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Neonatal acute stress by novelty in the absence of social isolation decreases fearfulness in young chicks

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Abstract
Two hours after hatching (Day 0), groups of chicks from both sexes were housed either individually (IND) or socially in pairs (SOC) for 24 h. On Day 1, for each of the two conditions, half of the chicks were individually exposed to early novelty for 10 min, which comprised being placed in a novel-cage with small pebbles glued to the floor. The other half (controls) remained in the home-cage (IND-C and SOC-C). Thus, the IND-N group was exposed to early novelty, and the SOC-N+I group was exposed to early novelty and social isolation. Subsequently, all groups were mixed and socially reared until reaching 15 days of age. At this time, chicks were exposed to open field (OF) and tonic immobility (TI) tests. The IND-N group showed a shorter latency to ambulate in the OF test, shorter immobility duration in the TI test, a reduced plasma corticosterone concentration and increased flunitrazepam sensitive-$GABA_A$ receptor basal forebrain density compared with other groups, indicating that a neonatal novelty induced lower fearfulness in young chicks. In contrast, the effect of neonatal novelty was abolished by a simultaneous effect of social isolation in the SOC-N+I group. Thus, early post-hatch life events such as early novelty could improve a bird's later ability to cope with new stressful events. In addition, it is possible that both novelty and social isolation act on different neurobiological processes.

Keywords: *Early novelty, early social isolation,* Gallus gallus domesticus, *later fearfulness, $GABA_A$ receptor, plasma corticosterone*

Introduction

The development of behavioral and endocrine responses to acute stress is greatly influenced by the early postnatal rearing environment in human infants (Denenberg 1964) and in rats (Meaney et al. 1996). These environmental effects persist throughout life, resulting in stable individual differences in stress reactivity. Early stimulation, such as neonatal novelty exposure, in rats decreases behavioral reactivity in the open field (OF) (Tang 2001) and enhances hypothalamic-pituitary-adrenal (HPA) function (Macri et al. 2004). Distress responses measured in chickens in an OF test at 17 weeks of age are related to their behavioral reactivity recorded at the age of 2 days (Webster and Hurnik 1990).

Caldji et al. (2000) compared the effects of handling or maternal separation in rats, during the first days of life, on behavioral responses to novelty and on two sites of $GABA_A$ receptor ($GABA_A$ R) expression. The results indicated that these early life events influence the development of the $GABA_A$ R system by altering the expression of fearfulness in adulthood. In chicks, there is evidence that neonatal environmental conditions induce transient increases in the flunitrazepam sensitive-$GABA_A$ R density due to the stress accompanying a food discrimination task (Salvatierra et al. 1997),

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a T maze task (Marín and Arce 1996) or imprinting (Salvatierra et al. 1994).

Novelty can be classified as a collative variable because the recognition of any stimulus as novel requires a comparison with events that have been experienced in the past (Gray 1979). Chicks housed in pairs have marked differences in the OF test and in plasma corticosterone levels with respect to chicks housed individually (Jones and Merry 1988). One-day-old chicks housed in pairs exhibit a latency to peck pebbles of about 2 min during the first session of pretraining on a food discrimination task, due to the handling and novelty (neophobia) accompanying the task (Salvatierra et al. 1997). In contrast, when chicks were individually housed and individually exposed to the first session of pretraining in the same task, a different behavioral reactivity and a difference in forebrain $GABA_A$ R density between individuals were observed, which remained stable until 15 days of age (Salvatierra and Arce 2001).

To understand the discordance described above, we studied if neonatal novelty or novelty and social isolation in chicks, during the first days of life, could have long-term effects on behavioral (OF and TI tests), endocrine responses (plasma corticosterone levels) and the flunitrazepam sensitive-$GABA_A$ R density in the forebrain when they were exposed to subsequent stressors.

