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Journal layout design by Victora Carriquiriborde

Letter from the Editors

It is a great honour to present the seventh volume of the Simon Fraser University Undergraduate Journal of Psychology. This journal provides a platform for undergraduate students to submit their work, as well as being an opportunity for them to learn about the steps involved in academic reviewing and publication. This is a learning process not only for the authors, but for the group of editors and managers of the journal. For this reason, we are grateful to have this opportunity to work together with the community and our fellow undergraduate and graduate students. Many psychology students aspire to continue their careers in an academic setting and this journal can provide students a step forward in that direction.

Twenty-twenty has been a very different and challenging year, we want to thank everyone that has made this edition possible. A special thanks to the undergraduate and graduate reviewers for their thoughtful feedback, hard work, and flexibility when adapting to this year's online review format. This journal would cease to exist without their contributions. We would also like to thank the SFU Digital Publishing team that helped us transition into an Open Journal System. We were able to produce something that we are proud of.

Above all, we would like to thank all the authors that submitted their work to the journal. This year we received 20 submissions from Simon Fraser University, the University of Windsor, the University of Ottawa, and University of the Fraser Valley. There were 16 critical review pieces, and four submissions of original research. We are pleased to be publishing four of the 20 initial submissions. We hope is that this was a positive learning experience for all authors, we encourage you to keep growing throughout your academic journey.

Lastly, we want to thank our readers. We hope that you learn something interesting from the work published here and that it may inspire you to pursue your own research.

Seana Semchishen, Sherene Balanji, & Victoria Carriquiriborde

The Managing Editorial Staff

The Effects of Probiotic Treatment During Puberty on LPS-induced Immune Response in Male and Female Mice

ATIQA PIRWANI, EMMA MURRAY, AND NAFISSA ISMAIL

UNIVERSITY OF OTTAWA

Puberty is a critical developmental period that is particularly vulnerable to stress and inflammation. In mice, exposure to an immune challenge (lipopolysaccharide; LPS) during puberty causes enduring effects on depression- and anxiety-like behaviour into adulthood. While the mechanisms underlying these effects remain unknown, the gut microbiome could play a role in mediating the immune system and can alter brain functioning. Thus, we investigated if colonizing the gut with beneficial microbes via probiotics could mediate the inflammatory response to pubertal LPS treatment, in 80 male and female CD1 mice. Sickness behaviour and pro-inflammatory cytokine mRNA expression via RT-qPCR were examined. LPS treatment increased sickness and inflammation in all mice. However, LPS-treated males showed more sickness behaviour, but less central cytokine mRNA expression compared to females and their control saline-treated counterparts. These effects were eliminated when the mice were treated with probiotics. In females, probiotic treatment reduced sickness behaviour, in a time-specific manner, and reduced cytokine mRNA expression in a region-specific manner following LPS treatment. Our results show that probiotics mitigate the LPS-induced immune response differently between males and females. These findings suggest that probiotics have a protective effect during puberty and may prevent the onset of mental health conditions like depression and anxiety.

Keywords: Stress, Kefir, Inflammation, Sickness Behaviour, RT-qPCR, Brain and Behaviour

Puberty: A critical period of development

Puberty is a developmental period that marks the transition from a non-reproductive state to a reproductive state, resulting in sexual maturity (Sisk & Foster, 2004). During this period, there is also rapid brain remodeling and reorganization (Levitt, 2003). These rapid and complex changes within the central nervous system (CNS) render puberty particularly vulnerable to exposure to stress and immune challenges (Holder & Blaustein, 2014; Kane & Ismail, 2017). More specifically, this exposure can have long-lasting effects on physical and psychological aspects of health, including increased susceptibility to mental illness, such as depression and anxiety (Queen et al., 2016; Holder & Blaustein, 2014). In rodent models, exposure to a variety of stressors, such as heat (Paris et al., 1973), immobilization (Paris et al., 1973) or shipping stress (Laroche et al., 2009) during the pubertal period results in long-term negative effects on reproductive capacity into adulthood. Moreover, social instability stress during adolescence increases anxiety-like behaviour and decreases social interaction in adult male rats (Green et al., 2012). Exposure to an immune challenge during puberty, like lipopolysaccharide (LPS), causes enduring reproductive effects such as reduced sexual receptivity and behavioural responsiveness to hormonal treatments in adulthood (Laroche et al., 2009). LPS may also influence non-reproductive effects including depression- (Ismail & Blaustein, 2013), anxiety- (Olesen et al., 2011), and Parkinson-like behaviour (Girard-Joyal & Ismail, 2017), as well as cognitive function in mice (Ismail & Blaustein, 2013). Exposure to LPS in female mice during puberty alters the behavioural response to ovarian hormones that would normally reduce anxiety-like and depression-like behaviour into adulthood (Olesen et al., 2011). These enduring effects of LPS are limited to the stress-sensitive pubertal period at 6 weeks of age in mice, as exposure to an immune challenge at ages younger or older than 6 weeks do not result in these enduring behavioural alterations.

Disruption of the Immune System via an Immune Challenge

LPS is a constituent of the outer membrane of gram-negative bacteria, which elicits an instant immune response that can be measured at both the molecular and behavioural levels (Kentner & Pittman, 2010; Kolmogorova et al., 2017). Molecularly, there are two types of cytokines that are produced in response to LPS: pro-and anti-inflammatory cytokines. Pro-inflammatory cytokines promote inflammation and sickness behaviour (IL-1β, IL-6 and TNFα), while anti-inflammatory cytokines (IL-4, IL-10) limit inflammation and sickness behaviour (Vilcek, 1998; Bluthé et al., 1999; Leon et al., 1999). Additionally, recent studies have shown that there are sex and age differences in the immune response to infections. Males display greater sickness behaviour at 30 minutes after LPS treatment in comparison to their female counterparts (Cai et al., 2016). Males also display higher levels of pro-inflammatory cytokines (TNFα, IL-1β, and IL-6) at 2 hours following LPS treatment in comparison to females (Sharma et al., 2018). However, adult female mice display the greatest increase in corticosterone (CORT) levels two hours following LPS treatment (Girard-Joyal et al., 2015). Age differences were also observed; pubertal mice seem to be more responsive when exposed to an acute stressor (physical or psychological), resulting in a longer hormonal stress response compared to adults (Goldman et al., 1973; Romeo et al., 2004; Vazquez & Akil, 1993). Age differences that occur in the stress response can be a result of the different levels of circulating gonadal hormones during puberty and adulthood. This is because gonadal hormones influence the peak and recovery time of the hormonal stress response in males and females, which could cause an overall effect on the stress response (Carey et al., 1995; Handa et al., 1994; McCormick et al., 1998, 2002; Redei et al., 1994; Viau, 2002; Viau & Meaney, 1991; Young et al., 2001). These sex differences in LPS effects may also be attributed in part to the prominent changes in circulating sex steroid hormones, which increase during puberty and influence the immune system. Taken together, there are important age and sex differences that influence the corresponding immune response. The gut microbiome may also play a role in influencing the immune system; however, the mechanism remains uninvestigated.

The Gut Microbiome

The gut microbiome is an important system that has the ability to influence the immune system and inflammatory responses (Rea et al., 2016; Dinan and Cryan, 2013; El Aidy et al., 2014; El Aidy et al., 2015; Moloney et al., 2014; Sampson and Mazmanian, 2015). In more recent years, studies suggest that our gut microbiota can influence central nervous system functioning, which can impact emotional among other kinds of behaviour(Kennedy et al., 2016; Fung et al., 2017; Tillisch et al., 2013; Savignac et al., 2014; Cryan & Dinan, 2012; Bravo et al., 2011). This is due to a bidirectional communication between the brain and the gut (Foster & Neufeld, 2013), commonly referred to as the gut-brain axis (Cryan & Dinan, 2012). It has been theorized that the intestinal bacteria may be a direct contributing factor to our mental health (Schmidt, 2015; Ng et al., 2018), and disruption of the gut-brain axis has been linked with the development of physical and neurological disorders (Ng et al., 2018; Kennedy et al., 2016; Bailey & Cryan, 2017). Studies conducted in germ-free (GF) mice lacking gut microbiota have provided support for the link between microbiota, brain chemistry, and mental health. Compared to normal mice, GF mice display a hyper-reactive HPA axis (Sudo et al., 2004) and increased anxiety-like (Heijtz et al., 2011; Neufeld et al., 2010) and depression-like behaviours (Naseribafrouei et al., 2014; Dinan & Cryan, 2013). However, microbiota colonization decreases anxiety-like behaviour and improves motor activity in GF mice (Heijtz et al., 2011). Overall, gut microorganisms strongly influence the immune system and CNS functioning. One emerging potential therapeutic agent for stress-related GI problems is probiotics.

Probiotics

Probiotics are living microorganisms that can be found in dietary supplements and food products and when ingested in sufficient amounts, provide health benefits to the host (Joint Food and Agriculture Organization/World Health Organization, 2001; Foster & Neufeld, 2013). The immune system can be influenced by probiotics resulting in limiting the production of pro-inflammatory cytokine and inflammation, which can therefore affect the endocrine and nervous systems (Desbonnet et al., 2008; Desbonnet et al., 2010). Thus, probiotics have anti-inflammatory and immune-regulatory properties and are suggested to also improve brain health through the mediation of the immune response (Kennedy et al., 2016). Recent research has also found that probiotics may influence the gut microbiota in neurological and psychiatric disorders (Fung et al., 2017). In naïve rats, the administration of the probiotic *Bifidobacteria infantis* results in a decrease in proinflammatory cytokines (IFN-γ, TNF-α, IL-6) in the blood in response to the forced swim test, a behaviour test used to induce a stress response and examine depression-like behavior (Desbonnet et al., 2008). Another study administered *L. plantarum* PS128 for 28 days to ELS mice and naïve mice. The researchers found that the probiotic decreased pro-inflammatory cytokines (TNF-α, IL-6) and increased anti-inflammatory cytokine (IL-10). In terms of CNS function, locomotor activity and anxiety were tested by open field test and depression was tested by the forced-swim test. Treatment of the probiotic resulted in an increase in locomotor activity, a decrease in anxiety-like behaviour in naïve mice, and a decrease in depression-like behaviour in ELS mice as well (Liu et al., 2016).

The objective of this study is to examine sex differences in the response to probiotic treatment during puberty on LPS-induced immune response by examining sickness behaviour and concentrations of pro-inflammatory cytokines in three different brain regions. LPS is expected to induce a strong immune response, which will be examined by monitoring sickness behaviour and measuring cytokine concentration and expression in pubertal male and female mice. Probiotics in this study are expected to mitigate LPS-induced immune response. Therefore, we hypothesized that mice exposed to probiotics would display less sickness behaviour and cytokine expression, in both mice that were given the immune compromising LPS and those given a control saline solution. Given that previous studies have found that there is a sex difference in response to an immune challenge, we hypothesized that male mice would display greater sickness behaviour, cytokine concentration, and expression compared to females following LPS treatment.

Materials and Methods

Animals

Forty male and forty female CD1 mice were obtained from Charles River Laboratories (St-Constant, Quebec) at three weeks of age. The mice were housed in pairs in polycarbonate Lexan cages (dimensions of 17 x 28 x 12 cm). Mice had *ad libitum* access to food, kefir or control skim milk. Water was not available during the probiotic or control treatment. Feeding bottles were weighed daily to record liquid consumption. There was no difference between kefir and control skim milk intake. Male and female mice were housed in separate rooms; that were maintained on a 14 h light/ 10 h dark cycle (lights off at 10:00am), a constant temperature of 24°C (±2 °C), and 45% humidity. A gradual induction of dusk and dawn was established over 1 h. The Animal Care Committee of the University of Ottawa approved all experimental procedures.

Probiotic treatment

Powdered kefir culture (provided by Lyo San Inc., Lachute, QC) with a lactic acid bacteria concentration of $3.0x10^9$ CFU/g was stored at -20°C. The probiotic kefir was prepared in accordance with Lyo San Inc, by mixing 5g of dry kefir culture in 1L of skim milk. The mixture was kept in an airtight container to inoculate at room temperature 23°C (±2 °C) for 24 hrs prior to being refrigerated at 4°C for a minimum of 8 hrs to end the reaction. A new batch of kefir mixture was prepared three times per week. Every 24 hrs, the treatment bottles were weighed and replaced with pre-weighed bottles. The feeding bottles with kefir mixture were vortexed twice a day in order to prevent clumping and maintain a liquid consistency. Additionally, the feeding bottles for the control group were also checked to maintain consistency. Forty mice (20 males:20 females) received the probiotic kefir and forty (20 males: 20 females) mice received skim milk as a control treatment.

Lipopolysaccharide (LPS) treatment

LPS (from Escherichia coli serotype O26:B6; No. L3755; Sigma- Aldrich Canada, Oakville, ON) was diluted in sterile saline (0.2 mg/ml). LPS was injected intraperitoneally at a dose of 1.5 mg/kg at 6 weeks of age. This dose of LPS treatment has been found to cause mild sickness that only lasts up to 48 hrs (Cai et al., 2016; Girard-Joyal et al., 2015; Ismail & Blaustein, 2013).

Treatments	Males (N=20)	Females (N=20)
Kefir		
LPS	10	10
Saline	10	10
Milk		
LPS	10	10
Saline	10	10

Groups (N=40)

Table 1. Experimental groups.

Sickness monitoring

Sickness behaviour was examined by observing the occurrence of four symptoms; huddling, piloerection, ptosis, and lethargy, as previous studies have found these symptoms to be indicative of sickness as previously described by Kolmogorova et al., (2017) at 30 min, 4 and 8 hrs following LPS or sterile saline treatment in our mice. Two observers, who were blind to treatment conditions, assessed the mice independently using a non-invasive and unbiased approach, as described in Kolmogorova et al., (2017). Each observer assigned a mouse with a sickness score ranging from 0 (no symptoms) to 4 (all four sickness behaviours observed). Sickness checks concluded at 8 hrs, when mice were euthanized.

