Research Article   **Open Access**

Multivariate Correlation Analysis Suggested High Ubiquinol and Low Ubiquinone in Plasma Promoted Primate's Social Motivation and IR Detected Lower Body Temperature

Yuka Shirakawa[1], Koki Mimura[1], Aya Senoo[1], Kenji Fujii[2], Takao Shimizu[3], Tadafumi Saga[3], Ikuko Tanaka[4], Yoshiko Honda[4], Hironobu Tokuno[4], Setsuo Usui[4], Toru Kodama[4], Wakako Tsugawa[1], Koji Sode[1], Shun Nakamura[1], and Mamiko Koshiba[1*]

[1]Tokyo University of Agriculture and Technology, Tokyo, Japan
[2]Kaneka, Tokyo, Japan
[3]Chino, Tokyo, Japan
[4]Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan

Abstract

Mental problems caused by various kinds of stress induce neurotoxic damage through biological mechanisms like oxidation or metabolic unbalancing. We evaluated the dietary supplementation of antioxidant and nutrition, ubiquinol with milk in normal isolated adult common marmoset (*Callithrix jacchus*) as a preliminary preclinical study. The primates were fed with milk with or without ubiquinol every day for three months and after a two-month-interval, the treatment conditions were alternated. Psycho-physiological state was evaluated by video-recording of social behavior, body temperature detection by a simple IR thermal camera and a blood glucose chip-sensor. Furthermore, social behavior data were information-processed by technology to integrate multiple factors, 'Behavior output analysis for quantification of emotional state translation' abbreviated as BOUQUET, which visualized a statistical partial space where the status of high ubiquinol and low ubiquinone in plasma strongly correlated with high frequency of social approaching behavior and lower body temperatures in a social meeting context. This analysis also suggested that high frequency of face direction to a peer correlated with the high ubiquinol-low ubiquinone and high variation of body temperature. Blood glucose seemed weakly relevant to alert behavior in this multiple correlation. These results imply that unbiquinol supplementation promotes social motivation. Finally, the result that the BOUQUET and the sensor systems revealed the implicit psycho-physiological information suggests its applicability in various toxico-psycho-pathological studies as quantitative manner.

Introduction

Recently, an etiological study of psychiatric disorders has suggested that the stress from complex socio-economical as well as toxico-chemical environment triggers inflammation and oxidative reaction to yield depression through neurotoxic impairment [1]. We need to develop early detection method of mental modulation which could develop severe mental disorder if no appropriate action was taken to prevent its progress. It is, however, still hard problem to quantitatively measure any mental modulation. We have previously developed a visualization method of multiple behavioral parameters correlation by BOUQUET that is abbreviated from 'Behavior output analysis for quantification of emotional state translation' [2,3]. The method is a kind of information processing based on principal component analysis of behavior parameters captured by video-recordings and makes it possible to extract socio-emotional features as a higher order correlation in a novel non-human primate model, common marmoset (*Callithrix jacchus*) [2,3]. The current study of primate psychological model also focused on their social response because any psychiatric diseases or mental problems certainly concern social emotion, cognition and other psychology [4,5]. Through observing the primate's social response, we evaluated effects of antioxidant ubiquinol dietary supplementation in social environment deficit individuals. Oxidative stress has been implied as one of psychiatric pathogen [6] and safe nutrient biomolecule, ubiquinol (reduced form of coenzyme Q10) [7-9] is frequently reported about the neural protection with anti-oxidation [10-12] and anti-inflammatory effect [13], additionally also clinical effect against type 2 diabetes [14]. We therefore challenged to search what was affected by this nutritive support in social behavior with psycho-physiological factors, blood glucose [15,16] and body temperatures [17,18] by a novel system of BOUQUET to objectively diagnose psychological modulation of the multiple kinds of parameters.