Materials and methods

Animals

Ninety six, newly hatched, mixed-sex chicks (*Gallus gallus domesticus*; Cobb strain; sex was determined on Day 0 according to the configuration of feathers of the wings and confirmed post mortem on day 15), were obtained from a commercial hatchery INDACOR (Córdoba, Argentina). Two hours after hatching (Day 0), 48 chicks were placed individually (IND) and another 48 chicks were placed in pairs (SOC) into white wooden pens of 24 × 20 × 20 cm (length × width × height) for 24 h. These pens were illuminated by an overhead red 25 W light and were maintained in a small room (2 × 2 m) with controlled temperature (28 ± 2°C), without food, but with water freely available.

Experimental design and procedures

On the morning of Day 1, half of the chicks of both the IND and SOC groups were individually transferred for 10 min to a novel cage, identical to the home-cage, for 10 min except for the scattering of small pebbles (of various colors and shapes), which had been glued to the floor as described previously for the food-pebble discrimination task (Anokhin and Rose 1991). Thus, the IND group was exposed to an early novelty (IND-N) that included a novel non-home cage and experimenter contact during the transfer (Salvatierra et al. 1997). In addition, the SOC group was also exposed to early novelty and experimenter handling as well as to social isolation during the stress session (SOC-N + I). The testing (novel) cage was placed in a contiguous room (3 × 3 m) with controlled temperature and illuminated by a lamp (60 W). The other half of the chicks from both the IND and SOC groups remained undisturbed during the novelty session and represented the non-stressed (control) groups (IND-C and SOC-C). Thus, the following four groups were formed: IND-N: Individually housed on Day 0 and exposed individually to novelty on Day 1, IND-C: Individually housed on Day 0, but without stress on Day 1 (control); SOC-N + I: housed in pairs on Day 0 and exposed individually to novelty on Day 1, and SOC-C: housed in pairs on Day 0, but without stress on Day 1 (control). Immediately after exposure to the early novelty, all birds were lightly marked on the head with a fast-drying color marker and were placed in the communal brooder. This contained a mixture of chicks of the same age from all four groups. The brooder was illuminated with a bright lamp (100 W) suspended immediately above it, and kept at a controlled temperature and humidity, for a 12 h:12 h dark:light cycle (lights on at 07.00 h) with food and tap water freely available. Chicks were socially reared in this way until 15 days of age (Day 15). Daily food replenishment and maintenance chores were performed at 09:00 h, with all experiments being carried out between 10:00 and 15:00 h. The procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals as approved by the Animal Care and Use Committee of the Universidad Nacional de Córdoba, and every effort was made to minimize animal suffering and to keep the number of animals used to a minimum.

Open-field test

On Day 15, eight chicks of each group (IND-N, IND-C, SOC-N+I, and SOC-C) were individually placed in the OF apparatus for 10 min. Each chick was randomly removed from a communal brooder by an experimenter, carried to a separate room, and placed in the center of a 98 cm × 98 cm OF apparatus with 70 cm high sides. The OF apparatus was made of white wood and the floor was marked off into 49 squares of 14 cm × 14 cm each. This was illuminated by a 100 W overhead bulb (Gallup and Suarez 1980). The following behaviors were recorded for 10 min: latency to ambulate, locomotor activity (number of squares crossed), latency for defecation, and number of defecations. Immediately after the test, all chicks were killed by conscious decapitation lasting less than 5 s. After testing, the floor of the open-field apparatus

was cleaned. Behaviors were monitored by a digital video camera suspended 1.5 m above the center of the apparatus. This monitoring system was set up in a separate room to avoid disturbing the birds.

Tonic immobility test

Another eight chicks of each group were submitted to the restraint of the tonic immobility (TI) test. This procedure followed that described by Jones (1986a). Briefly, on Day 15, a chick was randomly removed from a communal brooder by an experimenter, carried to a separate room, and individually placed on a bare table. Then, it was turned onto its right side, facing away from the experimenter, and TI was induced by manually restraining the bird for a 15 s induction period. The following measures were recorded: number of inductions (15 s periods of restraint) necessary to attain TI lasting for at least 20 s and also the duration of TI, i.e., until the bird righted itself. Immediately following this, chicks were sacrificed by decapitation lasting for less than 5 s. If the bird failed to meet the 20 s immobility criterion, it was returned to the box for 30 s, after which the procedure was repeated. If TI could not be induced after five attempts, the chick was assigned a duration of 0 s. A maximum score of 30 min was specified as the criterion for ending the TI duration. All OF and TI tests were completed between 10:00 and 15:00 h.