Euthanasia and tissue collection

Mice were euthanized at 8 hrs following saline or LPS treatment with an intraperitoneal injection of Euthanyl (pentobarbital) (prepared from Euthansol; Merck Animal Intervet Canada Corp; Kirckland, Quebec). Brains were extracted and frozen using liquid nitrogen and stored in aluminium foil in -80°C for further cytokine analysis. Brain samples were later sliced and dissected to collect the prefrontal cortex (PFC), the hypothalamus and the hippocampus following the schematics from *The Mouse Brain Atlas in Stereotaxic Coordinates* (Franklin & Paxinos, 1997).

Real-time qPCR

Messenger RNA (mRNA) was extracted from fresh frozen brain tissue using Isol-RNA lysis Reagent (Cat. No. 2302700, Fisher Scientific). Extracted RNA was exposed to DNAse to remove any genomic DNA prior to cDNA synthesis using the QuantiTect Reverse Transcription kit (Cat. No. 205311, Qiagen). cDNA aliquots were obtained from the extraction to be used in the following qPCR reactions. Relative gene expression was measured using the RealMasterMix Fast SYBR kit (Cat. No. 1725201, Bio-Rad) in 10 µL reactions on a CFX96TOUCH real time PCR machine. All primers were ordered through Integrated DNA Technologies. The efficiency of the primers was determined using the slope of the relation between RNA quantity and cycle thresholds (CT) using Bio-Rad software. All primer pairs in this experiment achieved reaction efficiencies between 90% and 110%. All primers were diluted to a final concentration of 0.3 µM for the real-time PCR reaction. The sequences for the primers were as follows:

Target Gene	Forward	Reverse
β-actin	GAACCCTAAG GCCAACCGTG	GGTACGACCAG AGGCATACAGG
IL-1β	TCTTGGGACT GATGCTGGTG	CAGAATTGCCAT TGCACA ACTC
TNFα	GCCTATGTCTC AGCCTCTTCTC	GCCATTTGGGA ACTTCTCATCC
IL-6	GCCTTCTTGG GACTGATGCT	GCCATTGCACA ACTCTTTTCTC

Table 2. Summary of Primer sequences.

β-actin is the housekeeping gene and was not significantly different amongst experimental groups; therefore, it was used as a reference for all samples. For each reaction, the quantitative threshold amplification cycle number (Cq) was determined, and the 2−ΔΔCq method was used to calculate the relative gene expression of each gene in question.

Statistical analysis

Sickness behaviour and cytokine measures were imported into IBM SPSS Statistics (version 22) for three-way analysis of variance (ANOVA) to examine the effects of sex (males or females), treatment (saline or LPS) and probiotic (kefir or milk). This was followed by pairwise comparisons using the Bonferroni correction, when appropriate. For all tests, the criterion for statistical significance was set to $p < 0.05$.

Results

Sickness behavior

LPS treatment induced sickness behavior in all mice (Fig. 1 and 2). Three-way mixed ANOVA revealed main effects of sex ($F_{(1,65)}=17.59$, $p < 0.01$, $\eta_p^2= 0.213$), LPS treatment ($F_{(1,65)}=2778.713$, $p < 0.01$, $\eta_p^2= 0.977$), and a sex × LPS treatment interaction ($F_{(1,65)}=15.57$, $p < 0.01$, $\eta_p^2= 0.193$). Pairwise comparisons revealed that all LPS-treated males displayed more sickness behavior than female counterparts at 30 min (mean difference; MD = 0.938, standard error; SE = 0.307, $p = 0.03$; MD=1.132, SE = 0.307, $p < 0.01$, respectively) and 4 h (MD = 0.472, SE = 0.141, $p < 0.05$; MD = 0.333, SE = 0.141, $p = 0.021$, respectively), regardless of probiotic treatment. LPS-treated males exposed to kefir showed more sickness behaviour at 2 hrs (MD = 1.063, SE = 0.273, $p < 0.01$), and 6 hrs (MD = 0.549, SE = 0.119, $p < 0.01$) compared to their female counterparts. LPS-treated females exposed to kefir showed significantly more sickness symptoms at 30 min (MD = 0.688, SE = 0.307, $p = 0.028$) but less symptoms at 6 hrs (MD = 0.486, SE = 0.119, $p < 0.01$) compared to LPS-treated females exposed to milk control condition.

Figure 1. Mean (±SEM) sickness score in 6-week-old male (n = 40) mice treated with saline or LPS and exposed to probiotics or milk control.

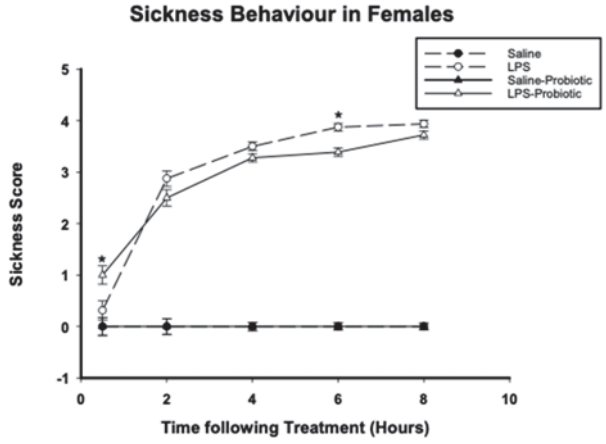

Figure 2. Mean (±SEM) sickness score in 6-week-old female (n = 40) mice treated with saline or LPS and exposed to probiotic or milk control. The asterisks (*) denote significant treatment differences between probiotics and the milk control. ($p < 0.05$) at specified time points.

Pro- Inflammatory Cytokines mRNA Expression in the Hypothalamus, Hippocampus and Prefrontal Cortex Following LPS Treatment

Interleukin-1 Beta (IL-1β) expression. In the hypothalamus, three-way ANOVA revealed main effects of sex ($F_{(1,34)}$=5.281, p = 0.028, η_p^2= 0.134) and LPS treatment ($F_{(1,34)}$=13.62, $p < 0.05$, η_p^2= 0.286) and a significant sex × LPS treatment interaction ($F_{(1,34)}$=5.35, p =0.027, η_p^2= 0.136) on IL-1β mRNA expression. Pairwise comparisons revealed that LPS-treated females displayed more IL-1β mRNA expression compared to their male counterparts in both the milk control (mean difference; MD = 15.726, standard error; SE = 6.863, p = 0.020) and kefir (MD = 15.726, SE = 6.863, p = 0.028) conditions. Additionally, LPS-treated females showed more IL-1β mRNA expression in the hypothalamus compared to saline controls, in both the kefir (MD = 22.97, SE = 6.571, $p <$ 0.05) and milk (MD = 17.36, SE = 6.863, p = 0.016) conditions.

A three-way ANOVA also found a main effect of LPS treatment ($F_{(1,35)}$=10.17, p =0.003, η_p^2= 0.225) on IL-1β mRNA expression in the hippocampus. Pairwise comparison showed that LPS-treated females display greater IL-1β mRNA expression than saline-treated counterparts, regardless of kefir (MD=36.13, SE=12.04, p = 0.005) and milk (MD = 27.39, SE = 12.63, p = 0.037) treatment.

In the prefrontal cortex (PFC), a three-way ANOVA displayed a main effect of LPS treatment ($F_{(1,35)}$=17.54, $p < 0.01$, η_p^2= 0.334) and a significant sex × LPS treatment interaction ($F_{(1,35)}$=9.25, p =0.004, η_p^2= 0.209) in IL-1β mRNA expression. Pairwise comparison showed that within the PFC LPS-treated females exposed to the milk control showed more IL-1β mRNA expression compared to their male counterparts (MD = 34.22, SE = 7.51, $p < 0.01$). This sex difference is absent in mice exposed to the kefir. LPS-injected females that were exposed to kefir showed less IL-1β mRNA expression (MD = 23.22, SE = 7.51, p =0.004) compared to milk controls. LPS-treated females showed more IL-1β mRNA expression compared to saline-treated controls (MD = 37.87, SE = 7.51, $p < 0.01$) in the milk condition only. This treatment difference is absent in mice exposed to kefir.

Tumor necrosis factor alpha (TNFα) expression. Within the hypothalamus, three-way ANOVA revealed main effects of sex ($F_{(1,38)}$ = 8.339, p = 0.006, η_p^2 = 0.180) and LPS treatment ($F_{(1,38)}$ = 25.74, $p < 0.01$, η_p^2 = 0.404) and a significant sex × LPS treatment interaction ($F_{(1,38)}$ = 6.218, p = 0.017, η_p^2 = 0.141) on TNFα mRNA expression. Pairwise

comparisons showed that LPS-treated females exposed to kefir displayed greater TNFα mRNA expression compared to their male counterparts (MD = 5.188, SE = 1.442, p = 0.01). In mice exposed to the milk control, LPS-treated males show more TNFα mRNA expression compared to saline-treated controls (MD = 2.930, SE = 1.374, p = 0.040). This treatment difference is absent in mice exposed to kefir. In both the kefir (MD = 5.951, SE = 1.442, p < 0.01) and milk (MD = 4.706, SE = 1.442, p = 0.002) conditions LPS-treated females show more TNFα mRNA expression compared to saline treated controls.

A three-way ANOVA also found a main effect of LPS treatment ($F_{(1,36)}$=12.37, p < 0.05, η_p^2= 0.256) and a significant probiotic treatment × LPS treatment interaction in TNFα mRNA expression in the hippocampus ($F_{(1,34)}$=6.467, p =0.015, η_p^2= 0.152). Pairwise comparison showed that LPS-treated females exposed to kefir show less TNFα mRNA expression (MD = 2.396, SE = 0.941, p = 0.015) compared to milk controls. Both LPS-treated females and males exposed to milk control displayed more TNFα mRNA expression compared to saline-treated counterparts (MD = 3.317, SE = 0.901, p < 0.05; MD = 2.143, SE = 0.859, p = 0.017, respectively). This effect of LPS treatment on TNFα mRNA expression in the hippocampus was absent in males and females exposed to kefir.

Within the PFC, a three-way ANOVA found main effects of sex ($F_{(1,34)}$=5.913, p =0.020, η_p^2= 0.148) and LPS treatment ($F_{(1,34)}$=11.639, p =0.002, η_p^2= 0.255) and a significant sex × LPS treatment interaction ($F_{(1,34)}$=7.028, p = 0.012, η_p^2= 0.171) for TNFα mRNA expression. Pairwise comparisons showed that LPS-treated females exposed to the milk control display more TNFα mRNA expression in the PFC compared to their male counterparts (MD = 13.580, SE = 4.011, p = 0.002). Moreover, LPS-treated females exposed to milk control also showed more TNFα mRNA expression in the PFC compared to saline-treated counterparts (MD = 16.684, SE = 4.011, p < 0.01). However, LPS-treated females exposed to kefir show less TNFα mRNA expression in the PFC compared to counterparts exposed to milk control (MD = 9.546, SE = 4.011, p = 0.023).

Interleukin-6 (IL-6) expression. In the hypothalamus, three-way ANOVA revealed a main effect of LPS treatment on IL-6 mRNA expression in the hypothalamus ($F_{(1,38)}$=16.92, p < 0.01, η_p^2= 0.308). Pairwise comparisons revealed that, in both kefir (MD = 6.334, SE = 2.73, p = 0.026) and milk conditions (MD = 8.733, SE = 2.61, p = 0.002) LPS-treated females showed more IL-6 mRNA expression compared to their saline-treated counterparts. LPS- treated males in the milk condition show more IL-6 mRNA expression (MD = 5.26, SE = 2.605, p = 0.051) to saline controls. This LPS-induced increase in IL-6 mRNA cytokine expression was eliminated with probiotic treatment. A three-way ANOVA also found a main effect of LPS treatment on IL-6 mRNA expression ($F_{(1,34)}$=15.49, p < 0.01, η_p^2= 0.313), in the hippocampus. Pairwise comparison revealed that in mice treated with kefir, LPS-injected females showed more IL-6 mRNA expression compared to their male counterparts (MD = 7.615, SE = 3.21, p = 0.023). Regardless of the probiotic treatment, LPS-treated females in the kefir (MD = 7.744, SE = 3.21, p = 0.021) and milk (MD = 8.859, SE = 3.21, p = 0.009) conditions showed more IL-6 mRNA expression compared to saline-injected controls. LPS-treated males in the milk conditions showed more IL-6 mRNA cytokine expression (MD = 7.744, SE = 3.208, p = 0.027) compared to saline-injected controls. Again, this LPS-induced increase in IL-6 mRNA cytokine expression was eliminated with probiotic treatment in the hippocampus of male mice.

Within the prefrontal cortex, three-way ANOVA found a main effect of LPS treatment on IL-6 mRNA expression ($F_{(1,31)}$=16.41, p < 0.01, η_p^2= 0.346). In mice treated with probiotics, LPS-injected females showed greater IL-6 mRNA expression compared to their male counterparts (MD = 7.908, SE = 3.787, p = 0.045). Pairwise comparison showed that regardless of the probiotic treatment, when treated with LPS females in both the kefir (MD = 13.94, SE = 3.79, p < 0.05) and milk (MD = 9.19, SE = 4.02, p = 0.029) conditions show more IL-6 compared to saline-injected controls.

