Peer-Social Network Development Revealed by the Brain Multivariate Correlation Map with 10 Monoamines and 11 Behaviors

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Abstract
Psychiatric disorders induced by social stress have suggested that monoamines play key roles in a whole brain network. To research an environmentally dependent neural network development of social psychology, we report our model animal study of peer-social behavior learning by screening the relevant monoamines in various brain regions in the light of social brain network. We have originally developed psycho-biological quantification batteries based on multivariate analysis over species, including the current subjects, domestic chicks (Gallus gallus). The chicks' peer-social environment was regulated as grouped and isolated from just after hatching. After two weeks, their acquired behavioral features were examined in a social meeting with unfamiliar peers and then, quantified 10 kinds of monoamines in their 13 brain regions at their steady state under off lighted conditions in day-time. The whole brain concentration map visualized regional difference of 10 monoamines. Furthermore, we attempted to map behavioral feature correlation with each monoamine in the whole 13 brain regions. These results showed that isolated chicks expressed (1) significantly higher Dopamine (DA) of left caudal forebrain, (2) moderately higher Norepinephrine (NE) of thalamus-midbrain correlating with immobile and alert behavior. While 3-methoxy-4-hydroxyphenylglycol (MHPG) as well as Normetanephrine (NM) correlated with alert-immobile behavioral feature, Homovanillic Acid (HVA) seemed to weekly correlate with active-affinity behavior. In addition, there existed lateral asymmetry of the correlation generally as left-alert and right-affinity in the lateral part of the brain. These correlations between social behavior and monoamines would possibly contribute to comprehend the developmental mechanism of social brain networks. These findings open a new way in constituting a functional map by multivariate analysis of social behavior and monoamines and their metabolites.

Keywords: Monoamine; Catecholamine; Serotonin; Social disability; Emotion; Memory; Developmental psychiatry; Molecular psychology; Peer interaction

Introduction
Social disabilities are the central symptoms in any psychiatric disorder. Clinical neuropharmacology [1-3] and various molecular researches have comprised a convincing evidence about critical role of monoaminergic neurons in socio-psychological function [4-9]. The neural circuits of limbic system and cortico-limbic system are neural substrates for physiological responses, behavior output, and socio-emotional subjective feeling [10-12], which are supposed to develop as a consequence of continuous adjustment of sensory-motor, emotional and cognitive systems in nervous systems during learning processes [9,13-16]. A deficiency of social experiences in childhood tends to induce severe underdevelopment in monoaminergic neural circuits because of the least experience of the learning [5,17,18]. Although we have much progress in the understanding of kinship interaction and pair bonding mechanisms [7,19], neurobiological mechanism of peer social interaction development is still uncertain. Domestic chicks have been used as an excellent model of social affiliation development since it is a precocial bird which is able to feed by itself from just after hatching and their very early neonatal experience or learning can be manipulated and evaluated [10-12,20-24]. We here report our domestic chick study of peer social behavior learning and the role of monoamines in this process. The goal of this study is an understanding of a social brain map in terms of monoamines levels and their correlation with social behaviors. The correlation mapping is widely used to reveal the inherent complexity of brain as a whole rather than just its individual parts, for example, a functional correlation of the volumetric change in longitudinal MRI study [25,26], task-related cingulated cortex sub-regions by an fMRI study [27], tractography and a resting state functional connectivity combined with graph-theory [28,29]. The social brain map described in this study is based on a multivariate analysis using correlation matrix [30,31] with eleven behavior parameters expressed at social meeting test with unfamiliar subjects and a particular monoamine with a total ten monoamines and their metabolites measured with each sub-region with a total thirteen brain regions from two experimental groups, a socially isolated group and an affiliated group. The statistical significance of the correlation was mapped in sub-regions, which revealed the region-specific modulation of monoamines.

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 regulates animal care and experimental guidelines of the Japan Neuroscience Society and NIH in USA. Fertilized eggs from domestic chicks (Gallus gallus domesticus), White Leghorn, Maria strain, were purchased from a local breeder, GHEN Corporation located in Gifu, Japan. They were incubated, and
hatched as described [32]. Two rearing conditions were set, grouped (Grp, n=6) and isolated (Iso, n=6). As Iso raised condition, each chick was isolated in a sound attenuated incubator surrounded by opaque walls with 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 females. Other conditions were essentially the same as described in [32]. The overview of experimental design is shown in Figure 1a.