In all cases, immediately after decapitation, the trunk blood from all chicks of each group was collected in tubes coated with ethylenetriaminetetraacetic acid (EDTA) for corticosterone measurement. Then, the brains were removed and the forebrain hemispheres quickly dissected on ice. Forebrain hemispheres are telencephalic structures neurochemically and functionally comparable to the mammalian neocortex, claustrum, and pallial amygdala, in addition to other pallial areas such as hippocampus (Reiner et al. 2004). A crude synaptosomal fraction was obtained as described below. All birds were dissected after they were killed to confirm their sex by inspection of the gonads.

Corticosterone determination

Plasma corticosterone concentrations were measured immediately after the OF or TI tests for all chicks. Another eight chicks from each group (IND-N, IND-C, SOC-N + I, and SOC-C) that had not been exposed to OF and TI tests were used in order to assess possible differences in basal corticosterone levels. Blood samples were centrifuged (3000 rpm for 20 min at 4°C), and plasma samples were collected and stored at −20°C until corticosterone was measured using a radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA, USA) according to the manufacturer's protocol. Both the intra- and inter-assay coefficients of variation were less than 10%. All the samples were assessed in the same assay in triplicate.

Preparation of crude forebrain synaptosomal fraction

The crude synaptosomal fraction was obtained essentially as described by De Robertis et al. (1961). Synaptosomes are fortuitous artifacts created by the pinching-off and self-sealing of the synaptic contact between two nerve cells during homogenization of brain tissue (De Robertis et al. 1961). All the procedures were carried out at 4°C. Briefly, the forebrain was homogenized in 20 volumes of ice-cold 0.32 M sucrose/g original forebrain tissue using a Potter glass–Teflon homogenizer, and centrifuged at 1000 *g* for 10 min. The supernatant was then centrifuged at 10,000 *g* for 20 min. After this, the pellets were resuspended in a solution containing 50 mM Tris–HCl buffer, at pH 7.4, obtaining a final concentration of 0.3 mg protein/ml (Lowry et al. 1951). These were immediately used for the binding assay.

[^{3}H]-flunitrazepam binding assay

The specific binding of [^{3}H]-flunitrazepam (85 Ci/mmol) was measured by a filtration technique. This was carried out in the presence of radioligand at final concentrations of 0.5, 1, 2, 3, 4, 5, 8, and 9 nM, at 4°C. Each assay was performed in triplicate using 1 ml aliquots containing 0.3 mg of protein from the synaptosomal fraction (Lowry et al. 1951). Non-specific binding was measured in the presence of 10 μM diazepam. After 60 min incubation, samples were filtered under vacuum through Whatman GF/B filters using a Brandel M-24 filtering manifold. These were then washed three times with 4 ml of ice-cold Tris–HCl buffer (50 mM, pH 7.4), and the radioactivity was counted in a LKB-1214-RackBeta counter at 60% efficiency. The K_d and B_{max} values were obtained by nonlinear regression using the equation for the hyperbola (one binding site): $Y = B_{max} \times X/(K_d + X)$, where B_{max} is the maximal binding, and K_d is the concentration of ligand required to reach half-maximal binding.

Statistical analysis

The results are expressed as group mean ± SEM. The behavioral data from the OF and TI tests were analyzed using two-way analysis of variance (ANOVA) with a 2 × 2 factorial arrangement of treatments (housing social condition, IND vs. SOC and stressor exposure novelty vs. control and their interaction). B_{max} and K_d values of $GABA_A$ R density were analyzed using two-way ANOVA with a 2 × 2 factorial arrangement of treatments (housing social condition, IND vs. SOC with OF or TI stressor, baseline vs. stress and their interaction). Plasma corticosterone concentrations

Table I. Open field behaviors in 15-day-old chicks exposed to novelty, or to novelty and social isolation on post-hatching Day 1.

