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A Sensitive Period of Peer-Social Learning

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Abstract

A critical period has been found in various sensory-motor learning system, for instance, vision, auditory, somatosensory, and language development. This report provides evidence that there may be a sensitive period also in peer-social learning. For consideration of brain toxicology during development, we should know the transient neurobiological process when gene-environment interaction is crucial for development and vulnerability to stress over lifelong. Here we developed an animal model (Gallus gallus domesticus) to study behavioral development of socialization with peers. By a multivariate analysis using principal components analysis, we found that there existed a sensitive period in which social environment was essential for peer social affiliation developmental. The behavioral features of this sensitive period learning were suggested in the head rotation and frequency of freezing in the meeting context with unfamiliar peers. This pre-clinical model of a sensitive period learning may contribute to develop orthomolecular therapy, behavioral and cognitive intervention for social disabilities in developmental psychiatry.

Keywords: Critical period; Psychological development; Social disability; Emotion; Memory; Psychiatry

Introduction

Developmental neuropathology of social disabilities, such as Autism Spectrum Disorders (ASD) is considering oxidative stress [1], a ubiquitous environmental neurotoxicant like methylmercury or dioxin [2], and medication such as valproic acid [3] as a causative factor especially during embryonic and neonatal development. Oxidative stress and chemical environment may influence the development of the nervous system particularly during vulnerable periods [4]. Various critical periods for sensory and motor learning have been found in developmental neuroscience, for instance, vision [5], tactile [6], auditory [7], locomotive controls by vestibular neurons [8], language [9], and social function [10]. However, it is uncertain when and how social affiliation and social communication capability are acquired through a series of sensory and motor learning, whose deficit is the core symptom of social disabilities in ASD. Domestic chick (Gallus gallus) studies have contributed to the formulation of a two process theory, namely CONSPEC and CONLERN [11]. CONSPEC proposes that widely divergent vertebrates possess similar domain-relevant biases toward faces, auditory, olfactory, biological motion, and self-propelled agency [11-14]. A chick's natural predisposition mechanisms adhere to CONSPEC. Additionally, attention biases lead to neuronal based development of species specific individual cognition or CONLERN. It is thought that chick, imprinting mechanisms correlate to CONLERN [11,12]. The neuronal substrates relevant to CONSPEC and CONLERN comprise the subcortical face-recognition route, which provides a developmental foundation for what later becomes the adult cortical 'social brain' network [11].

Here we developed an animal model (Gallus gallus domesticus) to study social affiliation development with peers and found the sensitive period for peer-social affiliation development. Domestic chicks are precocial birds, and we could therefore focus on peer interaction without having to consider parenting. To study the juveniles' social learning with peers, we set two social environmental conditions at home. A subject was reared in a group from hatching to two weeks of age developed tight social bonds in the group (Grp). By contrast, when a chick was socially isolated from the time of hatching (Iso), it froze upon meeting other chicks. We video-recorded a subject chick behavior towards unfamiliar reference peers, which were separated by either an opaque or transparent wall. By a multivariate analysis using principal component analysis (PCA) that we named as BOUQUET (Behavior output analysis for quantification of emotional state translation) [15,16], we had preliminarily found that there might exist a sensitive period in which social experience with peers was essential for their socialization during a few weeks after birth [17]. In this report, we evaluated the existence of a sensitive period for peer-socialization and searched the most sensitive period during two weeks after birth.

Materials and Methods

Animals

This experimental protocol was approved by the Ethics review Committee for Animal Experiments of Tokyo University of Agriculture and Technology, TUAT (19-19) that follows the animal care and experimental guidelines of Japan Neuroscience Society and NIH, in USA.

Fertilized eggs from domestic chicks (Gallus gallus domestics), White Leghorn, Maria strain, were purchased from a local breeder, GHEN Corporation (Gifu, Japan). They were kept in a dark incubator (Showa Furanki) at 37.7 degrees centigrade with approximately 50% humidity and automatic rolling every hour. On embryonic day 21 (E21, the start of incubation was defined as E 1), which usually coincided with one day before hatching (the day of hatching was defined as post natal day 1, P1), five to fifteen eggs were moved to incubator-boxes as Grp raised condition ((width) × (depth) × (height) in mm: 450 × 450 × 450) surrounded by opaque walls to avoid communication between the other sets of birds with the air circulation. Iso raised condition, each chick was isolated in a sound attenuated incubator surrounded opaque

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walls with the air circulation (230-270 × 250-270 × 220-300 mm). Iso birds were regarded as peer-social deficit models without any visual or auditory interaction. To minimize compared conditions, we observed only males. Each incubator box was kept at around 30 plus-minus 2 degrees centigrade, with a light bulb (10 watt). Constant lighting was maintained from E21 until P3, after which a 12h dark-light cycle was set. Chicks were transferred using a small opaque container during daily incubator cleaning and feeding the optimized volume of experimental food and distilled water at 9-10 am.

