Brain Monoamine Asymmetry in Chicks Subjected to a Separation-Stress Procedure with Litter Substrate

Takashi Bungo¹,², Eriko Nakasai¹, Minami Takawaki¹, Hiroshi Tanizawa¹ and Shin-Ichi Kawakami¹

¹Laboratory of Animal Behavior and Physiology, Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima 739–8528, Japan
²Japanese Avian Bioresource Project Research Center, Hiroshima University, Higashi-Hiroshima 739–8528, Japan

Activation of the right hemispheric neurotransmitter systems is related to negative emotion and stress in mammals, but this relationship is not fully known in birds. The effect of the presence of sawdust litter on behavior and brain monoamine laterality in isolation stressed chicks (6 days old) was investigated. Although there was no significant difference in peep, movement distance in litter group chicks (n=7) was significantly longer than that in non-litter group chicks (n=6) during 15 min isolation test (P<0.01). The laterality index (a measure of central monoamine laterality indicating the predominance of right over left hemispheric activity) of serotonin (5-HT) in intact chicks (no stress manipulation; n=8) was higher than those in chicks with and without litter substrate (P<0.01). The value of the dopamine (DA) metabolite ratio in intact chicks was lower than that in the non-litter group chicks (P<0.05), but was not low in the litter group chicks. In contrast, there was no significant difference in the levels of the 5-HT metabolite ratio among the groups. In addition, the laterality index of the ratio correlated negatively with the number of peeps in chicks with sawdust litter (P<0.01). It has been suggested that emotional processing in the right hemisphere might be optimal with DAergic and/or serotonergic lateralization, whereas either a decrease or an increase in this lateralization might be reinstated in animals suffering stress. In addition, the present results imply that sawdust litter may attenuate isolation stress that induces anxiety in chicks, and that the laterality index of the DA metabolite ratio may be effective in estimating positive emotional change.

Key words: chick, dopamine, laterality, sawdust litter, 5-HT

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Introduction

Hemispheric asymmetry and laterality of the brain function in humans is well known (e.g., Glick et al., 1982). Anatomical and behavioral asymmetries have also been shown in non-human vertebrates (Denenberg, 1981; Nordeen and Yahr, 1982; Rogers et al., 2013). Neurochemical studies revealed that the activation of the right hemispheric neurotransmitter systems is related to negative emotion and stress (Alonso et al., 1997; Verstynen et al., 2001; Andersen et al., 2002; Rogers et al., 2013). Physical or psychological stress increases levels of dopamine (DA) and 5-HT in the right hemisphere (Andersen and Teicher, 1999; Sullivan et al., 2009).

Likewise, the avian brain is structurally and functionally latedized across a wide range of sensory, perceptual and motor abilities (Andrew, 1983; Andrew and Brennen, 1983; Rashid and Andrew, 1989; Rogers, 2008; Rogers et al., 2013). The right hemisphere seems to be involved in spatial analysis and responses to novelty, while the left hemisphere seems to be involved in the selection of cues allowing stimuli to be classified into categories (Andrew and Brennen, 1983; Rashid and Andrew, 1989; Vallortigara et al., 1996; Vallortigara, 2000; Rogers, 2008; Rogers et al., 2013). However, understanding of monoaminergic lateralization in the avian brain affecting emotions remains incomplete.

Emotion in livestock has been the great concern of animal scientists and producers because it is important in understanding animal welfare. Although most past studies for emotion focused on stress, interest has shifted to emotional transfer or positive emotion (Désiré et al., 2002; Špinka, 2012). Désiré et al. (2002) proposed an analysis of the emotional repertoire of farm animals based on the relationship between behavioral and physiological responses and the evaluative process of an event: (a) the intrinsic characteris-
tics of an eliciting event (suddeness, novelty, pleasantness); (b) the degree of conflict of that event with an individual’s needs or expectations; and (c) an individual’s coping possibilities offered by the environment. We decided to investigate this novel environment with and without stimulus of pleasantness. Chickens innately prefer environments such as a litter floor that gives them the opportunity for ground scratching (Nicol et al., 2009). This behavior is very important to the bird because the higher intensity, duration, and incidence of ground scratching indicate better bird welfare (Bracke and Hopster, 2006). Thus, we tried to apply sawdust litter as a stimulus of pleasantness in the present study.

The aim of this present series of experiments was to establish in isolated chicks, (1) whether the presence of sawdust litter affects behavioral response, (2) whether the presence of sawdust litter alters brain monoamine asymmetry, and (3) whether there is a relationship between monoamine asymmetry and behavior.

