Serotonin depletion amplifies distinct human social emotions as a function of individual differences in personality

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Abstract

Serotonin is involved in a wide range of mental capacities essential for navigating the social world, including emotion and impulse control. Much recent work on serotonin and social functioning has focused on decision-making. Here we investigated the influence of serotonin on human emotional reactions to social conflict. We used an innovative computerised task that required mentally simulating social situations involving unjust harm and found that depleting the serotonin precursor tryptophan – in a double-blind randomised placebo-controlled design – enhanced emotional responses to the scenarios in a large sample of healthy volunteers (n = 73), and interacted with individual differences in trait personality to produce distinctive human emotions. Whereas guilt and shame were preferentially elevated in highly empathic participants, annoyance was potentiated in those with high trait psychopathy and more impulsive participants. Effect size of serotonin depletion on emotion was medium to large (the largest was for shame, η_p^2 = .190). Our findings show how individual differences in personality, when combined with fluctuations of serotonin, may produce diverse emotional phenotypes. This has implications for understanding vulnerability to psychopathology, determining who may be more sensitive to serotonin-modulating treatments, and casting new light on the functions of serotonin in emotional processing.

Introduction

A unified function for serotonin (5-hydroxytryptamine; 5-HT) has, perhaps unsurprisingly, proven to be elusive. It is hypothesised to have a role in many psychiatric disorders (Dayan & Huys, 2009), and is implicated in a wide range of mental functions including aversive processing, impulse control, and social behaviour (Cools et al. 2008). These domains can be viewed under a unified framework by considering how serotonin impacts Pavlovian (stimulus-outcome, including emotional) and instrumental (stimulus-response-outcome; behavioural) processes that underlie both social and non-social functions (Gesiarz & Crockett, 2015). Much recent work on the relationship between serotonin, aversive processing, and human social behaviour has focused on instrumental action in the context of behavioural economic games and moral dilemmas (Crockett et al. 2008, 2010a, 2010b). Here we examined emotional reactions to social scenarios depicting unjust harm.

Studies of healthy volunteers have primarily employed two techniques to investigate serotonin function: acute tryptophan depletion (ATD), a dietary technique that temporarily lowers brain serotonin levels by depleting its biosynthetic precursor, tryptophan (Bel & Artigas 1996; Biggio et al. 1974; Crockett et al. 2012a; Hood et al. 2005; Nishizawa et al. 1997), and treatment with single doses of selective serotonin reuptake inhibitors (SSRIs). SSRIs are generally assumed to increase extracellular serotonin, however it should be noted that single rather than chronic doses can paradoxically decrease serotonin in projection areas (Cools et al. 2008; Nord et al. 2013). ATD studies have revealed disinhibition of retaliatory behaviour in the face of perceived injustice (Crockett et al. 2008), modelled using the Ultimatum Game (UG) which is defined in Figure 1, whilst single dose SSRI has had pro-social effects (Crockett et al. 2010a).

Morally relevant social emotions, meanwhile, lie at the interface between moral standards and socially appropriate behaviour (Tangney et al. 2007). Moral standards prohibit behaviours that are likely to have negative consequences for the well being of others. Emotions are in part Pavlovian in nature, and while ATD has been shown to modulate

Pavlovian processes in non-social domains (Crockett et al. 2012b; Hindi Attar et al. 2012), here we tested the influence of ATD on emotion in a social context. We asked whether ATD would enhance morally relevant negative emotions evoked by social scenarios in which a person was unjustly harmed: these served as Pavlovian cues. Using an innovative task, we prompted participants to reflect on the situations and specifically assessed emotions involving annoyance, guilt, shame, and feeling "bad". Reporting one's emotional reactions after mentally simulating hypothetical social scenarios implicitly calls on autobiographical memories, which should result in a diversity of subjective experiences tied to personal qualities and expectations about social behaviour. We therefore also tested the influence of three personality traits – empathy, psychopathy, and impulsivity – on how serotonin would affect emotion.

Research on moral emotions has focused mostly on the self-conscious negative emotions guilt and shame (Tangney et al. 2007), depicted in Figure 1. Guilt often relates to a negative appraisal of a specific behaviour, whereas shame tends to involve a negative evaluation of the self (Tangney et al. 2007). Whilst guilt is part of the diagnostic criteria for depression (APA 2013), proneness to shame most consistently relates to an array of psychiatric conditions, including symptoms of depression (Ashby et al. 2006; Crossley & Rockett 2005; Stuewig & McCloskey 2005), anxiety (Crossley & Rockett 2005), post-traumatic stress disorder (Andrews et al. 2000; Brewin et al. 2000; Feiring & Taska 2005; Leskela et al. 2002; Orsillo et al. 1996), eating disorders (Murray et al. 2000; Sanftner et al. 1995), as well as more specific symptoms such as low self-esteem (Feiring et al. 2002), suicidal ideation (Bryan et al. 2013), anger (Harper & Arias 2004; Tangney et al. 1996), and aggression (Tangney et al. 1996). Importantly, guilt is thought to become maladaptive primarily when it is fused with shame (Tangney et al. 2007).

Evidence from patients with damage to the ventromedial prefrontal cortex (vmPFC) and incarcerated individuals with psychopathy provides a plausible connection between moral emotions and social behaviour, outlined in Figure 1. The vmPFC is an area central to emotion regulation (Schiller & Delgado, 2010), with dense serotonergic innervation (Hornung, 2010). Individuals with vmPFC damage also show increased retaliatory

behaviour to unfairness on the UG (Koenigs et al. 2007), which mirrors the ATD findings (Crockett et al. 2008; 2010b). This is furthermore analogous to the UG results from psychopathic individuals (Koenigs et al. 2010), where vmPFC dysfunction is a feature (Motzkin et al. 2011), as is reduced guilt (Blair 2010). Impaired moral behaviour following damage to the vmPFC has meanwhile been conceptualised as a manifestation of diminished guilt (Krajbich et al. 2009). The implication here is that guilt is related to inhibition of anti-social behaviour, as modelled by restraint in behavioural economic games like the UG. Studying the effects of ATD on guilt could therefore inform how moral emotion and behaviour are integrated.

Proneness to guilt consistently correlates with empathy, which refers to the ability to share the affective experiences of others (Tangney et al. 2007). Empathy, while not a discrete emotion, is a morally relevant emotional capacity (Tangney et al. 2007) and trait (Davis 1980). Guilt appears to foster reparative action, promote empathy (Tangney et al. 2007), and increase altruistic acts (O'Connor et al. 2007). Elevated empathy, moreover, has been correlated with severity of depression, and proposed as a risk factor for its development (O'Connor et al. 2002). Importantly, empathy is classically absent in psychopathy (Blair et al. 1996). These relationships from the literature are summarised in Figure 1.

We were especially interested in empathy because moral decision-making in individuals high in trait empathy has been shown to be particularly sensitive to manipulations of serotonin (Crockett et al. 2010a). Furthermore, the serotonin 2A (5-HT2A) receptor agonists lysergic acid diethylamide (LSD), psilocybin, and 3,4-methylenedioxy methamphetamine (MDMA), have all been shown to enhance empathy (Dolder et al. 2016; Hysek et al. 2014; Pokorny et al. 2017). To extend these findings we therefore tested the hypothesis that ATD would interact with trait empathy to amplify morally relevant social emotion. Given the consistent correlations between guilt-proneness and empathy (Tangney et al. 2007), we predicted guilt would be the most likely emotion to be affected.

Conversely, psychopathy is characterised by emotional dysfunction reflected in reduced guilt and empathy (Jones et al. 2010), and an increased risk for aggression, outlined in Figure 1. Aggression can be either goal-directed (e.g. a premeditated crime), or reactive: an explosive, impulsive response to frustration (Blair 2010). Many psychiatric conditions increase the risk for reactive aggression, however psychopathy is unique in that there is an increased risk for both reactive and goal directed aggression (Blair 2010). Aggression is in turn traditionally associated with low serotonin (Deakin 2003), including evidence from studies of violent offenders (Linnoila et al., 1983). We explored whether psychopathic traits are likewise related to morally relevant emotions, and whether ATD modulates this relationship. We predicted that psychopathic traits might have the most pronounced effect on feeling annoyed, which invokes the notion of frustration.

