Review Article

• **Title**

Susceptible period of socio-emotional development affected by constant exposure to daylight

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Abbreviations

AChE: acetylcholine esterase

ADHD: attention deficit hyperactive disorders

ASD: autistic spectrum disorders

ODD: oppositional defiant disorder

ChAT: choline acetyltransferase
DAT: dopamine transporter
DD: constant dark
DIA: dehydroascorbic acid
DTA: diphtheria toxin α subunit gene
ipRGC: intrinsically photosensitive retinal ganglion cells
GSH: Glutathione
LD: light-dark cycle per 12 hours
LHb: lateral habenula
LL: constant light
mAmy: medial amygdala
NOS: nitric oxide synthase
PCA: principal component analysis
SCN: suprachiasmatic nuclei
SERT: serotonin transporter
sPVZ: subparaventricular nucleus
VLPO: ventrolateral preoptic area

Abstract

As a diurnal experimental primate, the common marmoset (*Callithrix jacchus*) has recently contributed to numerous kinds of studies of neurobiological psychiatry as an essential pre-clinical model. The marmoset is capable of having offsprings within one or two years after birth. Thus, we can observe how the primate learns and develops psycho-cognitive functions through experiences in experimental environment for a much shorter period compared to that of humans.

Longer daylight exposure artificially may affect psychological development of children. In our research, we focus on raising marmosets under constant daylight (LL) from birth until various ages. In order to quantitatively evaluate the development of higher-ordered psychological functions, we designed a system of socio-behavioral tests and multivariate correlation analysis methods, called as the Behavior Output Analysis for Quantitative Emotional State Translation (BOUQUET) based on principal component analysis. With reference to the call and typical body movement expressed during a particular social context and after some developmental experiences, we statistically inferred the emotional features of the subjects.

In the current literature, we review our published results showing increased alert behaviors by constant light, and then, attempted to extend our additional analysis to seek age-dependent susceptibility to constant light. We then present the neurobiological mechanisms with reference to previous research reports. The current review suggests possible existence of a susceptible period earlier than three to five month-old in the environment-induced developmental disorder model, supposedly like attention deficit hyperactive disorders (ADHD) or oppositional defiant disorder (ODD).

Keywords

A susceptible period learning

Circadian rhythm

Socio-emotional behavior

Alert call and whole body motion

Multivariate analysis

Social sensory cues

1. Introduction

In etiological consideration of mental disorders, we need to study developmental processes of the neuronal circuit formation in early life. The typical psychological development of children is thought to occur progressively through diversification of the neuronal circuit by interaction between their own biological bases and surrounding environment. In childhood, our sensitivity to the environment seems higher and the response is simpler like examples of primitive reflexes that are transiently appeared and disappeared in neonates. Emotional behaviors such as ‘crying’ seem to be more easily triggered by physiological conditions in preschool-age children. Acute and chronic environmental stimuli in early life must crucially affect the neuronal circuit development of psychological function leading to form future higher-order circuitry responsible for more complex mental processing.

If the initial development is suppressed by deficiency in genetics of the biological basis and in the environmental factors, it may cause susceptibility to social stress in a later developmental stage and complex co-morbidities of mental disorders like attention deficit hyperactive disorders (ADHD), autism spectrum disorders (ASD), oppositional defiant disorder (ODD), or schizophrenia.

Among the many critical environmental factors for socio-emotional development after birth, we focused on light-induced circadian rhythm (Senoo et al., 2011). It is a common key factor which includes the sleep-awake cycle and its disturbance as seen in various psychiatric disorders. We selected a common marmoset (*Callithrix jacchus*) as the primate model of the study due to its particularly superior psychological function such as cooperative communication (Flack, 2013) and altruisms (Burkart et al., 2007), which is supposedly affected by its environment within one year from birth to early adulthood, as well as its rich socio-communicative behaviors. Such behaviors include multiple kinds of calls, emotional and social approaching or discrimination of typical motion (Pistorio et al., 2006, Yamaguchi et al., 2010, Senoo et al., 2011, Koshiba et al., 2011, 2013a, Cross et al., 2004).

