Stressor-Induced Changes in Open-Field Behavior of Japanese Quail Selected for Contrasting Adrenocortical Responsiveness to Immobilization[1]

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ABSTRACT The effect of no treatment (undisturbed controls; CON) or 5 min exposure to immobilization stress (STR) before assessment of open-field behavior in Japanese quail lines selected for high (high stress; HS) or low (low stress; LS) plasma corticosterone (B) response to a similar immobilization stress was examined. During a 5-min test period in an open field, the following behavioral measurements were made: the latency to ambulate (LAMB), the number of open-field floor sectors entered (SECTORS), and freezing behavior (total time spent in complete silence and inactivity, apart from slight movements associated with respiration; FREEZE). A further measure of ambulation across time (ambulation rate; ARATE) was calculated using the formula: ARATE = (SECTORS/[(300 s test ceiling) − LAMB)]. The LS-quail showed higher ($P < 0.04$) ARATE and lower ($P < 0.05$) FREEZE behavior than did their HS counterparts. When compared with the CON, quail exposed to STR showed decreased ARATE ($P < 0.02$) and numbers of open-field SECTORS entered ($P < 0.04$). More importantly, the LS-CON exhibited a higher ($P < 0.05$) mean ARATE, less FREEZE behavior, and increased numbers of SECTORS entered than did the other 3 treatment groups (LS-STR, HS-CON, and HS-STR), all of which showed similar responses for these variables. The LS-CON vs. HS-CON findings support our contention that selection for reduced adrenocortical responsiveness has been accompanied by a concomitant decrease in underlying fearfulness (exemplified herein by heightened activity in the open field in LS-CON). We further show here that this relationship was eliminated by application of an acute stressor before open-field testing of LS quail, wherein a reduction in activity similar to that found in both HS quail treatment groups was evident in comparison to the LS-CON.

Key words: Japanese quail, corticosterone, open-field, stress

INTRODUCTION

Although activation of the hypothalamic pituitary adrenocortical (**HPA**) axis is considered to be an adaptive response to stress, prolonged stimulation of the HPA axis that accompanies certain fear and distress states can exert many deleterious effects on the performance and welfare of fowl. Negative consequences of fear and stress include energy wastage, decreased growth, feed conversion, egg production, and product quality, delayed maturation, compromised hatchability, decreased immunocompetence, developmental instability, the development of feather pecking, and the enhanced likelihood of injury, pain, and even death (Jones, 1996; Faure and Mills, 1998; Jones and Hocking, 1999). Although several strategies have been offered to combat these negative outcomes, genetic selection remains one of the most rapid and reliable methods of eliminating harmful traits and promoting desirable ones, thereby improving animal productivity and well-being (Mench, 1992; Craig and Swanson, 1994; Jones, 1996; Faure and Mills, 1998; Jones and Hocking, 1999). Indeed, selection of Japanese quail for a reduced adrenocortical responsiveness to brief immobilization (low stress or **LS** line; Satterlee and Johnson, 1988) has been accompanied by a number of intuitively desirable changes, such as a nonspecific reduction in stress responsiveness to many different stressors (measured by both plasma corticosterone and heterophil:lymphocyte ratio responses), reduced fearfulness (measured by tonic immobility, open-field, avoidance of novel objects and experimenters, restraint-induced changes in struggling behavior, and timidity by hole-in-the-wall emergence box tests), greater sociality (as seen in runway and social proximity tests), less developmental instability, higher body and liver weights, less severely compromised bone strength, accelerated puberty and heightened early egg lay in females, and accelerated puberty and reproductive

advantages in males that persist well into adulthood (Satterlee and Johnson, 1985; Gildersleeve et al., 1987; Satterlee and Johnson, 1988; Satterlee and Roberts, 1990; Jones et al., 1992a,b, 1994, 1999, 2000, 2002, 2004; Satterlee et al., 1993; Jones and Satterlee, 1996; Satterlee et al., 2000; Marin et al., 2002b; Satterlee et al., 2002; Marin and Satterlee, 2003, 2004; Satterlee and Marin, 2004).

