

ORIGINAL ARTICLE

Inflammasome signaling affects anxiety- and depressive-like behavior and gut microbiome composition

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The inflammasome is hypothesized to be a key mediator of the response to physiological and psychological stressors, and its dysregulation may be implicated in major depressive disorder. Inflammasome activation causes the maturation of caspase-1 and activation of interleukin (IL)-1 β and IL-18, two proinflammatory cytokines involved in neuroimmunomodulation, neuroinflammation and neurodegeneration. In this study, C57BL/6 mice with genetic deficiency or pharmacological inhibition of caspase-1 were screened for anxiety- and depressive-like behaviors, and locomotion at baseline and after chronic stress. We found that genetic deficiency of caspase-1 decreased depressive- and anxiety-like behaviors, and conversely increased locomotor activity and skills. Caspase-1 deficiency also prevented the exacerbation of depressive-like behaviors following chronic stress. Furthermore, pharmacological caspase-1 antagonism with minocycline ameliorated stress-induced depressive-like behavior in wild-type mice. Interestingly, chronic stress or pharmacological inhibition of caspase-1 *per se* altered the fecal microbiome in a very similar manner. When stressed mice were treated with minocycline, the observed gut microbiota changes included increase in relative abundance of *Akkermansia* spp. and *Blautia* spp., which are compatible with beneficial effects of attenuated inflammation and rebalance of gut microbiota, respectively, and the increment in Lachnospiracea abundance was consistent with microbiota changes of caspase-1 deficiency. Our results suggest that the protective effect of caspase-1 inhibition involves the modulation of the relationship between stress and gut microbiota composition, and establishes the basis for a gut microbiota–inflammasome–brain axis, whereby the gut microbiota via inflammasome signaling modulate pathways that will alter brain function, and affect depressive- and anxiety-like behaviors. Our data also suggest that further elucidation of the gut microbiota–inflammasome–brain axis may offer novel therapeutic targets for psychiatric disorders.

Molecular Psychiatry (2016) **21**, 797–805; doi:10.1038/mp.2016.46; published online 19 April 2016

INTRODUCTION

Increasing evidence suggests an involvement of neuroinflammatory pathways in the etiopathophysiology of major depressive disorder (MDD) and antidepressant response.^{1,2} Depressive symptoms are underlined by increased levels of proinflammatory cytokines (that is, interleukin (IL)-1 β and IL-6), decreased levels of anti-inflammatory cytokines (that is, IL-4 and IL-10) and are associated with polymorphisms in inflammation-related genes.^{3–5} IL-1 receptor type-I and its ligands are expressed in brain areas relevant to stress response,^{6–8} and IL-1 β signaling is fundamental in mediating the deleterious neurobehavioral and neuroendocrine responses to stress and adaptation.^{9,10} Chronic stress or IL-1 β administration triggers depressive-like behavior.¹¹

A variety of stressors activate the inflammasome through the NLRP3 or P2X7 receptors, resulting in caspase-1 maturation that processes and releases bioactive IL-1 β and IL-18.^{12,13} Caspase-1 and

NLRP3 mRNA are increased in blood cells of depressed patients,¹⁴ suggesting that the inflammasome is a key mediator by which physical and psychological stressors contribute to the development of depression, leading to the ‘inflammasome hypothesis’ of depression.¹⁵ If that proves to be correct, caspase-1 inhibiting compounds may have antidepressant effects. Minocycline is a semisynthetic tetracycline antibiotic that inhibits caspase-1 and caspase-3 transcription and has anti-apoptotic, anti-inflammatory and neuroprotective properties as well as acute antidepressant-like effects.^{16–22}

Caspase-1 knockout (*cas1^{-/-}*) mice are overtly normal, despite having undetectable IL-1 β and low IL-1 α levels.²³ They have decreased systemic inflammatory response and increased survival to lethal endotoxin doses when compared with wild-type (wt) mice.^{23,24} This is underlined by reduced inflammation-induced brain transcription, decreased inflammasome assembly and consequently decreased circulating IL-1 β and IL-18.^{23,24}