Materials and Methods

Animals

The experimental protocol was approved by the Ethics Review Committee for Animal Experiments of Tokyo University of Agriculture and Technology, TUAT (20-21) that follows the animal care and experimental guidelines of Japan Neuroscience Society and NIH in USA. Immediately after birth, marmoset babies were isolated from their parents, and were fed on milk until weaning. They were housed individually in transparent plastic cages (22 × 14 × 14 cm) with paper sheets on the cage floor. Each cage was placed in a light-sealed incubator illuminated by a fluorescent lamp maintaining constant temperature (32 degree Celsius). Approximately 25 days after birth, each animal

was transferred into a stainless-steel grid cage (40 × 27 × 22 cm), and then put into larger cages (55 × 74 × 77 cm) afterward (26-30 degree Celsius, relative humidity 10-60%). After weaning, 40 g of formulated pelleted diet (CMS-11M, CLEA, Japan) with 0.03% of ascorbic acid (Nacali Tesque), 36 IU of palmitic retinol, 180 IU of cholecalciferol, 0.144 mg of tocopherol acetate (Kyoritsu), 1.5% of pure honey (Katoen, Japan) in 15 ml of distilled water (Millipore) were given throughout the remainder of the experiment. Home cage cleaning and feeding was at 9-10 am every day. The subject of Figure 1 was a 4-5 year-old female and the two subjects of Figure 2 were a 1.5-2 year-old female (A) and male

Figure 1: Plasma ubiquinol and ubiquinone at steady states or ubiquinol intake.

The concentrations of plasma ubiquinol (reduced form of coenzyme Q10, blue) or ubiquinone (oxidized form of coenzyme Q10, pink) was measured over 11 weeks in an adult female marmoset at the dose of ubiquinol specified in the figure. The result suggested the correlation between the logarithmic intake and plasma concentrations in both forms of coenzyme Q10 (see text).

Figure 2: A longitudinal cross-over evaluation between ubiquinol or vehicle supplementation visualized the correlation of high ubiquinol – low ubiquinone with social motivation by BOUQEUT.

a. The trajectories of two marmosets, A (female) and B (male) through four times of behavioral tests in a 3D time space

b. The 16 factor loading positive vectors projected from the average center (see more detail in text)

c. The top view of the 3D time space at each time point level with distribution ellipses of A and B

d. The raw data of behavioral and physiological parameters during a longitudinal cross-over ubiquinol test. Behavior test was performed four times and plasma ubiquinol/ubiquinone was measured at 9 time points over 11 weeks. The left panel is for the subject A and the right for the subject B. In the correlation analysis, the plasma data at the behavior test time point was used.

(B). The reference peers for behavioral tests included another female and two other males.

Blood sampling and administration of ubiquinol and vehicle

The tail hair was partially removed and sterilized with 70% of ethanol. Blood (200 micro L) was sampled with a needle and a syringe with 50 micro L of heparin solution in saline (10000 U/ml) from the tail vein before ubiquinol administration every day. The sample was immediately transferred into plasma separation column and centrifuged at 4 degree Celsius, at 15,000 rpm for 10 minutes, and then stored in -80 degree Celsius less than a few months.

The administration of 4, 40 or 400 [mg/kg weight / day] of ubiquinol (Kaneka QH, reduced form of coenzyme Q10) was set during the cleaning and feeding time at 9-10 am. The ubiquinol solution was taken from the −80°C stored stock solution of 40, 400 or 4000 [mg/mL] just before feeding and diluted in 1 mL of formulated milk (Haihai, Wakodo) in vehicle of corn oil (biochemistry experimental grade, Wako). The milk (1 ml) was given to each subject by 1ml plastic syringe (Terumo).

HPLC for ubiquinol and ubiquinone

The concentrations of ubiquinol and ubiquinone in plasma were determined by high-performance liquid chromatography (Alliance 2695 separations module, Waters, USA). To 50 micro L of plasma, 250 micro L of 2-propanol was added and mixed for 10 seconds. After a centrifuge at 10,000 rpm for three minutes, 50 μL of the supernatant liquid was analyzed by HPC-ECD (Nanospace SI-2, Shiseido Co., Ltd., Tokyo, Japan) with a octadecyl silane column (250 × 4.6 mm ODS-A A-303, YMC Co., Ltd., Kyoto, Japan) and reduce column (15 × 4.0 mm, RC-10, Shiseido Co., Ltd., Tokyo, Japan) at 30°C at +600mV. The mobile phase consisted of methanol/hexane (35/65, v/v) and 50 mM of sodium perchlorate at a flow rate of 0.6 ml/mi [9]. Statistical analysis for Figure 1 was performed using Microsoft Excel for linear regression and Pearson's correlation.