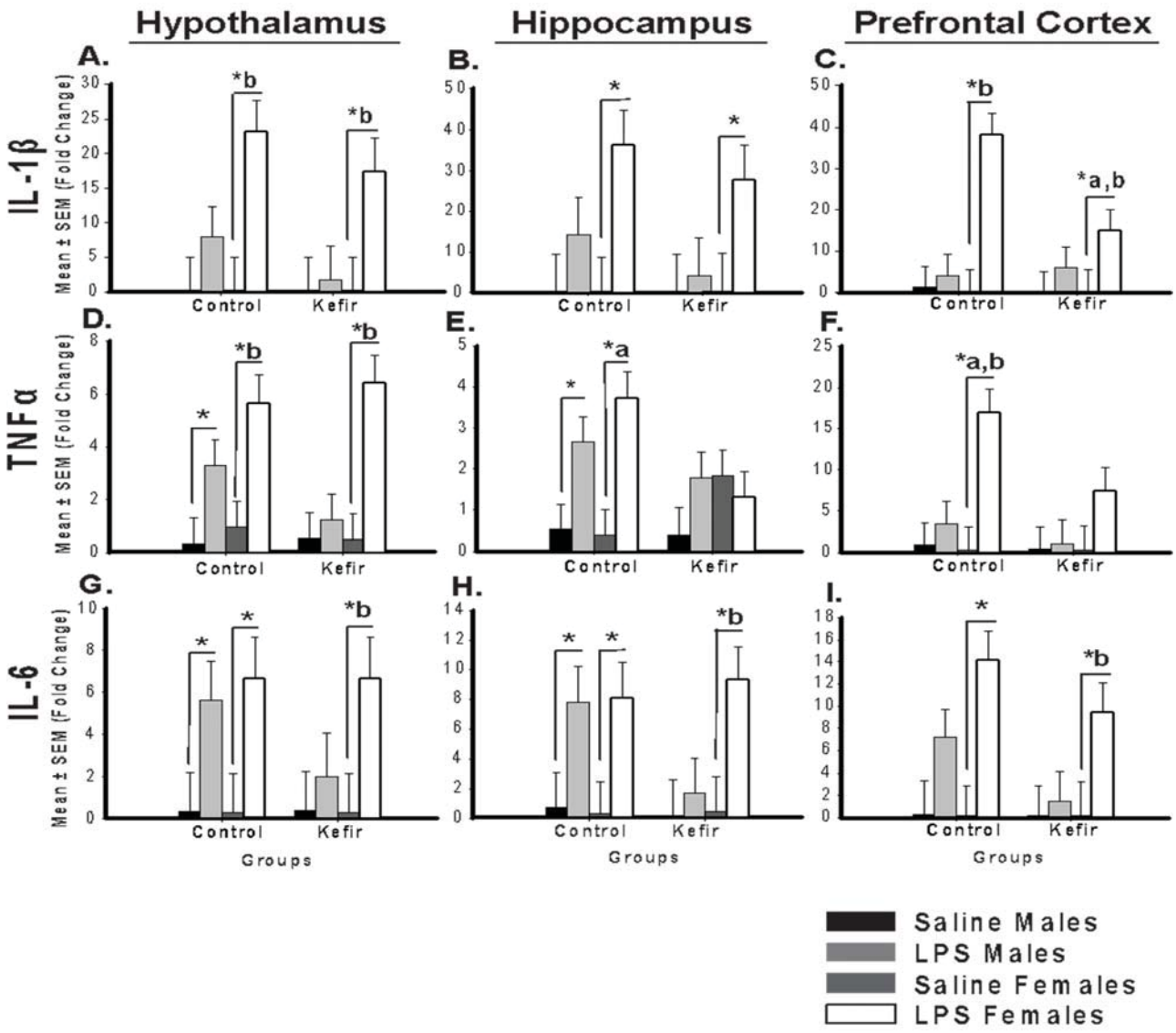

Figure 3. Mean (± SEM) fold change of IL-1β (A, B, C) TNFα (D, E, F) and IL-6 (G, H, I) mRNA expression in the hypothalamus, hippocampus and prefrontal cortex in 6-week-old male and female mice, 8 h following LPS treatment. The asterisk (*) denotes a significant difference between saline or LPS treatment conditions ($p < 0.05$). The (a) denotes a significant difference of probiotic treatment within the same sex. The (b) denotes a significant difference of LPS treatment between sexes.

Discussion

The gut microbiome exerts a strong influence on the immune system. However, the effect of probiotics on the immune response during puberty, a vulnerable period in development, was unknown. The current study examined sex-specific responses to LPS and the possible mitigating properties of probiotic treatment on the immune response. Here, we observed that exposure to LPS during puberty induced an increase in sickness behaviour and pro-inflammatory mRNA expression within the hypothalamus, hippocampus and the prefrontal cortex of both male and female mice. Similar to previous findings (Girard-Joyal et al., 2015; Cai et al., 2016), males displayed greater sickness behaviour compared to their female counterparts in a time-specific manner. Conversely, females displayed greater central mRNA expression of pro-inflammatory cytokines. Probiotic treatment lessened the sickness behavior in a time specific manner

and pro-inflammatory cytokine mRNA expression in a region-specific manner.

Exposure to an immune challenge such as LPS causes a robust immune response that is seen at both physiological and behavioral levels (Bilbo & Schwarz, 2009; Kentner & Pittman, 2010). The severity of LPS-induced sickness behaviours varies over time following infection in a sex-specific manner. Males tend to show greater and prolonged sickness behaviours (Girard-Joyal et al., 2015; Cai et al., 2016). Our results are consistent with previously published work (Cai et al., 2016; Sharma et al., 2018; Murray et al., 2019) and demonstrate that LPS induces greater sickness response in pubertal male mice in comparison to their female counterparts. Male mice showed more sickness at 30 min and at 4 hrs following LPS injection compared to their female counterparts. This sex difference is likely due to gonadal steroid hormones (Foo et al., 2016; Chrousos, 2010; Pittman, 2011). According to Cai et al. (2016), gonadectomized mice displayed significantly more sickness symptoms compared to their sham-operated counterparts 24 hrs following treatment, suggesting that gonadal hormones play a role in decreasing the severity of sickness behavior. Testosterone is a known immune suppressor (Wichmann et al., 1997; Kane & Ismail, 2017; Foo et al., 2016; Alexander and Stimson, 1988; Cutolo et al., 1996; Danel et al., 1983; Roberts et al., 2001; Wunderlich et al., 2002), while estradiol varies in its function. Estradiol can function as an immune suppressor (Kane & Ismail, 2017; Foo et al., 2016; Schuurs & Verheul, 1990; Razmara et al., 2007) or as an immune enhancer by mediating cytokine levels (Grimaldi et al., 2005; Orbach & Shoenfeld, 2007). Females are behaviorally and potentially immunologically protected from some types of inflammatory disease, which is thought to be due to the anti-inflammatory properties of estradiol and progesterone (Bekhbat & Neigh, 2018; Czlonkowska et al., 2006). Both testosterone and estradiol suppress inflammation at the physiological level, and our results indicate that these hormones also have the potential to impact stress-induced inflammation in the brain.

Pro-inflammatory cytokines, like IL-1β, TNFα and IL-6, are known to promote inflammation (Vilcek, 1998; Bluthé et al., 1999; Leon et al., 1999; Sharma et al., 2018; Bekhbat & Neigh, 2018; Tonelli et al., 2008). We hypothesized that cytokine mRNA expression would differ depending on sex and exposure to probiotics. More specifically, due to the increased sickness behavior displayed by male mice, we predicted that males would also display greater inflammation within the brain in response to LPS treatment. In the current study, LPS treatment caused a significant increase in pro-inflammatory cytokines, IL-1β, TNFα and IL-6 mRNA expression, in the hypothalamus, hippocampus and prefrontal cortex in both males and females, compared to saline controls. Contrary to our predictions, females displayed greater central cytokine mRNA expression, which may allude to enduring LPS-induced depression-like behaviour (Murray et al., 2019). Specifically, pubertal females that were treated with LPS showed greater IL-1β mRNA expression in the hippocampus and greater IL-6 mRNA expression in the prefrontal cortex. However, our findings are consistent with previously published work showing that women experience stronger pro-inflammatory responses during infection and are also at a greater risk to developing depression and anxiety disorders compared to men (Engler et al., 2016). Moreover, women tend to react with a stronger inflammatory and innate immune response to infections (Engler et al., 2016; Furman et al., 2014; Klein et al., 2010; Marriott & Huet-Hudson, 2006; Villacres et al., 2004; Verthelyi, 2001; Weinstein et al., 1984). Taken together, the robust central cytokine response in pubertal females alludes to an increased sensitivity to stressors, which could result in an enduring detrimental effect on mental health.

Due to evidence suggesting the gut microbiome influences stress and inflammation (Cryan & Dinan, 2012; Dinan & Cryan, 2013; El Aidy et al., 2015; El Aidy et al., 2014; Moloney et al., 2014; Sampson & Mazmanian, 2015), we hypothesized that mice treated with probiotics would show reduced sickness behavior and cytokine mRNA expression, in both sexes. LPS-injected female mice treated with probiotics showed significantly more sickness symptoms at 30 minutes but less symptoms at 6 hrs compared to LPS-treated females not

exposed to probiotics. These findings are consistent with published work (Bouman et al., 2005; Darnall & Suarez, 2009) and show that females tend to have a more vigorous initial cellular and humoral immune reaction and recover quicker from infections compared to their male counterparts. It is arguable that female mice further benefited from probiotics causing an earlier onset of sickness symptoms as an adaptive behavioural response, which enhanced their adaptive psychophysiological mechanism (Dhabhar, 2014) to overcome sickness.

Intestinal bacteria as well as probiotics have immunomodulatory properties (Desbonnet et al., 2008; Desbonnet et al., 2010). In the current study, male mice displayed increased expression of TNFα and IL-6 in the hypothalamus and hippocampus; however, this effect was reduced with probiotic treatment. Overall, males had a reduced central cytokine response compared to females and probiotic treatment led to a cytokine reduction in both males and females, eliminating sex differences. Probiotic treatment also caused a reduction in LPS-induced inflammation in female mice. Specifically, in the hippocampus TNFα and IL-6 were reduced following probiotic treatment in females, which suggests that beneficial microbes obtained from probiotics mediate the inflammatory response. Our findings indicate that treatment with probiotics preceding an immune challenge decreases the immune response at 8 hours post-infection. These findings are consistent with other published results. A study conducted in rats found similar beneficial effects of probiotics on inflammation; elevated pro-inflammatory cytokines induced by maternal separation were restored to normal levels after subsequent treatment with the probiotic *Bifidobacterium infantis* (Desbonnet et al., 2010). A human study conducted on patients of major depressive disorder found that following probiotic treatment, TNFα and IL-6 both decreased in concentration (Dowlati et al., 2010). This effect can be explained by probiotics having a mitigating effect on the HPA axis (Bravo et al., 2011; Gareau et al., 2011). Taken together, probiotic treatment reduces pro-inflammatory responses in both sexes in a cytokine- and region-specific manner.

Our findings in the current study were consistent with previously conducted research. Specifically, male mice display greater sickness behaviour compared to their female counterparts. Female mice display greater central mRNA expression of pro-inflammatory cytokines, and an immune challenge following probiotic treatment results in a decrease in the immune response. Despite these consistencies the study was not without its limitations. We were primarily concerned with the central cytokine response and did not explore the possibility of other peripheral effects. Studies have shown that peripheral cytokines also play a role in LPS-immune response and can impact mental health (Cai et al., 2016; Sharma et al., 2018). Moreover, we did not examine LPS-induced damage to the gut, which may have had an impact on the immune response. The current study only examined pubertal mice, as puberty is a critical period in development, however there are age-related differences in response to LPS-treatment (Girard-Joyal et al., 2015; Cai et al., 2016), therefore it would be beneficial in a future study to look at pubertal and adult mice in tandem to determine the effects probiotic treatment has on both sex and age.

It has already been established that the gut plays an important role in responding to an acute sickness. Since the CNS is connected to the gut via the vagus nerve, this nerve becomes of special importance too (Forsythe et al., 2010). Bravo et al. (2011) treated healthy mice with a probiotic, *L. rhamnosus*, to examine the effects probiotics had on anxiety- and depressive-like behavior. The results showed that the probiotic did have an alleviating effect, but only when the vagus nerve was intact. Therefore, it would be an interesting future study to further investigate the role that the vagus nerve plays on the immune response.

Finally, the probiotic that was analyzed in the current study was kefir, which contains a mixture of bacteria (Rosa et al. 2017). In order to identify the mechanism through which kefir mitigates LPS-induced inflammation, future studies should examine specific strains of bacteria, such as *lactobacillus* and determine the role that specific strains have on the immune response.

Conclusion

Pubertal exposure to LPS results in enduring negative programming consequences on the developing brain (Ismail et al., 2011; Laroche et al., 2009), but the mechanism underlying these effects remains unknown. The current study elucidated the impact of the gut microbiome on acute immune responses and gave insight into enduring sex-specific alterations in behavior. These findings further advance our understanding of the mechanism underlying sex-specific pubertal immune responses that are influenced by the gut microbiota. Our results also show that pubertal probiotic treatment can mitigate LPS-induced inflammation within the brain. Research on the effects the gut microbiome on the brain and behaviour is relatively new and our current study provides insight on the modulating effects of the gut microbiome on the immune system. The decrease in LPS-induced inflammation following probiotic treatment is likely protective against enduring alterations on behaviour. Additionally, the sex-specific responses to the immune challenge highlight the importance of considering sex in neuroimmunological studies. This study also encourages future research in the field of probiotics to further investigate the influence of the gut microbiome on the brain, particularly during critical periods in development such as puberty. Taken together, probiotics consumption during puberty could prevent enduring stress-induced negative outcomes on mental health in adulthood such as depression and anxiety.

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The Attributions of Students' Confidence Judgments and Related Feedback Preferences

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Much research has demonstrated that low performers tend to be prone to overconfidence, while high performers are disposed to underconfidence. Still, students' attributions for their confidence judgements and how their judgements relate to academic attitudes, such as feedback preferences, remains undetermined. Undergraduate students in eight introductory psychology classes made confidence judgements for their psychology midterm exam, then reported their attributions for the estimate. One week later, students received their exam score back, assessed how their actual performance compared to their expectations, and ranked their feedback preferences. Consistent with past work, low performers were overconfident and high performers were slightly underconfident. Overconfident students made significantly more internal and external attributions than underconfident students. The most influential attributions for both groups were the perceived difficulty and relevancy of exam questions. Additionally, a significant negative relationship between confidence judgement bias and feedback preferences suggests that as students become underconfident their preference for fewer feedback increases. These results indicate that overconfident learners are more motivated to provide explanations for their confidence judgements, possibly due to cognitive dissonance between their expected ability and actual ability. Contrary to expectations, overconfidence did not have a relationship with maladaptive feedback preferences. Future work would benefit from using alternative methodologies, such as using open-ended questions or a think-aloud protocol.