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Received 21 September 2015; revised 19 February 2016; accepted 22 February 2016; published online 19 April 2016

The microbiota–gut–brain axis is a complex multiorgan bidirectional signaling system between the microbiota and the brain that plays a fundamental role in host physiology, homeostasis, development and metabolism.²⁵ Growing evidence shows reproducible and consistent effects of microbial states on mouse behavior, supporting a role for microbiota in modulating behavior.^{26–28} Differences in anxiety-related behaviors are commonly reported in mice with altered gut microbiomes, implicating the role of gut microbiota in stress and depression.^{29,30} Casp1^{-/-} mice display depressive-like behavior and anorexia after peripheral but not central LPS administration and differ in gut microbiota composition compared with wt mice.^{31–33} Therefore, our primary and secondary hypotheses were, respectively, (1) that decreased caspase-1 activity would result in decreased depressive-like behavior and (2) that caspase-1 inhibition using intraperitoneal minocycline administration and chronic restraint stress would result in changes in the gut microbiome. The null hypothesis was that there would be no difference in these parameters between casp1^{-/-}, wt and minocycline-treated mice.

MATERIALS AND METHODS

Procedures were approved by the Animal Ethics Committees of the Australian National University and the South Australian Health and Medical Research and are in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes (8th edition, 2013). Male mice (C57BL/6J background, wt, $n = 81$; casp1^{-/-}, $n = 20$) aged 60–90 days were obtained from the Australian Phenomics (Canberra, ACT, Australia) or the Bioresources Facilities (Adelaide, SA, Australia). Genetic caspase-1 deficiency was confirmed by genotyping in experimental mice (Supplementary Figure S1). Littermates were group housed (Green Line IVC Sealsafe PLUS mouse, Tecniplast, Varese, Italy) in a temperature-specific (22C ± 1 °C) and light-specific (12 h cycles, lights on at 0700 h) pathogen-free room with water and standard regular chow *ad libitum*. Animals were assigned and randomized as described in the Supplementary Materials and Methods. The investigators were not blinded to group assignment. Behavioral phenotyping was performed between 0900 h and 1600 h. Animals were given 30 min of habituation to the behavioral testing room. Tests were performed from the least to the most invasive to minimize the influence of prior test history (in order: rotarod, elevated plus maze, marble burying test, open field test, sucrose preference test, novelty suppressed feeding and forced swim test; see Supplementary Materials and Methods for details).³⁴ Following chronic restraint stress this order was reversed for a bell-shaped stress exposure (Supplementary Figure S2).

Chronic restraint stress

After baseline behavioral testing, animals were submitted to restraint stress for 21 days. Every day, mice were placed in a horizontal resting position inside a well-ventilated (12 holes, 0.5 mm diameter) 50 ml falcon tube at 1000 h and after 4–6 h they were unrestrained.

Pharmacological caspase-1 inhibition with minocycline

The wt animals were treated with either saline (0.2 ml, intraperitoneally, $n = 27$) or minocycline (LKT Laboratories, St Paul, MN, USA; 5 mg kg⁻¹ per day in 10 ml kg⁻¹ saline, intraperitoneally, $n = 27$). Treatment lasted for the same duration of the restraint procedure (21 days).

Respirometry

Minocycline- or saline-treated restrained animals were individually housed in the Promethion Metabolic Monitoring System (Sable Systems International, Las Vegas, NV, USA) for 48 h to assess the effects of minocycline on exploratory behavior, food intake, energy expenditure and volume of oxygen inhaled and of carbon dioxide exhaled at baseline and after chronic restraint.