Behavior	Chick groups			
	IND-N	IND-C	SOC-N + I	SOC-C
Latency to ambulate (s)	149 ± 20 (8)*	228 ± 16 (8)	227 ± 15 (8)	263 ± 13 (8)
Locomotor activity (no. of squares crossed)	63 ± 5	35 ± 7	43 ± 6	47 ± 11
Defecations (no.)	1.52 ± 0.2	1.71 ± 0.3	1.27 ± 0.4	1.07 ± 0.3
Defecation latency (s)	192 ± 17	232 ± 20	229 ± 27	235 ± 22

Each value represents the group mean ± SEM. The number of tested chicks is indicated in parentheses. IND-N, individually housed on Day 0 and individually stressed (novel environment) on Day 1; IND-C, individually housed on Day 0 and no stress on Day 1; SOC-N + I, housed in pairs on Day 0 and individually stressed (novel environment) on Day 1; SOC-C, housed in pairs on Day 0 and no stress on Day 1. no., number; s, seconds.
*$P < 0.01$ compared to other groups (Newman–Keuls post hoc test).

were analyzed using a three-way ANOVA with a $2 \times 2 \times 2$ factorial arrangement of treatments (housing social condition, IND vs. SOC; stressor exposure, novelty vs. control; with OF or TI stressor, baseline vs. stress, and their interaction). Whenever ANOVA indicated significant effects ($P < 0.05$), a pairwise comparison of means by the Newman–Keuls test was carried out. In all cases, the assumptions of the ANOVA (homogeneity of variance and normal distribution) were attained. In all statistical analyses a *P*-value < 0.05 was considered to represent a significant difference between groups.

Results

Open field behavior of 15-day-old chicks exposed to acute stress by novelty alone, or to novelty and social isolation on Day 1

Table I shows the OF behavior of each chick group. A two-way ANOVA revealed independent significant effects of housing condition ($F(1,28) = 12.38$, $P < 0.001$) and early novelty on the latency to ambulate ($F(1,28) = 12.93$, $P < 0.001$). However, no interaction between these parameters was observed ($F(1,28) = 1.80$, $P = 0.19$). The Newman–Keuls test showed that the IND-N group exhibited a shorter latency to ambulate than the IND-C (by 34%, $P < 0.01$), SOC-N + I (by 34%, $P < 0.01$) and SOC-C groups (by 43%, $P < 0.01$).

A two-way ANOVA did not reveal any effect of housing condition ($F(1,28) = 0.33$, $P = 0.56$) or early novelty ($F(1,28) = 2.63$, $P = 0.11$) with no interaction between them being found ($F(1,28) = 3.03$, $P < 0.09$) with respect to locomotor activity (number of squares crossed). In addition, a two-way ANOVA of the number of defecations did not reveal a effect of housing condition ($F(1,28) = 0.18$, $P = 0.67$) or early novelty ($F(1,28) = 1.13$, $P = 0.26$) with no interaction between them observed ($F(1,28) = 0.19$, $P < 0.67$), and a two-way ANOVA of the latency to defecate did not reveal any effect of housing condition ($F(1,28) = 0.87$, $P = 0.36$) or early novelty ($F(1,28) = 1.13$, $P = 0.29$) or interaction between them ($F(1,28) = 0.60$, $P = 0.44$).

Tonic immobility responses of 15-day-old chicks exposed to acute stress by novelty alone, or to novelty and social isolation on Day 1

The results are shown in Table II. A two-way ANOVA revealed a significant interaction between housing condition and early novelty on immobility duration in the TI test ($F(1,28) = 7.18$, $P < 0.01$). Newman–Keuls tests showed that the IND-N group exhibited 2.5-fold shorter immobility duration than the IND-C group ($P < 0.04$), SOC-N + I ($P < 0.02$) and SOC-C groups ($P < 0.01$).