Another important consideration of the study on an animal model is the quantitative and objective methods to evaluate the socio-emotional behavior and higher-order psychological states in developing common marmosets from neonate to adulthood (Koshihara et al., 2011, 2013a). As a basic approach, we used multi-dimensional correlation analysis, mainly, the principal component analysis (PCA) and time-series regression analysis. The neuronal networks of higher ordered psychological function is supposedly re-wired one another by multiple domains of different functions formed in different stages during development. The complex networks may be able to be estimated mathematically with quantitative information processing by such multivariate analyses. In our previous studies, the complex functional domain correlation was visualized by multivariate analysis using multiple but extracted simple factors in behaviors, biological molecules and physiological information. We call the method as 'Behavior Output Analysis for Quantitative Emotional State Translation (BOUQUET)' which allows us to hypothetically visualize 'higher-order neuro-psychological modulation'. The BOUQUET method has been repeatedly used to evaluate common marmosets and also human groups with ASD and their typical developing siblings. We further used the BOUQUET method to analyze environment-induced developmental disorder models in domestic chicks. Their social acute and chronic experience were tested on visual, auditory, tactile and olfactory perception of peers and parents (Koshihara et al., 2011, 2013a-e. Mimura et al., 2013, Shirakawa et al. 2013a-b).

In the current review, we referenced our previous report with regards to the developmental socio-psychology under 'environmental constant light' in common marmosets (Senoo et al., 2011). This study was motivated by a recent social issue of longer light exposure especially on preschool-age children that is well-known about the sensitive age-stage for multiple kinds of learnings (Ito, 2004). In this report we analyzed twenty-five parameters derived from the behavioral data taken from videos of twenty-four common marmosets from birth to adult. We summarized that the primates exposed by constant light during early development had typical alert body-motion and more calling behaviors at meeting contexts with unfamiliar peers. The

behavioral types of active and alert emotion might be interpreted as models of ADHD or ODD either of which is diagnosed with irritable phenotypes by DSM-IV (Drabick and Gadow, 2014). Alert emotion is rather categorized in lower-ordered function that may be internalized in adults to avoid careless expression in social contexts. The LD subjects, supposedly regarded as the control group comparing to LL seemed to express their higher-ordered function as suppression of the lower-ordered functions at peer-social contexts in adult more clearly than in juvenile. The behavioral developing pattern that could be visualized in the multivariate correlation structure by BOUQUET might be interpreted one model of developmental disabilities of peer-social adjustment in LL group. In this review, we, furthermore, added new analyses of the same data to consider any susceptible period of more specific age to form the character to express rather emotionally negative behavior, supposedly caused by neuro-psychological network formation of social emotion in limbic systems (Amaral et al., 2007, Machado et al., 2003, Lesch et al., 2012, Happé et al., 2014, Leppänen et al., 2009). Finally, according to the relevant reports in developmental psychiatry, the three key terms are, susceptible period learning, circadian rhythmicity, and expected supporting tools for treatment.

2. Effects of constant light during early life to socio-psychological behavior development and susceptibility to earlier arrhythmic periods of locomotion and sensory context dependent on socio-behavioral modulation around weaning age

In common marmoset (*Callithrix jacchus*), we developed socio-psychological developmental model and multi-dimensional analysis which we call BOUQUET, to imply complex neuro-psycho-cognitive mechanisms generally (Koshiba et al., 2011). We applied it to examine the impact of longer exposure to daylight in early life (Senoo et al., 2011). The subjects were fed milk by humans until weaning (Tokuno et al., 2012) and were isolated from any other marmosets from birth until adult age. We simplified the social experience of animals for the purpose to examine directly the effects of early atypical light zeitgeber on psychological