16S rRNA analysis

Please see Supplementary Materials and Methods for a detailed explanation of the methods used for the 16S rRNA analysis. Briefly, fecal pellets were collected with autoclaved toothpicks, placed in 1.5 ml tubes, snap-frozen on

dry ice and stored at -80 °C. Following DNA extraction, fecal microbiota profiling was performed by paired-end 16S rRNA gene amplicon sequencing, based on the Illumina MiSeq platform (Australia and New Zealand, VIC, Australia) to a depth of ~40 000 reads per sample. Sequence data processing was performed as previously described.³⁵

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences version 22.0 for Windows (SPSS, Chicago, IL, USA) using a general linear model for repeated measures. The effects of genotype, stress, treatment and their interaction were explored and the significance set at $P < 0.05$. Sphericity of the variances of the groups was assessed with Mauchly's sphericity test. If the assumption of sphericity was violated, the Greenhouse–Geisser correction was generated. Effect size was reported as partial eta-squared (η_p^2). Significant stress × genotype or stress × treatment interaction was unpacked as described previously.³⁶ Comparison of microbiota composition between groups (β -diversity) was performed using Bray–Curtis similarity matrices in PRIMER (v6, PRIMER-E, Plymouth, UK). Matrices were generated from sample-normalized, square-root transformed, relative operational taxonomic unit abundance. Community-level changes were assessed for significance using one-way permutational multivariate analyses of variance (PERMANOVA) tests with 9999 random permutations and at a significance threshold of $P < 0.01$. The contribution of individual taxa to between-group variation was assessed by similarity percentage analysis, as previously reported.³⁷ Where specific bacterial taxa were identified as contributing to change in microbiota composition, variation in their relative abundance was further assessed through Mann–Whitney *U*-tests between groups. Differences of median relative abundance between groups were assessed using Hodges–Lehmann estimator.

RESULTS

Our primary outcome measure was the assessment of depressive-like behavior in the forced swim test. Secondary outcome measures included anxiety-like behavior, changes in the sucrose preference test, locomotor activity, gut microbiome and respirometry. Analyses and results of behavioral tests results are available in Supplementary Tables S1–S3.

Caspase-1 deficiency decreases depressive and anxiety-like behaviors

Our results showed that caspase-1 deficiency decreased depressive- and anxiety-like behaviors. In the forced swim test, the total floating time was lower in casp1^{-/-} compared with wt mice ($F_{1,45} = 117.04$, $P < 0.0001$, Figure 1a and Supplementary Table S1). At the same time, swimming and climbing behaviors were higher in casp1^{-/-} mice compared with wt (respectively $F_{1,45} = 117.10$, $P < 0.0001$, and $F_{1,45} = 38.69$, $P < 0.0001$). Anxiety-like behaviors had a significant main effect of genotype in 4 tests: (1) elevated plus maze, (2) novelty suppressed feeding, (3) marble burying and (4) open field tests. We found a significant main effect of genotype in the elevated plus maze open to closed arms time ratio ($F_{1,45} = 4.16$, $P = 0.047$, Figure 1b), indicating an anxiolytic phenotype in casp1^{-/-} mice. Accordingly, in the novelty suppressed feeding, casp1^{-/-} mice showed decreased latency to eat in a novel environment following fasting ($F_{1,43} = 32.17$, $P < 0.0001$, Figure 1c). In the marble burying test, which is considered predictive of anxiolytic compounds,³⁸ we observed a decreased number of marbles buried by casp1^{-/-} mice ($F_{1,45} = 11.55$, $P = 0.001$, Figure 1d). Moreover, casp1^{-/-} mice displayed a decreased number of fecal boli during the open field test ($F_{1,45} = 4.72$, $P = 0.035$, Figure 1e), whereas no differences were observed for the time spent in the center area of the arena, another measure of anxiety-related behavior ($F_{1,45} = 0.05$, $P = 0.826$). In the sucrose preference test, casp1^{-/-} mice displayed an increased preference for a 1% sucrose solution ($F_{1,33} = 5.52$, $P = 0.025$, Supplementary Table S1), suggesting greater hedonic behavior.