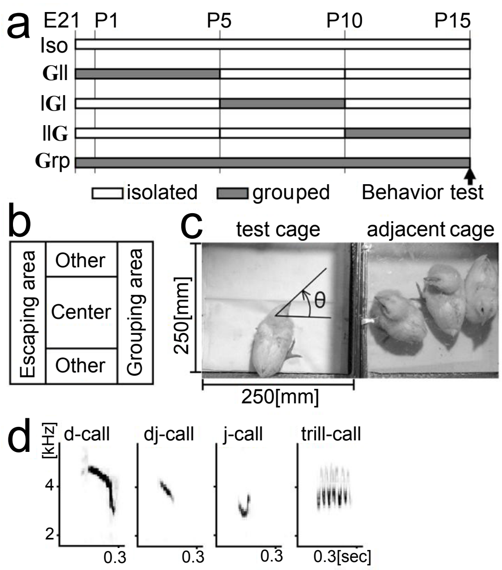
According to Figure 1a, we regulated each subject to one of five rearing conditions, Iso (subject number: 7), GII (8), IGI (5), IIG (7), Grp (7) from embryonic E21 through P15. We avoided social interaction with chicks, since handling can induce stress or affiliation effects on chicks [11,14,18].

Behavioral test

A behavioral test per subject was once conducted on P15 in the different sound proof room from the raised room. The subject chick was put into one of the two transparent metal net boxes covered with plastic sheets (Figure1c, 250 (w) x 250 (d) x 250 (h) mm) and the three unfamiliar reference grouped chicks (no gender control) into another. The two boxes were placed adjacent to each other, separated only by a masking board. The box containing the subject chick was covered with a transparent acrylic board, in order to reduce olfactory cues from the reference birds and researchers, and also to increase the specificity of call recordings from subject birds through a microphone in the test box. Subject chicks underwent the following four serial peer-social contexts: context 1; initial isolation period with no reference chicks, and a masking board in place, context 2; presented with acoustic only cues, ensured by a separation board, context 3; the reference chicks were presented with both visual and acoustic cues, after removing the separation board, context 4 (final); second period of isolation. We analyzed the response only context 3. Each context lasted for 2 minutes. All behavior was recorded using a top video camera (SONY handycam) with an external microphone in the test box.

Behavioral analysis

The recorded WMV files were transferred into WAVE and JPEG files per second using TMPGEnc-4.0XPress software (Pegasys Inc., Tokyo). The subject's behavior in context 3 was analyzed for this study. In the 15 parameters of Figure 1e, the x, y coordinate of head centre and forehead (in most cases, beak head) position (Figure1c) were sampled and used to calculate horizontal Velocity (V), face horizontal angle, its rotation velocity (delta Theta) and local preference to define social distance (four equally divided areas, Figure 1b) by Excel (Microsoft, USA). Freezing (Freeze) was defined as V less than 25 mm/second for more than 5 seconds. The parameter "facing to peers" defined as beak angle to adjacent cage within 30 degrees (beak-to-separating wall) refers to subject chick's preferred head position towards the reference chicks. We further defined pecking behavior, floor (pk-floor) and cage wall (pk-wall) expressed as frequency of pecking behavior per specified time. We used free software (Syrinx, version 2.4i) distributed by Dr. John Burt (University of Washington) to analyze chick calls. Spectrograms were used to identify four call types (Figure 1d; d- (distress), dj- (transient), j- (joyful), and trill-calls) by analyzing morphology of the first component [19]. Except trill-call, we defined the three call types based on the frequency change of the first component over time, as follows: First, we read the first time point (t1) where the frequency was maximum/minimum (f1), and the second time point (t2) where the frequency minimum/maximum (f2). Next, we calculated (delta) f=(f2-f1). Finally, if (delta) f<-2, we designated this call as a D-call. If (delta) f > 0, as a J-call, and if -2 < (delta) f< 0, as a dj-call.