Moreover, a two-way ANOVA did not reveal any effect of housing condition ($F(1,28) = 0.14$, $P = 0.71$) or early novelty ($F(1,28) = 0.63$, $P = 0.43$) with no interaction between them being found ($F(1,28) = 0.001$, $P < 0.99$) with respect to the number of immobility inductions.

Table II. Tonic immobility responses in 15-day-old chicks exposed to novelty alone, or to novelty and social isolation on post-hatching Day 1.

Behavior	Chick groups			
	IND-N	IND-C	SOC-N+I	SOC-C
Number of inductions	1.48± 0.18 (8)	1.85 ± 0.5 (8)	1.66 ± 0.35 (8)	2.00 ± 0.65(8)
Immobility duration (s)	343 ± 50*	859 ± 115	855 ± 114	845 ± 97

Each value represents the mean ± SEM. The number of tested chicks is indicated in parentheses. no., number; s, seconds.
*$P < 0.01$ compared to other groups (Newman–Keuls test).

Table III. Plasma corticosterone concentrations after Open Field or Tonic Immobility tests on 15-day-old chicks exposed to neonatal novelty or, to novelty and social isolation on post-hatching Day 1.

	Chick groups			
	IND-N	IND-C	SOC-N + I	SOC-C
Basal (non-stressed)	9.86 ± 2.31	9.40 ± 0.34	8.46 ± 2.03	7.78 ± 1.30
TI test (stressed)	15.59 ± 1.24$^{+\star}$	37.51 ± 4.08^{+}	37.95 ± 1.64^{+}	34.37 ± 3.16^{+}
OF test (stressed)	12.19 ± 1.00$^{+\star}$	31.39 ± 3.22^{+}	33.39 ± 1.12^{+}	30.45 ± 2.06^{+}

Each value represents the mean ± SEM of plasma corticosterone concentrations (ng/ml) ($n = 8$ experiments on different chicks per group). $^{+}P < 0.001$ compared with basal concentrations in IND-C, SOC-N+I, and SOC-C groups, respectively. $\star P < 0.001$ compared to the same condition in other groups (Newman–Keuls test).

Plasma corticosterone concentrations after OF and TI tests on 15-day-old chicks exposed to acute stress by novelty alone, or to novelty and social isolation on Day 1

The OF test included a novelty stress and social isolation, while chicks submitted to TI induction suffered additional stress due to manual restraint (Table III). A three-way ANOVA on plasma corticosterone concentrations revealed a significant interaction for housing condition (IND vs. SOC), early novelty (control vs. stress) and OF exposure (non-stressed vs. stressed chicks) ($F(1,56) = 16.91$, $P < 0.0001$). Newman–Keuls tests revealed that the basal corticosterone concentration did not differ among groups. Immediately after, the OF test, Newman–Keuls tests revealed a fourfold increase of corticosterone levels in the IND-C ($P < 0.01$), SOC-N + I ($P < 0.01$), and SOC-C groups ($P < 0.01$) compared to basal levels, but this increase was not observed within the IND-N group.

Similarly, the results of TI stress (Table III) showed a significant interaction for housing condition (IND vs. SOC), early novelty (control vs. stress) and TI exposure (non-stressed vs. stressed chicks) ($F(1,56) = 15.15$, $P < 0.002$). Newman–Keuls tests showed that basal corticosterone concentration did not differ among groups. In addition, Newman–Keuls tests also revealed a significant fourfold increase in the IND-C ($P < 0.01$), SOC-N + I ($P < 0.01$), and SOC-C groups ($P < 0.01$) compared to their basal concentrations. This increase was not observed within the IND-N group.

Acute effects of both TI and OF tests on the flunitrazepam sensitive-GABA$_A$ R density on 15-day-old chicks, exposed to novelty alone, or to novelty and social isolation on Day 1

$GABA_A$ R density measurement was only carried out in the groups either exposed to early novelty on Day 1 (IND-N) or exposed to early novelty in addition to social isolation (SOC-N + I), as described in "Materials and methods" section.