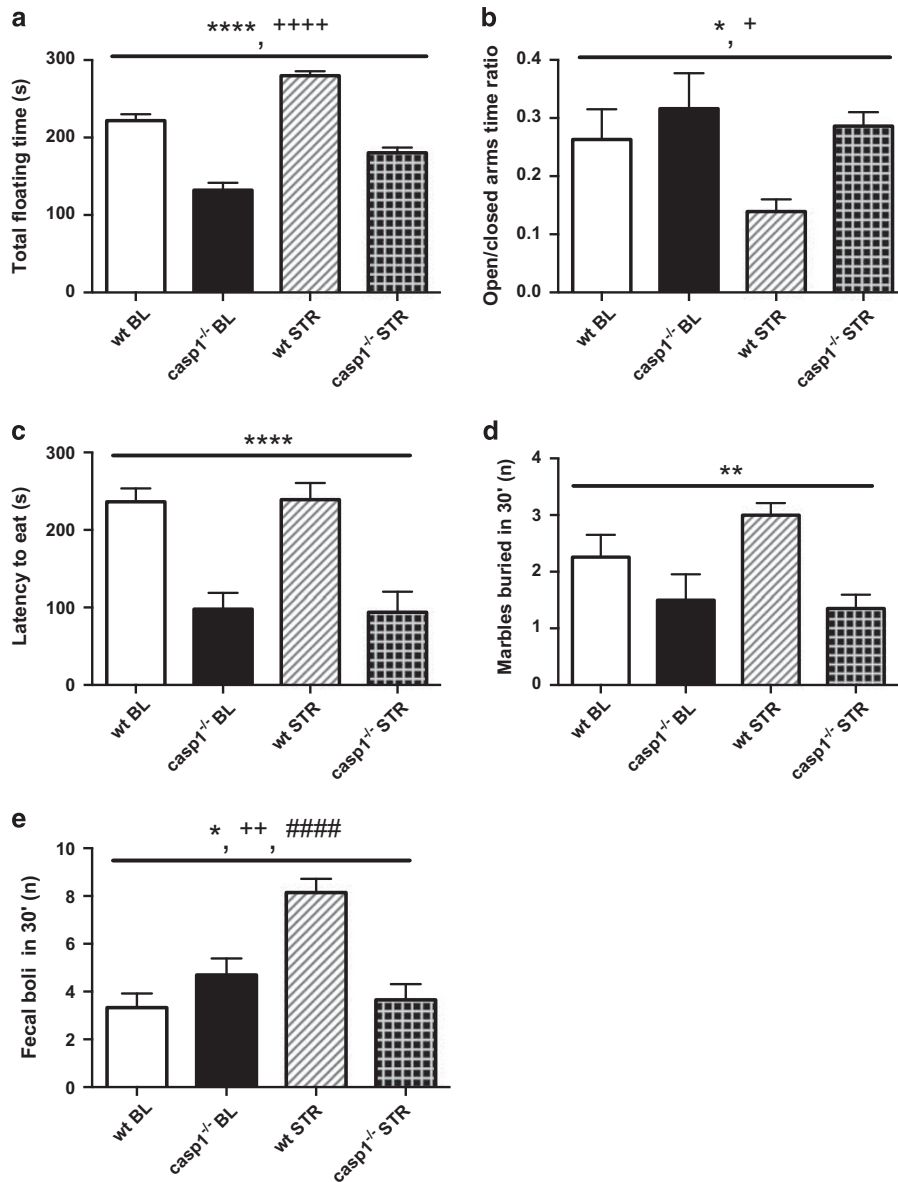


Figure 1. Caspase-1 (*casp1*) deficiency decreases anxiety-like and depressive like behavior and affects chronic restraint stress response. (a) *Casp1* knockout (*casp1*^{-/-}) mice displayed decreased floating time in the forced swim test in comparison with wild-type (wt) mice and (b) displayed decreased anxiety-like behavior as measured by the open/closed arms time ratio in the elevated plus maze. (c) In the novelty suppressed feeding test, *casp1*^{-/-} mice showed significantly decreased latency to feed following 16 h of fasting but not water deprivation. (d) Moreover, *casp1* deficiency resulted in less marbles buried in the marble burying test. (e) In the open field test, we observed a decreased number of fecal boli as a result of *casp1* deficiency as well as a different response to chronic restraint stress. Data are presented as mean ± s.e.m. Genotype effect: **P* < 0.05, ***P* < 0.01, *****P* < 0.0001; stress effect: +*P* < 0.05, ++*P* < 0.01, ++++*P* < 0.0001; genotype × stress effect: ####*P* < 0.0001. BL, baseline; STR, after chronic restraint stress paradigm; wt, wild type.

Caspase-1 deficiency affects chronic restraint stress response

Our results suggest that *casp1*^{-/-} mice had an attenuated response to chronic stress. We found a significant (genotype × stress) interaction for swimming and climbing time in the forced swim test (respectively $F_{1,45} = 7.02$, $P = 0.011$, and $F_{1,45} = 8.60$, $P = 0.005$). The wt mice showed a greater decrease in swimming time (70%, $F_{1,45} = 45.48$, $P < 0.0001$) than *casp1*^{-/-} mice (14%, $F_{1,45} = 