behavior modulation in social sensory contexts. In this study, we compared responses during social behavior tests and the analytic results of BOUQUET in three groups, LD (light-dark cycle per 12 hours, control condition), constant light (LL), and constant dark (DD) as another extreme condition (Fig.1a). Their development was diagnosed by the social behavior test once or twice for each individual with at least a two-month interval to maintain unfamiliarity between animals in the social behavior test. The test consists of four serial contexts of social meeting each other of two isolated subjects in a transparent test cage under regulation of their visual (v), auditory (a) and olfactory (o) interaction (Fig.2b). Each individual met three unfamiliar peers conditioned with different lighting environment and all the data were averaged, which was expected to avoid social pair-specific effects. They were tested at either of three age-stages, P30-50 [postnatal-day], P100-150 (around/after weaning) or P180-420 (Fig.1a, red-bar, Fig.2a, b (left schemes)). Fig.2c shows the reported general summary represented by each arrowed regression vector, DD (black), LD (light blue) and LL (pink) in two dimension coordinates of the component 1st and 2nd calculated by PCA with correlation matrix. The origin of the two-dimensional plane was the central average of all the twenty-four parameters except the parameter for age. Instead, age-dependent modulation is represented by the vector (the arrows in Fig.2c). The directed trajectory can be seen together with these factor loading positive vectors (simple radial lines from the average center), which suggest distribution patterns of parameters. The six representative factor loadings in the dimension of the 1st (c1) and 2nd (c2) can be presented by each radial gray and red vectors from the average center with labelled by 'z-high' or 'z-low' as preference at higher or lower position in the test cage, 'dTheta/dt' or 'dPhi/dt' as head horizontal rotation velocity to the cage horizontal coordinate or relative head horizontal rotation velocity to another individual, respectively, and two vectors, 'e-call' and 'shake' close to the 1st component. Consequently, comparing to extremely different DD, suggesting modulation of not only higher but also lower cognitive function, the LL vector was observed slightly different from LD but was certainly characterized by two explanatory variables of alert behaviors (Fig.2c,

Senoo et al., 2011, Koshiba et al., 2013a, Cross and Rogers, 2006, Kato, et al., 2014). The behaviors were ‘shake’ and ‘e-call’ more but there are no other parameters like head motion in space or affinity call (not shown).

In this chapter, we furthermore add two supplemental analyses to seek any age-dependent modulation in the same data. The first approach was assessed comparisons between LD and LL of three subjects each. A total of six animals and their locomotive activities from their birth to approximately postnatal-day 120 by an infrared detecting device (NS-AS01; Neuroscience, Tokyo) set at the home cage ceiling were recorded. These recorded subjects are called as RA (Rest-Activity recorded for 24hours, Fig.1a). Each sample data of an LD or an LL subject is represented in a double plotting graph of Fig. 1b or c, respectively. The abscissa and vertical axes show 48 hours and 150 days, respectively, with plots sized according to activity average per thirty minutes. We then extracted three kinds of parameters from each acquired graph. Firstly, the activity rhythm was unclear during approximately a month after birth and the period was defined qualitatively as ‘arrhythmic periods (arprd)’ that was summarized in Table 1. Any arprds of the LL subjects were longer than LD ones. The other two parameters, inclinations of linear regression of resting plots (activity = null) for running period 1 (rp1: P40-80), or rp2 (P80-120) were arbitrarily defined like the examples of formula (Fig.1c) to characterize free-running in the LL graphs. The results showed that the inclinations are clearly different between groups LD and LL, however it is hard to see any commonality in group LL.

	arprd [day]	rp1 [hour/day]	rp2 [hour/day]
RA-LD : female	31	0.00	0.00
RA-LD : male	35	0.00	0.00
RA-LD : male	23	0.00	0.00
RA-LL : male	39	0.77	0.20
RA-LL : male	47	-0.27	-0.03
RA-LL : male	44	0.76	-0.46

Table 1

Assessment of rhythmicity parameters of home-cage-locomotion in LD and LL monkeys detected by infrared recorders.