Table IV shows that a two-way ANOVA (early novelty × OF exposure) on B_{max} revealed a significant interaction between early novelty and OF exposure ($F(1,20) = 22.1$, $P < 0.001$). Newman–Keuls tests showed that B_{max} in the IND-N group was increased by 25% ($P < 0.001$) in stressed chicks compared to controls, immediately after the OF test. Furthermore, the B_{max} in the SOC-N + I Group was increased by 45% ($P < 0.001$) compared to its control (not stressed). Differences in K_d values in all cases were not observed.

Furthermore, a two-way ANOVA (early novelty × TI exposure) revealing a significant effect of early novelty ($F(1,20) = 41.2$, $P < 0.0001$) and TI exposure ($F(1,20) = 459.5$, $P < 0.0001$) on B_{max}. However, no interaction between them was observed ($F(1,20) = 2.47$, $P = 0.13$). The Newman–Keuls test showed that the B_{max} in the IND-N group was 34%

Table IV. Acute effects of both tonic immobility and open field tests on the flunitrazepam sensitive-$GABA_A$ receptor density in forebrain of 15-day-old chicks, exposed to novelty, or to novelty and social isolation on post-hatching Day 1.

Group	Stressor on day 15	Duration (min)	B_{max} (fmol/mg protein)	Increase (%)	K_d (nM)	Increase (%)
IND-N	Non-stressed	0	1243 ± 13 (6)$^{+}$	–	1.85 ± 0.2	–
	TI	30	1664 ± 36 (6)*	34	1.92 ± 0.1	4
	OF	30	1551 ± 23 (6)$^{\#}$	25	2.13 ± 0.4	15
SOC-N+I	Non-stressed	0	1073± 20 (6)	–	1.85 ± 0.1	–
	TI	30	1563 ± 29 (6)*	46	1.84 ± 0.2	−1
	OF	30	1552 ± 21 (6)$^{\#}$	45	2.16 ± 0.3	17

Each value of B_{max} and K_d represents the mean ± SEM of values obtained by non-linear regression of experimental data from saturation curves. The number of independent experiments is indicated in parentheses.
$\star P < 0.001$ and $^{\#}P < 0.001$ compared to respective control (non-stressed chicks). $^{+}P < 0.001$ compared to SOC-N+I control (non-stressed chicks) (Newman–Keuls test). The procedures for the OF and TI tests are described in "Materials and Methods" section.

greater ($P < 0.001$) with respect to control (non-stressed chicks). Furthermore, in the SOC-N + I group, the B_{max} in stressed chicks was significantly greater (46%, $P < 0.001$) than in non-stressed chicks. However, no differences in K_d values were observed. Interestingly, the Newman–Keuls test also revealed significant differences ($P < 0.001$) between the IND-N group and the SOC-N + I group, in non-stressed chicks, suggesting that early novelty enhances $GABA_A$ system activity, thereby improving the bird's ability to cope with new stressful events.

None of the analyses revealed a significant effect of sex for any measure.

Discussion

In the present study, we hypothesized that an early stress by novelty or by novelty and social isolation in chicks could have long-term effects on the behavioral and endocrine responses, also on the forebrain GABAergic neurotransmitter system when exposed to subsequent stressors. In the present experimental design, both the IND-N and IND-C groups were individually housed during Day 0, with only the IND-N group being exposed to neonatal novelty on Day 1. In addition, the SOC-N + I and SOC-C groups were housed in pairs on Day 0, with only the SOC-N + I group being individually exposed to early novelty and social isolation on Day 1.

The 15-day-old chicks of the IND-N group exhibited a shorter latency to ambulate in the OF test than other groups, indicating that they were less fearful and that early novelty induced a lower fearfulness at this age. No significant differences were observed in the latency to ambulate between the SOC-N + I and SOC-C groups, indicating that early stress by social isolation abolished the effect of early novelty. Although no significant differences were observed in the other behavioral parameters analyzed in the OF test, an increased tendency to ambulate in chicks of the IND-N group was observed. Thus, an increased locomotor activity appears to be associated with the reduced fear reactions induced by novelty stress exposure. These results are in agreement with Marín and Martijena (1999) who observed a decrease in the tendency of chickens to ambulate and escape from an OF test after acute stress by partial water immersion. Furthermore, the results coincide with previous reports indicating that administration of anxiogenic β-carboline increased the ambulatory latency of hens in an OF test (Moriarty 1995). In addition, an anxiolytic drug such as diazepam decreased the ambulatory latency in the OF test (Salvatierra and Arce 2001). Our results suggest that birds retain information concerning the early novelty during the first hours of life by showing greater adaptation to a subsequent stressful situation.