While some, but not all, aggression can be impulsive, aggression and impulsivity are distinct. Indeed, discrete serotonergic circuits modulate aggressive versus impulsive behaviour in mice (Nautiyal et al. 2015). ATD can induce "waiting impulsivity" (diminished action restraint whilst waiting for a reward) and "impulsive choice" (accepting small immediate rewards over larger delayed ones) in healthy individuals (Crockett et al. 2010b; Worbe et al. 2014). More impulsive choice has been correlated with increased aggressive impulses to perceived injustice on the UG, which was heightened further by ATD (Crockett et al. 2010b). We therefore asked whether trait impulsivity on the Barratt Impulsiveness Scale (BIS; Patton et al. 1995) was related to increased annoyance following ATD.

To test our hypotheses we employed ATD in a double-blind, randomised, placebo-controlled, between-groups design, in healthy volunteers. We predicted that ATD would enhance negative emotion overall, and that individual differences in empathy, psychopathy, and impulsivity would influence how ATD modulated the profile of emotion. In line with the traditional disconnection between psychopathy and empathy, we predicted that there would be dissociation between how trait empathy and psychopathy interact with neurochemical status to modulate annoyance, guilt, shame, and feeling bad. Given the established connection between serotonin and impulsivity (Bevilacqua et al.

2010; Dalley & Roiser 2012; Worbe et al. 2014), and retaliatory behaviour (Crocket et al. 2010b), we also hypothesised that high trait impulsivity would be related to increased feelings of annoyance, which would be further potentiated by ATD in these individuals.

Results

Seventy-three participants completed the study: thirty-seven underwent depletion (20 males), while the remaining 36 received placebo (19 males).

Effects of ATD on mood ratings

Mood ratings were unaffected by ATD. We collected rating data from 65 participants (n = 33 depletion) on how happy or sad they were feeling before the task, after depletion had taken effect, and these ratings did not differ from those of participants in the placebo group ($t_{(63)} = -0.727$, $p = .47$).

How serotonin depletion modulates emotional ratings overall

We tested whether serotonin depletion potentiated emotions overall, irrespective of individual differences. To do this we performed repeated measures analysis of variance (ANOVA) incorporating all four emotions measured. Serotonin status (ATD versus placebo) was the between-subjects factor, and the within-subjects factors were emotion (annoyance, guilt, shame, and feeling bad), agency (agent or victim of harm), and intentionality (intentional or unintentional harm). Indeed, serotonin depletion potentiated emotion overall ($F_{(1,71)} = 5.959$, $p = .017$, $\eta_p^2 = .077$), shown in Figure 3 and Table 1. There were no interactions with agency or intentionality (all $p > .05$): these factors were subsequently seldom shown to be significant moderators and we only discuss below those instances in which they had influence. Our core results in this study however came from analyses of how individual differences interacted with the serotonin-depleted state to modulate emotion, which now follows.

Correlations between trait measures

First, we ensured that our trait measures (see Methods) of interest were not correlated with one another. Scores on the impulsivity and psychopathy scales were not correlated ($r_{(73)} = -.113$, $p = .342$). Empathy scores were likewise not correlated with impulsivity ($r_{(73)} = .094$, $p = .427$) or psychopathy ($r_{(73)} = .015$, $p = .897$).

How trait empathy modulates emotional effects of serotonin depletion

Given prior evidence that single dose SSRI had a more pronounced effect on social behaviour in highly empathic participants (Crockett et al. 2010a), a central question in our study was whether the serotonin-depleted state and empathic trait interacted to influence emotion. Indeed, we found that the self-conscious emotions guilt and shame were more sensitive to serotonin depletion in individuals with high trait empathy. We analysed emotional ratings via analysis of covariance (ANCOVA), using empathy and serotonin status (ATD versus placebo) as between-subjects factors, and agency (agent or victim) and intent (intentional or unintentional) as within-subjects factors. For guilt ratings, this revealed a significant serotonin-by-empathy interaction ($F_{(2,70)} = 4.278$, $p = .018$, $\eta_p^2 = .109$). Guilt ratings were significantly higher in more empathic individuals following serotonin depletion, seen in Figure 4a and Table 1. For shame, the results were even stronger, with a highly significant serotonin-by-empathy interaction ($F_{(2,70)} = 8.207$, $p = .001$, $\eta_p^2 = .190$). Individuals with higher trait empathy scores reported feeling more ashamed overall, and this relationship was even stronger following serotonin depletion; ratings of shame increased even more with higher trait empathy, shown in Figure 4b and Table 1. There was also a significant three-way interaction between serotonin status, empathy, and agency ($F_{(2,70)} = 4.411$, $p = .016$, $\eta_p^2 = .112$). Feelings of shame were exacerbated by serotonin depletion, with increasing trait empathy, especially when the participant imagined being the victim of harm. The ANCOVA on annoyance revealed a marginally significant serotonin-by-empathy interaction, ($F_{(2,70)} = 3.098$, $p = .051$, $\eta_p^2 = .081$). The ANCOVA on feeling "bad", finally, yielded a non-significant trend for a serotonin-by-empathy interaction ($F_{(2,70)} = 2.648$, $p = .078$, $\eta_p^2 = .070$). Here and below, we followed up ANCOVAs yielding significant two-way interactions with simple linear regression analyses to test whether trait scores predicted emotional ratings (collapsed

across agency and intentionality). We reported instances where a significant trait-emotion relationship was present in either the placebo or depletion conditions. Level of trait empathy significantly predicted the extent of guilt ratings following depletion ($\beta = .016$, $p = .019$) and not on placebo ($\beta = .007$, $p = .118$). Meanwhile, empathy predicted shame ratings following both depletion ($\beta = .027$, $p = .007$) and on placebo ($\beta = .020$, $p = .010$), denoted in Table 1. Guilt and shame, the two self-conscious emotions measured by the task, were most affected by the interaction between trait empathy and the serotonin-depleted state. Serotonin therefore induced a distinct emotional profile in highly empathic individuals.

How trait psychopathy modulates emotional effects of serotonin depletion

Trait psychopathy also interacted with the tryptophan-depleted state to modulate emotion. ANCOVA with serotonin status (ATD versus placebo) and psychopathy as between-subjects factors, and agency and intentionality as within-subjects factors revealed a significant serotonin-by-psychopathy interaction for feelings of annoyance ($F_{(2,70)} = 4.022$, $p = .022$, $\eta_p^2 = .103$). With increasing trait psychopathy, individuals felt even more annoyed following serotonin depletion, seen in Figure 5a and Table 1. Next, we assessed guilt using the same ANCOVA approach: there was no serotonin-by-psychopathy interaction ($F_{(2,70)} = 2.265$, $p = .111$, $\eta_p^2 = .061$). There was no serotonin-by-psychopathy interaction for shame ($F_{(2,70)} = 1.369$, $p = .261$, $\eta_p^2 = .038$) nor for feeling bad ($F_{(2,70)} = 1.213$, $p = .303$, $\eta_p^2 = .033$).

How trait impulsivity modulates emotional effects of serotonin depletion

Trait impulsivity, measured at baseline, interacted with the serotonin-depleted state to modulate emotion. First, we assessed feelings of annoyance using ANCOVA with serotonin status (ATD versus placebo) and impulsivity as between-subjects factors, and agency (agent or victim) and intent (intentional or unintentional) as within-subjects factors: there was a serotonin-by-impulsivity interaction. Individuals higher in trait impulsivity felt more annoyed in reaction to the task scenarios and this was potentiated by serotonin depletion ($F_{(1,70)} = 3.417$, $p = .038$, $\eta_p^2 = .089$), shown in Figure 5b and Table 1. We performed the same ANCOVA instead for guilt: there was no serotonin-by-

impulsivity interaction ($F_{(1,70)} = .451$, $p = .639$, $\eta_p^2 = .013$). The same was true for shame: no serotonin-by-impulsivity interaction ($F_{(1,70)} = .342$, $p = .712$, $\eta_p^2 = .01$). The ANCOVA for feeling bad, meanwhile, revealed a significant serotonin-by-impulsivity interaction ($F_{(1,70)} = 4.524$, $p = .014$, $\eta_p^2 = .114$). Higher trait impulsivity was associated with feeling significantly less "bad" about harm in the social scenarios on placebo, though not following serotonin depletion. Accordingly, greater trait impulsivity was predictive of the degree to which participants felt less "bad" when on placebo ($\beta = -.038$ $p = .030$), and this relationship was not present following depletion ($\beta = -.025$, $p = .171$).