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Behavioral factors	contents	unit
head movement		
V	head horizontal moving velocity	mm/sec
⊿θ	head horizontal rotation velocity	degree/sec
θ<30°	frequency of facing to peers	time/sec
Freeze	frequency of freezing	time/sec
local preference		
LP-C	local preference (LP) of Center	sec/sec
LP-E	LP of Escaping area	sec/sec
LP-G	LP of Grouping area	sec/sec
LP-O	LP of Other area	sec/sec
pecking behavior		
pk-floor	frequency of peck at floor	time/sec
pk-wall	frequency of peck at wall	time/sec
pk-self	frequency of peck at self body	time/sec
vocal call		
d-call	d-call frequency	time/sec
dj-call	dj-call frequency	time/sec
j-call	j-call frequency	time/sec
trill-call	trill-call frequency	time/sec

Figure 1: Experimental designs to explore a sensitive period of peer-social learning
a. Five developmental conditions of peer-social environment
Besides the bipolar sets, isolated (Iso) and grouped (Grp), three kinds of five-day grouped with ten-day isolated conditions (GII, IGI, and IIG) were evaluated
b. Parameters of local preference to interpret a subject's social distance to the reference peers (see also e)
c. The scene of a behavioral test on P15
d. Four types of chicks' typical calls
e. Fifteen parameters to be extracted from video-recorded behavioral data

Statistics

Statistical analysis was performed using free software R for PCA, multivariate hypothesis testing, Wilks' lambda distribution and one-way ANOVA. In order to integrate 15 behavioral parameters by BOUQUET [15,16], we used PCA based on a correlation matrix. The calculated PCA scores were plotted in a two dimensional (2D) plane defined by the 1st and 2nd PCA components. To compare behavioral patterns among the different groups, we applied the second PCA to each group of data and fitted the covariance as an ellipse, where the center of the approximation ellipse was the average of the plots, the long axis was derived from the 1st component eigenvector multiplied by squared of the eigenvalue and the short axis from the 2nd component one of sub-PCA, using the variance-covariance matrix. The statistical difference tests between groups of the score plots were performed by Wilks' lambda. To know the approximate contribution of each parameter for the 1st PCA on the 1st and 2nd components projection plane, the factor loading vector was visualized only plus direction with minus vector omitted, then multiplied by our setting maximum value of graph to be compared with the projected data plots.

Results

Chicks had been reared under the five rearing conditions (Figure 1a) and tested for the acquired social behavior in a meeting context with three unfamiliar peers in the adjacent cage (Figure 1c) on postnatal day 15th. The behavior parameters (Figure 1e) were extracted from video data and the representative parameters were compared among the five experimental groups (Figure 2a). The values of locomotive parameters, 'V', 'delta Theta', 'Freeze' and distress call ('d-call') clearly differed between two extremes, Grp and Iso, and showed gradational difference among the other three groups, GII (grouped during P1-5), IGI (grouped during P6-10) and IIG (grouped during P11-15). Grp birds expressed more activity than ISO birds. Interestingly, IGI as well as Grp values significantly differed from Iso (+ in Figures 2a,2c and 2f) as well as GII (* in Figures 2a,2c and 2f), in the parameters, head rotation velocity 'delta Theta' and 'Freeze' marked (black arrows). On the while, IIG values were similar to Grp values in locomotive activity (V) and Freeze, but more weakly different from GII in delta Theta.

Furthermore, we analyzed behavioral features by multivariate correlation based on PCA and visualized each PCA score-distribution of three groups, GII, IGI and IIG by ellipse approximation in comparison with typical groups, ISo and Grp (Figure 2b). The plane could be interpreted by the major factor loading vectors having relative length of more than 0.7 (Figure 2e). The origin of the plane coordinate was average of a factor loading vector and only positive direction of the