In the same PCA scores shown in Fig.2c, we compared each behavioral pattern represented by ellipse regression. The significant difference of the averaged scores over contexts between LL and LD groups had been already confirmed only at the third age-stage (P180-420) in our previous report (Senoo, et al., 2011), also shown in the probability as 0.004 acquired by Wilks' lambda test in Fig.2a. The regression from the longer and shorter axes of an ellipse was acquired by another PCA with variance-covariance matrix and the 1st and 2nd factor loadings to both ways from the average center. These ellipses were compared between group LD and LL in three age-stages, P30-50, P100-150, or P180-420, or in the three sets of social sensory contexts, v-a+o- (gray), v+a+o- (light blue) and v+a+o+ (light green) in Fig. 2a. In addition, context-dependent modulation per group was assessed by showing general similarity to the initial context v-a+o- as the percentages of ellipse superimposed area in Fig. 2b. Consequently, contextual modulation looks the clearest from context v-a+o- to v+a+o+ in LD at the oldest stage of P180-420 ($p=0.02$, Wilks' lambda). Differences between LD and LL were furthermore confirmed in the first component of PCA as higher in LL than LD at contexts, v+a+o+ at P100-150 and all contexts at P180-420 ($p<0.05$, two sample t-test). It may suggest that the LL-like characters of alert behaviors, 'e-call' and 'shake' more, had already emerged in the second day-age-stage from postnatal 100 to 150, the latter juvenile stage after weaning. In addition, the ellipse similarity assessment seems to suggest a certain modulation in LD at P100-150 from context v-a+o- (gray) to v+a+o- (light blue) marked gray arrows (Fig.2a, upper-middle and Fig.2b, middle) whose direction may be explained by the factor loading positive vectors of Fig.2c from higher position (z-high) in the context of acoustical social interaction (v-a+o-) to lower (z-low) in the multisensory integration context of visual and auditory meeting (v+a+o- and v+a+o+) in both groups LD and LL. Interestingly, comparing to the LD's crucial

modulation from the auditory context to the auditory plus visual cues of unfamiliar peers (Fig.2a, upper-right, only significant as $p < 0.05$ by Wilks' Λ), LL patterns (Fig.2b, pink lines) seemed less alternative and kept the tendency also in the latter age-stage, P180-420. It is merely no more than speculation, but may be possible to discuss group LLs' disability of self-adaptive control at different social sensory inputs in a susceptible period for socio-emotional learning although furthermore examinations like age-screening studies are needed. It may be possible to refer also to sensory sensitivity in children of developmental disabilities (Corbett et al., 2009).

In another common marmoset study about socio-emotional learning in parental and peer-interactive environment, the developmental regression line of social-rich juvenile revealed an inflection period around P100 in the same BOUQUET analytic visualization (Koshiba et al., 2013a), consistently. Other diurnal and rich-communication species, domestic chicks, were suggested to have a susceptible period for their peer-social learning around postnatal a week, supported by our studies of social behavior analysis, brain anatomy, monoaminergic expression and anti-oxidant pharmacology (Koshiba et al., 2013b, d. Mimura et al., 2013a-c, Shirakawa et al., 2013a). In the BOUQUET visualization of age-dependent linear trajectory as socio-emotional development in chicks, an inflection has been confirmed around the susceptible period although it indicated not in social-rich but rather social-deficit subjects. Our cross-species observation suggests the presence of a susceptible period when environmental factors affect the developmental trajectory. These models may contribute to understand a mechanism in developmental psychiatry.

3. Neurobiological basis in disturbances of circadian rhythm for socio-emotional development

It has been an important issue to understand the emotional and cognitive consequence of the desynchronization of biological rhythms in the clinic and during child-rearing, especially

concerning the life-long effects of developmentally early photo-experience on activity-rest and sleep-awake rhythms. As described in the previous sections, we attempted to find an answer of the issue in a common marmoset model. In this section, to understand the causality of the socio-emotional behavior of the marmoset and the bio-psychiatric significance of our finding, we review recent progresses in neurobiology of circadian regulation of physiological rhythms and effect of the irregular solar cycle on emotional and cognitive function.