Blood glucose sensing

We measured blood glucose utilizing a blood glucose meter, GLUCOCARDARKRAY-Factory, Inc., Shiga, Japan. The meter enabled us to collect small volume of blood (less than 30 μl). We also used less-stress blood sampling technique by softly contacting animal abdomen to caregiver's abdomen while covering animal eyes with soft cloth.

Behavioral test

A subject in a transparent cage was exposed to animals kept in another transparent cage under a series of social context as follows in this order: (i) isolation (a−v−o−) covered fully with a transparent box, separated from the other cage by an opaque board with no conspecific present, (ii) plus acoustic cue (a+v−o−; with other animals present in other cage), (iii) plus visual (a+v+o−; the board was removed), (iv) plus olfactory (a+v+o+; the coverage of the test cage was removed), and finally going back to the first condition (context procedure). The duration per context including loss time was 50 seconds. The (iv) a+v+o+ expected as the most motivated context was analyzed for this study. To keep subjects as unfamiliar as possible, the possibility of meeting the same animal was limited less than ten times. Under fluorescent light in open space, we performed video-recording of an isolated subject (condition (i)) in the cage made of transparent, perforated vinyl chloride plates (29 × 29 (bottom) × 45 (height) cm), at 357–554 lx inside the apparatus.
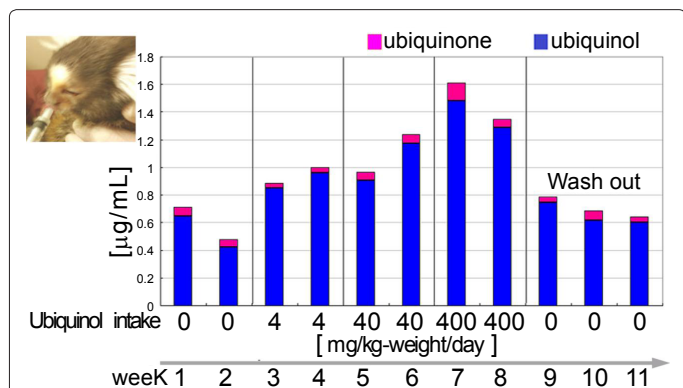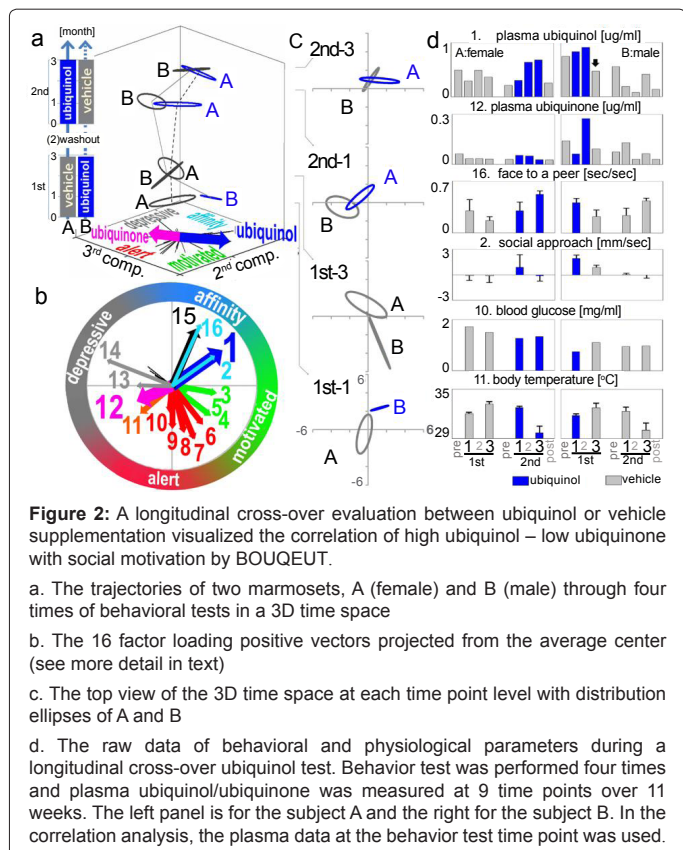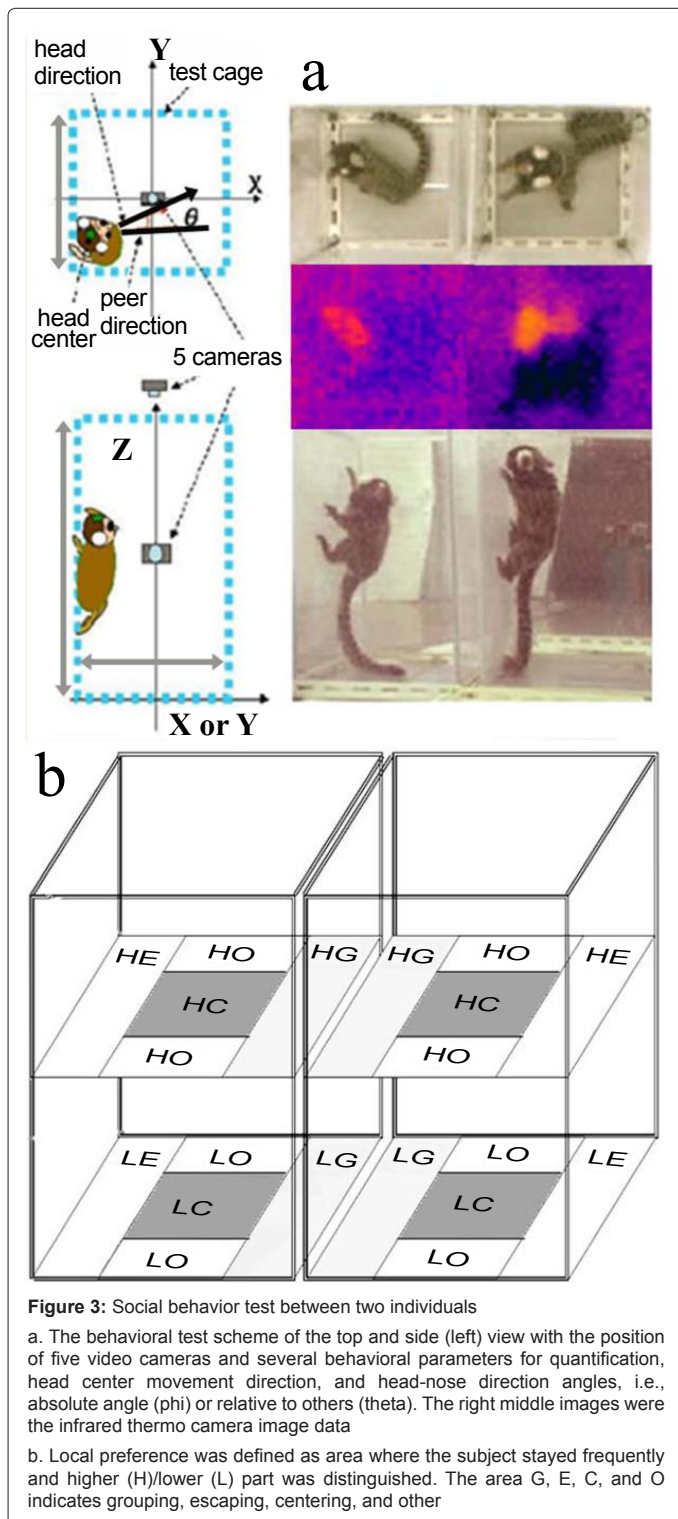
A simple infrared thermo-graphic camera (TP-L0260EN, CHINO, Japan) at the top and five digital video cameras (Qcam Pro 900, Logicool) and HDD video camera (HDR-CX560V, SONY, Japan/ GZ-HM400, victor) recorded the animals from the top and all four sides. Subjects were allowed to move freely within the cage. Vocal orientation assessment required 2 different microphones set to compare each sound wave length and frequency.

