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Anxiety Behaviour Displayed in C57BL/6J Mice Consuming Coffee and Cocoa, but not Observed in Mice Consuming Japanese Green Tea in a High Fat Diet Induced Obesity Model

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ABSTRACT

It is now acknowledged that certain foods may affect mood and behavior. Recently, the food industry has seen a rise in foods containing beverages such as green tea cake, ice-cream, coffee biscuits as per the traditional chocolate as a food and cocoa beverage. Green tea has been suggested to exert numerous health properties, including anxiolytic properties (Vignes *et. al.* 2006), as does cocoa (i.e. migrane) (Savi *et. al.* 2002). Numerous studies have studied the consumption of these beverages (i.e. cocoa, coffee and green tea) for their anti-oxidant effect but few studies have included them in a feed format. In this study, mice were allotted into a 16 week treatment of a high-fat, high carbohydrate diet of varying treatments including *1*) control diet (21% fat, 36.1% sucrose), *2*) 2% cocoa (21% fat, 36.1% sucrose), *3*) 2% coffee (21% fat, 36.1% sucrose) and *4*) 2% green tea (21% fat, 36.1% sucrose). Subjects were offered the diets in a non-beverage feed format and fresh diet was offered daily. At week 16, the cocoa group mice displayed quicker entry time to dark areas, a greater ($p<0.05$) amount of time spent in the dark box area and higher ($p<0.05$) number of peeking events when compared with the control group. In addition, the coffee group mice displayed a higher ($p<0.05$) re-entries from the light to the dark box area (i.e. anxiety like behavior/ agitation), whereas the green tea group did not display the same level of anxiety like behavior seen in the cocoa and coffee groups. This result may suggest an anxiolytic offset effect against caffeine in green tea versus coffee or another phytochemical component or body compositional changes when consuming a high fat, high sucrose diet.

Key words: Anxiety, Agitation, Behaviour, Cocoa, Coffee, Green tea, Light/ dark box.

INTRODUCTION

More beverages are now being included in food products. Green tea cakes (i.e. mocha cake from Japan, Korea and China), green tea and coffee ice creams, chocolate and coffee cake are notable examples of the use of anti-oxidant containing beverages included in a food format, typically added at 0.5-2% of the food weight. Further, many health-food supplements/ nutraceuticals are being sold promoting the effect of the beverage presented as a dry powder i.e. green tea tablets, green coffee bean or cocoa extract. These extracts are noted for their lipolysis effects. The question to be answered is; are the health effects (i.e. lipolytic, anxiolytic effect) seen in the consumption of these beverages the same as eating them? A diet high in saturated fats has been linked to numerous physiological health concerns, including obesity and type II diabetes (Bjermo *et. al.* 2012), but also depression

and anxiety, where consumption of a vegetarian diet may improve mood (Beezhold & Johnston 2012). The effect of consumption of different beverages on behaviour (i.e. anxiety, depression) has also been documented to be both beneficial and adverse (i.e. inclusion of green tea; anxiolytic properties) (Vignes *et. al.* 2006) and cocoa drink; migrane/ headaches (Savi *et. al.* 2002). During this study, the effect of a high-fat (i.e. butter fat) diet and certain beverages containing antioxidants were added to a diet and fed to mice that have a pre-dependency to obesity. Obesity has been linked to mood and anxiety disorders possibly through the increase in protein oxidation in the frontal cortex (Souza *et. al.* 2007). Further, weight loss is associated with positive changes in psychological well-being (Swencionis *et. al.* 2013), which may be personal perception or related to a physiological function (i.e. higher lean mass) or direct effect dietary phytochemical components (i.e. GABA(A) receptors and EGCG found in green tea). It has been observed that anti-oxidants (i.e. chlorogenic acid, catechins) may also have anxiolytic effects (Bouayed *et. al.* 2007). Moreover, L-theanine found in green tea has been observed to have relaxing effects in humans experiencing anticipated anxiety, but not a direct anxiolytic effect (Lu *et. al.* 2004). Moreover, it is also possible higher lean mass may reduce the incidence of anxiety like behaviour as a secondary effect of green tea consumption.

The aim of the study was to observe if feeding green tea and other anti-oxidant containing beverages as wholefoods in a high fat diet changes behaviour, especially fear and anxiety in mice after the confounding factor of obesity was removed.

Methods

Animal Studies

Prior to experimentation, La Trobe University animal ethics committee approval was granted. All experiments were performed with mice of 4 weeks of age (21–22 g). Adult C57BL/6J mice were used as their phenotype is obesity prone (Alexander 2006). The mice were n = 48 mice divided equally into four dietary treatment groups. The mouse were housed in Perspex boxes measuring (0.7m x 0.25m x 0.25m), with water and food recorded and replenished daily. Their weight was determined bi-weekly. At the end of the 18 week experiment, animals were sacrificed via injection of Nembutal (200mg/kg).