Disturbances of circadian rhythm due to abnormal lighting conditions may cause pathogenesis of various psychiatric disorders, such as insomnia, depression, and mental maldevelopment (Germain et al., 2008; Lewy 2007; Kohyama 2009). It is possible that such disorders are caused by genetic, neuronal and hormonal mechanisms regulating sleep, body temperature, and mood cycles (Cambras et al., 1998; Connolly et al., 1983, Diez-Noguera et al., 1990, Schelstraete et al., 1992; Suchecki et al., 1998, Tononi., 2001, Mistlberger et al., 2004; Toki et al., 2007, Yerushalmi, et al., 2006, Bunney et al., 2008, LeSauter et al., 2009). Touchette et al. (2009) and Kohyama (2009) reported the correlation of sleep disturbance in children with developmental deficits (e.g., ADHD and ASD). Constant light and the perinatal photoperiod imprinting desynchronize the circadian rhythms of suprachiasmatic nuclei activity (Ohta et al., 2005, Ciarleglio et al., 2009). Some reports also suggest the correlation of aggression and manic behavior with abnormal free-running cycles caused by corticosterone (Carere et al. 2003), neuronal nitric oxide synthase (NOS) (Kriegsfeld et al., 1999) and one of the circadian clock genes (CLOCK) (Roybal et al. 2007). These pathological conditions suggest the underlying physiological mechanism of circadian rhythmicity in hypothalamic suprachiasmatic nuclei (SCN) as a causality of psychiatric disorders.

3.1 Light induces the synchronization but does not generate the circadian rhythm itself of individual SCN neurons.

The neuronal circadian clocks are composed of populations of circadian pace-maker neurons organized to drive coherent daily rhythms in behavior and physiology. The central pace-maker neurons in SCN are responsible for the circadian clock of motor activity. Exposure to constant light (LL) disrupts overt rhythms and induces circadian arrhythmicity in mammals and other species. By imaging molecular rhythms of individual clock neurons in explanted SCN neurons, Ohta et al. showed that LL condition desynchronizes clock neurons, but does not compromise their ability to generate circadian rhythms (Ohta et al, 2005). Furthermore, the perinatal photoperiod has lasting effects on the circadian rhythms expressed by clock neurons as well as on mouse locomotive behavior. The perinatal photoperiod sets the responsiveness of the biological clock to subsequent seasonal changes in photoperiod (Ciarleglio CM et al, 2011), suggesting that circadian visual pathways are capable of the kind of developmental plasticity seen in cortical visual pathways (Morishita et al., 2008). The precise molecular mechanism of circadian imprinting, however, is uncertain.

3.2 Existence of several oscillators in SCN and the regulation of a particular physiological rhythm by each oscillator, like splitting between right-left brain hemispheres.

De la Iglesia et al. (2000) showed that LL condition induced rhythm "splitting", in which a single daily period of physical activity (usually measured as wheel running) dissociates into two stably coupled components about 12 hours apart. Wheel-running activity was continuously recorded in male golden hamsters maintained in LL. About 60% of their hamsters developed stably split activity rhythms after 6 to 8 weeks. Brains of individual animals at various circadian times were processed using in situ hybridization with antisense cRNA probes to each *Per* subtype. It was found that the two oscillators underlying the split condition correspond to the left and right sides of the SCN (Fig. 3).

Rats exposed to an artificially short 22-hour (22h) LD cycle express two stable motor activity rhythms with different periodic lengths in individual animals (de la Iglesia et al, 2004).

They suggested that these two motor activity rhythms reflect the separate activities of two oscillators in the anatomically defined ventrolateral and dorsomedial SCN subdivisions. They also showed the dissociation of slow wave sleep and core body temperature within individual animals exposed to the same 22h LD cycle (Cambras T et al, 2007). Azzi et al., (2014) recently reported that DNA genome-wide methylation profiling revealed global alterations in promoter DNA methylation in the SCN as a mechanism to drive circadian clock plasticity that correlated with 22h LD cyclic environment. Behavioral, transcriptional and DNA methylation changes were reversible after prolonged re-entrainment to 24h LD cycle. Infusion of a methyltransferase inhibitor to the SCN suppressed periodic changes.