Figure 3: Social behavior test between two individuals

a. The behavioral test scheme of the top and side (left) view with the position of five video cameras and several behavioral parameters for quantification, head center movement direction, and head-nose direction angles, i.e., absolute angle (phi) or relative to others (theta). The right middle images were the infrared thermo camera image data

b. Local preference was defined as area where the subject stayed frequently and higher (H)/lower (L) part was distinguished. The area G, E, C, and O indicates grouping, escaping, centering, and other

Abbreviation	Content of parameter for PCA	Unit
Ubiquinol	Olasma ubiquinol	Ug/ml
V	Social approach (velocity to a peer)	Mm/sec
LP-LO	Lp of low-O area	sec/sec
LP-LG	Local preference (LP) of low-G area	sec/sec
Sy-close	Frequency of synchronized approaching	sec/sec
Sp-away	Frequency of synchronized avoidance	sec/sec
e-call	e-call (alert) frequency	sec/sec
ө ave	Head direction to a peer	°
Shake	Shaking (alert) frequency	sec/sec
postBG	Blood glucose after behavioral trest	mg/ml
IR ave	Body temperature measured by infrared camera	°C
Ubiquinol	Plasma ubiquinone	Ug/ml
Ssp-away	Frequency of super-spontaneous avoidance	sec/sec
LP-LC	Lp of low-C area	sec/sec
IR sd	Standard deviation of body tempearature	°C
ө<45°	Frequency of face to peer	sec/sec

Table 1: The behavioral and physiological parameters for PCA used in this study.

Behavioral and infrared image analysis

Body temperature data were automatically computed as the maximum valued spots by our applications integrated with CHINO's and R with our manual compensation partially. According to our previous study, we analyzed multiple parameters by BOUQUET [2,3]. The recorded WMV files of the initial 30 seconds were transferred into WAVE and JPEG files per second using TMPGEnc-4.0XPress software (Pegasys Inc., Tokyo). In the 16 parameters of Table, the x, y coordinate of head centre and forehead position (Figure 3) were sampled and used to calculate horizontal velocity, face horizontal angle, its rotation velocity (delta Theta) and local preference to define social distance (eight equally divided areas, Figure 3b) by Excel (Microsoft, USA). Vocal spectrogram visualized by Syrinx (kindly provided by Dr. John Burt at University of Washington, USA) was used to define 17 call types. The calls were divided into three categories: p-call, purportedly social antiphonal affinity expression but including 'anxious-like' meaning (phee only); e-call, seemingly 'strained' (egg, highegg, bass, high, higheggbass, or strong alert call 'gugaga') and t-call, supposedly 'feeling affinity' (trill, peep, short, short combination, trillphee, twitter, twitterhead, U, tsik, tsik-string. Approach and avoidance behaviors were grouped in four categories, i.e., sp-close, sy-close, sp-away, and sp-away with face orientation angle defined within 180° toward or away from the conspecific. In this report, we used sy-close, sp-away and ssp-away. Sy-close is the synchronized approaching behavior while the conspecific first moved to the area or was already within 30 units of the border of 100 units. Sp away is the spontaneous retreat behavior while the conspecific was within 30 units. Ssp-away (supreme spontaneous-away without viewing peer) is similar to sp-away, with the exception that the starting point was over 30 units distal from the border. Shake is bilateral shifting movement of the upper body, representing typical alert behavior. These video-recorded behavior parameters were sampled at 1 Hz and used as each ratio versus total duration for principal components analysis (PCA).

