line differences affecting memory that are independent of age or exposure to management stressors, 2) line differences in how quickly individuals learn the RAM task, or 3) line differences in fear and distress responsiveness due to bird capture, handling and transfer to the RAM, or to the RAM testing per se.

Of the 3 alternative possibilities for explaining the present RAM data just listed, a seemingly strong argument could be made that the stress line impairment in spatial memory (HS > LS) found on d 1 of RAM testing might simply reflect the greater underlying fearfulness (HS > LS) that is known to exist between the quail stress lines. Indeed, HS quail have consistently shown greater fear than their LS counterparts in multiple tests of fear or timidity, or both [e.g., tonic immobility (Jones et al., 1992b; Satterlee et al., 1993; Davis et al., 2008), open field (Jones et al., 1992a; Satterlee and Marin, 2006; Kembro et al., 2008), emergence from a hole-in-the wall box (Jones et al., 1999), and struggling in a crush cage (Jones and Satterlee, 1996; Jones et al., 2000) tests]. In each of these tests, the positive association between increased adrenocortical activation and heightened fearfulness in HS quail has been interpreted using the hypothesis that fear is associated with increased silence and inactivity. Thus, one is tempted to conclude that the reduced exploration (TOTCHOICES) and less successful obtaining of food baits (PELLETS) by HS quail in the present RAM studies might be simply a reflection of the well-known increased fearfulness (i.e., reduced locomotion) in birds of this line considering



**Figure 3.** Mean ( $\pm$ SE; vertical bars) time required by low-stress (LS) and high-stress (HS) quail to make a choice to enter an arm of a baited 8-arm radial arm maze (TIME/CHOICE) on d 1 (baseline day), 2 (acute stress day), and 3 (acute stress recovery day) of testing. Note: this variable was calculated by dividing the total time for completion of a test session by the total number of maze arms entered using the criteria that a test trial was terminated when all 8 arms of the maze were visited, when 16 entries were made, or at the end of 600 s (test session ceiling time), whichever came first. Means with different letters are significantly different at  $P < 0.05$ .

that they were placed in a novel (potentially frightening) environment, the RAM. However, unlike in RAM testing, training to the apparatuses used in each of the tests of fear mentioned above (tonic immobility, open field, hole-in-the-wall emergence, and struggling tests) was not used. Theoretically, the 7 d of RAM training should have greatly reduced fear of the RAM test apparatus. Also, there was no food reward (environmental relaxing feature) used as a stimulus in food-motivated subjects in our previous tests of fear and none of those



**Figure 4.** Mean ( $\pm$ SE; vertical bars) number of feed pellets (PELLETS) eaten by low-stress (LS) and high-stress (HS) quail within a baited 8-arm radial arm maze on d 1 (baseline day), 2 (acute stress day), and 3 (acute stress recovery day) of testing. Note: this variable was determined at the end of a full 10 min (600 s) of time spent in the maze. Means with different letters are significantly different at  $P < 0.05$ .

tests required solving a maze. Therefore, we believe the poor performance of HS quail in the food-baited win-shift RAM tests more reflects impairment in memory function rather than known line differences in underlying fearfulness.

Also at first glance, the possibility that line differences exist in how quickly individuals learn the RAM task (i.e., HS birds may have simply lagged behind LS ones in reaching asymptotic levels of performance in the RAM, the second alternative explanation hypothesis listed above) seems to be another strong candidate to explain the observed impairment in short-term memory of HS quail on the baseline test day of testing. However, in view of certain behavioral outcomes on d 2 and 3 of RAM testing, this explanation for our data remains guarded (see acute stress day and acute stressor recovery day discussions below). This leaves then the idea that the present line memory differences observed might be independent of age or exposure to management stressors (alternative hypothesis 1 above). Unfortunately, this possibility will have to also remain unaddressed until an additional study that tests short-term memory responses in young LS and HS quail is conducted. Determining whether hippocampal neurogenesis has been affected differentially in young LS and HS quail would also be worthwhile.