Keywords: Confidence judgement, attributions, estimation, performance, feedback preferences, university students

"The demand for certainty is one which is natural to man but is nevertheless an intellectual vice." – Bertrand Russell, Unpopular Essays (1950)

Nearly a century later, Bertrand Russell's words ring as true as when they were first scribed. Since Russel first wrote this line in his Unpopular Essays (1950), there has been much evidence gathered to determine the prevalence and causes of the intellectual vice he was describing; that is, overconfidence. Much work has shown that students are prone to overconfidence. As students mature into self-regulated learners throughout their university education, they are faced with many situations where they must appraise the certainty with which they know something. Whether studying for or writing a test, students are constantly making appraisals of what they do or do not know. Indeed, the occurrence of self-appraisals extends beyond the aforementioned examples to nearly all learning processes. Students' appraisals of what they know play a causal role in their study behaviours (Metcalfe, 2009). For example, a student studying for a test needs to accurately monitor their knowledge to successfully navigate the material that they are learning. If they are overconfident about what they have learned, they may terminate their study prematurely. Conversely, if they are underconfident, they may misallocate their study time, spending redundant time on already learned material. High accuracy when appraising one's knowledge has been linked to greater academic achievement (Bol, Hacker, O'Shea, & Allen, 2005). In short, learners' confidence judgments play an important role in effective learning. It is important to explain the factors that influence these confidence judgments and to further our understanding of how confidence judgements fit into the learning experience.

Learners can often exhibit a poor understanding of their actual knowledge and ability (Dunning, Heath, & Suls, 2004; Ehrlinger, Johnson, Banner, Dunning, & Kruger, 2008; Hacker & Bol, 2019). A consistently observed phenomenon is that, as a function of actual performance, the poorest performers show the greatest overconfidence while the best performers are slightly underconfident, a la the Dunning-Kruger effect (1999). The causes of this effect, and overconfidence in general, have been postulated to include factors such as metacognitive ability, motivational factors, and cognitive errors/heuristics (Anderson, Brion, Moore, & Kennedy, 2012; Dunning et al., 2004; Ehrlinger et al., 2008; Ehrlinger & Dunning, 2003). However, perhaps as equally important as these drivers of biased estimates is how students understand their estimates.

The attributional style of students may be an important, yet insufficiently investigated, influence on their estimations of performance. To remedy the lack of evidence, this study investigated the connection between students' postdiction estimates of exam performance, their actual performance, and their attributions in support of their predictions. Additionally, this study inquired about the potential relationship between students' estimate bias and their feedback preferences, furthering existing inquiry on the connection between students' estimates and their ensuing academic behaviours. The relations between these variables can provide a greater understanding of the means to overcoming the consequences associated with inaccurate self-appraisals of performance.

Background

To understand self-appraisals, learners' estimations of performance are compared to their actual performance. This comparison produces two measures: accuracy and bias (Gutierrez & Price, 2017). Accuracy reflects how well the learner has judged their performance compared to their objective performance. Bias indicates the direction of errors present in the learner's accuracy. A positive bias score indicates overconfidence, as their estimate is greater than their performance, while a negative bias score reflects underconfidence, as their estimate is lower than their performance.

Students' academic performance is one of the greatest predictors of their confidence judgements (Dunning et al., 2004; Hacker, Bol, Bahbahani, 2008, Kruger & Dunning, 1999). Despite the large relationship between performance and estimates of performance, an examination of the distribution of these data points reveals that high performing students often exhibit slight

underconfidence while low performing students exhibit a large amount of overconfidence. This effect has been replicated across several domains and with different measures; commonly known as the Dunning-Kruger Effect (Ehrlinger et al., 2008).

Attributional Style

As proposed by Bol and Hacker (2012), learners' attributional style can factor into the formation of one's estimates, thus influencing overconfidence and underconfidence. Zimmerman's (2008) model of self-regulated learning provides a useful conceptual framework for understanding the role and formation of confidence judgements in learning. This model of self-regulation proposes that learners use a personal feedback loop comprising social, environmental, and personal information about one's performance to guide successive efforts towards learning. The personal feedback loop involves three stages: forethought, performance, and self-reflection. While learners make attributions at each stage of the feedback loop, the current study is interested in the attributions that learners make during the self-reflection stage when they engage in self-judgement and consider reasons for their judgements. Self-judgements include the process of setting a standard of performance and judging whether one meets that standard. Based on that judgement, learners make attributions for the causes of their performance, and with confidence judgements, their estimated performance. However, it is not the case that these two mental events are linear. There may be a reciprocal relationship instead, where learners' judgements about their performance are affected by their attributions, and vice versa. For example, lower-achieving students may use their attributions as a defensive mechanism to preserve feelings of self-worth and academic identity, thus leading to overconfident appraisals of their performance (Hacker et al., 2008).

Work from several studies has illuminated some connections that attributions have with judgements of performance. Bol, Hacker, O'Shea, and Allen (2005) completed one particularly illustrative study. The researchers measured the attributional styles of university students' prediction and postdiction estimates for a final exam. Their results showed that students' attributional styles were associated with their actual performance and estimation accuracy on the final exam. Task-centered attributions were predictive for overconfident predictions, and student-centered testing attributions were linked to underconfident predictions. Hence, students' judgements of performance are indeed linked to personal and environmental attributions, such as study behaviour or testing conditions.

Similar studies have confirmed and expanded upon the relationships that attributions have with students' confidence judgements. Attributional style appears to differ as a function of achievement level. Hacker and colleagues (2008) found that high performers' attributional style was not predictive for their exam predictions and postdictions, however, low performers' attributional style significantly predicted their pre-and-postdicted estimates. Thus, for low performing students, their judgements of performance may be alterable by their beliefs about their performance. An examination of open-ended attributions revealed that some high performers wrote that their underestimates stemmed from a lack of confidence in their performance. Hence, high performers' underestimates may be linked to insecurities regarding their academic ability. Performance level appears to moderate students' explanations for their appraisals of performance.

While there are tendencies for certain attributional styles to emerge between low and high performers, when students are asked to express freely the factors they believe influence their estimates, they often give a mixture of both task and environmental factors (Dinsmore & Parker, 2013). Students' responses to an open-ended question inquiring about what influenced their confidence judgements for a reading comprehension test described a mix of attributions, including prior knowledge, characteristics of the text, and guessing. Interestingly, the participants with the most biased estimates provided multiple attributions more often than their less biased counterparts.

In sum, these studies describe the

factors to which learners attribute their confidence judgements. Overconfident and more biased learners are increasingly more likely to make external attributions and to say that multiple factors influenced their confidence judgements. Conversely, the usually less biased and slightly underconfident learners make attributions directed towards themselves, citing their preparation or lack of confidence as responsible for their conservative confidence judgements.

Feedback

Students' confidence judgements and reasons for their judgements do not occur in a vacuum. An equally important part of understanding the basis of confidence judgements is determining how these appraisals relate to other academic behaviours. For example, when students are overconfident in a poor performance and make external attributions, it can lead to learned helplessness, a state where they accept their inability to improve (Hacker & Bol, 2019). While there are many ways learned helplessness could manifest as maladaptive academic attitudes, this study is interested in learners' feedback preferences, as maladaptive feedback preferences may take the form of disinterest or lack of engagement with feedback.

A seminal meta-analysis and review highlighted engagement and receptivity as one of the most important variables when considering what makes feedback effective (Hattie, 2015; Hattie & Timperley, 2007). Quality feedback identifies the gaps in one's knowledge and provides strategies or information to help the learner fill in the knowledge gap. Though, without engagement, even the most perfectly tailored feedback may fall on deaf ears and blind eyes. Given the defensive role that overconfidence has been purported to have, we might expect that overconfident learners would be unwilling to engage with feedback that identifies their shortcomings. On the other hand, underconfident learners may feel empowered by their better than expected performance, thus being encouraged to further their learning through engagement with feedback.

Within the domain of emotional intelligence (EI), overconfident students are significantly less willing to report interest in improving their EI ability when compared to those who are underconfident (Sheldon, Dunning, & Ames, 2014). The overconfident participants were more likely to question the accuracy or the relevancy of the EI test used in their study. Thus, the overconfident business students appeared to exhibit learned helplessness, as they expressed a maladaptive approach to disconfirming feedback. While this report is not the first to have established that negative feedback provokes negative reactions to the feedback (see Brett & Atwater, 2001), Sheldon and colleagues claim to be the first to provide a motivational account for overconfident students' reluctance to engage with the feedback. However, it remains to be seen whether confidence judgements play a role in students' academic feedback preferences.

Based upon the aforementioned research, three hypotheses were formed. Work by Dunning and Kruger (1999), and subsequent studies (Dunning et al, 2004; Ehrlinger & Dunning, 2003; Ehrlinger et al, 2008) replicating their findings, suggests that in this study an unskilled and unaware effect should be present in quartile comparisons between students' estimates of performance and actual performance. The lowest quartile of performers should exhibit the greatest overestimation, while the highest quartile of performers should slightly underestimate.

The second goal of this study is to examine students' attributions for their estimates of performance. I predict that students who overestimate will attribute external attributions as most relevant to their estimate, whereas those who underestimate will attribute internal attributions as most pertinent. In addition, student's estimation bias should be related to their feedback preferences. Specifically, I expect that the more overconfident learners are, the more they will prefer not to receive feedback beyond their exam score.

Methods

Participants

Students from eight Psychology 101 sections at the University of the Fraser Valley

were provided with the opportunity to take part in this study in exchange for participation credit in their class. Of those offered the chance, 215 psychology undergraduate students volunteered for this research. The University of the Fraser Valley's Human Research Ethics Board approved this study.

Materials

Performance

Performance was operationalized as students' midterm percentage mark. Students were split into quartiles based on their actual exam performance. This split reflects the method used to determine the presence of a Dunning-Kruger effect (Ehrlinger et al., 2008; Kruger & Dunning, 1999). Eight psychology 101 sections wrote their midterm, which examined the topics covered in the first four weeks of class. Five sections were taught by Instructor A and three sections were taught by Instructor B. Sections taught by the same instructor received identical exams, while exams differed between instructors. The exam material tested was equivalent between sections. The number of questions on the exam differed between instructors. An independent groups t-test demonstrated that student exam scores did not differ significantly between lecturers.

Questionnaire One

The first questionnaire measured difficulty, estimated performance, and estimate attributions.

Difficulty. Students assessed exam difficulty by rating the exam from 1 (very difficult) to 5 (very easy).

Estimated Performance. Students offered a postdiction estimate for their exam performance by answering the following question: "How well do you think you did on the test? Please estimate the percentage you expect to receive: _____% out of 100." Participants' estimated scores were subtracted from their actual scores to create a bias score. Positive bias scores indicate overestimates, and negative scores indicate underestimates.

Estimate Attributions. Participants expressed which attributions they believed influenced their estimates by rating their agreement with seven Likert-type items (e.g., "The test covered the things we covered in class") ranging from -2 (strongly disagree) to 2 (strongly agree). Items were either internal (three items; e.g. "The studying I did was relevant to the exam content") or external (four items; e.g. The test content covered the content in the textbook readings) attributions. External attributions focused on influences outside the student's control. External attributions included test difficulty, test question relevancy, lecture helpfulness, and textbook helpfulness. Internal attributions were factors within the student's control, including the time spent studying, relevancy of studied materials, and the student's academic expectations (i.e., how they have performed on previous tests). Positive scores showed that the attribution applied to their estimate, and negative scores indicated that the attribution was irrelevant.

Questionnaire Two

The second questionnaire measured students' expectations and feedback preferences.

Expectations. Once they received their exam mark, students were asked to rate how their mark compared to their expected exam score, from 1 (far exceeds expectations) to 3 (meets expectations) to 5 (far below expectations).

Feedback Preferences. Students were asked to give their preferences for feedback by ranking four feedback options from 1 (least preferred) to 4 (most preferred). The feedback options were only receiving their exam score, going over the exam as a class or by themselves, or meeting with the instructor to review the exam.

Procedure

One week before their midterm exam and this study, students were informed about the nature of the research. Upon arriving for their midterm exam students were provided with a research booklet containing the difficulty question, confidence judgement estimation, and estimation attribution questionnaire. Students then wrote their

midterm exams. Once they finished, students opened the research booklet, signed the informed consent, and completed Questionnaire One.

One week after writing their midterm exam, students attended their scheduled psychology class. At the start of the class, they received their midterm exam scores and a second research booklet, asking for their ratings of expectations and feedback preferences. Students examined their exam score and then completed Questionnaire Two.

Results

Hypothesis 1: The Unskilled and Unaware Effect

To verify the normalcy of the current sample and replicate previous findings, students' exam scores were compared to their estimated exam score; first by examining the relationship in general, then by performance quartile. Overall, participants overestimated their exam performance. Students estimated their exam percentage to be 69.73%, while the actual mean exam percentage was 63.31%, an overestimate of 6.49%.

To determine whether there was an unskilled and unaware effect, I followed the practice described by Kruger and Dunning (1999) and split participants into quartiles based on exam performance (see Figure 1). Consistent with Kruger and Dunning, those in the bottom quartile (n=53) showed the greatest overestimation, as their expected percentage was 61.89% while their actual percentage was 43.58%, an overestimation of 18.23%. The top quartile (n=54) slightly underestimated their percentage as 78.02% when their actual percentage was 82.44%, an underestimate of -4.42%.

The students who overestimated their exam score (n=109) rated expectations as not being met (M=3.86, SD=.80), whereas those who underestimated their score (n=58) rated their expectations as being closely met, with an lean towards exceeding expectations (M=2.83, SD = 1.01). A Spearman's Rho correlation analysis between estimation bias and expectation described a large relationship ($r_s(171) = .60$, $p < .05$).