Diets

There were four diets composed. The standard diet was a 21% fat, 19% protein, 49% carbohydrate, 0.15% cholesterol semi-pure rodent diet (Specialty Feeds, WA, Australia). The other four diets consisted of the standard diet with the exception that they contained 2% of the following; bournville cocoa (Cadbury, TAS, Australia), coffee (Vittoria, NSW, Australia) and powdered Japanese green tea (Good Young, TAIPEI, Taiwan) in replacement for 2% carbohydrate (i.e. 34.1% instead of 36.1% sucrose used). For a more detailed outline of dietary composition, please see table 1 in appendices.

Light/ Dark Box test for anxiety

At week 15 of the study, the mice were placed in a wooden box with two domains; total; 43cm (h) x 27.2cm (w). The light chamber measured 19.5cm x 27.2cm and was uncovered. On the other hand, the dark chamber measured 9.3cm x 27.2cm and was covered with a lid. The light source was 40cm away from the entrance to the dark area, and emitted a light source of PAR = 4.2 at the mouse (i.e. PAR - Photosynthetic Active Radiation units $\mu mol/m^2/s$). The mice were initially placed inside the light area of the box and were video monitored for 10 minutes whilst in silence in an enclosed room. They were monitored for initial entry into dark area, the number of entries into either section (re-entries) or other behavioural characteristics were recorded.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) software (Version 12; Chicago, IL, USA) was used for statistical analysis. A one-way ANOVA test at a $p<0.05$ and
$P<0.01$ was applied.

RESULTS

The ingestion of 2% cocoa with a high fat, high carbohydrate diet causes anxiety-like behaviour

As it can be seen from figure 1, the cocoa group display increased ($p<0.05$) anxiety behaviour as demonstrated by their total time spent in the dark box and peeking events.

The ingestion of 2% coffee causes agitation and re-entries from light to dark box areas

As displayed in figure 2 the coffee group had increased ($p<0.05$) re-entries from the light to dark area and thus agitation.

The ingestion of 2% green tea did not cause significant increase in peeking nor increased re-entry from light to dark box areas

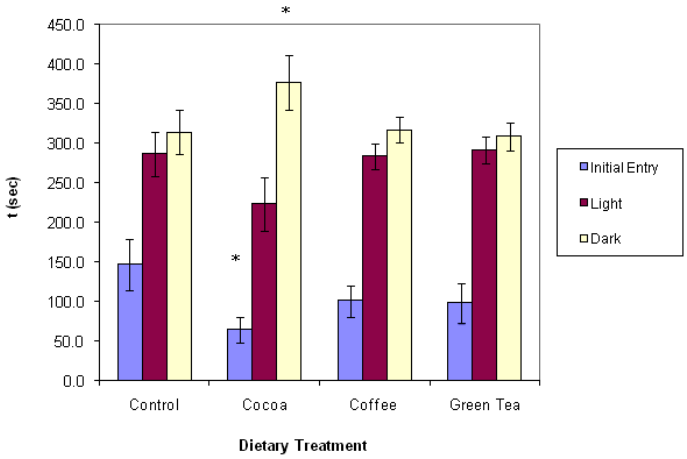
As displayed in figure 2 the green tea group had lower but non-significant peeking events from the light to dark area when compared to the coffee and cocoa groups and thus displayed non-significant reduced anxiety-like behaviour.

The ingestion of 2% green tea reduces body weight, adiposity and increase lean mass

As displayed in figure 3, consumption of 2% green tea in a high fat diet results in increased lean mass and reduced body weight and adipose tissue.

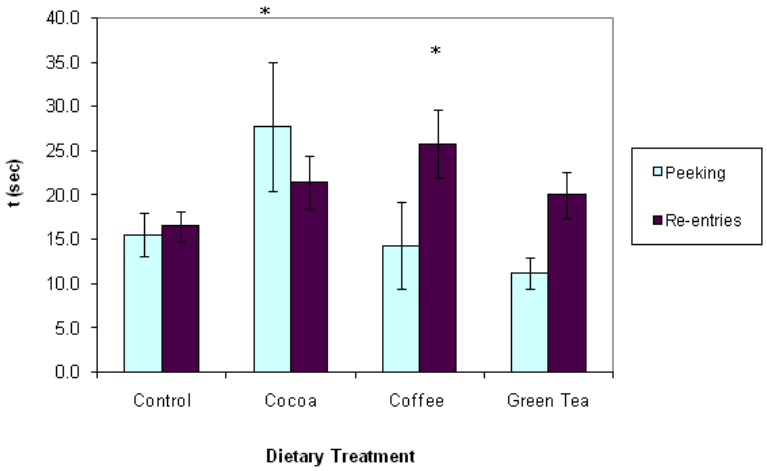
DISCUSSION

Mice who display fear/ anxiety migrate towards darker rather than lighter areas (Heredia *et. al.* 2013), as evident in the cocoa group, with agitation a symptom of anxiety (i.e. coffee group). It may be probable that the phytochemical caffeine