Principal Components Analysis

We also explored whether there was any structure underlying our task measurements that was not detectable by our prior analyses. To do this, we used principal components analysis (PCA) on the 16 outcome variables from the task. The validity of this PCA was confirmed (see Supplementary). We then interpreted how the task measurements from our experiment clustered into the 4 principal components. Component 1 centres on annoyance with others for having done harm to oneself – in other words, outward frustration. The predominant theme of component 2 is inward frustration, or annoyance with oneself for having harmed another. Components 3 and 4 centre on the self-conscious negative emotions guilt and shame. Component 3 captures these emotions when the participant is the agent, component 4 when the participant was the victim of harm. We then used the estimated factor scores for each individual to assess how serotonin depletion modulated the constructs captured by the 4 components. ANOVA with serotonin status (ATD versus placebo) as between-subjects factor, and the 4 components as within-subjects factors, revealed a significant serotonin-by-component interaction ($F_{(3,213)} = 3.165$, $p = .025$, $\eta_p^2 = .043$). There was no main effect of serotonin depletion ($F_{(1,71)} = 2.187$, $p = .144$, $\eta_p^2 = .030$). Follow up t-tests revealed the values of component 2 ($t(71) = 2.124$, $p = .037$) and component 4 ($t(71) = 2.290$, $p = .025$) were each significantly greater following depletion relative to placebo, as seen in Figure 6. Inward frustration, or annoyance, for having harmed another – captured by component 2 – was potentiated by serotonin depletion. When the victim of harm in the task, self-conscious negative emotion was also exacerbated by serotonin depletion (component 4).

Blood Analysis

The ratio of tryptophan to large neutral amino acids (TRP:LNAAs; valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine) was calculated, as this is thought to be most reflective of the extent of brain serotonin depletion (Fernstrom, 1979). We then performed a t-test on the change in the TRP:LNAA ratio between samples taken at baseline and approximately 4.5 hours following administration of the mixture. Plasma levels were unavailable for two participants: one due to a staff processing error, and one due to difficulty with venepuncture. We achieved a robust depletion of tryptophan ($t_{(60)} = -19.163$, $p = 3.01 \times 10^{-27}$).

Summary of results

Results are summarised in Table 1. Serotonin depletion enhanced emotion overall. Examining individual trait differences revealed a deeper story. Guilt and shame were significantly enhanced by serotonin depletion in highly empathic participants, which was a distinctive emotional profile. High trait psychopathy following serotonin depletion, meanwhile, was associated with enhancement of annoyance only. High trait impulsivity also enhanced the effects of serotonin depletion on annoyance.

Discussion

Using an innovative test that cued autobiographical memories, we showed that serotonin depletion heightened emotional reactions when mentally simulating social scenarios involving unjust harm. Whilst emotion was enhanced non-specifically at the group level, harnessing baseline individual differences revealed that personality traits play a critical role in shaping which distinctive types of emotions are affected by serotonin depletion. A key result was that individuals high in trait empathy showed a distinct profile of both enhanced guilt and shame following serotonin depletion. This contrasted with how variation in trait psychopathy (classically associated with lack of empathy and guilt), as well as impulsivity, influenced the relationship between serotonin depletion and emotional reactions: only annoyance was potentiated. This dissociation, in other words,

mirrored the antithetical nature of empathy and psychopathy. Previous studies have shown that traits can influence the magnitude of effects of serotonin manipulations on social behaviour (Crockett et al. 2010a, 2010b); we now show that traits modulate the quality, as well as the magnitude, of social emotion following serotonin depletion. Traits contribute to an individual's model of the world, and therefore shape prior expectations about social interactions: we propose the influence of traits on how serotonin modulated emotions can be thought of as constituting biological "priors".

Emotions prepare the body for action or inaction (Tooby and Cosmides, 2008). Our findings on the serotonergic modulation of social emotion converge with the literature on social decision-making, and we propose that our results represent a Pavlovian influence that can shape social behaviour (Gesiarz & Crockett, 2015). The deployment of empathy has additionally been described as having a Pavlovian character, which can shape behaviour triggered by cues signalling harm (Gesiarz & Crockett, 2015). Whilst we did not measure behaviour, there are some intriguing parallels between our findings on emotional reactions to unjust harm, and studies of retaliatory behaviour to unfairness, as assessed using the Ultimatum Game (see Figure 1; Crockett et al. 2008, 2010b), which we highlight below. Indeed, the UG has been studied under serotonin depletion and in relation to empathy, psychopathy, and impulsivity.

Empathy was of central importance to our analysis. This was motivated by the observation that social behaviour in highly empathic participants was especially sensitive to single dose SSRI administration: these individuals showed the greatest reduction in retaliatory behaviour to unfairness (Crockett et al. 2010a). Critically, we found that individuals high in trait empathy had a distinct profile of enhanced emotion: guilt and shame were amplified. Empathy and guilt are consistently correlated (Tangney et al. 2007), and guilt may even promote empathy (Tangney et al. 2007). Feelings of guilt have been associated with real-life altruistic acts (O'Connor et al. 2007). Guilt has been proposed to restrain antisocial behaviour as modelled by laboratory tests: a diminished sense of guilt is thought to contribute to dysfunctional social behaviour following vmPFC damage (Krajbich et al. 2009), and is also a feature of psychopathy (Blair 2010).

Guilt and shame clustered together when we assessed the influence of empathy, which we did not observe with trait psychopathy or impulsivity. Our PCA additionally revealed that guilt and shame grouped together, and that component was elevated by serotonin depletion regardless of personality traits. This was particularly true when the victim of harm, as was the case for shame in high trait empathy. Guilt and shame are distinguishable yet can overlap (Tangney et al. 2007), which is also true of their neural correlates (Wagner et al. 2011). Shame-free guilt is seen as possibly adaptive – for instance by promoting reparations – whilst proneness to shame is seen as a risk for, and is indeed associated with a wide range of psychopathology (Tangney et al. 2007). Importantly, guilt is thought to become maladaptive primarily when it is fused with shame (Tangney et al. 2007). Guilt overlaid with shame is most likely a source of rumination (Tangney et al. 2007). The hippocampus is involved in the experience of shame (Bastin et al. 2016), and failure of hippocampal serotonin is suggested to contribute to rumination (Deakin 2013). SSRIs, meanwhile, improve hippocampal function in depression (Dale et al. 2016). Individuals with hippocampal lesions, moreover, appear to show heightened reactive emotionality congruent with their behaviour in moral decision-making tasks, which is antithetical to the pattern seen with vmPFC lesions (McCormick et al. 2016). While the relationship with our results is merely speculative, hippocampal dysfunction is a feature of numerous psychiatric conditions, as is social dysfunction: a recent framework proposes these two well established phenomena can be unified through the purported role of the hippocampus in organising social information (memories), via relational maps that support simulations of social outcomes (Schafer & Schiller 2019).

Indeed, there are reported links between elevated empathy and depression (O'Connor et al. 2002). Individuals sensitive to distress in others may be more likely to experience personal distress, and this has been highlighted as a vulnerability factor for depression (O'Connor et al. 2002, 2007). At the same time, there is evidence for diminished deployment of theory of mind – non-affective perspective taking – in depression (Wolkenstein et al. 2011), and this combination raises the possibility that sensitivity to

distress in oneself and others may become misattributed or inappropriately directed inward.

Whilst mood was unaffected in our study, consistent with the literature on ATD in healthy individuals (Bell et al. 2005), ATD can transiently reinstate low mood in depressed individuals successfully treated with an SSRI (Bell et al. 2005). By using trait measurements and a task that elicited emotions, however, we were able to uncover a pattern reminiscent of depression: more guilt and shame in the highly empathic under serotonin depletion. Indeed, this task has already been used to detect possible latent vulnerabilities in a healthy population with trait paranoia (Savulich et al. 2018). We propose that empathy, which produced a qualitatively unique emotional profile under ATD, may represent an important proxy for sensitivity to changes in serotonin.