Behavioral test
A behavioral test per subject was once conducted within 11:00 a.m. and 3:00 p.m. hours on P13 in the different sound proof room from the raised room. The details of the test conditions were essentially the same as described in [32]. The 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. Each context lasted for 2 minutes. All behavior was recorded using a top video camera (DVD VIDEO CAMERA NTSC-DC40 (Canon)) 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 Version 4.6.3.268 (Pegasys Inc., Tokyo). The subject’s behavior in context 3 was analyzed for this study. In the 10 parameters of Figure 1b, the x, y coordinate of head centre and forehead (in most cases, beak head) position (Figure 1c) were sampled and used to calculate horizontal Velocity (V), head horizontal moving velocity (mm/sec).

![Figure 1](image1)

**Figure 1:** Experimental designs and quantified parameters from behavioral test.

a. The experimental procedure for comparison between group-reared chicks (Grp) and socially isolated chicks (Iso) (see details in Materials and Methods).

b. The list of behavioral factors extracted from the video data during behavior test. The definition of each behavior factor and the test condition were described in detail under Materials and Methods.

c. Social meeting test of a subject (left) and three reference peers which were unfamiliar to the test subject (right). The test comprised of four contexts (2min each) and only third context (visually and acoustically interactive) was illustrated here and the behavior data during the context was used for further analysis.

d. The definition scheme of local preference (LP-C, E, G and O) was illustrated, which related with personal distance [33,34] and emotional/cognitive response to other environmental condition.

e. Each spectrogram example of two kinds of vocal calls: a distress call and an intermediate call. The detailed definition was described in Materials and Methods.

![Figure 2](image2)

**Figure 2:** The multivariate analysis of eleven behavioral factors and the correlation analysis with behaviors and a monoamine concentration in a brain sub-region.

a. The data distribution of Grp (right) and Iso (left) behavior during the behavior test in the context 3. Two experimental chick groups were prepared and tested with social interaction behavior at postnatal day 13. The behavior factors were extracted from the video recording, quantified, and the feature vectors of the behavior were computed by principal components analysis as described in Materials and Methods. The principal scores of each subject was plotted in a plane. x axis is the first component score and y axis is the second component score. The plot was assigned to each group and the distribution of the plots (n=6) in either group was demarcated by variance ellipse (asterisk: significantly different by Wilks’ lambda with the F and p values).

b. The eleven factor loading vectors in the projected plane a. The factor loading vector was calculated as the correlation of behavior eigen vector with the first or second component of principal components analysis giving the value between -1 and +1 (0 means no correlation) after normalization by eigen value of each component. Only positive value was illustrated in the panel.

c. Illustration of four emotional quadrants in the feature space of the behavior. The clustering of the factor loading vectors and emotional expression with each behavior gave four clusters in the feature space and we labeled each cluster by emotional behavior, like active (green), immobile (orange), affinity (blue), and alert (red). The quadrant border was arbitrarily assigned. The correlation of a monoamine (in this case MHPG, a metabolite of norepinephrine) in a brain sub-region (in this case #1, left lateral frontal, Figure 3f) with behaviors was based on Pearson product-moment correlation coefficient (R) computed using the monoamine concentration and the first component score or second component score of each subject, which was derived by PCA of behavior factors (Fig.2a). Thus obtained monoamine vector was color coded according to the location of the vector in four emotional quadrants. The color density indicates as ‘0.3<R<0.6’ (shorter length vector, light) or ‘R>0.6’ (longer vector, dark).

horizontal angle, its rotation velocity (delta Theta) and local preference to define social distance (four equally divided areas, Figure 1d) by Excel (Microsoft, USA). The parameter “facing to peers” was defined as the angle between the beak direction and the perpendicular line to the separating cage wall within 45 degrees (beak-to-separating wall), in reference to where the chicks were placed. We further defined the pecking cage wall (pk-wall) expressed as the frequency of pecking per specified time. Chick calls were classified into four types (Figure 1e) by spectrogram as described in [32].

**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 11 behavioral parameters, we used PCA analysis based on a correlation matrix. The details of PCA analysis and the assignment of behavior to emotional state (BOUQUET method) were described in [30-32]. 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 (Figures 2c and Figure 3g).