The representative parameters are shown in Table 1. Total 33 were integrated in Principal Component Analysis (PCA) by correlation matrix (Microsoft Excel and free software R) as the 1st PCA. The longitudinal behavioral development was represented in 3D space (X, Y: the 2nd and 3rd component of the 1st PCA, Z: day-age) using software Origin 7.5 (Origin Pro, USA). We introduced a variance approximation ellipse whose center is the average of the PCA score plots per condition,

and whose long or short axis equals the factor loading vectors extended from the average after the second PCA for the 1st PCA scores by variance–covariance matrix.

Results

Pretest to determine appropriate intake condition for the common marmoset

In order to decide appropriate dose and delivery of ubiquinol for common marmoset, we pretested to give an adult female marmoset ubiquinol with milk at three doses (4, 40 and 400 [mg/kg-weight/day]) continuously for two weeks per each dose and measured the plasma concentration of ubiquinol and ubiquinone once per week. As baseline control we also measured them at two weeks before and three weeks after (washout) the intake duration (Figure 1). The ubiquinol percentages in total plasma coenzyme Q10 were seen higher during intake in the average per condition as (0) 89.6, (4) 96.0, (40) 94.3, (400) 94.0, (0) 92.7 [%], respectively. Comparing to the steady states before intake, the concentration of plasma ubiquinol increased as (4) 1.70, (40) 1.94, (400) 2.60 and (0) 1.23 (times versus steady state), whereas the times of plasma ubiquinone were less as (4) 0.62, (40) 1.03, (400) 1.54, (0) 0.83 (times versus steady state). The summarized ubiquinol intake showed high correlation with logarithmic concentration of plasma ubiquinol by the linear regression and Pearson's correlation as,

(plasma ubiquinol)=$0.4504 \times \log_{10}$ (intake ubiquinol) + 1.3573 (R^2=0.938), and plasma ubiquinone was similar as,

(plasma ubiquinone)=$0.4596 \times \log_{10}$ (intake ubiquinone) + 0.3298 (R^2=0.996).

The plasma ubiquinol showed plateau at the first week of intake of 400. It decreased after stopping intake, precipitously at the first week then followed by gradual decline with reaching the initial concentration. Any marmoset in the current study preferred to drink the milk from syringe. The body weight was not correlated with the intake volume (data not shown). Consequently, we determined the intake does at 4 mg/kgwt duration 3 month and 2 month washing out interval. The dose is equivalent to appropriate dose for human [9].

Parameter correlation structure on the partial space defined by 'high ubiquinol–low ubiquinone'

We examined two isolated adult marmosets, a female and a male with the ubiquinol or vehicle (corn oil) dietary administration in milk for two terms of three months. After a two-month-interval followed the 1st term, we swapped the condition in the 2nd term (Figure 2a, vertical time axis from bottom to top). Plasma was repeatedly sampled from tail vein every month from one month before the 1st term to one month after the 2nd term in total 9 times and measured for uniquinol/ubiquinone (Figure 2d). To evaluate the socio-emotional effect of ubiquinol dietary supplementation, we recorded the subjects' responding behavior to reference marmosets and the body temperature at our designed social meeting set (Figure 3 [2,3]) with measurement of blood glucose four times at 1 and 3 month of both terms. All the analyzed parameters are listed in Table 1 with its code number and the raw data were bar-graphed in Figure 2d. The dependency of plasma ubiquinol on intake was observed, but the plasma ubiquinone less correlated with the intake of ubiquinol (Figure 2d). Blood glucose showed lower level during ubiquinol intake in both subjects.