The immobility duration in the TI test was significantly shorter in the IND-N group compared to other groups, suggesting that the IND-N group was less fearful and that early novelty induced a lower fearfulness at this age. These results are consistent with previous reports indicating that the systemic administration of β-carboline increased, and diazepam decreased the immobility duration respectively (Moriarty 1995; Salvatierra and Arce 2001). No significant differences were observed in immobility duration between the SOC-N + I and SOC-C groups, indicating that social isolation abolished the effect of early novelty. Several studies have indicated that early environmental enrichment such as the incorporation of conspicuous novel objects and colored foods into the home cages reduces emotionality and increases exploration in an OF (Jones 1982, 1986b). Hence, this early novelty protocol might not be interpreted as dangerous for inexperienced chicks, with the later fearfulness being reduced, as measured in the OF and TI tests.

Basal plasma corticosterone levels did not differ among groups, suggesting that early life novelty did not induce differences in HPA function at 15 days of age. However, plasma corticosterone levels immediately after the OF and TI tests lasted for a significantly shorter time in the IND-N compared to the increments induced in other groups. Thus, this reduced adrenal activity may have long-term consequences, suggesting a lower fearfulness at 15 days of age. Cavigelli and McClintock (2003) reported that adult corticosterone dynamics are associated with a response to novelty in infant rats. However, no significant differences were observed in plasma corticosterone levels between the SOC-N + I and SOC-C groups in the present study, indicating that social isolation abolished the early novelty effect. In agreement with our results, Jones and Merry (1988) reported that individual testing of chicks that had previously been housed in pairs showed they displayed higher plasma corticosterone levels than chicks tested in pairs.

In the present report, forebrain benzodiazepine receptor density was used to express the $GABA_A$ R density, as the FNZ-binding site is located in the α subunit of the $GABA_A$ R (Primus and Kellogg 1991). In order to study insertion of receptors into the postsynaptic membrane, we investigated whether the *in vitro* measurement of $GABA_A$ R recruitment into synaptosomes, induced by acute stress *in vivo* was modulated by early novelty and early social isolation. Exposure to the TI and OF tests increased the $GABA_A$ R density in synaptosomes from the forebrain of 15-day-old chicks in both the IND-N and SOC-N + I groups compared to control chicks (non-stressed), similar to that induced by novelty in rats (Bodnoff et al. 1987), by novelty accompanying a food discrimination task (Salvatierra et al. 1997), and by partial water immersion of chicks (Martijena et al. 1992; Cid et al. 2008). It is possible that acute stress stimulates protein phosphorylation and consequently increases the velocity of vesicular transport, their fusion with

membranes and final receptor insertion at the membrane surface (Cid et al. 2008). Nevertheless, a greater B_{max} value in non-stressed chicks (control) of the IND-N group compared to the SOC-N + I Group was observed. These findings might relate to underlying differences in anxiety and/or fear associated with early novelty, and increased forebrain $GABA_A$ R density might indicate reduced fearfulness. It has been previously reported that an early-life mild stimulation, such as neonatal novelty exposure, decreases behavioral reactivity (Tang 2001) as well as increasing synaptic plasticity (Tang and Zou 2002) long after the initial neonatal stimulation. Thus, the present data indicate that, for 15-day-old chicks, exposure to an early novel environment induces reduced fearfulness compared to chicks stressed by social isolation in addition to novelty. Consequently, in the latter the early effect of novelty was abolished by a simultaneous effect of social isolation.

Hence, we conclude that early life events such as exposure to a novel environment improved the response to the negative impact of later environmental change and the young chick's ability to cope with new stressful events.

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