In the behavioral tests just cited that document differences in fearfulness between the LS quail line and its contrasting line selected for exaggerated adrenocortical stress responsiveness (high stress or **HS** line; Satterlee and Johnson, 1988), where appropriate, we have interpreted test results using the widely held belief that greater inactivity is indicative of greater fearfulness (see later). Thus, in the open-field study of Jones et al. (1992a), the observations that chicks of the LS quail line showed less freezing and ambulated sooner than did their HS counterparts were construed to mean that 1) an open-field (novel environment) elicited less fear in LS quail, and 2) fearfulness and adrenocortical activation are positively associated (since LS quail consistently show reduced plasma corticosterone (B) release in response to many different nonspecific systemic stressors). In that study, however, no attempt was made to deliberately stress the animals beyond the stressors that would be unavoidably associated with open-field testing (e.g., bird capture and transport to the test apparatus, novelty of the test environment, and isolation distress). Therefore, it was assumed that the observed line differences in behavior were directly related to suspected differences in adrenocortical activation between the lines in response to the novelty of the open field.

Additional evidence that a positive relationship likely exists between adrenocortical activation and heightened fearfulness also exists in stressed, genetically unremarkable birds. These studies present a theme that subjecting animals to a negative experience increases their subsequent fear behavior responses to a second threat (i.e., the unavoidable stressful elements of the behavioral test per se). For example, short-term stressful stimulation, such as electric shock, loud noise, or social separation, prolongs tonic immobility in domestic fowl (Jones, 1986). Similarly, prior exposure to an electric shock (Gallup and Suarez, 1980) or a partial water immersion (Marin and Martijena, 1999) before placement in an open-field also decreases the tendency of chickens to ambulate and escape from this situation. Thus, a reduced kinetic activity appears to be associated with increased fear reactions induced by prior short-term stress exposure. In all likelihood, this relationship in random bred birds is likely at least partially driven by stressor-induced increases of plasma B (see previous discussion). Therefore, herein, we wondered whether the use of an acute stressor would further enhance the fear behavior differences documented in our quail stress response lines wherein open-field behaviors were previously measured only in undisturbed quail (Jones et al., 1992a). To test this suspicion, we essentially repeated our earlier study treatments (the LS and HS control groups herein) and added an assessment of the potential to yet further alter fear behaviors during open-

field testing in the quail stress response lines via the use of deliberate immobilization in a crush-cage for 5 min (a similar stressor regimen to that used in the selection program) followed by 55 min of respite before open-field testing. Because sex differences in stress susceptibility (Marin et al., 2002a) and open-field behavior (Jones, 1977; 1978; Jones and Faure, 1982) have been reported, we only tested females from each line.

MATERIALS AND METHODS

Animals and Husbandry

Female Japanese quail from generation $(G)_{32}$ of the LS and HS lines of Satterlee and Johnson (1988) were used in the present study. The complete genetic history of the lines, up to G_{31}, is discussed elsewhere (Marin and Satterlee, 2004).

The study quail were taken from a larger population of an approximately 300 mixed-sex bird hatch. Egg incubation, chick brooding, feeding, and lighting procedures were similar to those described by Jones and Satterlee (1996) with the exception that, at hatch, 288 chicks were leg-banded and housed in mixed-line groups of 48 birds (24 LS + 24 HS) per compartment in each of 6 compartments ($102 \times 64 \times 20$ cm; length × width × height) of a model 2S-D, 6-deck Petersime (Petersime Incubator Co., Gettysburg, OH) brooder battery modified for quail. To maintain the line identity of each bird, leg bands were replaced with permanent wing bands at 21 d of age.

At 4 wk of age, birds were sexed by plumage coloration, and 48 females of each line were individually and randomly caged in two 4-tier cage batteries (Alternative Design Manufacturing and Supply, Inc., Siloam Springs, AR; each battery contained 48 laying cages). Cages measured $50.8 \times 15.2 \times 26.7$ cm (length × width × height). Coincident with placement in cages, birds were given a laying ration (21% protein, 2,750 kcal of ME/kg) and water ad libitum and subjected to a daily cycle of 14 h light (280 to 300 lx):10 h dark until the end of the study (at 14 wk of age). The chicks then remained undisturbed, apart from routine maintenance, until testing began.