Materials and Methods

All experiments were conducted in accordance with the regulations of the Animal Experiment Committee of Hiroshima University.

Animals

Day-old male layer chicks (Julia light) were obtained from a local hatchery (Akita Co. Ltd., Hiroshima, Japan) and housed in wooden cages with wire-mesh floor (18×25×20 cm) at a population density of 3 chicks per cage. The birds were maintained in a room with 24-h lighting and at a temperature of 30°C. They were given free access to a commercial starter diet (Nichiwa Sangyo Co. Ltd., Kobe, Japan) and water during the pre-experimental period.

Experimental Procedures

Birds (6 days old) were isolated from their flock and placed in one of two types of isolation box (25×40×30 cm), which were made of black polypropylene with floors covered with shavings to a depth of approximately 3 cm or without shavings. They were handled gently and carried into either isolation box (only one chick was chosen at random in each cage). Throughout the 15-min test, the behavior of all treatment chicks were recorded with a digital video recorder mounted on the top. The videos were analyzed to ascertain the number of peeps and movement distance. Total movement distance during tests was measured using observer software. At the end of each test, chicks were bled by cardiac puncture and blood was collected into heparinized tubes. For studies requiring measurements of brain monoamines, the telencephalon was dissected with the aid of a chick brain atlas (Kuenzel and Masson, 1988). The collected blood (0.5 ml) of each bird was centrifuged for plasma, which was stored at −20°C until analysis. Brain tissue was stored at −80°C until analysis. Blood and brain tissue were also collected from intact (non-treated) chicks immediately after they were removed from their home cage. The number of birds used for data analysis is shown in each table.

Plasma Levels of Glucose and Free Fatty Acid (FFA)

The plasma concentrations of glucose and FFA were measured using a commercial kit (Glucose CII-Test Wako and NEFA C-Test Wako, Wako Pure Chemical Industries Ltd., Osaka, Japan).

Measurement of Monoamines in Brain Tissues

Tissue was homogenized in ten volumes of 0.1 N HClO4. The homogenates were centrifuged at 13,000 rpm for 15 min, and supernatants were filtered through a 0.22 μm filter before injection (100 μl) into a high performance liquid chromatography system (Tozoh, Tokyo, Japan) with a 150×2.1 mm ODS column (CA-5ODS, EICOM, Kyoto, Japan) for the measurement of monoamines and their metabolites. Column temperature was kept at 40°C by a thermocontroller (TSK CO-8000; Tozoh). The solvent delivery system (TSK CCPD; Tozoh) contained 2.5 mM 1-octanesulfonic acid sodium salt (SOS), 20 μM Na2EDTA and 15% methanol in a 0.1 M phosphate buffer solution (0.1 M NaH2PO4: 0.1 M Na2HPO4= 1000:85). The pH of the buffer was adjusted to 3.0 with H3PO4. The buffer was filtered and degassed (TSK SD-8022; Tozoh) and the flow rate adjusted to 180 μL/min. The electrochemical detector (TSK EC-8020; Tozoh) was set at 750 mV and peak heights were measured using a computer integrator. All values were corrected for actual recovery based on the extraction rate of the internal standard, isoproterenol.

According to the method of Andersen and Teicher (1999), lateriality index was calculated using the following equation: laterality index = (Right−Left) / [(Right+Left)/2], with a positive number indicating greater transmitter content in the right hemisphere.

Statistical Analysis

The data were analyzed using the commercially available package, StatView (Version 5, SAS Institute, Cary, USA, 1998). Blood parameters and laterality indices were evaluated by one-way ANOVA, and the differences between treatments were determined by the Tukey-Kramer multiple comparisons procedure. Frequencies of peep and movement distances were evaluated by the nonparametric test of Mann-Whitney and Student-t test, respectively. Correlations between the frequency of peep and the laterality indices were analyzed using Spearman’s rank correlation coefficient analysis. Results are presented as means±S.E.M.

Results

Effect of the Presence of Litter Substrate on Behavior

Table 1 shows the effect of the presence of sawdust litter on peep and movement distance in isolated chicks. Although there was no significant difference in the frequency of peep,

| Table 1. Effect of the presence of litter substrate on peep and movement distance |
|-----------------------------------------------|
|                  | NLG (6)   | LG (7)  |
| Peep (frequency) | 239.0±94.8 | 175.6±78.9 |
| Movement distance (m) | 4.72±1.15 | 16.83±3.00** |
|                  | **P<0.01.  |          |

LG, floor covered with sawdust; NLG, floor without sawdust. Data were presented as means±SEM. Sample size is in parentheses.
The relationship between peep frequency and the laterality index of DA metabolite ratio in chicks is shown in Fig. 1. Statistical analysis showed a negative correlation ($r = -0.991, P < 0.05$) between peep and the laterality index in the litter group chicks, but not in the non-litter chicks.