It should also be noted that stressor intensity (and therefore B response) is one of the classifying factors used in the review of Sandi and Pinelo-Nava (2007) to explain how stress might affect memory function. In fact, an inverted-U relationship between B levels and hippocampal neuron firing rates has been demonstrated (Diamond et al., 1992); it suggests although both low and high increases in B levels seem to decrease hippocampal neuron firing rates, moderate elevations in B may do the opposite, increase firing rates. The average blood B response to brief mechanical restraint in our HS quail typically exceeds basal B levels by 10-fold or more, and, in some HS individuals, 25-fold and higher B responses have been observed (D. G. Satterlee, unpublished data). This supports the hypothesis that stressor-induced greatly exaggerated B release in HS quail may be associated with decreased hippocampal firing rate and thus compromised short-term spatial memory as seen herein. In contrast to our d 1 baseline test day HS quail memory deficit findings, but in keeping with the inverted-U relationship between B and hippocampal neuron firing rates postulated by Diamond et al. (1992), Pravosudov (2003, 2005) and Pravosudov and Omanska (2005) have found that implant-induced moderate long-term chronic elevation of B by 150% above baseline in mountain chickadees facilitates feeding and food-caching, a behavior that “at least partially relies on spatial memory for successful cache retrieval.” However, it also deserves note that domesticated Japanese quail have been selected for maintenance under confinement rearing and ad libitum feeding conditions. Thus, unlike chickadees, wintering parids that naturally

cache food, *Coturnix* do not store their food (Papini, 2002) and would therefore not rely heavily on spatial memory for food retrieval.

### Acute Stress Day

In 3 of the 4 variables measured (NUMCOR8, TIME/CHOICE, and PELLETS), the chronic stress line impairment in spatial memory performance (HS > LS; seen at 255 d of age on the baseline test day) could not be further altered by application of a single acute stressor the following day (5 min of immobilization at 256 d of age; d 2 of RAM testing, acute stress day). Of these 3 variables, we consider NUMCOR8 and PELLETS to be the most important indicators of spatial memory performance. In contrast to our NUMCOR8, TIME/CHOICE, and PELLETS findings, line differences were no longer evident in TOTCHOICES on the acute stress day of RAM testing. Collectively, our acute stress day findings are somewhat surprising because they differ in many ways from numerous studies that have examined the effects of acute stressors on spatial memory in both mammals and birds. For example, in rats tested in a radial arm water maze, acute predator (cat) exposures that dramatically increase plasma B have been associated with considerable impairment of short-term hippocampus-dependent spatial memory tasks, as well as processes involved in memory consolidation and retrieval (Diamond et al., 1999; Park et al., 2008). In addition, in the rat study used to help in the design of the present experiments (Stillman et al., 1998), the use of both brief normothermic-restraint and cold-restraint treatments decreased TOTCHOICES and NUMCOR8 and increased TIME/CHOICE RAM measurements in comparison to normothermic-unrestrained (control) responses. This study even detected differences in the severity of the 2 acute stressors in impairing spatial memory, cold restraint being more potent than just normothermic-restraint treatment, suggesting that the effects of acute stressors on memory impairment can be additive and, assumedly, related to the effects of greater B release on neurodegradation that would be expected in the combined restraint plus cold stressor treatment. It deserves noting, however, that the rat studies cited above dealt with the effects of acute stressors on cognitive responses of nonselected (randombred) animals. In contrast, our HS birds are genetically predisposed toward exaggerated adrenocortical stress responsiveness. Thus, our findings of no further decreases in the short-term memory of HS quail after acute stress, as reflected in the TOTCHOICES, NUMCOR8, and TIME/CHOICE variables on d 2 of RAM testing (acute stress day), may mean that the hippocampus brain areas that affect spatial memory in HS quail were so affected from previous lifelong exaggerated B stress responses that they could not be affected further by an acute stressor treatment (5 min of restraint). In other words, it may be that, due to habitual exaggerated B responses in

HS quail, permanent hippocampal neuron degeneration has occurred, changes consistent with decreased neuron volumes or firing rates, or both, that affect short-term memory as has been demonstrated by studies that support the GC cascade hypothesis of brain aging and neurodegeneration (Landfield et al., 1978; Sapolsky et al., 1986; Kerr et al., 1991; Landfield and Eldridge, 1991; McEwen et al., 1997; He et al., 2008). This explanation must, however, remain guarded because we did not conduct any brain neurological studies. Furthermore, this theory would not explain why we did not find any additional decrease in RAM memory responses of LS quail after subjecting low line birds to the same acute stressor. It could also be, however, that LS birds are so rife with hippocampus neurons that affect short-term memory that small perturbations from B release cannot produce sufficient enough neurodegradation to momentarily negatively affect this memory system. Regardless of why LS quail are resistant to the effects of B release during acute stress, this represents yet another intuitively desirable trait that has accompanied selection for reduced adrenocortical responsiveness.