Treatments

At 5 wk of age, the open-field behavior (see later) of 46 quail from each of the 2 lines was measured after their exposure to 1 of 2 treatments. Two LS and 2 HS birds either died or escaped from their cages between 4 and 5 wk of age (the week following housing into laying cages); therefore, since no attempt was made to replace birds that died or escaped, a remainder of 46 of the original 48 females per line was available for study at 5 wk. Unstressed chicks (n = 23 LS or HS controls; **CON**) remained undisturbed in their home cages until they were individually captured and hand-carried approximately 8 m to a separate room for open-field testing. Quail from stress-treatment groups (n = 23 LS or HS; **STR**) were similarly individually captured and then immediately restrained

in a metal hatching basket for 5 min. The hatching basket used served as a crush cage device identical to that used in the selection procedure of Satterlee and Johnson (1988). Following STR treatment, STR-quail were immediately returned to their home cages. After 55 min of respite (1 h after the application of crush cage stressor exposure), STR quail were tested in the open-field. Thus, there were 4 test groups (LS-CON, LS-STR, HS-CON, and HS-STR).
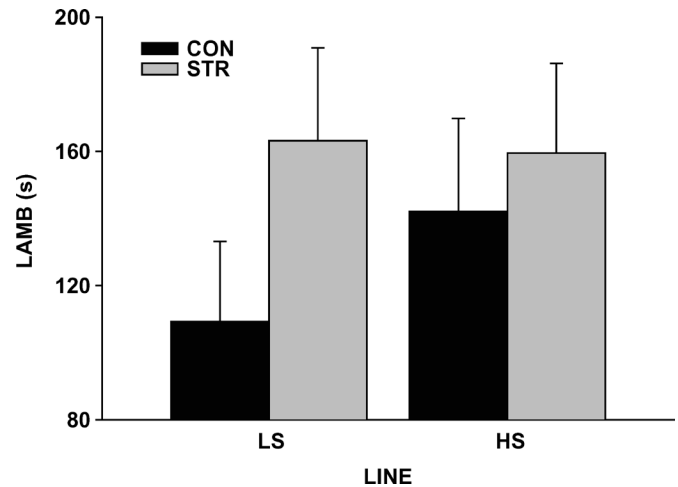
Open-Field Test

At 5 wk of age, open-field testing was conducted over 2 consecutive days between 0800 h and 1500 h daily (approximately one-half of the birds being tested each day). Each bird was tested individually and once only, and its behavior was video recorded. Testing order of individuals was randomized over the 4 treatment groups and 2 test days.

The open-field apparatus consisted of a wooden box measuring $60 \times 50 \times 60$ cm (width $\times$ length $\times$ height, respectively). The walls of the open-field were painted matte white. If defecation occurred during a test, following completion of that test, the floor was wiped clean before reuse for the next test. The test room was maintained at an ambient temperature and light intensity similar to that in the home cages.

To begin a test, each chick was placed near the midpoint of the open-field floor, and its behavior was recorded using a closed circuit television system with a video camera suspended approximately 1.8 m directly above the open-field. This arrangement made certain that the experimenter was completely hidden from the chick's view during testing. The test ceiling for observation of open-field behaviors was 5 min. During subsequent replay of the video recordings on a television screen, the projected floor image of the open-field was divided on the screen into 30 sectors of approximately equal area. The following behavior patterns were scored: the latency to ambulate (**LAMB**; s), number of floor sectors entered (**SECTORS**; no), and the time spent freezing (**FREEZE**; s). The FREEZE behavior was defined as complete silence and inactivity, apart from slight movements associated with respiration. A measure of ambulation across time (ambulation rate; **ARATE**) was also calculated using the formula: ARATE = (SECTORS/[(300 s test ceiling) − LAMB].