### Table 2. Effect of the presence of litter substrate on plasma glucose and free fatty acid (FFA) levels

|                 | Intact (8)             | NLG (6)          | LG (7)           |
|-----------------|------------------------|-----------------|-----------------|
| Glucose (mg/dl) | 261.3±6.0$^a$          | 301.6±7.3$^b$   | 287.3±2.5$^b$   |
| FFA (mEq/l)     | 322.7±45.9             | 270.0±17.1      | 291.1±16.7      |

LG, floor covered with sawdust; NLG, floor without sawdust. Values are means±SEM of the number of chicks in parentheses. Means with different letters are significantly different at $P<0.05$.

### Table 3. Laterality indices of telencephalic dopamine, 5-HT and their metabolite ratios in chicks

|                 | Intact (8)             | NLG (6)          | LG (7)           |
|-----------------|------------------------|-----------------|-----------------|
| DA              | 0.061±0.022            | 0.039±0.009     | 0.070±0.022     |
| HVA/DA          | 0.051±0.046$^a$        | 0.210±0.012$^b$ | 0.175±0.014$^{ab}$ |
| 5-HT            | −0.042±0.024$^a$       | −0.230±0.023$^b$| −0.189±0.040$^b$|
| 5-HIAA/5-HT     | −0.004±0.018           | 0.047±0.018     | 0.070±0.032     |

LG, floor covered with sawdust; NLG, floor without sawdust; DA, dopamine; HVA, 4-hydroxy-3-methoxyphenylacetic acid; 5-HT, serotonin; 5-HIAA, 5-hydroxyindole, 3-acetic acid. Values are means±SEM of the number of chicks in parentheses. Means with different letters are significantly different at $P<0.05$.

**Movement distance in chicks with litter was significantly longer, about three times than without litter ($P<0.01$).**

**Effect of the Presence of Litter Substrate on Plasma Glucose and FFA Levels**

The effect of the presence of litter substrate on plasma glucose and FFA concentration is shown in Table 2. Although there was no significant difference between groups on the level of plasma FFA, the isolated chicks with and without litter substrate exhibited a higher level of plasma glucose than the intact chicks ($P<0.05$).

**Effect of the Presence of Litter Substrate on Brain Monoamine Asymmetry**

Table 3 shows the effect of isolation treatment on the laterality index of telencephalic dopamine (DA), serotonin (5-HT) or their metabolite ratios. Although there was no significant difference in the laterality index of DA among the groups, the value for 5-HT in intact chicks was higher than that in chicks with and without litter substrate ($P<0.01$). The laterality index of the DA metabolite ratio in intact chicks was lower than that in the non-litter group chicks ($P<0.05$), but was not lower in the litter group chicks. In contrast, there was no significant difference in the levels of the 5-HT metabolite ratio among the groups.

**Relationship between Peep and Laterality Index of DA Metabolite Ratio**

The relationship between peep frequency and the laterality index of the DA metabolite ratio in chicks is shown in Fig. 1. Statistical analysis showed a negative correlation ($r = -0.991, P<0.05$) between peep and the laterality index in the litter group chicks, but not in the non-litter chicks.

**Discussion**

Environmental stimulation greatly affects various behaviors in rodents (Olsson and Dahlborn, 2002). We found that a floor covered with sawdust significantly increases movement distance in isolated chicks (Table 1). Although there was no systematic attempt to quantify other behavioral measurement, the presence of litter seemed to induce ground pecking and scratching without freezing in isolated chicks. Likewise, compared to the barren environment of conventional battery cages, access to straw or feathers can improve the welfare of laying hens, because these substrates enrich the environment and support important hen behavior, such as ground pecking and ground scratching (Dawkins, 1989). The effect of enriched rearing on exploratory behavior has been examined in birds: ostriches raised with enrichment and support important hen behavior, such as ground pecking and ground scratching (Dawkins, 1989). The effect of enriched rearing on exploratory behavior has been examined in birds: ostriches raised with enrichment plant materials and sticks showed more exploratory behavior than controls (Christensen and Nielsen, 2004). Thus, it was thought that sawdust stimulated exploratory behavior with pecking and scratching, and as a result, chicks in the litter group appeared to move around more actively.