3.3 Importance of glucocorticoid level and its rhythm for sleep and neuronal development

Another intriguing experimental paradigm was the ultradian cycle exposure. LeGates et al. exposed mice to an ultradian cycle consisting of 3.5h light and 3.5h dark (T7) (LeGates TA et al, 2012). They showed that T7 aberrant light cycle did not affect the architecture or the total sleep levels in comparison to a 12h:12h light-dark (T24) control cycle. T7 cycle maintained daily a corticosterone rhythm, but the overall levels of corticosterone increased. Despite normal circadian and sleep structures, these animals showed to increase their depression-like behaviors and impaired hippocampal long-term potentiation and learning. A small population of retinal ganglion cells express the photopigment melanopsin (intrinsically photosensitive retinal ganglion cells: ipRGCs) and the cells were ablated in *Opn4*-driven DTA (diphtheria toxin α subunit gene) mice. These mice still retain more than 95% of RGCs and are capable of image formation. The increased depression-like behavior, learning deficits and hippocampal LTP decrement observed in the T7 cycle in wild-type animals were not observed in diphtheria toxin exposed mouse because of specific depletion of melanopsin expressing cells. These results indicate that the negative influence of the aberrant light cycles on behavior requires ipRGCs. It is of note that ipRGCs not only project to SCN, subparaventricular nucleus and ventrolateral

preoptic area to regulate circadian rhythms and sleep, but also to limbic regions such as the lateral habenula and the medial amygdala (Fig. 4). The equivalent marmoset neuronal network regulating circadian rhythm, physiological response, and emotional reaction was presented based on cholinergic and monoaminergic neural substrate histology (Fig.4b).

As circadian rhythm regulation relevant to emotional and cognitive function, glucocorticoid activity oscillated in synchrony with circadian rhythm. Diurnal peaks and troughs are closely coupled with the active and inactive phases of the circadian cycle. Recent studies indicate that glucocorticoids have rapid effects on dendritic spine development and plasticity in the mouse somatosensory cortex (Liston C et al, 2013). On the other hand, prolonged exposure to glucocorticoids causes postsynaptic dendritic spine loss and branch atrophy in diverse cortical and subcortical areas. Using transcranial two-photon microscopy, Liston et al. showed the circadian glucocorticoid oscillations for the formation and maintenance of new spines after motor skill learning, acting in part through a newly identified, non-transcriptional signaling pathway (phosphorylation of LIM kinase-cofilin) (Liston C et al, 2013). On the other hand, chronic glucocorticoid exposure causes spine loss and memory impairment.

3.4 Oscillation of reduction-oxidation states and the rhythms of neuronal activity in SCN

Importance of non-transcriptional regulation on neural activity is also reported in redox state regulation of SCN neurons (Wang et al, 2012). The metabolic rhythm of redox state is thought under the control of clock genes and the glutathionylation (the capability of glutathione (GSH) uptake by the disulfide bond in proteins, that is, oxidized state of the protein) peaked in early night and was lower (reductive state of the protein) in midday in mouse brain slice. Similarly, it oscillates with the cycle of ascorbic acid-dehydroascorbic acid (DIA), an important antioxidant and neuroprotective buffer in the brain. Interestingly, the membrane potential of neurons oscillates high (up-state) in midday and low (down-state) in early night, which is under the regulation of potassium ion-leak channel. The channel activity of the patch-clamped SCN

neuron was directly modulated by DIA or GSH in opposite way, attenuation and activation, respectively. The cross-talk between energetic and neuronal states may bridge cellular metabolisms and systematic physiology.