Statistical Analyses

Data were subjected to ANOVA with a 2×2 factorial arrangement of treatments. The factorial was made on the effects of line (HS vs. LS) and stress treatment (CON vs. STR). The LAMB, SECTORS, and ARATE variables were transformed to square roots to better fit the ANOVA assumptions (normality or homogeneity of variance or both). The FREEZE values were transformed to ranks (Shirley, 1987). The LSD tests were used for posthoc analyses. A P-value of <0.05 was considered to represent significant differences.
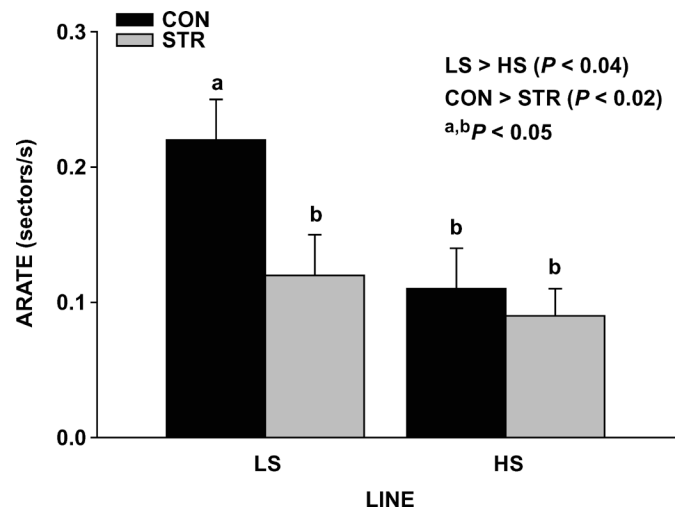

Figure 1. Mean (±SE; vertical bars) latency to ambulate (LAMB) responses in an open field of undisturbed (CON) or stressed (STR; 5 min of immobilization 1 h before testing) quail selected for either a reduced (LS; low stress) or exaggerated (HS; high stress) plasma corticosterone response to brief restraint.

RESULTS

Line × treatment effects on mean LAMB, ARATE, FREEZE, and SECTORS responses of quail tested in the open-field are depicted in Figures 1, 2, 3, and 4, respectively.

Despite an approximately 28% decrease in LAMB of CON compared with STR quail, a nearly 11% decrease in LAMB in LS quail compared with HS ones, and a particularly robust 33.2% decline in LAMB of LS-CON compared with LS-STR quail, neither of the main effects (line or treatment), nor their interactive effect, significantly affected mean LAMB responses (Figure 1). The ANOVA results, however, showed that LS quail had a
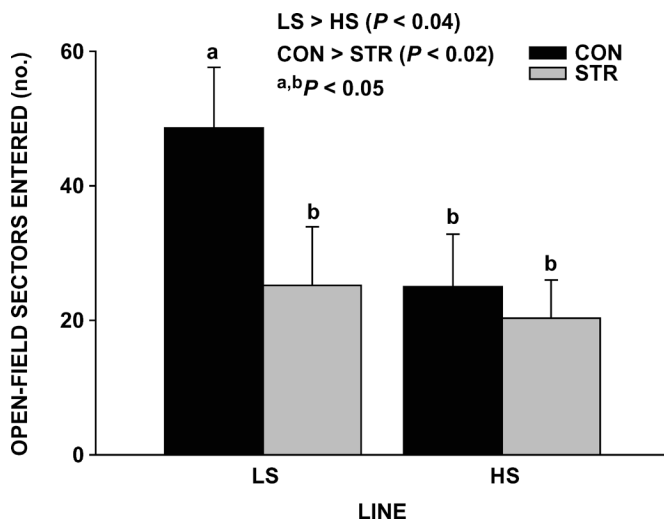

Figure 2. Mean (±SE; vertical bars) ambulation rate (ARATE) responses in an open field of undisturbed (CON) or stressed (STR; 5 min of immobilization 1 h before testing) quail selected for either a reduced (LS; low stress) or exaggerated (HS; high stress) plasma corticosterone response to brief restraint.
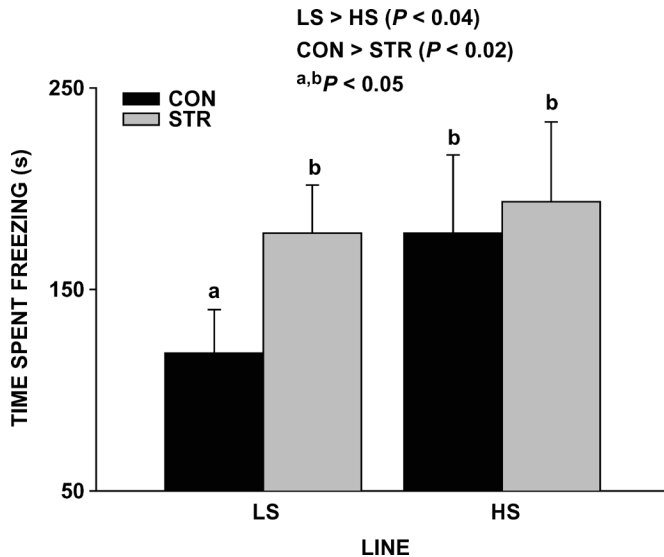
Figure 3. Mean (±SE; vertical bars) cumulative freezing (FREEZE) behavior responses in an open field of undisturbed (CON) or stressed (STR; 5 min of immobilization 1 h before testing) quail selected for either a reduced (LS; low stress) or exaggerated (HS; high stress) plasma corticosterone response to brief restraint.