Hypothesis 2: Estimation Attributions and Estimation Bias

The relationship between students' estimation attributions and their estimation bias was examined by investigating the present associations and by comparing the attributional styles between underconfident and overconfident students. The differences between students who overestimated and those who underestimated were examined using an independent-groups t-test (see Table 1 and Figure 2). No assumptions were violated for the t-test. Results demonstrated

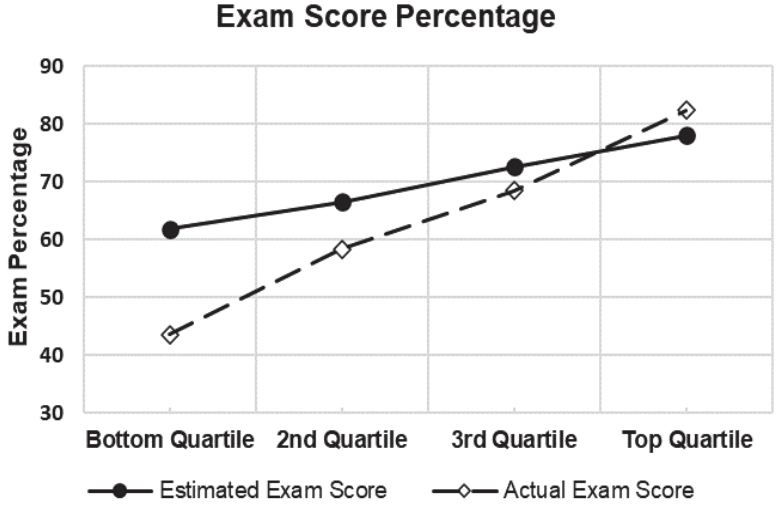

Figure 1. Graph Representing the Unskilled and Unaware Effect. Students' estimated and actual percentage on a psychology exam, as a function of their actual psychology exam performance quartile.

	Overestimate		Underestimate		*t*(195)	95% CI		Effect Size (Cohen's d)
	M	*SD*	*M*	*SD*		Lower	Upper	
Internal	1.85	2.21	.80	2.61	2.89*	.33	1.76	.43
External	4.11	2.70	2.6	2.96	3.51*	.66	2.36	.53

Table 1. T-test Results for the Relevancy of Attributions Between Overestimators and Underestimators. *CI=Confidence Interval of the Difference.*
**p<.05*

that students who overestimated (n = 136) made significantly more internal and external attributions than those who underestimated (n = 60). The finding that those who overestimated made more external attributions was consistent with the prediction; however, these students surprisingly also made more internal attributions. Furthermore, underestimators were less likely to rate both internal and external attributions as relevant to their score.

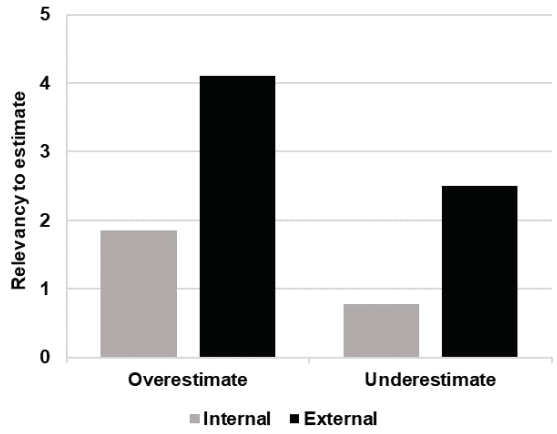

Figure 2. The Relevancy of Attributions to Students' Estimations. Students' internal and external attributions for their exam estimate as a function of their estimation bias. Underestimators. CI=Confidence Interval of the Difference *p<.05

An observation of the most frequent attributions showed that both groups made the same top three attributions but in distinct orders. Those who overestimated attributed the relevancy of the lecture material to the test (M = 1.25), then equally relevant was the relevancy of the textbook to the test (M = 1.01) and the judged appropriateness of the test difficulty (M = 1.00). Students who underestimated most strongly attributed the relevancy of the textbook (M = .88), then the relevancy of lectures (M = .82), and then appropriate test difficulty (M = .50). Intriguingly, both groups' most ardent attributions were external.

Bivariate Pearson's correlations revealed that students' bias scores exhibited small associations with both external and internal attributional styles (respectively, $r(205) = .20$, $p < .05$ and $r(205) = .16$, $p < .05$). Thus, as students' bias moved toward overestimation, their internal and external attributional style scores increased, indicating increased relevancy of the attributions to their estimate.

Feedback Preferences and Estimation Bias

Finally, a one-way ANOVA examined the relationship between students' estimation bias and their preferences for four types of feedback for their exam performance. An observation of the mean of students' feedback preferences revealed that their preferences did not differ as a function of estimation bias (see Figure 3). The top-ranked feedback options were, first, reviewing the exam answers as a class (M = 3.06, SD = .92) and second, reviewing the exam answers privately (M = 2.94, SD = .94). These two options reflect the exam feedback practices of most classrooms, revealing that students prefer the status quo feedback practices.

Interestingly, a Spearman's Rho correlation analysis determined that there was a significant negative relationship between estimation bias and preferring to only receive one's exam score as feedback, $r_s(174) = -.17$,

$p < .05$. This finding runs contrary to the hypothesized outcome; it suggests that as students' bias trends towards overconfidence, their preference for minimal feedback decreases.

A Spearman's Rho correlation analysis demonstrated that students' expectations were moderately negatively associated with their preference to only receive their exam score as feedback, $r_s(182) = -.31$, $p < .05$. This finding implies that as students' expectations reflected increased disappointment (i.e., exam score not meeting expectations) their preference to only receive their exam score decreased.

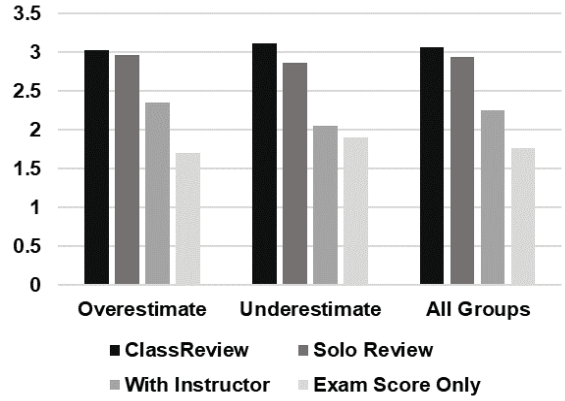

Figure 3. Students' Ranked Feedback Preferences

Discussion

It is a well-established phenomenon that students' estimates of performance only partially align with their actual performance (Dunning et al., 2004). Usually observed, is that the poorer an individual's performance, the more biased their estimates are (Kruger & Dunning, 1999; Ehrlinger et al., 2008; Hacker et al., 2008). These biased estimates create both indirect and direct costs on performance. Performance suffers directly as students make worse decisions on their exams, while biased estimates leading to poorer study choices can impair their future performances (Bjork et al., 2013).

The current research set out to answer the following questions: to what do students attribute their estimations, and do these internal and/or external attributions exhibit any trends? Further, what is the relationship between students' estimation bias and their future study attitudes, such as their preferences towards feedback?

To begin answering these questions, this research replicated the Unskilled and Unaware Effect (Ehrlinger et al., 2008; Kruger & Dunning, 1999). Low performers had the least amount of insight into their actual performance, as they greatly overestimated their actual performance by multiple letter grades. Their overestimation often reflected the belief that they had passed their exam when in reality they had failed. On the other hand, high performers slightly underestimated their actual performance.

Students' estimation biases appear to have factored into the expectations they created for their actual exam performance, especially for those who overestimated. The large relationship observed between estimation bias and expectation scores means that the appraisal students made after their exam directly relates to how they interpret their actual performance. If a student overestimates their performance, they're more likely to experience disappointment when they learn of their exam scores. In contrast, those who underestimate are better aligned in their expectations.

Beyond replicating the Unskilled and Unaware effect, I looked into what attributions students make for their estimates. Specifically, I assessed whether internal and external attributions were different between those who over- or underestimated. Results from the attribution questionnaire showed that students who overestimated made significantly more internal and external attributions than those who underestimated. This finding opposed the hypothesis that those who underestimated would make more internal attributions. Still, this finding validates previous work. Students who made biased estimates and then asked to make open-ended attributions for their estimates were more likely to attribute multiple causes for their confidence judgement than their more accurate peers (Dinsmore & Parker, 2013). These findings support the idea that low performers' attributional style plays a role in the formation of overconfidence as a defense mechanism (Hacker & Bol, 2019; Hacker et al., 2008). Overestimation, especially in sizeable amounts, relates to an increase in the

number of attributions students make, such that overestimated poor performance creates more of a need for explanation than an underestimated good performance.

One explanation for this effect may be that the students who overestimate while making numerous attributions are experiencing cognitive dissonance. Their expectations about their current abilities and knowledge do not align with reality, thus creating a dissonant discrepancy. This dissonance may drive their additional explanations. Indeed, overconfidence is at least partially driven by an enduring motivation to view the self as an accurate perceiver (Blanton, Pelham, DeHart, & Carvallo, 2001; Ehrlinger & Dunning, 2003). For example, students made estimates for 13 quizzes over the course of a semester. Instead of improving the accuracy of their estimates over the weeks, the students desired final grade was a better predictor of their estimates than previous performances (Serra & DeMarree, 2016). Thus, the motivation to be obstinate about one's academic identity appears to be strong. The current study supports these findings, as when participants were confronted with evidence to suggest that they do not measure up to their academic self-beliefs, their performance feedback spurred on numerous explanations to ease the discomfort of inaccuracy.

The fact that students who underestimated made fewer attributions is interesting. If contrasted with overconfident learners' use of attributions as a protective mechanism, it may be that the underconfident learners do not feel pressured to bolster their academic identity with a plethora of attributions. Their performance tends to be higher, and surpasses their estimations; thus, there is no need to 'explain away' a good performance. In sum, it appears that evidence suggests that attributional style may play a larger role for overconfident learners rather than the underconfident (Hacker et al., 2008).

Intriguingly, the most highly cited attributions were the same between those who over- or underestimated. Both groups judged the appropriateness of the test difficulty and how well the test questions reflected what was taught in the class and textbook as most influential in their estimate. Yet, overconfident learners' thought these attributions were more important to their estimation than underconfident learners. These facts lend themselves to an alternative interpretation of the fact that underconfident learners made fewer attributions than the overconfident learners. It may be that the current attributional items did not sufficiently capture the actual attributions for underconfident students. According to answers responding to open-ended questions, at least some high performing underconfident students believe that making conservative estimates is influenced by insecurity in their academic identity (Hacker et al., 2008). Thus, overconfident and underconfident students may use attributions in different ways for the same end goal: to protect their academic identity. The evidence for the underconfident side of the argument still needs more support beyond the anecdotal evidence cited.

An increase in the number of attributions could have a two-fold impact on the future efforts and performance of a student who overestimated their score. One, if the student made many internal attributions, they may feel in control of their ability to improve, leading to more effort and potentially better performance. Still, an increase in the number of external attributions may contribute to self-handicapping, as they may not believe they can control future outcomes.

The link between confidence judgements and feedback preferences produced surprising results. I expected that low performing overconfident students would prefer minimal feedback. However, the opposite was true. The less overconfident students' confidence judgements became, the more they preferred to only receive their exam score as feedback. Students who were disappointed in their exam scores, having been overconfident in their performance, were more receptive to meeting with their instructor to go over their exam. These findings contrast results suggesting that overconfident business students were less likely to report interest in improving their emotional intelligence after disappointing feedback (Sheldon et al., 2014). The different outcomes may be due to different methodologies; however, it could also be the case that the

importance of the domain skill to the respective sample was different. Sheldon and colleagues' (2014) participants were graduate-level business students, aware of the importance of emotional intelligence to their future career prospects as business managers. If these business students felt that they were lacking in emotional intelligence, it may have posed a great threat to their business acumen identity. Whereas a disappointing grade in a first-year psychology exam may pose little threat to the academic identity of the current studies' undergraduate students. Additionally, business students have been found to be more overconfident than those who are studying the sciences or humanities (Schulz & Thoni, 2016). Hence the divergent results between the business and psychology students may be accounted for by innate characteristics of the respective samples.

The results from the current study are more optimistic than Sheldon and colleagues (2014) indicate. Instead of suggesting that overconfident performances are linked to learned helplessness, the opposite appears to be supported. When students are confronted with a grade that does not meet their expectations, they may be prompted to engage with feedback by meeting with their instructor. Although, overall, the students preferred the feedback choices of going over the exam questions as a class or going over the exam answer key by themselves. Both options closely reflect the current common practice in their university classes. As such, it appears that students find the current feedback practices preferable. This may be because class review feedback provides sufficient anonymity for the overconfident and disappointed students, who desire feedback but might feel embarrassment or shame about sitting down one-on-one with an professor. While underconfident, yet pleasantly surprised students may not feel the need for further explanation.

Limitations and Future Directions

The internal validity of this study is limited by the choice to study participants in a classroom setting. Multiple variables are beyond the current studies' measures and control. For example, the amount of student interaction between classroom sessions may have influenced the expectations of students. Students often gather after a test to compare answers. As such, other test takers may provide participants with corrective insight into their actual performance, which was not accounted for when they completed their questionnaire. However, the lack of internal validity is offset by high external validity. As this research took place in the classroom, it is reasonable to suspect that the measured attitudes indicate the real-life experiences of university students. Yet, the external validity came at a cost, as due to efforts to limit the size of the questionnaire to prevent further disruption of the exam environment, demographics information was not collected. Future should prioritize collecting this information since demographics such as gender, age, and intended major could factor into confidence judgements.

Other limitations may direct fruitful future work. As is common with much of the research in psychology, the participants of this study were first-year psychology students. Future work would benefit from studying other groups, such as students further along in their studies and/or in other academic fields. It might be expected that more experienced university students would have more adaptive responses to their performance, i.e. preferring greater feedback and focusing their appraisals on controllable internal factors.

Drawing on past work investigating students' estimation attributions, this investigation provided students with a list of potential attributions for their estimates (Bol et al., 2005; Hacker et al., 2008). The scope and variability of students' attributions was limited by using a questionnaire. It would be useful to use open-ended measures, such as a think-aloud protocol or self-generated answers, to examine the organic tendencies of students' attributions. As noted in past work, open-ended responses would probably be an assortment of internal and external attributions (Dinsmore & Parker, 2013).

To further establish the role that students' estimation attributions play, future work may want to determine how these attributions link to actual behaviour. Researchers may consider converging a behavioural measure with students' attributions to determine whether attributions

reflect actual causes of performance. For example, one might try converging a measure of study time with students' attributions regarding the impact that studying had on their performance.