Conversely, psychopathic individuals classically have impairments in guilt and empathy, and an increased risk for aggressive behaviour, especially following frustration (Blair 2010; Jones et al. 2010). This is consonant with our results. We found that the emotional profile following serotonin depletion in healthy individuals high in psychopathic traits dissociated from what we observed in the highly empathic: annoyance was instead amplified following unjust harm. This result is furthermore in line with existing literature on social decision-making: clinically psychopathic individuals show an analogous pattern of behaviour on the UG (Koenigs et al. 2010) to the disinhibited aggressive impulses seen in ATD studies of healthy volunteers (Crockett et al. 2008), that is also quantitatively similar to how individuals with vmPFC lesions behave on the UG (Koenigs et al. 2010). Critically, vmPFC damage is associated with impaired emotion regulation, and individuals with such lesions tend to exhibit anger and irritability particularly following frustration in their personal lives (Koenigs et al. 2007). Diminished structural and functional connectivity between the vmPFC and amygdala in clinically psychopathic individuals (Motzkin et al. 2011) is indeed thought to be a central mechanism underlying the condition. Interactions between these structures are furthermore sensitive to ATD in healthy individuals viewing facial signs of aggression (Passamonti et al. 2012). That serotonin depletion made participants high in trait psychopathy more annoyed by social

injustice may be relevant for understanding how serotonin affects the emotional basis of retaliatory behaviour to unfairness (Crockett et al. 2008, 2010b). This view is supported by work showing that such behavioural reactions are associated with self-reported anger in healthy volunteers (Pillutla & Murnighan, 1996). Trait anger and psychopathy in violent offenders indeed appears to reflect 5-HT1B receptor levels (da Cunha-Bang et al. 2016), which moreover fits with the vast literature implicating serotonergic dysfunction in aggression (Bevilacqua et al. 2010; Deakin 2003; Frankle et al. 2005).

Whilst some aggression can be impulsive (Bevilacqua et al. 2010), aggression can also be goal-directed (e.g. planning a crime), which is more closely associated with psychopathy (Blair 2010). Aggression and impulsivity are in fact distinct phenomena. Dissociable serotonergic circuits, both involving the 5-HT1B receptor, have been identified that modulate aggressive versus impulsive (impaired waiting and action restraint) behaviour in mice, for instance (Nautiyal et al. 2015). In contrast to the construct of impulsivity, psychopathy inherently applies to social processes. Indeed, ATD can induce (waiting and choice) impulsivity in healthy individuals outside of the social realm (Crockett et al. 2010b; Worbe et al. 2014). Critically, trait psychopathy as measured here was not correlated with trait impulsivity. We found annoyance was also potentiated following serotonin depletion in individuals higher in self-reported trait impulsivity. This aligns with work showing impulsive choice in healthy individuals correlated with retaliatory behaviour, both of which increased in tandem following serotonin depletion (Crockett et al. 2010b). Our evidence therefore suggests that another distinct baseline trait – impulsivity – when combined with alterations in serotonin function can modulate emotional reactions, which may in turn promote maladaptive behaviour. This sensitivity of impulsivity to serotonin depletion is consistent with a wide literature of strong ties between impulsivity (Crockett et al. 2010b; Dalley & Roiser 2012; Winstanley et al. 2004; Worbe et al. 2014), as well as impulsive aggression (Bevilacqua et al. 2010; da Cunha-Bang et al. 2016; Deakin 2003; Frankle et al. 2005), and low serotonin.

The individual differences we observed in response to a challenge of brain serotonin are likely in part related to the relative contribution of the multiple serotonin subsystems in

the brain. Deakin (2003), importantly, has outlined how preferential dysfunction in the median or dorsal raphe nuclei, which innervate, among other regions, the hippocampus and prefrontal cortex, respectively, is putatively related to phenotypes as divergent as depression and antisocial personality disorder, respectively; both nuclei project to the amygdala. Recent data underscore the complexity of serotonin subsystems, revealing that even within the dorsal raphe there are subsystems that have distinct and at times opposing functions: both activate to reward but have opposing responses to aversion (Ren et al. 2018).

A limitation of our experiment is we did not measure serotonin (5-HT) directly: we measured plasma tryptophan levels following depletion, as tryptophan is the amino acid precursor of serotonin and ATD has been shown to produce transient reductions in central serotonin function in humans (Nishizawa et al. 1997). Whilst the validity of ATD as a method to manipulate central serotonin has been questioned (van Donkelaar et al. 2011), this position has been rebutted on the basis of considerable evidence (Crockett et al. 2012a). Consonant results between human studies employing ATD, and rodent experiments that induce profound serotonin loss using the neurotoxin 5,7-DHT, bolster the case that ATD reduces central serotonin. A prime example comes from studies of waiting impulsivity, which can be induced in humans following ATD (Worbe et al. 2014), and in rats after serotonin depletion via 5,7-DHT (Winstanley et al. 2004). It is also important to note that our task did not measure positive emotions. While ATD is associated with evoking negative biases (Cools et al. 2008), consistent with our results, future work will be required to clarify whether positive emotions to social scenarios would be blunted or potentiated by ATD.

Our data from an innovative test, that required drawing on autobiographical memories to mentally simulate cued social scenarios, demonstrate that there are important individual differences in the way serotonin influences how we react emotionally to social injustice. This should not come as a surprise given the intricacy of the serotonin systems and the complexities of human emotion and behaviour. Whilst serotonin depletion potentiated the magnitude of emotion non-specifically at the group level, personality traits played a

critical role in shaping which distinctive types of emotions were affected. There was a qualitative dissociation in the way trait empathy, relative to psychopathy and impulsivity, amplified social emotion following serotonin depletion. Previous ATD studies on social cognition, by contrast, examined behavior rather than emotion and found changes in the magnitude but not the quality of effects (Crockett et al. 2008; 2010b). We propose that traits in conjunction with the memories our task evoked represent biological priors, which prime individuals to have different emotional reactions in the social world. Our data indicate serotonin would affect the gain. Given emotions are a prescription for action (Tooby and Cosmides, 2008), it follows that our results could represent how serotonin impacts social behaviour via underlying emotional responses, positioned at the nexus of a social Pavlovian influence over action (Pavlovian action selection). When considering apparent paradoxes in the serotonin literature (Cools et al. 2008; Deakin 2003) and designing future studies, it is critical to note that the quality and magnitude of effects of a single serotonin manipulation can depend on personality. These data additionally inform the neurochemical basis of psychopathology associated with excessive emotions such as guilt and shame. Our findings on the interaction between the serotonin depleted state and personal attributes could help inform which individuals are particularly vulnerable to pathological emotional reactions, and who may be more amenable to serotonin-modulating treatments, with implications for psychiatric classification in frameworks such as the Research Domain Criteria (RDoC; Cuthbert and Insel, 2013).

Methods

Participants.

Seventy-three healthy participants (39 males, mean age 24.6) completed the experiment. Participants were medically healthy and screened to be free from any psychiatric disorders, using the mini international neuropsychiatric interview (MINI; Sheehan, et al., 1998). Individuals who reported, during screening, having a first-degree relative (parent or sibling) with a psychiatric disorder were also excluded. Other exclusion criteria included neurological disorders, pregnancy, current use of any regular medication excluding contraceptive pills, past use of neurological, psychiatric, or endocrine medication, use of St. John's Wort, regular consumption of over 38 units of alcohol per

week, consumption of more than five cigarettes per day, use of cannabis more than once per month, use of other recreational drugs besides cannabis more than five times in the lifespan, cardiac or circulation problems, respiratory issues including asthma, gastrointestinal disorders, kidney disorders, thyroid problems, head injury, a bleeding disorder, and diabetes. Participants gave informed consent before the start of the study and were paid for their participation.

General Procedure

The protocol was approved by the Cambridge Central Research Ethics Committee (16/EE/0101), and the study took place at the National Institute for Health Research / Wellcome Trust Clinical Research Facility at Addenbrooke's Hospital in Cambridge, England. Participants arrived in the morning of the study day having fasted for at least 9 hours beforehand, and completed a baseline 16-item visual analogue scale (VAS) to assess mood and other feelings including alertness. The VAS was also completed during the middle and end of the day. Participants then gave a baseline blood sample, and ingested either a placebo or tryptophan depletion drink. In the afternoon, participants gave a second blood sample, approximately 4.5 hours after ingesting the drink, and completed the Moral Emotions task (described below), along with several other tasks that will be reported elsewhere. Participants additionally attended a short afternoon session the day before, with no pharmacological manipulation, where they completed baseline questionnaires and three tasks, which will not be reported here.