We then conducted multiple correlation analysis in all the parameters by BOUQUET. The 3-dimension graph of Fig.2a shows the horizontal plane of the 2nd and 3rd PCA scores and the vertical axis of the experimental duration for 8 months. This horizontal plane was selected as the highest correlation of plasma ubiquinol that could be confirmed by the longest factor loading vector (1) (Figure 2b). Interestingly, the plasma ubiquinone vector (12) showed moderately negative correlation with plasma ubiquinol (1). Furthermore, these 16 factor loading vectors were clustering in radial, possibly comprising four kinds of psychological states [2,3], motivated (green) versus depressive (gray) or social affinity (blue) versus alert (red) characterized by the parametric own features. Each distribution of subject A (female) or B (male) plots was approximated by the variance ellipse per behavior test and the ellipse center (average value) was connected by line, yielding behavior state trajectory over the behavior tests (total four times). The top view of each time can be seen in Figure 2c. Any ellipse patterns of ubiquinol intake condition or vehicle emerged closely to the 1st or the 3rd quadrant of this plane coordinate. This computed information to limit the state as high ubiquinol (1) and low ubiquinone (12) in plasma revealed the correlation of high frequency of social approach (2) with plasma ubiquinol in Fig.2b. Furthermore, high face to a peer (16) (socially motivated behavior) moderately correlated with low body temperature (11) and highly with variable nature of body temperature (15).

Discussion

In this study, we showed that marmoset behavior improved in social domain by cross over supplementation of ubiquinol (reduced form of CoQ10) in concomitant with plasma level ubiquinol increment. The importance and significance of this finding is three ways. First, the finding paved the way to introduce common marmoset in toxico-pharmacological study as an animal model in relation to stress induced mental modulation, depression, and other psychiatric disorders. The common marmoset is a small New World primate that, "because of its size, availability, and unique biological characteristics, has attracted considerable attention as a potentially useful animal model in fields such as neuroscience, stem cell research, drug toxicology, immunity and autoimmune diseases, reproductive biology and regenerative medicine" [19,20]. Second, the finding provided the evidence that BOUQUET method was useful to capture subtle behavior change over 11 week ubibuinol supplementation. The method is a multi-variate analysis and an information processing based on principal component analysis of behavior and physiological parameters captured by non-invasive video-camera and infrared thermo-camera recordings. We have previously published the application of this method to marmoset socio-emotional behavior analysis [2,3]. This study is the first report of making use of this method captured by thermo-camera and showed the possibility to extend the use for the combination with other bio-sensing method, which allows to integrate useful physiological parameter for diagnosis and prediction of mental health condition possibly leading to more severe symptom. Lastly, as far as we know, this is the first report on ubiquinol supplementation to marmoset model. In the literature, so far supplementation of ubiquinone, but not ubiquinol has been reported to be effective in mouse model of Alzheimer's Disease [21], Huntington's disease [22], locomotor activity [23], and functional performance in rat [24]. This study introduced ubiquinol to marmoset model. Although the animal number was not enough in this study, we could find a subtle difference of socio-emotional behavior in cross-over supplementation paradigm. Thus, it is expected to extend and apply this method as a general tool for toxicological study.

References

1. Leonard B, Maes M (2012) Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression. Neurosci Biobehav Rev 36: 764-785.

2. Koshiba M, Mimura K, Sugiura Y, Okuya T, Senoo A, et al. (2011) Reading marmoset behavior 'semantics' under particular social context by multi-parameters correlation analysis. Prog Neuropsychopharmacol Biol Psychiatry 35: 1499-1504.

3. Senoo A, Okuya T, Sugiura Y, Mimura K, Honda Y, et al. (2011) Effects of constant daylight exposure during early development on marmoset psychosocial behavior. Prog Neuropsychopharmacol Biol Psychiatry 35: 1493-1498.

4. Cusi AM, Nazarov A, Holshausen K, Macqueen GM, McKinnon MC (2012) Systematic review of the neural basis of social cognition in patients with mood disorders. J Psychiatry Neurosci 37: 154-169.

5. Lieberman MD (2007) Social cognitive neuroscience: a review of core processes. Annu Rev Psychol 58: 259-289.

6. Kulak A, Steullet P, Cabungcal JH, Werge T, Ingason A, et al. (2013) Redox dysregulation in the pathophysiology of schizophrenia and bipolar disorder: insights from animal models. Antioxid Redox Signal 18: 1428-1443.

7. Kitano M, Watanabe D, Oda S, Kubo H, Kishida H, et al. (2008) Subchronic oral toxicity of ubiquinol in rats and dogs. Int J Toxicol 27: 189-215.