Conclusion

To become effective learners, students must be able to make accurate confidence judgements about what they do and do not know. These results support claims that the lowest quartile of performers have difficulty assessing their knowledge, as demonstrated by vast overconfidence. These overconfident learners attribute their estimations to a combination of external and internal forces. Indeed, the frequency of their attributions may act to protect their academic identity. Dissimilarly, top performers are slightly underconfident. Those who underestimated their performance made fewer attributions for their estimates than those who overestimated. Although, the most frequently cited attributions did not differ between over-or-underconfident students. The top feedback preferences between students did not differ. Still, there appeared to be a trend for those who underestimated, to prefer minimal feedback. As such, the idea that overconfidence produces learned helplessness was not supported.

Given these results, underconfident learners may want to be mindful that they do not let their surpassed expectations interfere with their desire to seek high-quality feedback. On the other hand, overconfident learners may be encouraged to learn that their biased estimates do not interfere with their feedback preferences. However, I would recommend that students still attempt to increase the accuracy of their confidence judgements, given the benefits that increased clarity can have for other actions, such as study behaviours (Bjork, Dunlosky, & Kornell, 2013).

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Serotonin: Origins, Roles, and Toxic Effects

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Through its imperative modulatory and regulatory role in supporting life, serotonin has heavily influenced a number of functions within the body since evolutionary beginnings. The discovery of this indolamine in the brain has led to much research in exploring its role within the synapse and receptors. The receptors themselves are associated with an array of phenomena and behaviours, which are also influenced by the locations in which the serotonergic projections innervate. The 5-HT_{1A},and 5-HT_{2A} receptors have primarily been the target of research, as they have been implicated in affecting psychological states such as empathy, anxiety, and depression; imperative life functions such as sleep and hunger; and basic psychological functions such as inhibition and control. Despite all the beneficial functions serotonin can contribute to, overstimulation of serotonin can lead to life threatening conditions such as serotonin syndrome. Dangerous but treatable if attended to in time, the syndrome provides a drastic example of how crucial serotonin's role is within the body. Given its impact in maintaining life, more research must be conducted to fill in the gaps of the current literature and to produce viable treatments for serotonin syndrome.

Keywords: serotonin, 5-HT_{1A}, 5-HT_{2A}, receptors, serotonin syndrome

Ancient and ubiquitous, the indolamine serotonin (also known as 5-hydroxytryptamine or 5-HT) has always been an asset for life, modulating and stimulating biological processes and functions (Turlejski, 1996). Appearing early in evolution, indolamines play a large role in the development and plasticity of cells (Turlejski, 1996). Serotonin holds an imperative role in fetal development of neuronal structures due to the ability of serotonergic fibers to modulate mitosis, dendritic elongation and spine appearance (Azmitia, 1999). Serotonergic fibers are innately very plastic in the mature brain, inducing regenerative sprouting responses on the serotonergic neuron when contacts with motoneurons are disrupted by damage of the spinal cord or forebrain (Azmitia, 1999). Serotonin specifically influences basic cell functions such as RNA synthesis, cAMP levels, mitosis, and cell and energy regeneration, making it imperative to sustaining and developing life (Turlejski, 1996). In researching the actions of the serotonin produced in the central nervous system, researchers are able to link neuroanatomical and neurophysiological bases to behaviours and phenomena such as empathy, clinical disorders, sleep, hunger, aggression, impulsivity, and more. This is imperative given the ubiquitous influence serotonin has on the body, driving the need for even more research to fill in the gaps of the current literature and to produce the most optimal treatments for these resulting afflictions.

Serotonin can act both peripherally and centrally within the nervous system. The literature has however focused primarily on the serotonin produced in the central nervous systems due to its role in affecting behaviour and psychological states (Turlejski, 1996). Serotonin was first discovered peripherally in the enterochromaffin cells of the gut of rabbits in the form of enteramine by Vittorio Erspamer in 1937, which was later identified as serotonin by Maurice Rapport, Arda Green, and Irvine Page in 1943 using blood from cows (Nichols & Nichols, 2008). A decade after this identification, Twarog and Page (1953) discovered serotonin's presence in the central nervous system by examining the brain tissue of dogs, rats, and rabbits. As a result of these efforts, a link between serotonin and lysergic acid diethylamide (LSD-25) was made, as a scaffold for the serotonin structure was found in the chemical structure of LSD (Nichols & Nichols, 2008). This was a significant finding, as it was one of the first neurochemical explanations for mental illness, as the serotonergic interferences brought upon by LSD alters the mind and causes 'mental disturbances' similar to those seen in a variety of psychiatric conditions (Nichols & Nichols, 2008). By linking the two, researchers were then prompted to produce the vast amount of research and literature on serotonin's role in the human brain seen today.

Though more than 90% of the serotonin in the body is located in the gut, research on the role of the gut in producing behavioural effects is currently in its infancy. It is only just recently that the brain-gut-microbiome axis has been implicated in psychological disorders (Ganci et al., 2019). Gut microbiota produced in the gastrointestinal tract contribute to the availability of peripheral serotonin precursors, which is then used in the synthesis of 5-HT in the central nervous system (Martin et al., 2018). By influencing the levels of serotonin throughout the body, various effects on behaviour and psychological effects can occur as a result (Martin et al., 2018). Clinical studies have also implicated gut probiotics with psychiatric symptoms such as obsessive-compulsive tendencies and increased scores on anxiety and depression measures, symptoms often associated with alteration in serotonin levels (Martin et al., 2018). These effects can all be attributed to the bidirectionality of serotonin's role in the peripheral and central nervous systems.

Chemically, serotonin is synthesized from the precursor indolamine, tryptophan; the structure itself involves tryptophan hydroxylase and aromatic amino acid decarboxylase (Azmitia, 2007). This comprises the base of serotonin, which is synthesized and released into the cell synapse to influence the cells involved, the receptors themselves, and ultimately behaviour (Azmitia, 2007). With 14 different receptor subtypes grouped into seven families, serotonin has the most receptor subtypes out of the classical neurotransmitters (Nichols & Nichols, 2008). The 5-HT_{1A} and 5-HT_{2A} receptors are the primary focus of research, as they are expressed in high concentrations within serotonin's neuroanatomical system and play

a large role in the psychological and behavioural phenomena discussed (Nichols & Nichols, 2008). The neuroanatomical system mentioned refers to the serotonergic projections of the raphe nuclei which vastly expand to the rest of the brain, impacting behaviour through its interactions with various brain structures (Hornung, 2003). The 5-HT_{1A} receptor is expressed in high concentrations within the amygdala, hypothalamus, hippocampus and septal area, while 5-HT_{2A} is expressed in high concentrations within the cortex, nucleus accumbens, and striatum (Nichols and Nichols, 2008). As these receptors are expressed in copious quantities in these specific anatomical regions, serotonin can produce varying behaviours and psychological phenomena depending on the location.

The Prefrontal Cortex, Aggression, and Impulsivity

The prefrontal cortex is implicated in acts of aggression and impulsivity due to its role in functions such as inhibition and control (Cetin et al., 2017). Serotonergic innervation due to projections of the dorsal raphe nucleus in the cortex influence these behaviours, as 60% of the glutamatergic and 25% of the GABAnergic neurons within the prefrontal cortex have 5-HT_{1A} or 5-HT_{2A} receptors (Santana et al., 2004). Research has associated 5-HT_{2A} in particular with these behaviours, showing that stimulation of these receptors increases dopamine release, regulating hyperactivity and thus increasing impulsivity seen in disorders like attention deficit hyperactivity disorder (ADHD) (O'Neill et al., 1999). Additionally, the presence of increased 5-HT_{2A} receptor concentration in the prefrontal cortex has been associated with impulsive aggression behaviours (Moeller et al., 1996). Autism, being associated with decreased ability to control actions and increased aggressive behaviour, is also implicated with tryptophan depletion (McDougle et al., 1996). In the study by McDougle et al. (1996), researchers found that an increase in behaviours associated with autism such as whirling around, hitting oneself, flapping arms, and more were worsened with depleted levels of tryptophan, which would result in decreased serotonin levels. The participants also reported feeling less calm and happy, and experienced increased anxiety, demonstrating the role of serotonin in influencing affect (Zmudzka et al., 2018).

Affect and Influences on Clinical Disorders

Serotonin plays a large role in the modulation of affect, feelings, emotions, or moods experienced in everyday life, as serotonergic projections deeply innervate structures involved with producing complex emotions such as the amygdala and the ventromedial prefrontal cortex (vmPFC) (Crockett et al., 2010; Jhangiani et al., 2014). Crockett et al., (2010) studied this relationship by enhancing the effects of serotonin in subjects using serotonin reuptake inhibitors (SSRIs) and measuring their emotional reactions to moral dilemmas (ultimatum games). These moral dilemmas were designed to elicit emotional responses, which previous research have linked to activity within the amygdala and vmPFC (Blair, 2008). Subjects given SSRIs and controls were instructed to pick between utilitarian outcomes (e.g. saving multiple lives) and aversive harmful actions (e.g. killing an innocent civilian). They found that enhanced aversiveness in harming others and thus prosocial behaviour can be promoted by serotonin.

As serotonin can modulate affect, it has also been implicated in clinical disorders such as anxiety and depression. Modulation of anxiety-like behaviour occurs as a result of serotonin's interaction with the 5-HT_{1A} receptors in the bed nucleus of the stria terminalis, which is a major output pathway of the amygdala (Zmudzka et al., 2018). Abnormalities such as decreased binding of 5-HT and short alleles of the 5-HTT gene on the human chromosome have been found to elicit symptoms of anxiety. The serotonin-transporter-linked polymorphism region (5-HTTLPR) genotype in particular is associated with increased trait anxiety and knocking out these genes in mice yield abnormal levels of anxiety behaviours (Hariri & Holmes, 2006; Holmes et al., 2003). Depression is also impacted by interactions with its 5-HT_{1A} receptors as well as its 5-HT_{2A} receptors, as both are utilized by antidepressants to aid in mitigating depressive symptoms (Celadaet al., 2004; Zmudzka et al., 2018). However, unlike anxiety, depression can be influenced

by serotonin action within the hippocampus as well. Depletion of 5-HT_{1A} receptors in the hippocampus can lead to a decrease in neurogenesis, which is associated with eliciting depressive symptoms (Zmudzka et al., 2018). Neurotrophins may play a role in this decrease, as Banerjee (2013) found that post-mortem individuals with major depressive disorder also showed decreased levels of neurotrophins, brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF). Selective 5-HT_{2A} receptor antagonists were also found to block the expression of stress-induced down-regulation of BDNF in the hippocampi of rats, providing further evidence of this mediatory relationship between serotonin and neurotrophins in the hippocampus (Vaidya et al., 1999).

The Hypothalamus, Sleep, and Hunger

Neural activity at the hypothalamus is also modulated by serotonin and controls thermoregulation, hunger, sleep, and more. As a precursor to melatonin, serotonin interacts alongside melatonin, being released at varying points throughout the sleep cycle (Ursin, 2002). Animal research investigating 5-HT_{1A} receptor agonists found that a response of serotonergic neurons within the dorsal raphe nuclei, similar to the activity of these neurons during wakefulness, was elicited (Guzman-Marin et al., 2000). The projections of the raphe nuclei innervating the hypothalamus and the cortex allow for this action, as these structures play a large role in modulating arousal and attention (Guzman-Marin et al., 2000; Ursin, 2002). Narcolepsy is directly affected by serotonin's role in wakefulness, as animal research has revealed that during cataplexy, a symptom which causes sudden episodes of muscle tone loss, the raphe nuclei are active and releasing higher levels of serotonin than normal during REM sleep (Wu et al., 2003). Hunger is also a factor controlled by serotonergic control over the hypothalamus. Serotonin receptors are involved in hunger modulation, as 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} agonists have been found to reduce food intake, while 5-HT_{2C} is the primary receptor for maintaining the homeostatic relationship between food intake and energy balance (Feijo, et al., 2010). The 5-HT_{2C} receptor is critical for mediation of serotonin's effect in the body's ingestive behaviours (Lam et al., 2008).

Serotonin Syndrome

As serotonin holds a heavy influence over many behaviours and phenomena, overactivation is dangerous and potentially life threatening. Serotonin syndrome is a condition occurring as a result of overactivation of 5-HT_{1A} and 5-HT_{2A} receptors (Boyer & Shannon, 2005). As the syndrome itself is defined as the presence of a toxic levels of serotonin in the brain, there is no singular diagnostic test for the condition; diagnosis must be conducted by a medical toxicologist (Boyer & Shannon, 2005). The condition is the consequence of excessive 5-HT agonism, affecting most if not all of the phenomena mentioned previously, impairing function of thermoregulation, mood, aggression, anxiety and depression levels, modulation of wakefulness, and more (Volpi-Abadie et al., 2013). This is due to the abundant axonal projections of the raphe nuclei, which innervate and modulate many structures of the brain in its role as the primary serotonin pathway (Volpi-Abadie et al., 2013). Symptoms range from mild to severe, depending on the level of serotonin present. Mild symptoms can consist of hypertension, tachycardia, tremor, and hyperreflexia, while moderate symptoms can consist of hyperthermia, pressured speech, mild agitation, and hypervigilance (Volpi-Abadie et al., 2013). Severe symptoms add to the symptoms of the previous severity levels with the possibility of experiencing delirium, seizures, renal failure, coma, or even death (Volpi-Abadie et al., 2013).

Approximately 75% of those diagnosed with the syndrome present symptoms within 24 hours of the initial use of medication, overdose, or change in dosage (Mason et al., 2000). Many psychopharmacological mechanisms are reported to cause the syndrome. Drug abuse, drug interactions with antidepressants like SSRIs or monoamine oxidase inhibitors (MAOIs), and more can cause mechanisms of serotonin syndrome like the inhibition of serotonin uptake, decreased serotonin synthesis, increased serotonin release, activation of serotonergic receptors, or possibly the inhibition of cytochrome P450 (CYP45), which would enable accumulation of

serotonergic drugs in the body due to decreased metabolism of drugs (Volpi-Abadie et al., 2013). The keys to managing the syndrome are to discontinue use of serotonergic agents, stabilizing and monitoring vital signs, providing oxygen, or sedating with benzodiazepines (Volpi-Abadie et al., 2013). If symptoms persist, the most effective treatment is to administer 5-HT_{2A} antagonist drugs (such as cyproheptadine and chlorpromazine), these interventions mitigate the more severe symptoms of the syndrome by blocking the activation of 5-HT_{2A} receptors (Nisijima et al., 2001).