significantly higher ($F_{(1, 92)} = 4,26$; $P < 0.04$) ARATE (Figure 2), shorter ($F_{(1, 92)} = 4,18$; $P < 0.05$) FREEZE behavior (Figure 3), and a trend, albeit nonsignificant, to enter more ($F_{(1, 92)} = 2,66$; $P = 0.11$) floor SECTORS of the open-field (Figure 4) than did the HS birds. Exposure to the immobilization stressor before open-field testing also decreased both the number of floor SECTORS entered ($F_{(1, 92)} = 4,90$; $P < 0.03$; Figure 4) and the ARATE ($F_{(1, 92)} = 5,26$; $P < 0.03$; Figure 2) of STR quail when compared with CON responses. There were also longer FREEZE times following STR treatment ($F_{(1, 92)} = 4,39$; $P = 0.04$).

Figure 4. Mean (±SE; vertical bars) number of sectors (SECTORS) entered in an open field of undisturbed (CON) or stressed (STR; 5 min of immobilization 1 h before testing) quail selected for either a reduced (LS; low stress) or exaggerated (HS; high stress) plasma corticosterone response to brief restraint.

Although no significant interactions were observed between the main effects (line and treatment) on any of the variables studied herein, posthoc LSD tests indicated that the LS-CON significantly ($P < 0.05$) entered a higher number of SECTORS (Figure 4), moved about the open-field at a higher ARATE (Figure 2), and exhibited shorter FREEZE time (Figure 3) than the other 3 treatment combinations (LS-STR, HS-CON, and HS-STR) that shared similar mean responses for each of these variables.

DISCUSSION

Fear is considered to compete with and oftentimes to overcome behavior patterns motivated by other systems (Hogan, 1965; Jones et al., 1991; Jones, 1996). In poultry, the open-field test has been used to assess fear and anxiety reactions with inactivity behaviors interpreted as being characteristic of exaggerated fearfulness (Faure et al., 1983; Jones et al., 1992a; Moriarty 1995; Jones, 1996; Marin et al., 1997). Although several other constructs such as exploration, social motivation, and predator avoidance have also been used to explain avian open-field behavior (Murphy, 1978; Gallup and Suarez, 1980), these constructs are not mutually incompatible, and fear probably represents a common motivational state. Indeed, many of the behavioral and physiological effects of social, environmental, and pharmacological manipulations are consistent with an interpretation based on the inactivity-fear hypothesis (Faure et al., 1983; Jones, 1996). In addition, although not always a straightforward relationship, there is growing evidence that adrenocortical activation is positively related to heightened fearfulness (Jones and Harvey, 1987; Jones et al., 1988; Cashman et al., 1989; Knowles and Broom, 1990; Jones, 1996).

Mixed-sex studies of LS and HS quail by Jones et al. (1992a) showed that undisturbed LS birds were more active in an open field, and therefore considered to be less fearful, than undisturbed HS birds. The contention of these authors that genetic selection of LS-quail for reduced plasma B response to brief immobilization in a crush-cage has been accompanied by a decrease in underlying fearfulness is supported by other behavioral tests of fear as well, tests that also make use of or do not necessarily use inactivity as an end point for interpretation. These tests include: tonic immobility (Jones et al., 1992a; Satterlee et al., 1993), avoidance of novel objects (Jones et al., 1994) and experimenters (Satterlee et al., unpublished findings), restraint-induced activity inhibition (Jones and Satterlee, 1996), and a timidity assessment by the hole-in-the-wall emergence box test (Satterlee and Jones, 1995; Jones et al., 1999).