Acute Tryptophan Depletion

Tryptophan is the amino acid precursor necessary to synthesize brain serotonin. Acute tryptophan depletion (ATD) is a widely used dietary manipulation, which results in a rapid decrease in the synthesis and release of serotonin (Bel & Artigas, 1996; Biggio et al., 1974; Crockett et al., 2012a). Participants were randomly assigned to receive either ATD or a placebo condition, in a double-blind, between-groups design. The depletion group received a drink that contained a balance of all the essential amino acids except for tryptophan. The placebo group received the same drink only it included tryptophan.

Blood plasma samples were collected to verify depletion and analysed using high performance liquid chromatography (HPLC).

Moral Emotions Task

We employed a novel task, part of the EMOTICOM neuropsychological testing battery (Bland et al. 2016; Savulich et al. 2018), to measure feelings of guilt, shame, annoyance, and feeling "bad". Using a touchscreen computer, participants were presented with cartoons of social scenarios – Pavlovian cues – in which someone was unjustly harmed, either intentionally or unintentionally. We then interrogated emotional reactions to these scenarios by asking participants to reflect, and report how they would feel if they were the victim or agent of harm. An example of one trial is depicted in Figure 2. There were 28 randomised trials, composed of 14 different cartoons, which were each presented twice – once where participants were prompted to identify as the victim, and once where they were asked to identify as the agent. The specific instruction was, "If this was you, please indicate below how you would feel by touching the line." The four different emotions were measured using four unnumbered touchscreen scales, with seven rungs to choose from, where the first rung was labelled "not at all", scored as 1, and the seventh labelled "extremely", scored as 7. Half of the scenarios involved an intentional harm. In the other half, the harm committed was accidental. The task was self-paced.

Individual characteristics

We assessed several personality traits and psychological symptoms primarily to ensure the placebo and depletion groups were matched. There were no differences in depressive symptoms ($t_{(71)} = -1.258$, $p = .212$) using the Beck Depression Inventory (BDI-II; Beck et al., 1996); trait anxiety ($t_{(71)} = -0.872$, $p = .386$) using the Spielberger Trait Anxiety Inventory (STAI; Spielberger et al., 1983); psychopathic traits ($t_{(71)} = 1.132$, $p = .261$) assessed with the Levenson Self-Report Psychopathy Scale (LSRP; Levenson et al., 1985); autistic characteristics ($t_{(71)} = -0.112$, $p = .911$) using the Adult Autism Spectrum Quotient (AQ; Baron-Cohen et al., 2001); empathy ($t_{(63)} = 0.442$, $p = .660$; Levene's test $F_{(71)} = 4.569$, $p = .036$) using the Interpersonal Reactivity Index (IRI; Davis, 1980); and impulsivity ($t_{(71)} = .444$, $p = .658$) assessed with the Barratt Impulsiveness Scale (BIS;

Patton et al. 1995). Participants in each group, additionally, did not differ in their years of education ($t_{(71)} = 0.634$, $p = .528$).

Statistics

Data were analysed using MATLAB (MathWorks) and SPSS (IBM). Homogeneity of variance in t-tests was verified with Levene's test, and degrees of freedom were adjusted when this assumption was violated. The Greenhouse-Geisser correction was used where applicable, in designs with within-subjects factors, to correct for violation of the sphericity assumption as determined by Mauchly's test.

Acknowledgements

This research was funded by a Wellcome Trust Senior Investigator Award (104631/Z/14/Z) to T.W.R. B.J.S. receives funding from the National Institute for Health Research (NIHR) Cambridge Biomedical Research Centre (Mental Health Theme). R.N.C.'s research is supported by the UK Medical Research Council (MC_PC_17213). J.W.K. is supported by a Gates Cambridge Scholarship. We would like to thank the staff at the NIHR/Wellcome Trust Clinical Research Facility at Addenbrooke's Hospital, where the study was conducted, and Rachel Kyd of the Cambridge University Hospital Research & Development Office for assistance with study approval.

Author Contributions

J.W.K, T.W.R., B.J.S., and A.M.A-S. designed research. J.W.K., F.E.A., and R.Y. collected data. D.M.C., A.P., and R.N.C provided medical cover. J.W.K. analysed data and wrote the paper with input from T.W.R. and R.N.C. All authors reviewed the manuscript.

Competing Interests Statement

T.W.R. discloses consultancy with Cambridge Cognition, Lundbeck, Mundipharma and Unilever; he receives royalties for CANTAB from Cambridge Cognition and editorial honoraria from Springer Verlag and Elsevier. B.J.S discloses consultancy with Cambridge Cognition, Greenfield BioVentures, and Cassava Sciences, and receives royalties for CANTAB from Cambridge Cognition. R.N.C. consults for Campden Instruments and receives royalties from Cambridge Enterprise, Routledge, and Cambridge University Press. J.W.K., F.E.A., R.Y, D.M.C., and A.P. declare no conflicts of interest.

Figures and Tables

Table 1. Summary of results on personality traits. ↑↑ indicates a significant enhancement of the emotion by ATD at high levels of the personality trait shown; + indicates a significant positive relationship between the personality trait and the emotion, under placebo; − likewise indicates a significant negative relationship. Emotions are collapsed across agency and intentionality.

	Annoyance		Guilt		Shame		Bad	
	Placebo	ATD	Placebo	ATD	Placebo	ATD	Placebo	ATD
Impulsivity		↑↑					−	
Psychopathy		↑↑						
Empathy				↑↑	+	↑↑		

Figure 1. Background information about relationships among constructs invoked. Dark blue solid arrows signify a positive relationship, light blue dashed arrows a negative association (see main text). vmPFC = ventromedial prefrontal cortex. Note that guilt and shame are distinct yet guilt can at times be laden with shame. We conceptualise frustration as closest to annoyance, measured by our task. Frustration here can also be related to anger (Koenigs et al. 2007; Pillutla & Murnighan, 1996). Note the form of aggression depicted is impulsive/reactive, but aggression can also be non-impulsive and goal directed (Blair 2010). The studies on retaliatory behaviour to unfairness employed the Ultimatum Game as a laboratory model (e.g. Crockett et al. 2008, 2010a, 2010b, Koenigs et al. 2007; Koenigs et al. 2010; Krajbich et al. 2009): One player, programmed by the experiment, proposes to split a sum of money with the study participant who can either accept or reject the offer. Both players are paid accordingly if the offer is accepted; however neither player is paid if the offer is rejected. Participants tend to reject offers that are less than 30% of the total sum even though such retaliatory behaviour to unfairness is costly. Healthy individuals reject more unfair offers under ATD (Crockett et al. 2008; 2010b) and accept more unfair offers on a single dose of an SSRI (Crockett et al. 2010a).

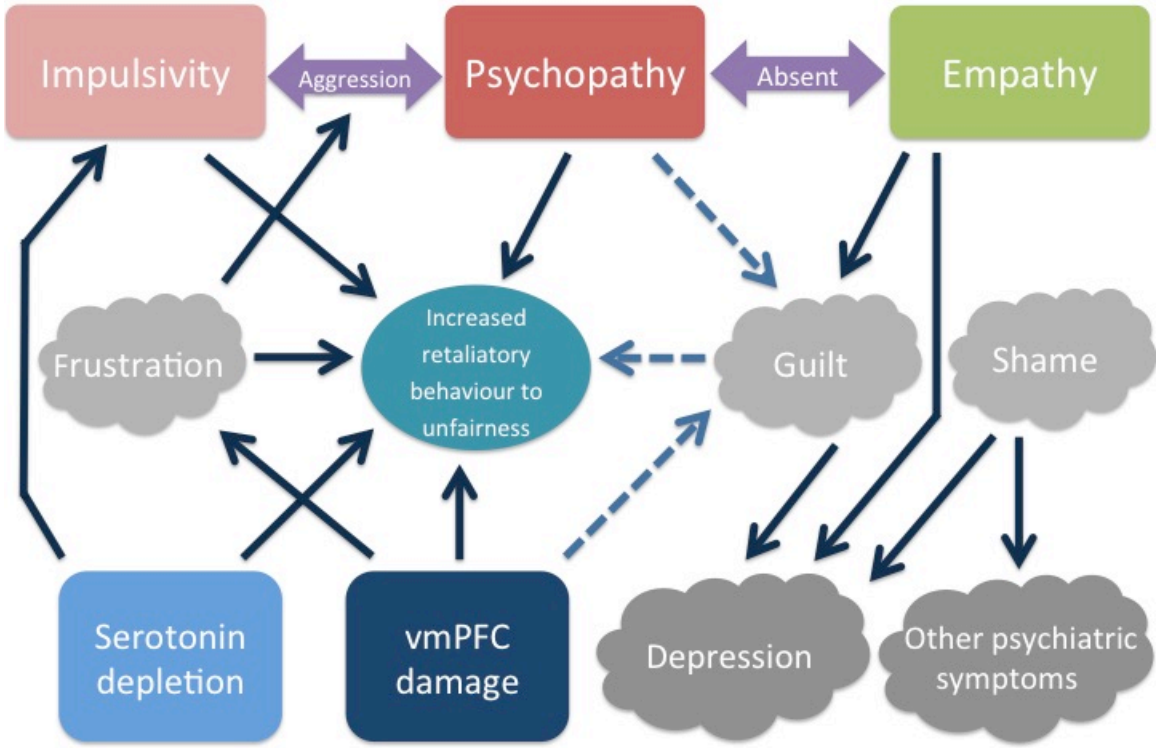

Impulsivity
Aggression
Psychopathy
Absent
Empathy
Frustration
Increased retaliatory behaviour to unfairness
Guilt
Shame
Serotonin depletion
vmPFC damage
Depression
Other psychiatric symptoms