5.33$, $P = 0.026$) following stress. Accordingly, wt animals displayed a greater reduction in climbing time (91%, $F_{1,45} = 33.33$, $P < 0.0001$) compared with *casp1*^{-/-} mice (64%, $F_{1,45} = 78.13$, $P < 0.0001$) following restraint (Figure 1a). We found a significant (genotype × stress) interaction for body weight changes ($F_{1,45} = 6.06$, $P = 0.018$)

that decreased in wt mice following restraint ($F_{1,45} = 14.24$, $P < 0.0001$, average Δ body weight = -1.3 g) but remained unchanged in *casp1*^{-/-} mice ($F_{1,45} = 0$, $P = 1$, average Δ body weight = 0 g). Furthermore, we found a significant (genotype × stress) interaction in the number of defecations in the open field test ($F_{1,45} = 30.93$, $P < 0.0001$, Figure 1d); *casp1*^{-/-} mice did not show an increase in this parameter following restraint ($F_{1,45} = 1.73$, $P = 0.196$) whereas wt mice did ($F_{1,45} = 48.98$, $P < 0.0001$).

Caspase-1 deficiency increases locomotion and locomotor skills

We found that caspase-1 deficiency increases locomotor activity in the open field test ($F_{1,45} = 10.54$, $P = 0.002$, Figure 2a). Moreover,

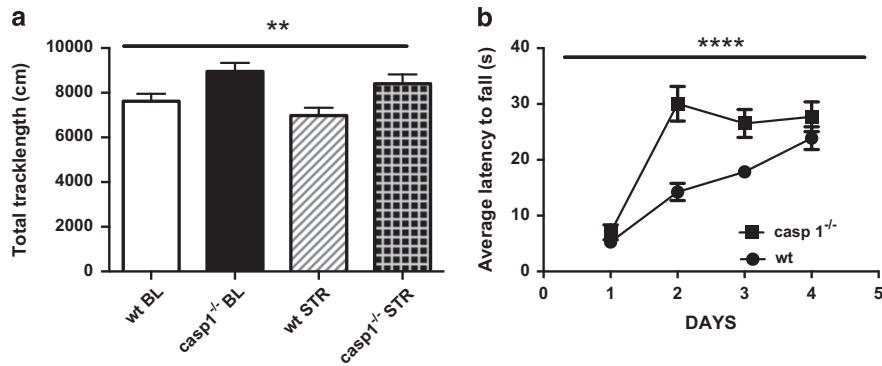


Figure 2. Caspase-1 (*casp1*) deficiency increases spontaneous locomotion and locomotory skills. (a) Casp1 knockout (*casp1*^{-/-}) mice had increased locomotor activity in the open field test when compared with wild-type (wt) mice and (b) acquired quicker the skills to perform the rotarod test. Data are mean \pm s.e.m. Genotype effect: $P < 0.05$; $**P < 0.01$; $****P < 0.0001$. BL, baseline; STR, after chronic restraint stress paradigm.