8. Kitano M, Mizuhashi F, Kubo H, Kishida H, Fujii K, et al. (2007) Evaluation of the mutagenic and genotoxic potential of ubiquinol. Int J Toxicol 26: 533-544.

9. Hosoe K, Kitano M, Kishida H, Kubo H, Fujii K, et al. (2007) Study on safety and bioavailability of ubiquinol (Kaneka QH) after single and 4-week multiple oral administration to healthy volunteers. Regul Toxicol Pharmacol 47: 19-28.

10. Yan J, Fujii K, Yao J, Kishida H, Hosoe K, et al. (2006) Reduced coenzyme Q10 supplementation decelerates senescence in SAMP1 mice. Exp Gerontol 41: 130-140.

11. Cleren C, Yang L, Lorenzo B, Calingasan NY, Schomer A, et al. (2008) Therapeutic effects of coenzyme Q10 (CoQ10) and reduced CoQ10 in the MPTP model of Parkinsonism. J Neurochem 104: 1613-1621.

12. Isobe C, Abe T, Terayama Y (2009) Increase in the oxidized/total coenzyme Q-10 ratio in the cerebrospinal fluid of Alzheimer's disease patients. Dement Geriatr Cogn Disord 28: 449-454.

13. Schmelzer C, Lorenz G, Rimbach G, Döring F (2009) In Vitro Effects of the Reduced Form of Coenzyme Q(10) on Secretion Levels of TNF-alpha and Chemokines in Response to LPS in the Human Monocytic Cell Line THP-1. J Clin Biochem Nutr 44: 62-66.

14. Mezawa M, Takemoto M, Onishi S, Ishibashi R, Ishikawa T, et al. (2012) The reduced form of coenzyme Q10 improves glycemic control in patients with type 2 diabetes: an open label pilot study. Biofactors 38: 416-421.

15. Beardsley G, Goldstein MG (1993) Psychological factors affecting physical condition. Endocrine disease literature review. Psychosomatics 34: 12-19.

16. Cherubini A, Lowenthal DT, Paran E, Mecocci P, Williams LS, et al. (2010) Hypertension and cognitive function in the elderly. Dis Mon 56: 106-147.

17. Herzog CJ, Czéh B, Corbach S, Wuttke W, Schulte-Herbrüggen O, et al. (2009) Chronic social instability stress in female rats: a potential animal model for female depression. Neuroscience 159: 982-992.

18. Nakamura K (2011) Central circuitries for body temperature regulation and fever. Am J Physiol Regul Integr Comp Physiol 301: R1207-1228.

19. Okano H, Hikishima K, Iriki A, Sasaki E (2012) The common marmoset as a novel animal model system for biomedical and neuroscience research applications. Semin Fetal Neonatal Med 17: 336-340.

20. Mashiko H, Yoshida AC, Kikuchi SS, Niimi K, Takahashi E, et al. (2012) Comparative anatomy of marmoset and mouse cortex from genomic expression. J Neurosci 32: 5039-5053.

21. Dumont M, Kipiani K, Yu F, Wille E, Katz M, et al. (2011) Coenzyme Q10 decreases amyloid pathology and improves behavior in a transgenic mouse model of Alzheimer's disease. J Alzheimers Dis 27: 211-223.

22. Hickey MA, Zhu C, Medvedeva V, Franich NR, Levine MS, et al. (2012) Evidence for behavioral benefits of early dietary supplementation with CoEnzymeQ10 in a slowly progressing mouse model of Huntington's disease. Mol Cell Neurosci 49: 149-157.

23. Xu J, Seo AY, Vorobyeva DA, Carter CS, Anton SD, et al. (2010) Beneficial Effects of a Q-terH Based Nutritional Mixture on Functional Performance, Mitochondrial Function, and Oxidative Stress in Rats. PLoS ONE. 5: e10572.

24. Sinatra DS, Sinatra ST, Heyser CJ (2003) The effects of Coenzyme Q10 on locomotor and behavioral activity in young and aged C57BL/6 mice. Biofactors 18: 283-287.