Figure 2. Moral emotions task schematic. Three example slides of a trial are shown. Feeling "bad" was assessed with a rating scale on a fourth slide.

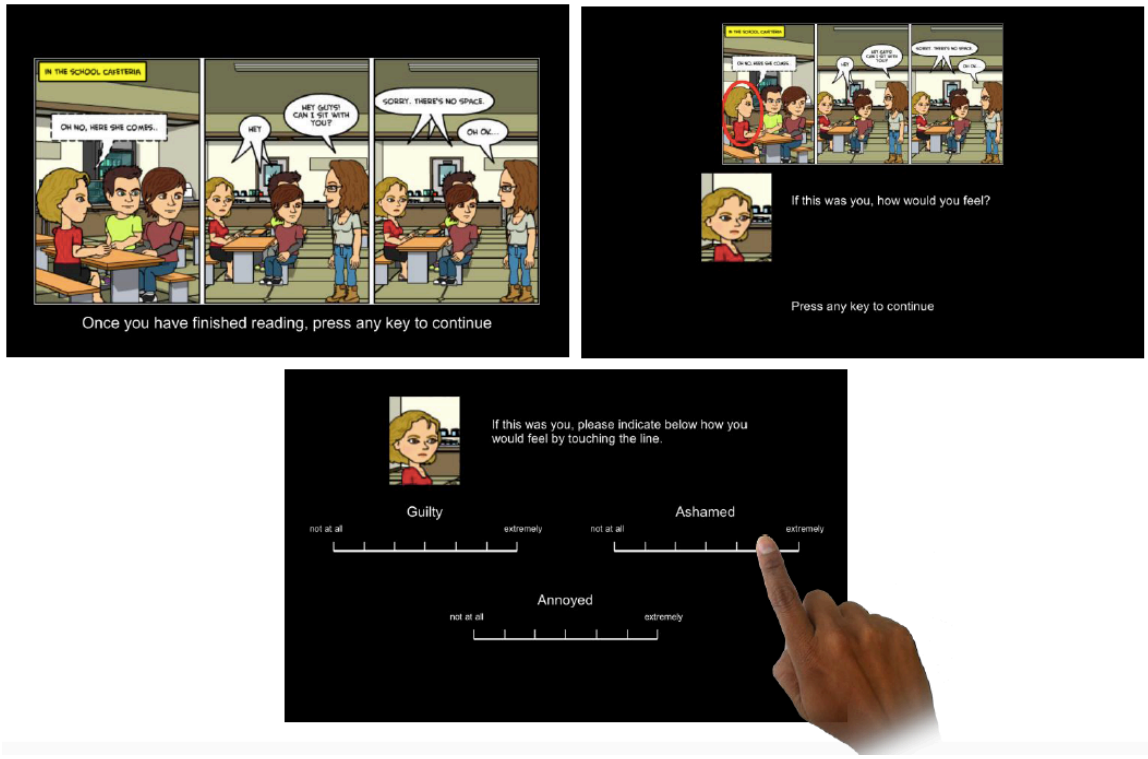


Figure 3. Effects of ATD on emotion: serotonin depletion enhanced emotion non-specifically overall (see main text). Each bar represents the average emotion ratings per group, collapsed across agency and intentionality. Error bars indicate 1 standard error. Asterisk indicates main effect, $p < .05$.

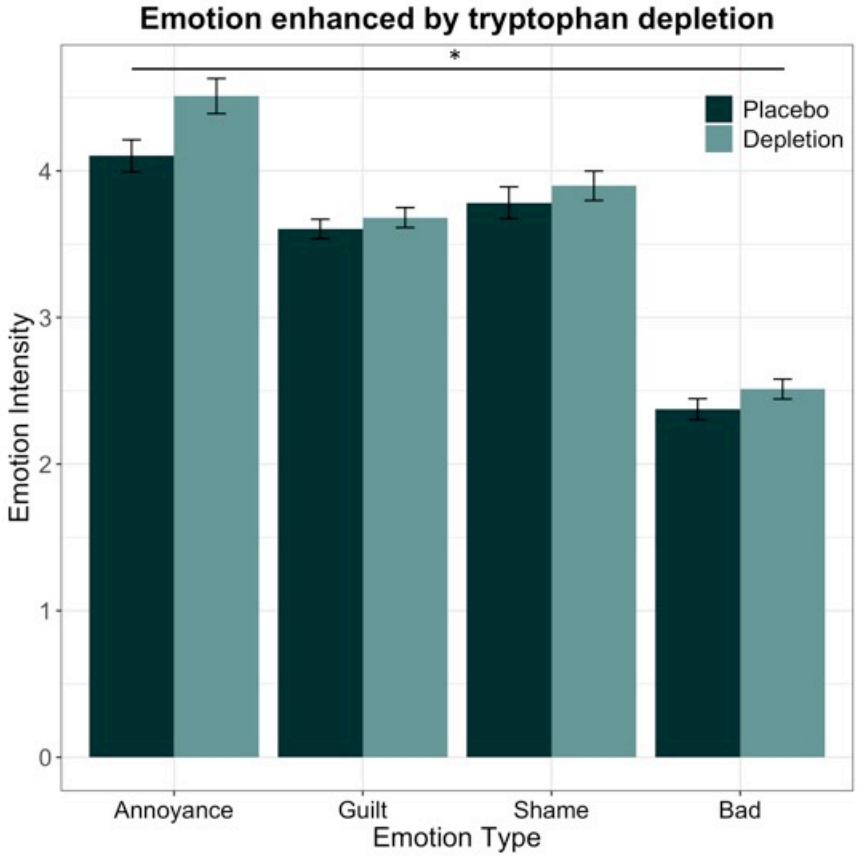


Figure 4. Effects of trait empathy on how serotonin depletion influences emotion. Shading indicates 1 standard error. Significance at $p < .05$ is denoted by #; Significance at $p < .01$ is denoted by ##. Each point represents the average emotion ratings for each individual, collapsed across agency and intentionality. **a)** The highly empathic reported more guilt following depletion relative to when on placebo. **b)** Shame was significantly elevated in individuals high in trait empathy, and this relationship was potentiated by serotonin depletion.

Figure 4a.