**Brain monoamine measurement by HPLC**
After leaving a subject in the home cage in darkness for 30 minutes.
within 11:00 a.m. to 3:00 p.m., animals were decapitated and the brain was taken out from the skull. The brain was dissected into 13 regions (Figure 3f) by a surgical blade on a 7% agar block in 0.1M of PBS on ice and the dissected tissues were put in tubes, then immediately frozen in liquid nitrogen, and transferred in -80 degree Celsius within 2 days. Each tissue was homogenized in 500 microL of 0.2M perchloric acid per 100 mg of tissue. After 30 minutes on ice, the samples were spun in a microcentrifuge at 20,000 G for 10 min at 0 degree Celsius. Samples of the supernatant were adjusted at pH 3 with 1M of CH3COONa by pH test paper (ADVANTEC, Universal pH1-11), filtered (13mmMillipore), then stored in – 80 degree Celsius. The samples were analyzed for 10 kinds of monoamines (Figure 3a) by HPLC (Eicom...
on postnatal day 14th (Figure 1a). First, a monoamine expression map was constructed (Figures 3b and 3c). Group difference was not widely segregated. The feature space was a PCA plane consisting of the first and second components as x and y axes. Secondly, a monoamine concentration in a sub-region was correlated with the first or second component scores of the same subject used for behavior analysis. Next, we calculated the Pearson correlation coefficient (R) with each component and its R value was plotted on the behavior feature plane as x or y coordinate values. Thereby, a monoamine in a sub-region can be related with behavior features in this study. These four emotional features are active, immobile, affinity, and alert. We successfully mapped the correlation of monoamines and emotional features in a whole brain. This map revealed reasonable connectivity of monoaminergic systems, DA to medial frontal region, NE to various brain sub-regions. First, we determined the behavior feature space where two groups were well segregated. The feature space was a PCA plane consisting of the first and second components as x and y axes. Secondly, a monoamine concentration in a sub-region was correlated with the first or second component scores of the same subject used for behavior analysis. Next, we calculated the Pearson correlation coefficient (R) with each component and its R value was plotted on the behavior feature plane as x or y coordinate values. Thereby, a monoamine in a sub-region can be related with behavior features in this study. These four emotional features are active, immobile, affinity, and alert. We successfully mapped the correlation of monoamines and emotional features in a whole brain. This map revealed reasonable connectivity of monoaminergic systems, DA to medial frontal region, NE to various brain sub-regions as an alert system, and 5-HT to mesolimbic system. The distribution of monoamines between two experimental groups seemed not much different, but with close examination a couple of difference was observed, for example, higher expressions of DA in left caudal-lateral forebrain and NE in right thalamus-midbrain regions in Iso subjects were statistically significant (Figure 3d). Another prominent result of the brain mapping was the finding of MHPG, a NE metabolite correlated with alert emotion in many brain regions and HVA, a DA metabolite correlated with active/affinity emotion in prefrontal and mesolymbic systems. Although the sample population in this experiment was limited, these results encouraged further study using a statistical brain mapping method to understand a functional brain network as a whole even without brain imaging technology and would be potentially useful for toxicological analysis especially when using animal models.

Discussion

In this study, we explored the possibility of linking micro (monoamines) and macro (behavior) physiology using a statistical brain mapping method and to comprehend the complexity and integrated nature of the social brain network as a whole instead of individual brain parts [10-12]. As the first step to see the applicability of a statistical brain mapping method, we prepared two experimental chick groups, socially isolated (Iso) and affiliated ones (Grp), analyzed their social behavior, and brain ten monoamines in thirteen brain sub-regions. First, we determined the behavior feature space where two groups were well segregated. The feature space was a PCA plane consisting of the first and second components as x and y axes. Secondly, a monoamine concentration in a sub-region was correlated with the first or second component scores of the same subject used for behavior analysis. Next, we calculated the Pearson correlation coefficient (R) with each component and its R value was plotted on the behavior feature plane as x or y coordinate values. Thereby, a monoamine in a sub-region can be related with behavior features in this study. These four emotional features are active, immobile, affinity, and alert. We successfully mapped the correlation of monoamines and emotional features in a whole brain. This map revealed reasonable connectivity of monoaminergic systems, DA to medial frontal region, NE to various brain sub-regions as an alert system, and 5-HT to mesolimbic system. The distribution of monoamines between two experimental groups seemed not much different, but with close examination a couple of difference was observed, for example, higher expressions of DA in left caudal-lateral forebrain and NE in right thalamus-midbrain regions in Iso subjects were statistically significant (Figure 3d). Another prominent result of the brain mapping was the finding of MHPG, a NE metabolite correlated with alert emotion in many brain regions and HVA, a DA metabolite correlated with active/affinity emotion in prefrontal and mesolymbic systems. Although the sample population in this experiment was limited, these results encouraged further study using a statistical brain mapping method to understand a functional brain network as a whole even without brain imaging technology and would be potentially useful for toxicological analysis especially when using animal models.

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References