In this point, the contribution of mitochondrial function to bipolar disorder is another interesting topic (Kasahara et al, 2006). In their report, mice with neuron-specific accumulation of mitochondrial DNA mutations showed mood disorder-like phenotype. The mutant mice exhibited characteristic behavioral phenotypes, a distorted day-night rhythms and a robust periodic activity pattern associated with estrous cycle, which were worsened by tricyclic antidepressant treatment (mania-like behavior and long-lasting irregularity) and improved by lithium, a mood stabilizer.

Our marmoset research using the modulation of lighting condition in early life and long time observation of individual animal behavior over more than one year suggested that the animal reared under the constant lighting (LL condition) in early life showed increasing of alert and aggressive behavior in adulthood compared with the animal reared under the regular day-night rhythm. Considering the neurobiological evidence in these other studies described above, LL condition may induce the "splitting" of SCN function in left and right brain hemisphere (Fig.3) and the modulation of SCN may be "imprinted" in early life through melanopsin-expressing retinal ganglion cells, glucocorticoid level and rhythm regulation, and reduction-oxidation rhythm in neuronal cells. The possible equivalent socio-emotional network in marmoset was also presented (Fig.4b). Further studies are required to understand the neurobiological basis of development of socio-emotional behavior.

The progress in the circadian rhythm research described above opened further possibility to investigate etiology of psychiatric disorders using animal models exposed to various aberrant environmental paradigm. One of the indispensable merits of animal model is the possibility to trace a long-term effect of early photo-experience until adolescence, adult, and

even elderly. This may allow us to understand the epigenetic base of stress vulnerability and various comorbidity of psychiatric disorders over development and aging, eventually leading to prevent a march of psychiatric disorders by modulating environment during a particular developmental stage (Blakemore et al., 2012).

4. Expected supporting tools to seek susceptible periods to disturbances of circadian rhythm for socio-emotional development

Earlier critical learning systems have been well-studied and numerous reported as ‘imprinting’ or susceptible learning systems (Lorenz, 1937). Susceptible period learning is one of the key concepts studied in developmental psychiatry. However, except a small number of reports (Plante et al., 2008, Weinstock, 2010, Evans, 2012, Klump, 2013, Lewis et al., 2014), there are not many reports describing developmental psychiatry with these direct key words about both susceptible period learning systems and circadian rhythms. This may be due to the necessity of longitudinal, patient, various and complex approaches to reveal any evidences. It might be caused by the limitation of rodent models for human neuro-psycho-cognitive function although the rodent studies have supported and will continually have much more potential. Common marmoset models must compensate the further study in psychiatry with application from rodents approach. To find developmental modulation in psychology and its mechanisms, special technologies to detect without psychological stress like actigraphy (Ancoli-Israel et al., 2003), infrared recorders used in the present study (Minematsu., 1991), and analytic tools for solution of complexity (Senoo et al., 2011, Koshiba, 2013a-e, Pantazatos. 2013), which is expected to improve furthermore to design novel diagnosis and treatment.

5. Conclusion

Constant light in early life in socio-emotional development of common marmoset showed increased alert behaviors. The susceptible period might exist earlier than three-five month-old, which we showed two tendencies: firstly, the 'arrhythmic periods (arprd)' of group LL was longer at around one month-old, and secondly, in group LL, less modulation of vertical positioning behavior from context auditory meeting to context auditory and visual meeting with an unfamiliar peer, which was prominently observed in group LD during postnatal-day 100-150. Through these analyses, we reviewed the potential of the visualization of BOUQUET by multivariate analysis with biological factors to understand development of higher-order psychological function.

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Figure Captions

Fig.1.

The photic and behavioral test conditions of each individual and the parametric definitions for assessment of rhythmicity.

a. The detailed lighting conditions of the experimental subjects, LD, LL, and DD. The red- and blue-filled bars represent the period regulated under constant light (LLprd) and dark (DDprd), respectively. The open bar represents the period of light-dark cycle per 12 hours (LDprd).

b and c. Typical example of LD (b) and LL (c) locomotive rhythm. Each rhythm was assessed by the linear regression. The locomotive rhythm was not clear until postnatal day P30 in the case of LD subject (b) and P40 in LL subject (c) and this arrhythmic period (arprd) was marked by a double-headed arrow. Under LL condition (c), the subject showed free running locomotive rhythm and the circadian rhythm changed around P80 over development. We defined P40-P80 as free-running period 1 (rp1) and P80-P120 as free-running period 2 (rp2) (see text in more detail).