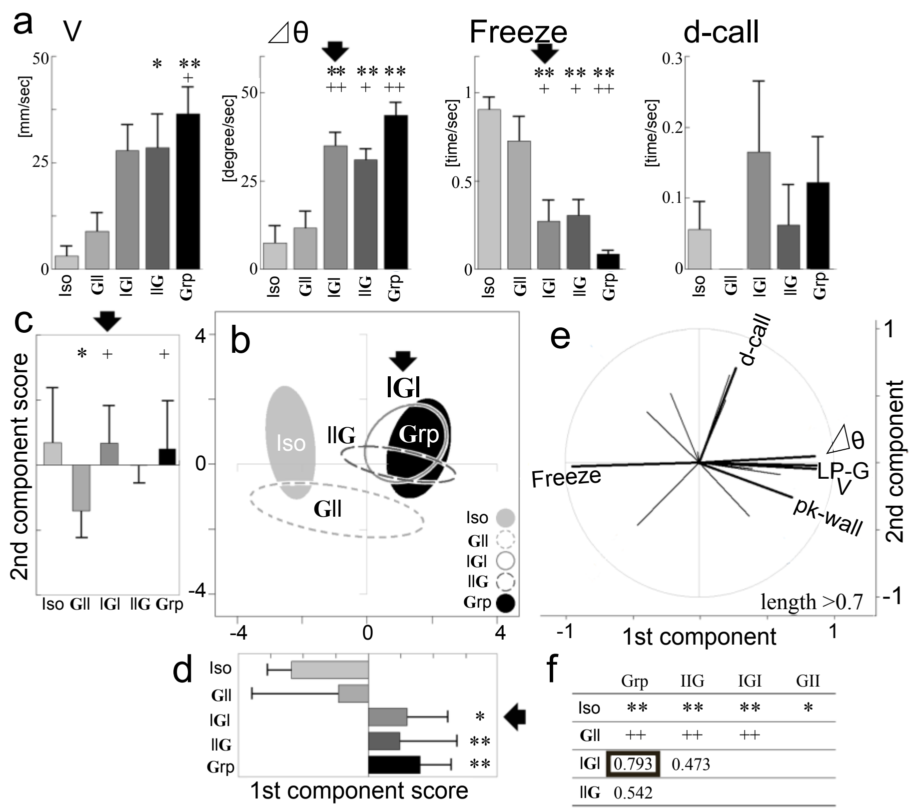

Figure 2: The single and multi-parametric analysis revealed a sensitive period for peer-social learning during P5-P10

a. The quantified behavioral difference of GII, IGI and IIG with comparing the bipolar types of Grp and Iso. Note the similarity of IGI to Grp in 'delta Theta', 'Freeze' (black arrows), parallelly, the similar features of GII to Iso. Statistical significance was evaluated by ANOVA and the significance with Iso or with GII was demarcated by symbol * or +, respectively

b-e. Multi-parametric correlation analysis, BOUQUET based on PCA

In the 1st -2nd component plane, five ellipses approximated each distribution of PCA scores (b) with the comparison of each component score (c and d). The fifteen factor loading vectors (shown only positive direction) were shown with each parameter having the longer correlation than 0.7 (e)

f. Wilks' lambda distribution analysis of the PCA scores. IGI were the most similar chicks to Grp comparing with either GII or IIG vector was represented. The 1st component negatively correlated with a negative parameter, 'Freeze' and positively with a positive valence vector cluster consisting of locomotion (V, delta Theta), affective social distance (LP-G), and a parameter motivated outside, pk-wall. The comparison of the first component scores was shown in the bar graph of Figure 2d. This graph showed similar feature of IIG/IGI to Grp (black arrow) comparing to either GII or IIG. With regard to the 2nd component, the longer factor loading parameter was seen only d-call (Figure 2e) and the comparison of the 2nd component scores showed that IGI, but not IIG was different from GII (+ in Figure 2c, black arrow) as like Grp was. Finally, we found IGI to be the highest similarity to Grp (0.793) by Wilks' lambda distribution analysis (Figure 2f, black edged square). In summary, the subjects of IGI resembled to Grp chicks in terms of combinatorial behavior parameters of call and locomotive activity in compared with either GII (no locomotive activity with call) or IIG (no call with locomotive activity).

Discussion

We explored a sensitive period for peer-social learning and found the period around P5-P10 in the animal model by multiple parametric analysis of behavioral quantification in the meeting context. This period seemed to comprise another sensitive period after a well-known critical period of imprinting system around P1-P3 [20] or may correspond to the one around P7 [21]. It is important to define quantitatively how sensory-motor functions contribute for early development of social brain network. We found that the acquired behavioral features were in the head rotation and frequency of freezing in the meeting context with unfamiliar peers. Both of these parameters may be of the common behavior over species, the former as a subject's motivation dependency, frequently seen in Grp and IGI, or the latter as fear or anxiety, expressed more in Iso and GII. These parameters negatively correlated each other in the factor loading vectors of Figure 2e that also explained strong correlation of head rotation with affective social distance (LP-G) simultaneously. The head rotation-less behavior, possibly translated as view-modulation less has been reported as the key dysfunction in social disabilities [22], which may be the case in Iso and GII chicks in our study. Freezing is supposed one of the most typical fear-response behaviors whose final output nuclei is periaquaductal grey regulated by ventral striatum (basal ganglia). Amygdale modulates ventral striatum, thus the lesion of amygdale in juveniles affected social development [23] and personal (social) distance [24]. Domestic chicks' diversified socio-emotional behavior has been frequently reported such as pecking and flapping in group like 'play' [25] or isolated context with panic calls [26]. Cholinergic and other neurotransmitter systems control chick basal ganglia. The neurotoxicology such as acetylcholine esterase inhibitor, Physostigmine suggests the relevancy to developmental disorders [27]. Thus, social affiliation development in chick suggests a common neural substrate, i.e., mesolimbic system which comprises a social brain network. In human, the network interacts with more complex cognitive system eventually yielding a theory of mind [28]. Therefore, our animal model and a statistical behavior measure may be relevant to evaluate development of a subcortical limbic system comprising a common neural substrate for social affiliation acquisition over species [29].

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