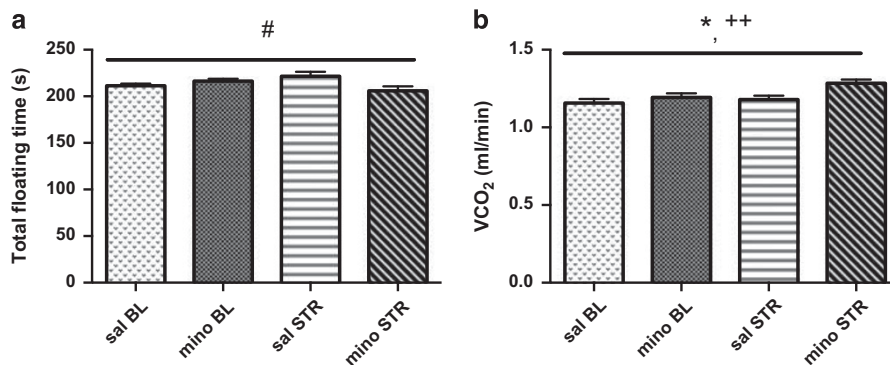


Figure 3. Caspase-1 antagonism affects chronic restraint stress response. (a) Minocycline treatment (mino) in wild-type (wt) animals during chronic restraint stress (STR) prevented stress-induced increased floating time in the forced swim test. (b) Respirometry measurement for volume of CO₂ exhaled revealed a significant effect of stress as well as treatment. Data are mean \pm s.e.m. Treatment effect: $*P < 0.05$; stress effect: $***P < 0.01$; treatment \times stress effect: $#P < 0.05$. BL, baseline.

casp1^{-/-} mice acquired skills more quickly than wt mice to perform in the accelerating rotarod test ($F_{1,45} = 15.35$, $P < 0.0001$, Figure 2b and Supplementary Table S2).

Chronic restraint stress increases anxiety-like and depressive-like behaviors

Chronic restraint stress (4–6 h per day for 21 days) increased the floating time in the forced swim test ($F_{1,45} = 66.92$, $P < 0.0001$, Figure 1a), whereas it decreased swimming ($F_{1,45} = 37.80$, $P < 0.0001$) and climbing behavior ($F_{1,45} = 109.52$, $P < 0.0001$). It also increased anxiety-like behavior in the elevated plus maze test, decreasing the time spent in the open arms ($F_{1,45} = 5.65$, $P = 0.022$) and the open to closed arms time ratio ($F_{1,45} = 4.55$, $P = 0.038$, Figure 1b), as well as in the open field test, increasing the number of defecations ($F_{1,45} = 12.74$, $P = 0.001$, Figure 1e). Furthermore, restraint decreased body weight gain ($F_{1,45} = 6.06$, $P = 0.018$) and food intake ($F_{1,43} = 5.75$, $P = 0.021$). Nevertheless, restrained mice showed an increase in ratio quotient ($F_{1,28} = 4.79$, $P = 0.037$). Following restraint, no changes were observed in the sucrose preference test ($F_{1,33} = 0.05$, $P = 0.817$, Supplementary Table S1) or in locomotor activity in the open field test ($F_{1,45} = 3.64$, $P = 0.063$, Figure 2a).