Using zebra finches selected for peak blood B response to 20 min of restraint in a cloth bag, Hodgson et al. (2007) compared their performance of a spatial associative memory task (the number of hook-and-loop fastener-secured felt flaps lifted by birds to uncover feed in circular wells beneath the flaps) to an unselected (control) line. Control finch performance was superior to that of their high B line birds. Unfortunately, the age of the test finches was not given. But, because their birds were adults at testing, we suggest that their line difference finding (high B line > controls in memory impairment) offers support to our contention that chronic (lifelong) stress in our HS quail (birds similarly selected for exaggerated adrenal stress responsiveness) underlies their decreased short-term memory. That said, however, Hodgson et al. (2007) also found that, after application of their selection stressor, all their finches, regardless of line, failed to perform the spatial associative memory task. This implies that, at least in zebra finches, an acute stressor, albeit presumably a very severe one (20 min of restraint in a cloth bag without any visual clues) that would likely be accompanied by very high releases of B, is capable of totally eliminating short-term memory abilities. However, it is also possible that the stress of such severe restraint may have simply made the birds just unwilling to perform the test situation. Regardless of how the cloth bag restraint stressor caused the finch of Hodgson et al. (2007) to not perform the spatial memory task, this finding is very unlike our findings on d 2 of RAM testing (acute stress day) that the application of a comparatively milder acute stressor (5 min of restraint without exclusion of visual input) immediately before memory testing largely does not alter the line memory difference (HS > LS) that we detected on the previous baseline test day and have proposed to be a result of chronic stress.

## **Acute Stressor Recovery Day**

Considering the d 2 (acute stressor day) findings that brief immobilization largely (see above and below) did not produce any further major changes in RAM memory responses beyond the line differences (LS > HS in spatial memory performance) found in all 4 measurements made on d 1 of testing (baseline test day), no further line differences in spatial memory responses would logically be expected, nor were any found, when the RAM tests were repeated for a third consecutive day (at 257 d of age; what was originally envisioned as an acute stressor recovery day). It deserves noting, however, that by the third day of RAM testing, the original line differences on d 1 in all 4 spatial memory parameters (i.e., HS quail showing inferior TOTCHOICES, NUMCOR8, TIME/CHOICE, and PELLETS responses than LS quail) had disappeared. This was largely due to what appeared to be gradual and continual slight improvements in most of these parameters across time in the HS quail. These findings argue back in the direction that B, in certain instances, might actually facilitate short-term memory. Another possibility to explain the d 3 results is that the HS quail may have lagged behind the LS birds in improving their daily performance during RAM training and had not, unlike LS quail, yet reached asymptotic levels (Stillman et al., 1998) by the beginning of recorded RAM tests. However, if behavioral ceilings had not been reached by the end of 7 d of RAM training, then only the statistical improvement in TOTCHOICE RAM performance on d 2 compared with d 1 in HS quail could be used to argue such a case. In fact, only slight numerical improvements in NUMCOR8, TIME/CHOICE, and PELLETS on d 2 were seen in HS quail and the SEM for these same 3 parameters across time (during all 3 d of testing) overlapped in HS quail. Although the RAM training period used herein was of the same length (7 d) and very similar in nature to that used by Stillman et al. (1998) in rats to produce maximum RAM performance before application of their restraint and cold-restraint treatments (see above), we did not measure the treatment variables during our training period and therefore cannot rule out a hypothesis of variable time requirements for both the LS and HS quail to reach asymptotic levels of RAM performance.

In summary, we found short-term memory responses in a food-baited RAM to be severely impaired in aged male HS compared with LS quail. It is possible that lifelong exposure to management stressors may have produced periodically higher plasma B releases in HS than LS quail that, in turn, impaired short-term spatial memory to a greater degree in HS quail. The observed line differences in RAM testing may also reflect 1) line differences affecting memory that are independent of age or exposure to management stressors, 2) line differences in how quickly individuals learn the RAM task, or 3) line differences in fear and distress responsive-

ness due to bird capture, handling and transfer to the RAM, or to the RAM testing per se. The stress line impairment in spatial memory performance (HS > LS) could not be further altered by application of an acute stressor.

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