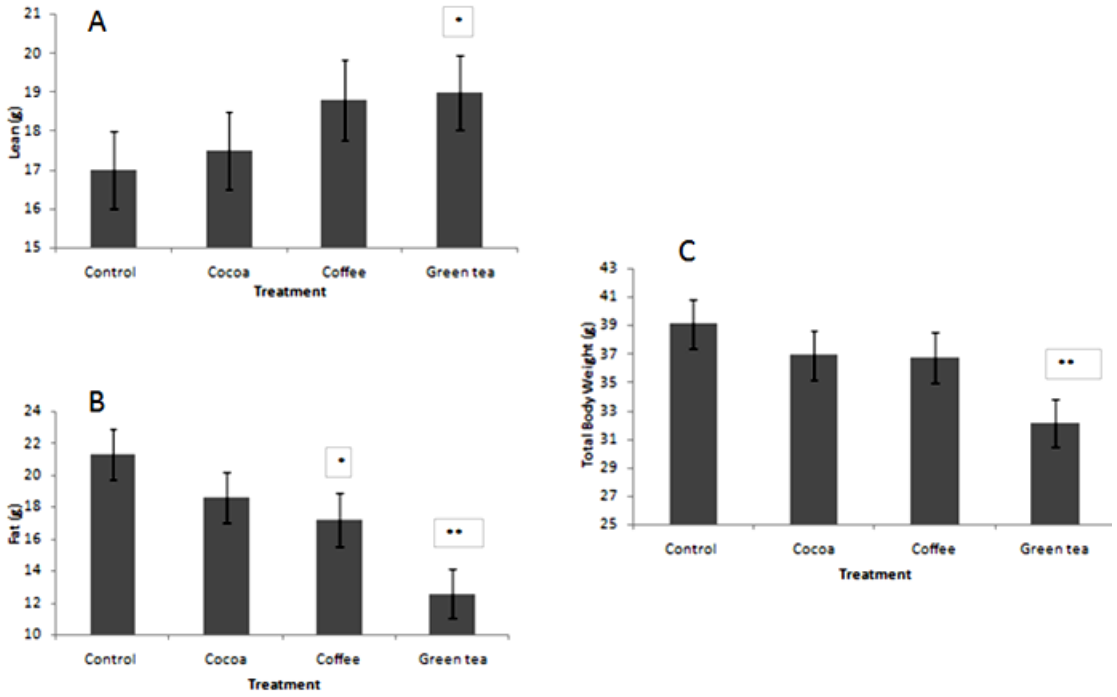
*Treatments versus 'Control' group at a level of $p<0.05$

Fig. 1: Light/ Dark Box test for fear and anxiety – Initial entry, time (seconds) spent in the light and dark area of the light/dark box



*Treatments versus 'Control' group at a level of $p<0.05$

Fig. 2: Light/ Dark Box test for fear and anxiety – Number of peeking events and re-entries



*Treatments versus 'Control' group at a level of $p<0.05$

Fig. 3: Body compositional components as a percentage total body weight. Figure (A) depicts changes in Lean body mass as percentage of body weight and Figure (B) depicts changes in body fat as percentage of body weight

may be responsible for this observation, but the anxiety like behavior was not seen in green tea (i.e. contains caffiene) group with intake on a high fat diet. Different dietary influences give rise to modulation in behavior; as observed in the cocoa consumption (Dallard *et. al.* 2001), who observed anxiety disorder in chronic cocoa users. Phytochemicals in cocoa include theaflavin, epigallocatechin gallate, resveratrol, and procyanidin, of which the former two are also present in green tea as well. This would suggest that another phytochemical in green tea is protecting the mice against a rise in anxiety like behavior. Although, 2% green tea in the diet did not significant reduce anxiety like behavior, there was no difference with the control animals. On the contrary, the present results exemplify a 2% shift in a diet can cause a large shift in behavior, in particular agitation (i.e. significantly high number of re-entries seen in figure 2 in the coffee group), caused by coffee consumption in a high fat and sucrose diet which is symptomatic of anxiety like behavior. The agitation behavior seen in the coffee group as demonstrated by their high number ($p<0.05$) of re-entries may be attributable to the caffeine content of the diet and absence of a regulatory phytochemical that maybe present in green tea. Further, caffeine consumption has been linked to restless leg syndrome/ anxious depressive disorder (Lutz 1978), however, the chlorogenic acid content of coffee may counteract this effect as Bouayed *et. al.* 2007 observed that chlorogenic acid (dose 20 mg/kg) decreases anxiety-related behaviors (i.e. activation of benzodiazepine receptor) and is also beneficial in oxidative stress. The green tea group did not display the same level of light avoidance as the other groups concerned & hence anxiety/ fear was not present in that group (i.e. as per the cocoa group) as they displayed the lowest number of peeking events, and lowest time spent in the light section. Although non-significant, these findings are aligned with Vignes *et. al.* 2006 who showed that EGCG (polyphenol) found in green tea are also ligands for the GABA(A) receptor benzodiazepine