As for research and diagnosis of serotonin syndrome, there are still gaps in the literature due to the nature of the syndrome itself. Due to the large variety of symptoms, variation in causes (which drugs, specifically) and the requirement of a toxicologist to diagnose, the true incidence of the syndrome is unknown (Werneke et al., 2016). Medical professionals who are less knowledgeable in the condition may put their patient in danger, as failure to diagnose can turn mild drug interactions into a deadly concoction (Werneke et al., 2016). Even those who are familiar with serotonin syndrome may overlook symptoms due to the lack of consensus on the existing criteria systems for diagnosis, or confuse the condition for neuroleptic malignant syndrome, which shares similar symptoms (Haberzettl, 2013; Werneke et al., 2016). A lack of standardized measures also exists within research, as scientists have found it difficult to model it consistently in animals (Haberzettl, 2013). Problems arise when looking at discriminability of symptoms since the animal symptoms differ across as well as within species (Haberzettl, 2013). The symptoms observed in a rodent will differ from another rodent and will differ even more compared to a human. Evaluation of a human model is only able to be made in case studies and by physician reports, and while animal models can assist in this research, the area still requires further research (Haberzettl, 2013).

Conclusion

Thanks to the discoveries in the mid-1990s by researchers examining cells, blood, and tissue, scientists today are able to further our understanding of serotonin and the implications of fluctuating levels or overdose. Through its role in the central and peripheral nervous system, serotonin influences a wide array of psychological and life-sustaining functions. The omniety of this indolamine, though helpful in modulating necessary functions such as affect, sleep, hunger, and aggression/impulsivity, can also cause severe symptoms when there is an overabundance. The recent research on the brain-gut-microbiome axis shows promise in contributing to the management and treatment of serotonin syndrome, as there is still much about this system that is currently unknown. Despite the current state of the field, the least researchers can do right now is to continue to expand the current knowledge on serotonin and elucidate where the projections of serotonin reach, what functions it affects and what treatments target the conditions resulting from high levels of serotonin while having the fewest side effects. Though a difficult task at a glance, much work and knowledge has been gathered since Erspamer in 1937, and given the advances in technology since then, it is most definitely within arm's reach.

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Diagnosing and Treating Mental Illness Across Cultures:

Systemic Racism in Clinical Psychology

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Diverse cultures have historically been underrepresented by psychological research (Arnett, 2008; Nielsen et al., 2017). Using western data and diagnostic criteria designed by western society leads to contemporary understandings of clinical diagnoses and psychotherapies that lack external validity beyond western society. Consequently, when immigrants from these diverse countries seek mental health services, they are disproportionately misdiagnosed and receive psychotherapies that are far less effective. The tools and training that clinicians are provided with do not effectively translate through different cultural lenses. Contemporary diagnostic instruments like the Diagnostic and Statistical Manual of Mental Disorders (DSM) need to include additional representative research to improve their sensitivity across cultures. Furthermore, psychotherapies need appropriate cultural adaptations that connect with cultural minority clients to become properly effective. Diagnostic manuals and empirically supported psychotherapies are culturally biased descriptions of clinical psychology which need cultural competence to accommodate the growing cultural diversity present within Canada and America. This literature overview details the extent of the underrepresentation in western psychological research. Subsequently, it presents a brief account of diverse cultural research demonstrating how mental health expression varies extensively by culture. Finally, expounding on these points demonstrates how the resulting DSM is not adequate for the countries that use it, and the resulting psychotherapies lack efficacy in their populations, perpetuating systemic racism in clinical psychology.

Keywords: Culture, expression, systemic racism, clinical psychology, DSM.

Globalization trends and projections are increasingly diversifying western countries. The U.S. Census Bureau (2017) has estimated that by 2045, non-Hispanic whites will comprise less than 50% of the U.S. population.[1] In Canada, 41.6% of the population are first or second-generation immigrants with 22.3% identifying as visible minorities (Statistics Canada, 2017). As such, cultural awareness is becoming essential for establishments in both the public and private sector. This awareness is paramount in health care to avoid severe misdiagnosis. For instance, people of African descent have a significantly lower white blood cell count, which is essential knowledge when conducting regular checkups or developing effective treatment plans (Reich et al., 2009). Similarly, in mental health care, different cultures display unique symptoms requiring diverse appropriate diagnostics, treatments, and interventions (Bhugra, 1997; Bredström, 2017; Goodmann et al., 2020; Hinton & Patel, 2017; O'Farrell et al., 2020; Zanon et al., 2020). These diverse cultures are severely underrepresented in psychological research, meaning much of the data obtained concerning mental illnesses cannot be properly generalized to diverse populations (Arnett, 2008; Henrich et al., 2010). This creates problems facilitating proper diagnoses and treatments. Clinical psychologists must expand diagnostic criteria, psychotherapies, and research to be cross-culturally representative to accurately diagnose and treat clients not identifying with western, educated, industrialized, rich, and democratic cultures. More specifically, psychologists need culturally competent revisions and additions to diagnostic manuals and treatment protocols to appropriately recognize and treat mood disorders.

This literature review investigates the severity of the aforementioned issues. Furthermore, this overview reveals how underrepresentation in research leads to improper diagnosis and psychotherapy in racialized populations. Countless western societal institutions are systemically inadequate for people of diverse cultures, which keeps the dominant culture at an unethical advantage throughout life (Morgan et al., 2018). This overview exposes how the field of clinical psychology contributes to systemic racism in North America.[2]

To preface, it is important to appreciate what institutions these arguments are addressing. The focus is specifically towards the modern Canadian and American context where the main diagnostic manual is the Diagnostic and Statistical Manual of Mental Disorders (DSM), published by the American Psychiatric Association (APA) (American Psychiatric Association n.d.; Paniagua, 2018). The DSM-5 is primarily used in Canada and America, whereas the International Classification of Diseases (ICD) published by the World Health Organization is typically used internationally (American Psychiatric Association n.d.; Paniagua, 2018). Nevertheless, the DSM has become an international standard for research and there are efforts to further align editions of the DSM and ICD (American Psychiatric Association, n.d.; Bredström, 2017). Therefore, although criticisms in this overview are aimed at the DSM for the North American context, its influence on the international stage should not be underestimated.

Underrepresentation

Arnett (2008) analyzed six reputable American Psychological Association journals representing established disciplines in psychology. He revealed the magnitude of psychological research sourced from western, educated, industrialized, rich, and democratic (WEIRD) samples. A dominating 96% of all sampled participants across the publications between the years 2003-2007 came from a WEIRD culture (Arnett, 2008; Henrich et al., 2010). In fact, 82% of all participants were specifically from the UK, Canada, Australia, New Zealand, or the US; the US alone being 68% (Arnett, 2008). Furthermore, over two

[1] Non-Hispanic white is a term used by the U.S. Census Bureau to refer to people who identify as white and are not from Mexico, Puerto Rico, Cuba, Central or South America (2019).

[2] Also known as 'institutional racism', describes when societal systems and practices reduce access to opportunities based on race (Morgan et al., 2018). Where 'systemic' is defined as "fundamental to a predominant social, economic, or political practice" (Merriam-Webster, n.d.-a) and racism, "the systemic oppression of a racial group to the social, economic, and political advantage of another" (Merriam-Webster, n.d.-b).

thirds of participants were undergraduate psychology students (Arnett, 2008). Even within a country, undergraduate students only represent a narrow socioeconomic status and education level making the majority of psychological research ungeneralizable to a global and cultural scale. Researchers like Arnett (2008) posit that psychology should not consider itself a human science when its samples only culturally represent 5% of the world.

More recently, Nielsen et al. (2017) conducted a follow-up study analyzing the top three most influential developmental psychology journals by impact factor between the years 2006 and 2010. In congruence with Arnett (2008), Nielsen et al. (2017) bluntly explains that of the 1,582 papers, 3% of participants represented 85% of the global population living in "Central and South America, Africa, Asia, the Middle East and Israel" (Nielsen et al., 2017, p. 34). They also reviewed papers from 2015 and similarly found that almost 93% of participant data came from WEIRD sources, showing little to no improvement in a decade (Nielsen et al., 2017). This demonstrates that despite increased awareness, there is a lack of evidence suggesting that research is being culturally expanded, perpetuating the issue of underrepresentation and leading to inappropriate methods for diagnosing and treating mental illness in diverse populations (Bhugra, 1997; Bredström, 2017; Goodmann et al., 2020; Hinton & Patel, 2017; Zanon et al., 2020). Psychological data is largely gathered from WEIRD sources which are then responsible for informing the diagnostic criteria in the DSM-5 and other instruments related to measuring mental health. It should not be assumed that the data that informs these diagnostic manuals can be generalized to all populations for diagnosis and treatment. Countries as diverse as Canada and the U.S. must provide mental health services that adequately accommodate the needs of the growing non-WEIRD populations. More research and training are required for clinical psychologists to understand, recognize, and treat cultural expressions of mental disorders.

Symptomatic Differences Across Cultures

There are inherent differences in the way various cultures display, describe, and experience symptoms of mood disorders (Goodmann et al., 2020; O'Farrell et al., 2020; Zanon et al., 2020). According to the DSM-5 there are variations of behavioural and emotional criteria that must be met in order to be diagnosed with a mood disorder (American Psychiatric Association, 2013). Symptoms of depression, for example, are primarily related to feelings and emotions such as depressed mood, diminished pleasure, feelings of guilt, and worthlessness. These diagnostic criteria have empirical support in western populations. However, research comparing symptoms cross-culturally has shown that the criteria is not universally applicable (Goodmann et al., 2020; Kleinman, 1977; O'Farrell et al., 2020; Ryder & Chentsova-Dutton, 2012; Zanon et al., 2020).

The somatization of mood and anxiety disorders is a well documented but poorly understood phenomenon that refers to physical expressions of psychological illnesses (Kleinman, 1982). Research in Chinese populations has demonstrated a tendency to experience and primarily describe somatic symptoms such as muscle pain or fatigue for mood disorders (Kleinman, 1982). In Kleinman's (1982) ground-breaking study on the topic, he observed a sample of 100 patients in China that had been diagnosed with neurasthenia. This currently controversial diagnosis listed in the DSM-II and ICD-10 was characterized as the 'exhaustion of the central nervous system', and related to fatigue and burnout (Cheung, 1998). Neurasthenia was a more culturally acceptable diagnosis at the time, particularly in China, because it was a disease of the nervous system emphasising somatic symptoms, as opposed to a strictly psychological disorder with cultural stigma. Kleinman's review found that 87% of the patients diagnosed with neurasthenia could also be re-diagnosed with depression, according to the DSM-III (Kleinman, 1982). Given China's low rates of reported depression, Kleinman and other researchers hypothesize that it gets conflated with other physical illnesses such as neurasthenia. This is due in part to the somatization of symptoms that are poorly accounted for by psychiatric diagnostic criteria (Dere et al., 2013; Kirmayer & Sartourius 2007; Kleinman, 1982). Different manifestations of symptoms and culturally different ways of communicating these symptoms, demonstrates the difficulty in

diagnosing depression cross-culturally.

Similarly, Lehti et al. (2009) conducted a qualitative study interviewing Swedish general practitioners (GPs) about their clinical experience in recognizing depression in patients from the Middle East and Eastern Europe. GPs are forced to develop intuition anecdotally as "diagnostic manuals gave little backing [and] screening-instruments, sometimes recommended and used, were not regarded as suitable for these patients" (Lehti et al., 2009, p. 5). The GPs elaborate by explaining that bodily symptoms, physical pain, or alcohol abuse can be common indicators of depression in this demographic, albeit not mentioned in the DSM-5's diagnostic criteria. Verbal and non-verbal communication of pain and distress varies by culture, resulting in increased confusion and further complicating signs that typically serve as indicators for GPs to shift their diagnosis to mental health, as opposed to bacterial, musculoskeletal, or otherwise (Lehti et al., 2009). The GPs are criticized by their national authorities for missing diagnoses, yet the standardized screening scales and national guidelines provided are not adequate for these populations (Lehti et al., 2009). Cultural differences in communication styles further exacerbate this problem (Lehti et al., 2009; Zanon et al., 2020). A practical example of clinical miscommunication is with the word '*dépression*' in Haitian Creole which differs from the western understanding of depression (Pierre et al., 2010). The word *dépression* in Haitian culture refers to feeling empty, distractibility, fatigue, and poor appetite, which can be attributed to anything from anemia, a fixation on stress, or even a curse (Pierre et al., 2010). Zanon et al. (2020) studied cultural communication on a macro level by testing the reliability of the Depression, Anxiety, and Stress Scale-21 across eight culturally diverse countries. They concluded that although the scale is used as a research standard, they could not find support that it reliably scored depression, anxiety, or stress across cultures. Likely due to culturally diverse biases, response style, and familiarity with western survey formats (Zanon et al., 2020). Thus, more culturally competent resources must be made available for such variances in communication.

These culture-specific examples are not only observed internationally. Even immigrants who are established in North America, and second-generation immigrants, exhibit many culture-specific symptoms and experiences throughout their lifetime (Misev & Phillips 2017; Wang 2019; Wong, 2017). An *'ataque de nervios'* is a culture-specific mental health syndrome that is most experienced in Latino cultures. It is characterized as short episodes of intense emotions, lack of physical or mental control, fainting, and dissociation (Hinton & Patel, 2017; Wong, 2017). Using extensive survey data, contrary to the hypothesis, Wong (2017) found that second and third generation Latino immigrants in America reported an increased likelihood of experiencing an *ataque de nervios*. Corroborating this phenomenon is Wang's (2019) study, where they found that Asian American second-generation adolescents were significantly less likely to seek mental health services compared to their peers. Wang's study detailed that these findings are heavily influenced by the parental views of western mental health care which inevitably get passed down to children. It is disingenuous for clinical psychology to assume that acculturation will eventually correct for the lack of cultural sensitivity put into research, the DSM, and psychotherapy.