In support of the Jones et al. (1992a) study that examined undisturbed quail stress line birds, CON-LS females herein showed a higher number of SECTORS entered, a higher ARATE, and less FREEZE behavior than did their CON-HS counterparts. This finding once again supports our basic hypothesis that LS quail are generally less fearful than HS ones. Most noteworthy, however, was the new finding that a mere 5 min of immobilization (a stres-

sor known to elicit a lower plasma B increase in LS than HS quail; Satterlee and Johnson, 1988; Satterlee et al., 2002), when applied 1 h before open-field testing, produced behavioral changes representing dampened activity states in all 3 of the variables mentioned previously in LS-STR quail when compared with LS-CON. These LS-STR quail outcomes were similar to those found for HS-quail regardless of stress treatment. We submit that the finding of an overall decrease in activity in STR-LS quail, when compared with the CON (undisturbed) LS-quail, is indicative of heightened fearfulness in STR-LS birds. We further suggest that, during open-field testing, although undetected herein, levels of plasma B in STR-LS quail were likely elevated sufficiently enough (due to the combined effects of the deliberate pretest 5-min immobilization stressor and the unavoidable stressors associated with open-field testing, such as capture, transport to the open-field, and placement in a novel environment) so as to cause the STR-LS quail to surpass a hormonal threshold needed to exhibit dampened activity behaviors. In other words, we believe elevated levels of plasma B provided the driving force for this observation. The same explanation for the observation of the similarly compromised activity behavioral outcomes observed in STR-LS quail and HS quail regardless of stress treatment can be used. It is reasonable to suggest that because of their inherent genetic predisposition toward exaggerated adrenocortical stress responsiveness, HS quail were very likely experiencing even greater elevations in levels of plasma B than both the LS-CON and LS-STR quail during open-field testing. Such elevations would stem from the open-field stressors (CON-HS quail) and the additive effects of these same stressors with the pretest 5-min immobilization stressor (STR-HS quail), all operating under the umbrella influence of the HS genome. Thus, the suspected even yet greater plasma B elevations would, theoretically, go well beyond the proposed threshold needed to dampen activity behaviors. Indeed, the hypothesis that increasingly greater plasma B responses likely occurred in HS-CON and HS-STR quail is supported, particularly for the STR-HS treatment group (that showed the greatest compromises in the activity barometers of SECTORS, ARATE, and FREEZE behaviors), by the recent report of Jones et al. (2000). In that study, not only did 5 min of crush cage restraint produce the typical (expected) effects of this stressor on adrenocortical activation (HS > LS) and immobility behavior (HS > LS), unlike LS quail, in which there were no detectable further effects of repeated restraint, the adrenocortical responses (plasma B measurements) of HS birds showed evidence of experience-dependent sensitization to repeated immobilization.

Clearly, the present studies also support the idea that subjecting animals to a negative experience increases their subsequent fear behavior responses to a second threat (Gallup and Suarez, 1980; Jones, 1986; Marin and Martijena, 1999). We further suggest that these findings of reduced kinetic activity being associated with increased fear reactions induced by prior short-term stress exposure in random bred birds is likely at least partially driven by prebehavioral testing, stressor-induced increases of plasma levels of B.

The reduction of fear and stress in poultry is important because of the deleterious effects of these states on animal performance and welfare (Hemsworth and Barnett, 1989; Mills and Faure, 1990; Jones 1996). Regardless of the mechanism(s) underlying the behavioral findings of the present study, the current findings, coupled with the numerous intuitively desirable behavioral and physiological characteristics previously shown to accompany selection for reduced adrenocortical responsiveness to stress (see Introduction), suggest that avian geneticists should consider use of the same or a similar selection regimen in the more economically viable commercial poultry species (chickens and turkeys) as a means of improving animal productivity and well-being.

In conclusion, we suggest that during open-field testing, levels of plasma B in STR-LS-quail were likely elevated sufficiently enough (from the additive effects of the deliberate pretest 5-min immobilization stressor and the unavoidable stressors associated with open-field testing) to surpass a hormonal threshold needed to induce dampened activity behaviors. Conceivably then, the interactive influences of HS genome with these same stressors would have produced yet higher levels of plasma B, responses that went well beyond the proposed threshold needed to induce heightened fearfulness. This explains why similarly compromised activity outcomes were found in both HS-quail groups.

ACKNOWLEDGMENTS

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