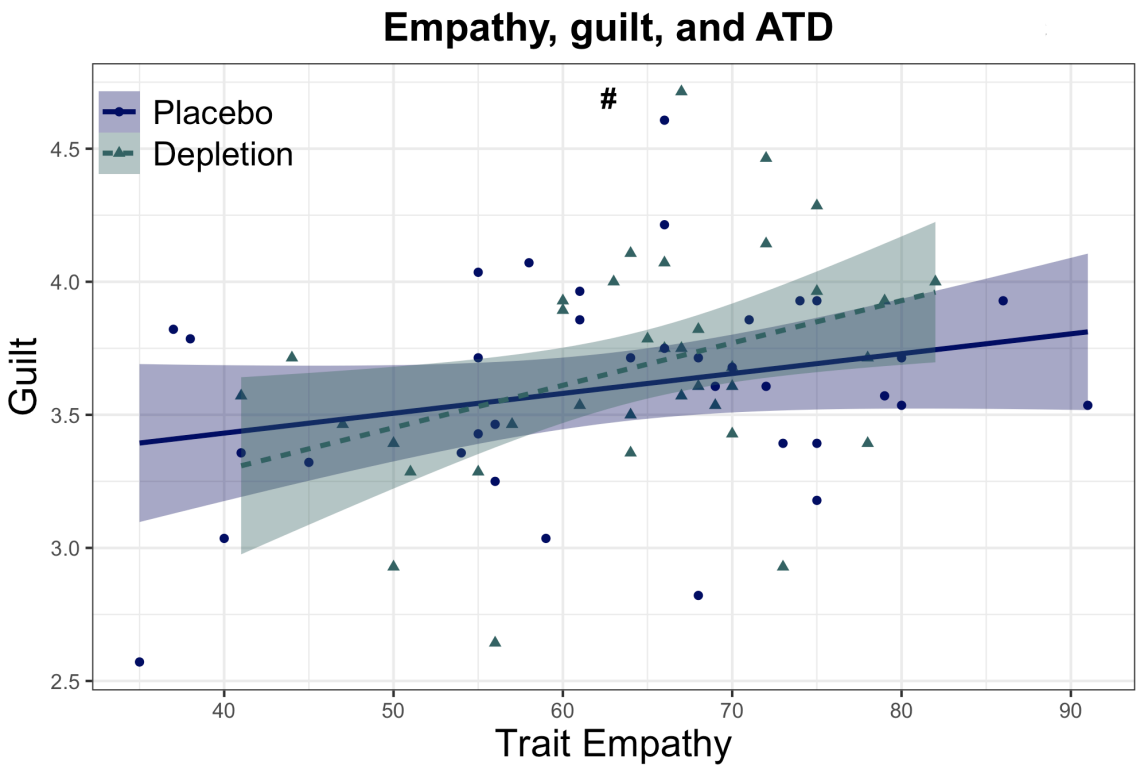


Figure 4b.

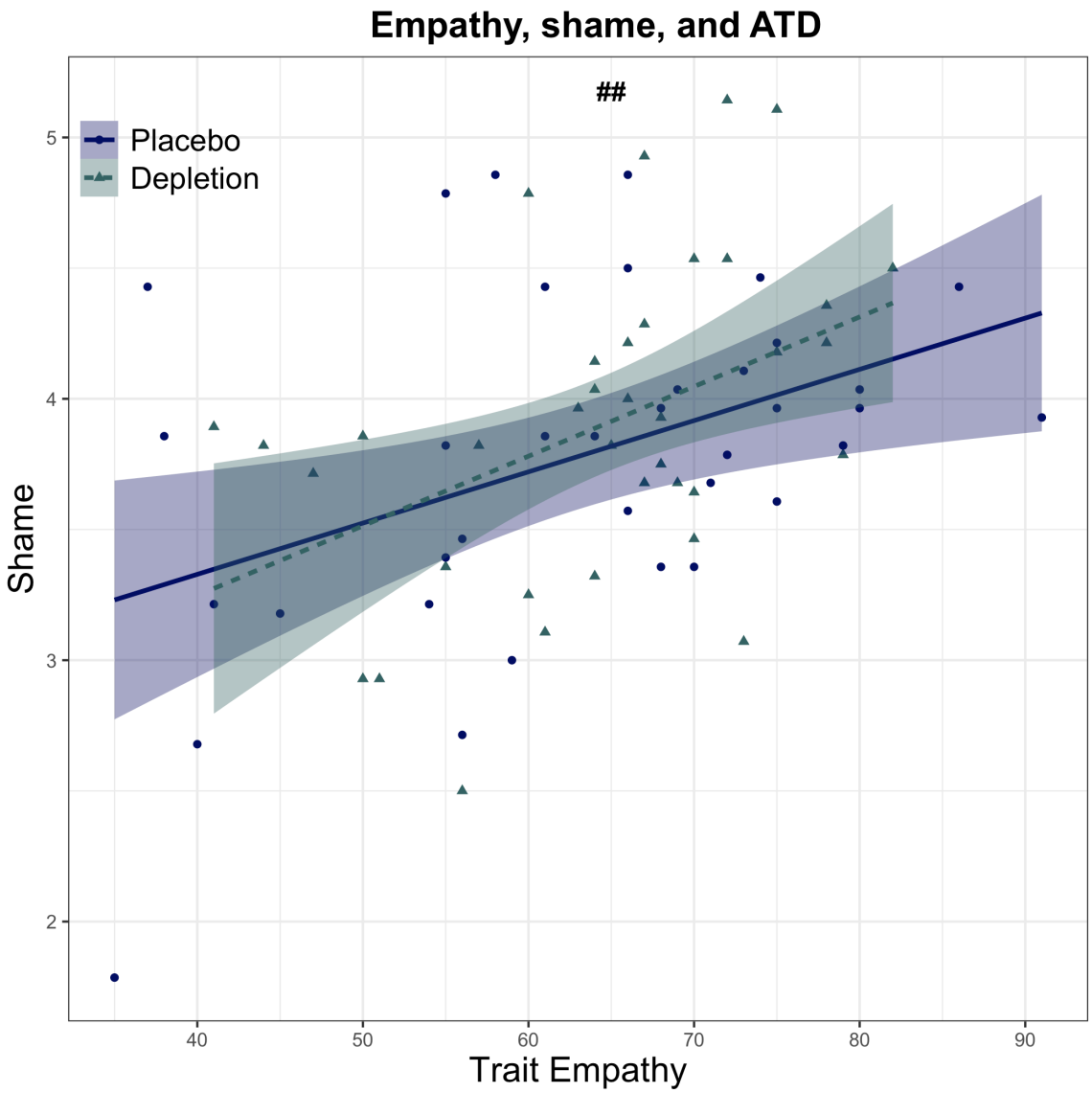

Empathy, shame, and ATD
##
Placebo
Depletion
Shame
Trait Empathy
5
4
3
2
40
50
60
70
80
90

Figure 5. How trait psychopathy and impulsivity modulate emotional effects of serotonin depletion. Shading indicates 1 standard error. Significance at $p < .05$ is denoted by #. Each point represents the average emotion ratings for each individual, collapsed across agency and intentionality. Annoyance was potentiated by serotonin depletion in **a)** high trait psychopathy and **b)** high trait impulsivity.

Figure 5a.