Many reports indicate that stressors increase parameters such as plasma glucose and FFA (e.g., Ricart-Jané et al., 2002; Bowers et al., 2004; Yanagita et al., 2011). In the present study, the level of plasma glucose in both the litter and non-litter groups was higher than that in the intact group, but the blood parameters of chicks with and without litter did not differ significantly (Table 2), suggesting that isolation treatment induce stress condition but sawdust litter may not reduce the increased levels of blood parameters for stress.
Antennal asymmetry is not possible when using these blood parameters.

Activation of the right hemisphere neurotransmitter systems is related to negative emotion and stress. Male rats exhibit a right-biased DA activation in the cortex and amygdala in response to tail pinch stress (Sullivan et al., 2009). Increased monoamine in the right prefrontal cortex is also shown to relate to anxiety-like behavior in rats (Andersen and Teicher, 1999). Many studies have shown functional asymmetries of the avian visual system wherein the left hemisphere is superior in discriminating visual features while the right hemisphere has an advantage in relational spatial orientation (Rashid and Andrew, 1989; Vallortigara et al., 1996; Tommasi and Vallortigara, 2004). Therefore, it is not surprising that asymmetric activity also exists for stress coping like visual information processing in chicks. In the present results, right DA activation might be enhanced by isolation because the laterality indices of the DA metabolite ratio in both groups had positive values (Table 3). Also, the laterality indices of 5-HT in both isolated groups were higher than those in the intact group ($P<0.05$). Although there was no significant difference in the levels of the 5-HT metabolite ratio among the groups, the laterality of the 5-HT metabolite ratio of the isolated groups taken together (those with and without litter substrate) and the intact group differed significantly when evaluated using the Student-$t$ test ($0.060 \pm 0.019 [n=13]$ vs. $-0.004 \pm 0.018 [n=8]; P<0.05$). Thus, right 5-HT activation might also be enhanced by isolation. These results indicate that scanning asymmetrical activity of the cerebral hemisphere might be useful in evaluating stress in chicks.

There was no significant difference between the intact and litter groups in the laterality indices (Table 3) and the value in the litter group tended to be lower than that in the non-litter group when evaluated using the Student-$t$ test ($P=0.084$), implying that sawdust attenuated isolation stress that induces anxiety in chicks. Thus, there is the possibility that the laterality index of the DA metabolite ratio may be effective in estimating emotional change. Further research is needed to investigate the relationship between monoamine asymmetry and positive emotion in chicks. Such investigations might have implications for animal welfare in poultry.

Distress vocalization is generally defined as peep emitted under a stress condition (Montevecchi et al., 1973) and social separation elicits distress vocalization as a typical response in chicks (Panksepp et al., 1997; Feltenstein et al., 2003; Saito et al., 2005; Yanagita et al., 2011). At first, we hypothesized that the number of peep correlated positively with the laterality index, and peep would be decreased by the presence of sawdust litter, which might mitigate isolation stress. Although the frequency of peep in chicks with and without litter substrate did not differ significantly (Table 1), the number of peeps correlated negatively with the laterality index of the DA metabolite ratio in the litter group, but not the non-litter group (Fig. 1). As for no significant difference in peep frequency, it is reasonable that the effect of litter might be not very significant, similar to the results of laterality indices of telencephalic monoamine (Table 3). The reason why lower asymmetric birds with sawdust litter had higher frequency of peep is not clear, but it appeared that sawdust litter would affect the relationship with brain activity and behavior of chicks to isolation. Further research is needed to research the situation to peep with any behaviors in chicks.

The present study revealed that the right hemisphere is important for animal welfare, and it will be possible to estimate emotional status in animals with observed behavioral asymmetry. Behavioral asymmetry may be reflected by hemispheric dominance and can be associated with neurochemical laterization (Sullivan et al., 1994; Cabib et al., 1995; Thiel and Schwarting, 2001). It has been shown that the preferred limb used to pick up food when an animal is in a relaxed state reflects the dominant hemisphere and may be an accessible measure indicating susceptibility to stress and tendency towards positive versus negative cognitive bias (Rogers, 2010, 2013). Hence, limb preference might be a useful measure for such tendencies in domesticated species.

Emotional processing in the right hemisphere might be optimal with DAergic and/or serotonergic lateralization, whereas either a decrease or an increase in this lateralization might be reinstated in animals suffering stress. The present study also contributes possible evidence on the role of DA systems in eliciting emotion by suggesting attenuation effect of litter substrate on DAergic lateralization in isolated chicks. Our data also provide clues to research for emotional transfer in animals. Clearly, further studies are necessary to investigate this suggestion.

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