site (i.e. sedative properties), and that EGCG can induce anxiolytic activity, resulting from an interaction with GABA(A) receptors (Vignes *et. al.* 2006), yet this finding was rebuked by (Heese *et. al.* 2009).

Further, there is an observed association between specific genes involved in lower risk assessment behaviour, oxidative stress metabolism, and anxiety-like behaviour/ symptoms (Souza *et. al.* 2007). A series of studies have observed that migraine and headache were attributed to many dietary influences but mainly chocolate (i.e. cocoa, butter fat, sucrose), oral contraceptives, and cheese (Savi *et. al.* 2002; Wöber *et. al.* 2006). Schuman *et. al.* 1987 observed that some individuals who "self-medicating" with chocolate were more likely to have personality traits associated with an atypical depressive syndrome (i.e. hysteroid dysphoria). Further, it has been observed that craving for chocolate produces increased activation of the medial orbito-frontal cortex and ventral striatum (Rolls and McCabe 2007). In major depression, there is reduced volume (i.e. reduced density of neurons and glia) in the 32% smaller medial orbitofrontal (gyrus rectus) cortex (Bremmer *et. al.* 2002), as the OFC appears cytoarchitectonically distinct functionally with respect to mood regulation, as depression severity correlates inversely with physiological activity in parts of the posterior lateral and medial OFC (Drevets 2007).The mechanisms for cocoa to evoke similar psychopharmacologic and behavioral reactions as drug, alcohol addicted individuals may be caused by the content of fat, sugar, texture, and aroma (Bruinsma and Taren 1999). Other researchers suggest that chocolate contains several biologically active constituents (methylxanthines, biogenic amines, caffeine, phenylethylamine, magnesium and cannabinoid-like fatty acids), all of which potentially cause abnormal behaviours and psychological sensations that parallel those of other addictive substances (Bruinsma and Taren 1999; Benton and Donohoe 1999).

Green tea on the other hand, has been shown to exert anxiolytic and cognitive beneficial effects for the elderly in both animal models and humans. In Wistar rats, orally administered 0.5% green tea extract for eight weeks improved learning and memory significantly with an accompanying decline in cerebrum acetylcholinesterase activity of the older rats when compared with young rats (Kaur *et. al.* 2008). Moreover, the anxiolytic effects of L-theanine with midazolam, a chief amino acid component of green tea, but not related to the GABAA receptor in Sprague-Dawley rats (Heese *et. al.* 2009). It may be probable that both L-theanine, unique to green tea is counteracting a significant anxiety like behaviour in the green tea group attributable to caffeine. L-theanine and caffeine have been observed to be responsible for the beneficial effect on cognition and mood mainly via inhibition of blood-pressure increases in high-stress-response adults (Yoto *et. al.* 2012). However, supplementation of epigallocatechin gallate (EGCG) in schizophrenic patients was not able to significantly reduce psychiatric symptoms in a placebo-controlled pilot study (Loftis *et. al.* 2013), suggesting the action of a phytochemical other than EGCG or caffiene. It may also be probable that rather than the sole action of a specific phytochemical, the difference in body composition displayed in the green tea group (higher lean tissue mass) as seen in figure 4, may be responsible for the reduced state of anxiety as per observations by Rosmond and Björntorp 1996 who found a higher use of anxiolytics with women of a higher BMI, especially abdominal obesity. Moreover, in patients with metabolic syndrome it has been revealed that total percentage body fat is associated with increased severity of anxiety and also depressive symptoms (i.e. body composition of higher lean mass reduces incident of anxiety/depressive symptoms) (Guedes *et. al.* 2013).

Lastly, confirmation with physiological markers of stress and anxiety are necessary to support behavioural observations in the mice as cortisol metabolite levels have been observed to be elevated in depression in men, but not related to adiposity (Katz *et. al.* 2000). Further research is required to elaborate on the anxiolytic effect of green tea phytochemical components *in vivo* in humans, that may be counter-acting anxiety behaviour seen in caffeine consumption via consumption of beverages such as cocoa or coffee, or in food products as composite foods. Moreover, the influence of body composition is needed, as it appears a lower body fat percentage and secondly

a lower BMI confer anxiolytic effect as well and the result may be attributable to body compositional changes rather than the action of specific phytochemicals.

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Declaration of Interest Statement

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