Fig.2

Social sensory cues to characterize age-specific behavioral modulation between group LD and LL

- a. Distribution pattern visualized by the ellipse regression according to PCA with variance-covariance matrix. The three developmental stages of each lighting condition (LD and LL) were characterized by the three combinations of sensory cues (grey, blue, and green, see below).
- b. The schemes of behavioral test through three contexts regulated in visual (v), auditory (a) and olfactory (o) social interaction for sixty seconds each. The combination of each sensory cue was color-coded. The similarity of behavior in each context was represented as the percentage of the superimposed ellipse area to that of the initial auditory only context (v-a+o-). The behavior of LD (blue) and LL (red) was significantly different at P100-150 (*, probability value = 0.037, Wilks' lambda).
- c. Age-dependent developmental trajectories of DD, LD and LL represented by arrows with factor loading vectors in the 1st and 2nd PCA components. It resulted in adult LL's characters suggested by two kinds of alert behaviors (e-call and shake).

Fig.3. A hypothesis of the rhythm "splitting" and asymmetric expression of a clock gene in SCN of marmosets triggered by constant 24-hour lighting.

De la Iglasia, et al. showed that male golden hamsters under LL condition for 6-8 weeks were induced "splitting" running wheel rhythm and cyclic expression of SCN *Per1* mRNA levels as left and right-sided circadian oscillation. Like shown in this hypothetical figure of marmoset SCN (MRminiSA, 2.0T-MRI, DS pharma biomedical) according to the brain atlas (Tokuno et al, 2009a-b, Watson CR et al, 2010), LL condition may cause a paired SCN into two oppositely phased oscillators.

Fig. 4. Modulation of corticosteron level and depression-like behavior by an ultradian cycle of lighting: from rodent to marmoset comparatively.

a) An ultradian cycle of lighting for two weeks (3.5h Light, 3.5h Dark, T7) kept normal corticosteron circadian rhythm, but induced higher serum level corticosteron in mice. At the same time, mice spent more time immobile than mice housed in the T24 cycle, and developed sucrose anhedonia and behavioral despair in the forced swim test (La Gates TA et al, 2012). On the other hand, anxiety-like behavior did not develop. Retinal ganglion cells expressing melanopsin (ipRGCs) is responsible for the depression like behavior presumable through direct connectivity with lateral habenula (LHb) and medial amygdala (mAmy). The circuit can be suggested that ipRGCs send neuronal signal to SCN, subparaventricular nucleus (sPVZ) and ventrolateral preoptic area (VLPO) regulating circadian rhythms and sleep.

b) Equivalent neuronal connectivity in the common marmoset regulating circadian rhythms

The molecular regulation of circadian rhythms has been mostly studied with genetically manipulated mouse model. The recent advance of marmoset research (Okano et al, Mashiko et al, Tokuno et al, 2012, 2009a,b), however, this enables us to tackle the neuronal basis of the rhythm regulation and its disorders relevant to psychiatric diseases by using the animal model having more complex neuronal modules and circuits. The marmoset nuclei equivalent with mouse ones regulating circadian rhythms and emotion are presented in these immunohistochemistry images edited from the digital atlas of the common marmoset (http://marmoset-brain.org/main/terms_table.html). The hypothalamus, amygdala, and habenula regions were arranged according to anteria-posterial axis of the atlas. Left-right integrated slices suggest cholinergic (ChAT: choline acetyltransferase, AChE: acetylcholine esterase) and monoaminergic (DAT: dopamine transporter, SERT: serotonin transporter) systems in these comparative circuits consistently, suggesting the regulation of emotional activity by circadian rhythms.

Figure 1

Figure 2

Figure 3

Figure 4