Minocycline treatment affects stress response and metabolic parameters

We found a significant (treatment \times stress) interaction in the floating time in the forced swim test ($F_{1,28} = 6.67$, $P = 0.015$,

Figure 3a and Supplementary Table S3). Saline- and minocycline-treated animals displayed similar floating times at baseline ($F_{1,28} = 2.35$, $P = 0.137$); however, minocycline-treated mice were less immobile than saline-treated mice following restraint ($F_{1,28} = 5.25$, $P = 0.030$). No differences were observed between restrained mice receiving saline or minocycline in locomotion, food intake, energy expenditure, body mass and volume of oxygen inhaled (not shown). We found a significant effect of treatment and stress on the volume of carbon dioxide exhaled (respectively $F_{1,28} = 5.64$, $P = 0.025$ and $F_{1,28} = 8.13$, $P = 0.008$, Figure 3b).

Chronic restraint stress affects the gut microbiome

Chronic restraint stress (4–6 h per day for 21 days) affected the gut microbiome compared with nonstressed animals (PERMANOVA $P = 0.0027$, $t = 2.3492$). Although the shallowest level of classification (that is, phylum level) only revealed a nonsignificant trend toward an increased ratio of Firmicutes to Bacteroidetes (Figure 4a), the deeper analysis did identify clear differences between the animal groups. In particular, the relative abundances of the genera *Allobaculum* (difference in median relative abundance -7.8% , $P < 0.0001$ Mann–Whitney *U*-test), *Bifidobacterium* (-4.6% , $P = 0.0002$), *Turicibacter* (-3.4% , $P < 0.0007$), *Clostridium* (-0.7% , $P < 0.0001$) and the family S24-7 (-5.8% , $P = 0.0021$) were all reduced in restrained animals, and the relative abundance of the family Lachnospiraceae was increased ($+0.3\%$,