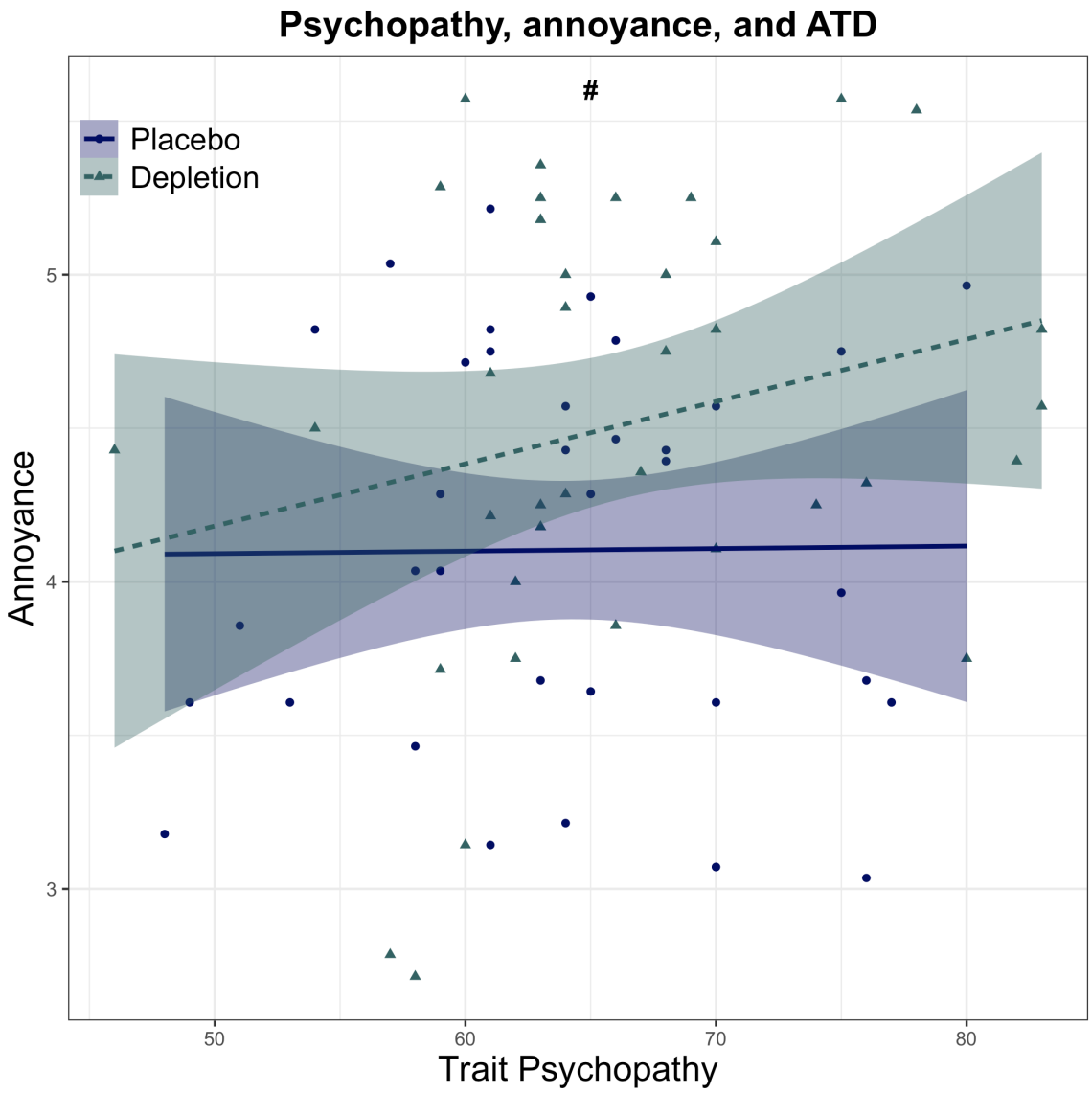


Figure 5b.

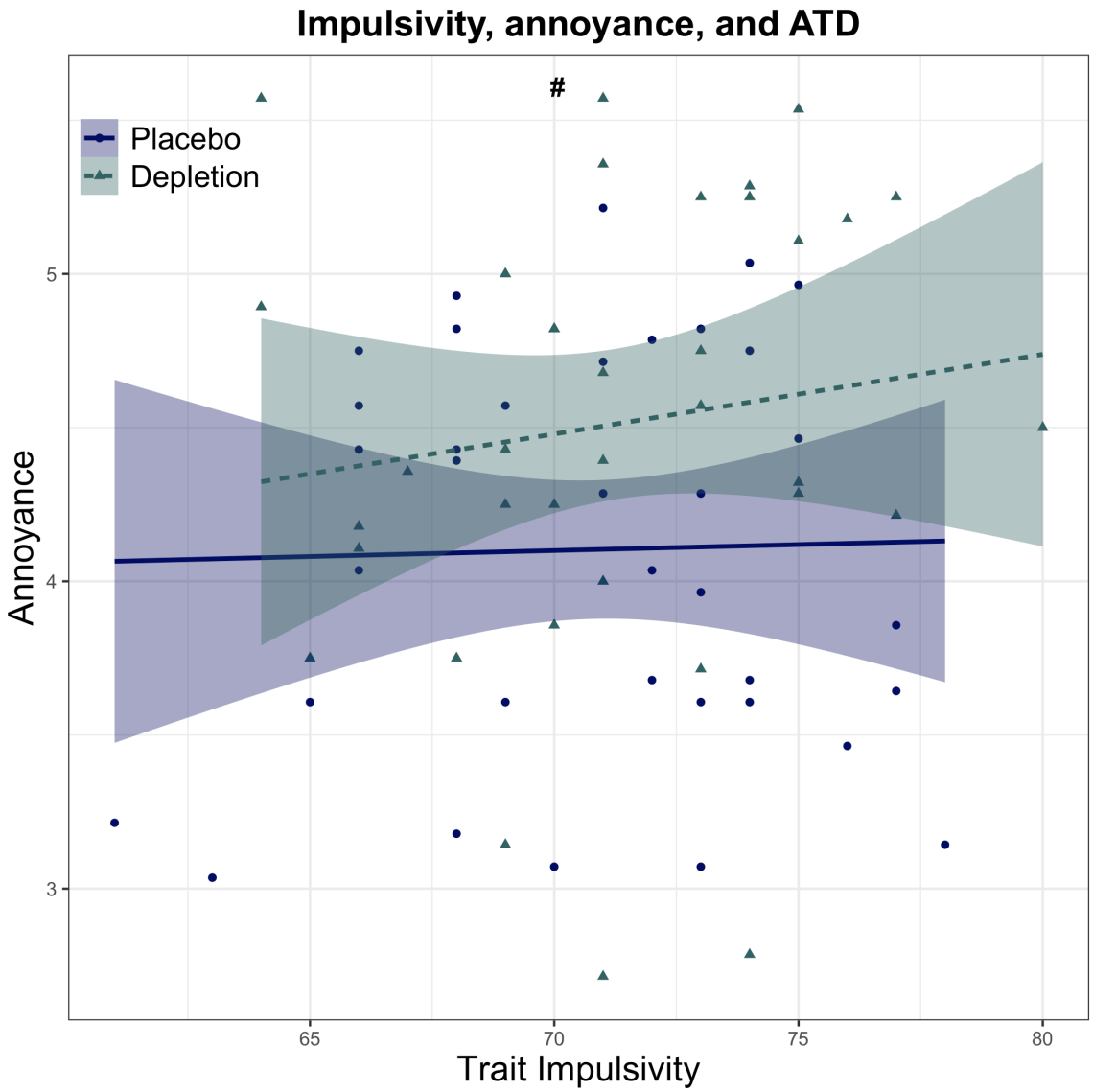
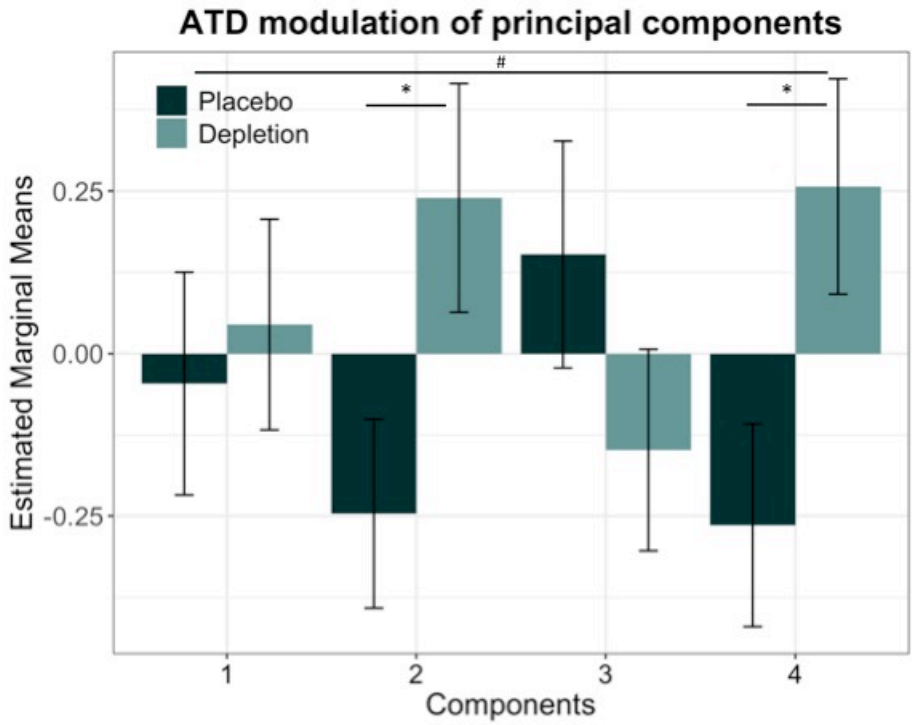

Impulsivity, annoyance, and ATD
#
Placebo
Depletion
Annoyance
5
4
3
65
70
75
80
Trait Impulsivity

Figure 6. ANOVA on four principal components from principal components analysis. Measures contained in components 2 and 4 were significantly increased by ATD (acute tryptophan depletion). For more thorough description of the principal components, see the Pattern Matrix in the supplementary materials. Error bars indicate 1 standard error. # indicates interaction at $p < .05$; asterisk indicates significant follow-up t-test at $p < .05$.



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