APA's Responses to Criticism

The APA is not oblivious to research on cross-cultural expressions of mental disorders and has implemented revisions and sections that claim to have dealt with cultural disparities. The proposed solutions and culturally relevant sections in the DSM-5 include three main sections. There is a brief dialogue of culture-specific expressions of symptoms under some disorders, an appendix on cultural concepts of distress previously named 'culture-bound syndromes', and a guided interview section on client-clinician cultural formulation (American Psychiatric Association, 2013). These revisions and additions are supposed to provide clinicians and researchers with adequate tools to appreciate mental illness across cultures within the DSM-5.

The dialogue highlighting culture-specific expressions, found under some disorders, typically adds various symptoms that are often recognized in foreign samples.

For instance, under the diagnostic criteria for panic disorder, the APA further lists 'culture-specific symptoms' including "tinnitus, neck soreness, headache, uncontrollable screaming or crying" (American Psychiatric Association, 2013). In the section on major depressive disorder, there is a disclaimer explaining the possible underdiagnosis in diverse cultures, also mentioning the common somatization of symptoms (American Psychiatric Association, 2013). The section goes on to indicate that 'insomnia' and 'loss of energy' are the most consistent official criteria across cultures (American Psychiatric Association, 2013).

In the appendix of the DSM-5 entitled 'Glossary of Cultural Concepts of Distress', psychologists can find narratives specifying local names and concepts for mental illness amongst different ethnic groups. Some examples they provide are '*dhat* syndrome' in southeast Asia, '*maladi moun*' in Haitian culture, and the term '*nervios*' in Latin America, for a total of nine examples (American Psychiatric Association, 2013). The appendix details what these terms of distress mean to an individual from that culture, and what DSM disorder they can relate to. This section was previously known as culture bound syndromes.

Lastly, the cultural formulation consists of a guided interview that is supposed to take place with patients to learn about their background and to relate their explanations of distress to the clinician (American Psychiatric Association, 2013). This section contains culturally relevant sample questions that the therapist can ask to develop an understanding of their client's mental health experience. The interview is accompanied by explanations regarding the reasons and goals for each question (American Psychiatric Association, 2013). These steps demonstrate that the APA has attempted to include adequate resources in the DSM-5 to avoid issues in cross-cultural diagnosis.

While these sections show improvement, they suffer from many inherent shortcomings. For instance, the short paragraph on culture-specific expressions following the 'official' symptoms is concluded by the statement, "such symptoms should not count as one of the four required symptoms" (American Psychiatric Association, 2013). This defeats the purpose of listing culture-specific symptoms because they are not acceptable for diagnosis. The diagnostic criteria in the DSM-5 is therefore only representative of the small percentage of the worlds population that has developed in a WEIRD culture. When symptoms of outside cultures are ignored, the overwhelming majority of the world is ignored, as over 85% of the global population remains virtually excluded from psychological research (Arnett, 2008; Nielsen et al., 2017). Listing symptoms representative of these underrepresented cultures, and then undermining their validity for diagnosis is counterproductive and diminishes their value, such racist language needs to be removed. Furthermore, the DSM uses rigid generalization such as 'Latin Americans' or 'Vietnamese' (American Psychiatric Association, 2013, p. 211), when it could benefit from using a more broadly intersectional lens to approach diagnosis (Bredström, 2017).

The 'Glossary of Cultural Concepts of Distress' in the appendix, previously known as 'culture-bound syndromes' is defined as "collective, shared ways of experiencing and talking about personal and social concerns" (American Psychiatric Association, 2013, p. 758). Ironically, that new definition seems to describe why 'regular' disorders are classified and categorized in the DSM at all. That is to say, all disorders characterized by the APA provide a collective understanding of a psychologically relevant concern, so why separate these ones in an appendix? Bredström (2017) criticizes the section on culture-bound syndromes' placement in an appendix, which makes them seem as 'other' and separate from the 'western' DSM disorders. The DSM is a manual contrived of its own culture-bound data and disorders, but instead of separating western cultural influence, it has become the standard. Moreover, disorders primarily found in western culture, like anorexia nervosa (Banks, 1992), are not listed under the appendix as 'cultural concepts of distress', doing so would be offensive and diminish the legitimacy of the illness (Bredström, 2017). Treating the distress of differing cultures as though they are separate perpetuates the problem. The pervasive labeling and racializing of 'other' cultures without any mention or distinctions for

western culture insidiously exposes the cultural biases present throughout the DSM-5 (Bredström, 2017). Watters (2013) points out that any omissions and placements in the DSM are of utmost importance, as it inadvertently governs everything from insurance coverage to legal ramifications (American Psychiatric Association, n.d.). Furthermore, it is disappointing that an appendix specifically referencing culture-bound syndromes was only able to include nine options to represent the rest of the world. The DSM requires improved cultural competency that addresses these mischaracterisations. Although these criticisms are specifically towards the DSM in the North American context, it is worth mentioning that many researchers have claimed the ICD-10 is arguably inferior to the DSM-5 concerning the emphasis of cultural considerations (Paniagua, 2018). These symptoms of systemic racism are seen globally.

Some would further argue that the DSM is made by western culture, for western culture, and should not have to be universally applicable throughout the cultures of the world. Further, if it studies its own people, and is only primarily used in Canada and America, it should be accurate enough for the culture it was made for. This short-sighted argument fails to appreciate the continual diversification of western countries that was addressed in the introduction. That is, almost half of Canadians were either not born in Canada or have parents who were not born in Canada (Statistics Canada, 2017). Furthermore, even for first- or second-generation immigrants who have lived in a new culture for many years, psychologists should be careful not to overestimate the acculturation of these populations. There are many systemic reasons that those minority groups are not being included in psychological research even though they live here. Minorities are typically underrepresented in societal institutions that may have language, financial, or educational barriers. Perhaps a new immigrant's previous education is not recognized in Canada and they are forced to take a lower paying job, and they are unable to pay for their children's university fees. With 67% of psychological research coming from undergraduate psychology students (Arnett, 2008), minorities do not have a representative chance to participate. It is not enough to say that American research should apply to all Americans, without looking at which Americans are truly being researched, and the significant percentage that are left unaccounted for. This is especially important for the DSM-5 which the APA claims to be a globally "authoritative guide to the diagnosis of mental disorders" (n.d.).

Psychotherapy for Ethnically Diverse Clients

After appropriately diagnosing a mood disorder, the clinician needs to provide appropriate treatment. An effective and common therapy for a variety of mental disorders is cognitive behavioural therapy (CBT). Nevertheless, when administering research-based practices of CBT, the same underlying issue prevails. As per their training, psychologists are administering therapies that have empirical support in WEIRD samples and assuming its efficacy for all clients (Bhugra, 1997). This may explain the disproportionately high therapy drop-out rate, and medication non-adherence rate among minorities in western countries, as when a client is not connecting with a therapeutic approach, they are unlikely to be motivated to continue (Bhugra, 1997). Research indicates that although CBT has some positive effects in non-WEIRD samples, it has been shown to be up to 4 times more effective when the treatment is culturally adapted to the client's cultural context (Benish et al., 2011; Crumlish & O'Rourke, 2010; Griner & Smith, 2006). These meta-analyses all found that culturally adapting therapy resulted in more effective treatment for mood disorders and PTSD (Benish et al., 2011; Crumlish & O'Rourke, 2010; Griner & Smith, 2006). In some instances, culturally adapted therapy meant placing a therapeutic emphasis on the somaticized symptoms often experienced in different cultures, demonstrating the therapist's understanding of the disorder is synonymous with the client's (Hinton et al., 2005). In others, it meant using culturally appropriate language, explicit beliefs, and known values (Griner & Smith, 2006). Cultural adaptation increases the positive expectancy of clients by using culture-specific communication styles and employing culturally appropriate CBT technique adaptations (Hinton & Patel, 2017).

Positive expectancy is crucial in therapeutic relationships as a client will be less likely to contribute the required time and energy into therapies they do not expect to work (Woodhead et al., 2012). The way to develop positive expectancy, however, significantly varies depending on the client's own belief and understanding of their problem, which is often informed and heavily influenced by culture (Hinton & Patel, 2017). For example, traditional Cambodian cultures do not typically refer to 'anxiety' or 'depression' as an illness, instead they use language such as 'thinking a lot' or 'working past one's energy stores' which can lead to dizziness and onset of a *khyâl* attack (Hinton & Patel, 2017). Latino cultures often believe that when limbs get shaky or the mind races it is a problem with nerves, or an *ataque de nervios* (Hinton & Patel, 2017). If the psychologist tells these clients that they will treat them for anxiety or depression, positive expectancy is significantly decreased as the client does not believe they have anxiety or depression, but rather a problem with nerves or an overexertion of energy. Similarly, a Chinese patient may be more willing to seek treatment for "the headaches clouding their mind" but not for depression (Kleinman, 1982). The clinician needs to acknowledge the client's symptoms and cultural understandings of the disorder and explain that their treatment will address their symptoms of dizziness, headaches, or fatigue. This strategy of clearly addressing the patients primary concern and belief increases positive expectancy (Hinton & Patel, 2017), which in turn decreases attrition and increases adherence (Bhugra, 1997). Correspondingly, the CBT techniques used must be culturally salient. For a Latino client with similar symptoms, an intervention could induce dizziness by spinning, and pair it with a more culturally positive activity, such as imagining the piñata game (Hinton & Patel, 2017).

Just as a client's communication style can lead to misunderstandings, so can the clinician's. Culturally adapting communication styles can be, for example, developing a knowledge of proverbs, sayings, or analogies used in that culture (Hinton & Patel, 2017). Many of these culture-specific figures of speech have therapeutically relevant morals, such as anger regulation, seeking help under stress, or expanding focus. These strategies increase therapeutic alliance and culturally relevant communication, promote positive affect, and increase the client's cultural self-esteem (Hinton & Patel, 2017). Some practical examples of this are, the Cambodian proverb 'If you don't become angry 1 time, it gains you 100 days of happiness' to encourage temper regulation; the Latino saying 'don't drown in a glass of water' to become less hyperfocused; or the African-American term 'rebound' referring to making a comeback from a perceived failure (Hinton & Patel, 2017).

It is important to acknowledge that not all non-western people and culture has come to Canada and America through immigration. Indigenous nations within the western world have deep roots of rich cultural heritage that many identify with today (Stewart, 2008). Psychotherapy assumes a western understanding of mental health and the self, which can create a disconnect for any differing culture (Kirmayer, 2007). Indigenous peoples living in Canada seek mental health treatment disproportionately less, and the lack of cultural competency from western psychology may be to blame (Stewart, 2008). They are subject to unique distresses and traumas as a result of ongoing colonialism that has attempted to disconnect them from their cultures, which they rely on for wellness and healing (Stewart, 2008). Canada's mental health services do not adequately ground its paradigms in the Indigenous experience to properly serve these populations (Stewart, 2008). Psychotherapy carries the assumption of the individualistic self, championing self-efficacy, which does not always align with Indigenous ways of knowing and being (Kirmayer, 2007). Mental health from Indigenous perspectives often focuses on wellness and healing, which are both required when balancing the four parts of the self: spiritual, emotional, physical, and mental (Mussell et al., 1993; Stewart, 2008). Stewart interviewed mental health professionals who work with Indigenous clients and social services. Their knowledge described four connected meta-themes to model mental health: community, cultural identity, interdependence, and a holistic approach. Incorporating Indigenous knowledge and values into therapeutic interventions may increase positive expectancy in those communities, leading to

an increase in the usage of mental health services, and healing (Stewart, 2008).

Conclusion

Clinical psychology suffers from gaps in scientific knowledge and understanding on effectively treating culturally diverse populations. This is not the fault of clinicians who simply follow the guidelines in the DSM-5 and the evidence-based therapies they are trained in. These manuals provided by the APA need to embrace culture as a factor in all disorders and within the entire field of psychology, instead of creating a distinction between WEIRD disorders and cultural variations. The western system of mental health care systemically prioritizes the health of western culture and neglects the health of ethnically diverse cultures (Arnett, 2008; Bhugra, 1997; Lehti et al., 2009; Nielsen et al., 2017). Inevitably, the resulting society leaves cultural minorities with poorer mental health (Bhugra, 1997; Stewart, 2008). Marginalizing these groups results in a disproportionate increase in treatable mood disorders, propagating significant, preventable, consequences for their family, career, and other aspects of life, further perpetuating a detrimental cycle (Bhugra, 1997; Morgan et al., 2018). This is how the field of clinical psychology contributes to systemic racism through its western centric research, diagnostic manuals, and therapies.

Moving forward, including ethnically diverse participants in research is of paramount importance to address the roots of the problem. A proportionate amount of research and data representative of the diverse population would increase accessibility and lead to a greater emphasis on updating diagnostic manuals and developing effective psychotherapies. When conducting psychological research, the standard is to gather basic demographic information such as age and sex. Cultural identity should become a basic permanent demographic question so that variables can always be compared for potential differences. The current situation in research is dire with only 5% of the world being accounted for in 2008 (Arnett) and little to no improvement since then (Nielsen et al., 2017). Such a situation calls for potentially drastic measures, for example the APA could implement diversity requirements for participants in order to pass the peer reviewal process. There could be a participant diversity quota that universities must satisfy by the end of the year. Some could call this extreme, but the concern has been raised about whether psychology can even be considered a human science with such a small field of view (Arnett, 2008). The APA should also integrate research being published internationally to develop a more culturally comprehensive understanding of clinical psychology for the diverse populations living and immigrating to Canada and America.

Nonetheless, there are strategies to increase cultural competence in the clinician population. This can mean increasing personal awareness of how diverse cultures display symptoms of mental disorders and how that differs from the west. It can also involve putting in the effort to learn more about the different cultural groups who reside in their area of practice. Learning every culture is a difficult task, however the clinician's community may have a couple of prominent cultures which would be worth learning about to ameliorate therapeutic experience. In the absence of culturally diverse empirical data, clinical and personal experience is valuable and can be shared amongst colleagues, leading to increased cultural awareness, relevant training, and even research. Culture is the lens through which people develop, see, and interpret the world making it always relevant when therapeutically targeting cognitions, behaviours, and emotions. In the same way, the DSM and CBT are both a product of culture; western culture. As such, they must be removed from the context of their own bias before they can be properly related and applied to diverse clients. An increased sensitivity will result in more accurate diagnoses and efficacious psychotherapies.

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