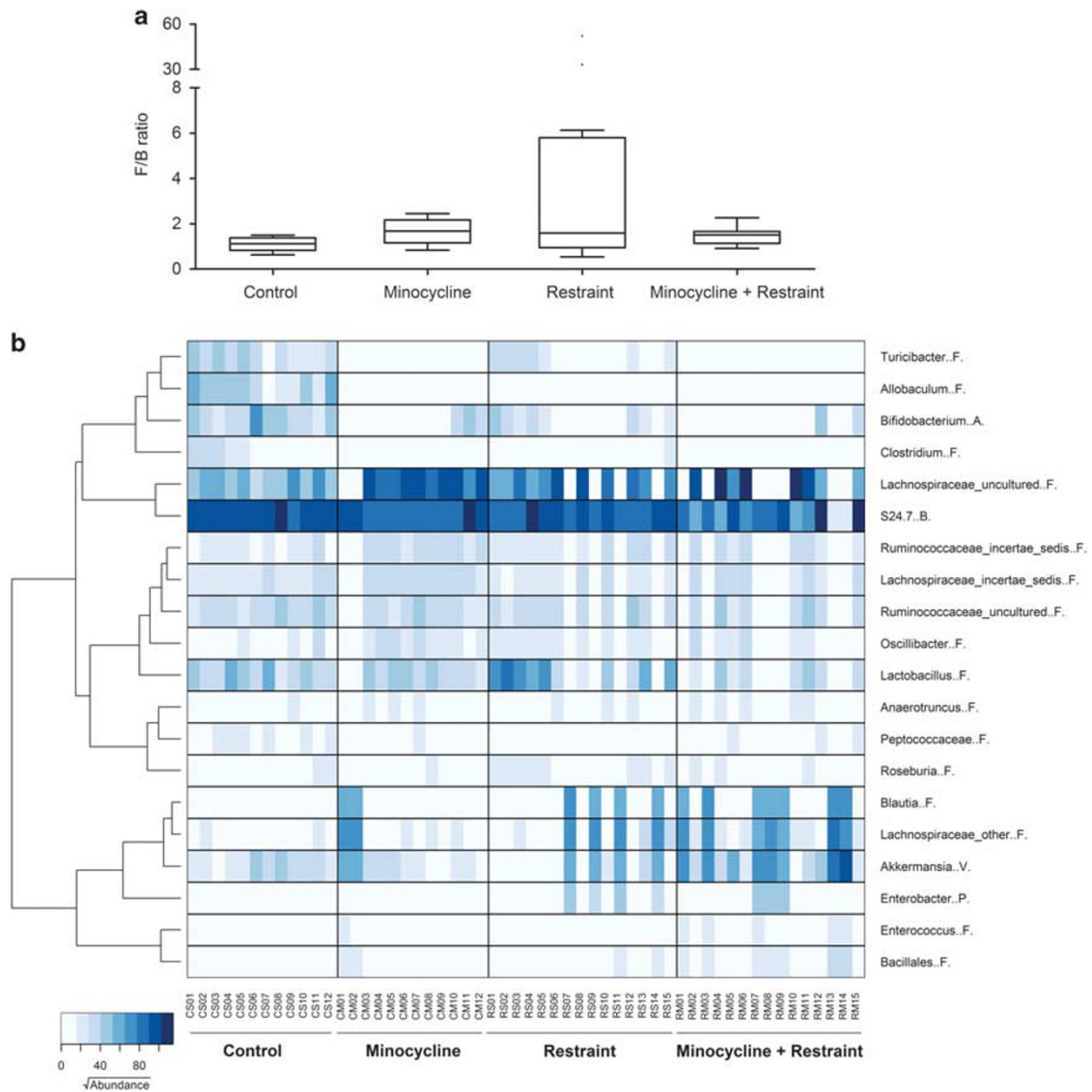


Figure 4. Minocycline treatment and chronic restraint stress affect the gut microbiome and chronic restraint stress changes the gut Firmicutes/Bacteroidetes (F/B) ratio. **(a)** Box and whiskers plot displayed the analysis of the differences of the main composition of the microbiota (Firmicutes to Bacteroidetes). Upper and lower quartiles defined the box with median midline, and the whiskers were assessed using Tukey's method. **(b)** Microbiota distribution at species level of taxon contributing to 97.5% of sample variations. Heatmap shows square root-transformed read counts for the 20 taxa determined by similarity percentage analysis. The dendrogram shows the clustering of genera based on Ward's hierarchical clustering method. Phyla are abbreviated as follows: Actinobacteria (A), Bacteroidetes (B), Firmicutes (F), Proteobacteria (P) and Verrococmicrobia (V).

$P=0.0244$). Variance in the relative abundance of these taxa accounted for >40% of intergroup variance.

Minocycline affects the gut microbiome

Minocycline treatment (5 mg kg^{-1} per day for 21 days) also affected microbiota composition compared with saline-treated controls (PERMANOVA $P=0.0001$, $t=3.0947$, Figure 4b) and, interestingly, in a manner very similar to that observed for restrained animals. In particular, minocycline-treated animals were also found to possess lower relative abundances of the genera *Allobaculum* (difference in median relative abundance -7.8% , $P < 0.0001$ Mann-Whitney U -test), *Bifidobacterium* (-5.8% , $P < 0.001$), *Turicibacter* (-4.2% , $P < 0.0001$), *Clostridium* (-0.7% , $P < 0.0001$) and the family S24-7 (-7.4% , $P=0.003$), and significantly high relative abundances of the family Lachnospiraceae

($+25.3\%$, $P=0.005$) and Ruminococcaceae incertae sedis ($+2.4\%$, $P=0.024$). Variance in the relative abundance of these taxa accounted for >67% of intergroup variance.

Effect of chronic restraint stress on the gut microbiome in combination with minocycline

Combining chronic restraint with minocycline treatment resulted in a microbiota composition that was different to that of nonrestrained saline-treated controls (PERMANOVA $P=0.0002$, $t=3.4593$, Figure 4b). There were also no significant differences in the shallow, phylum-level profiles produced from mice receiving each treatment alone or in combination (using a PERMANOVA threshold of $P < 0.01$), and at deeper levels of analysis, the significant reductions in the relative abundances of both *Turicibacter* and *Bifidobacterium* spp. were also observed in mice receiving

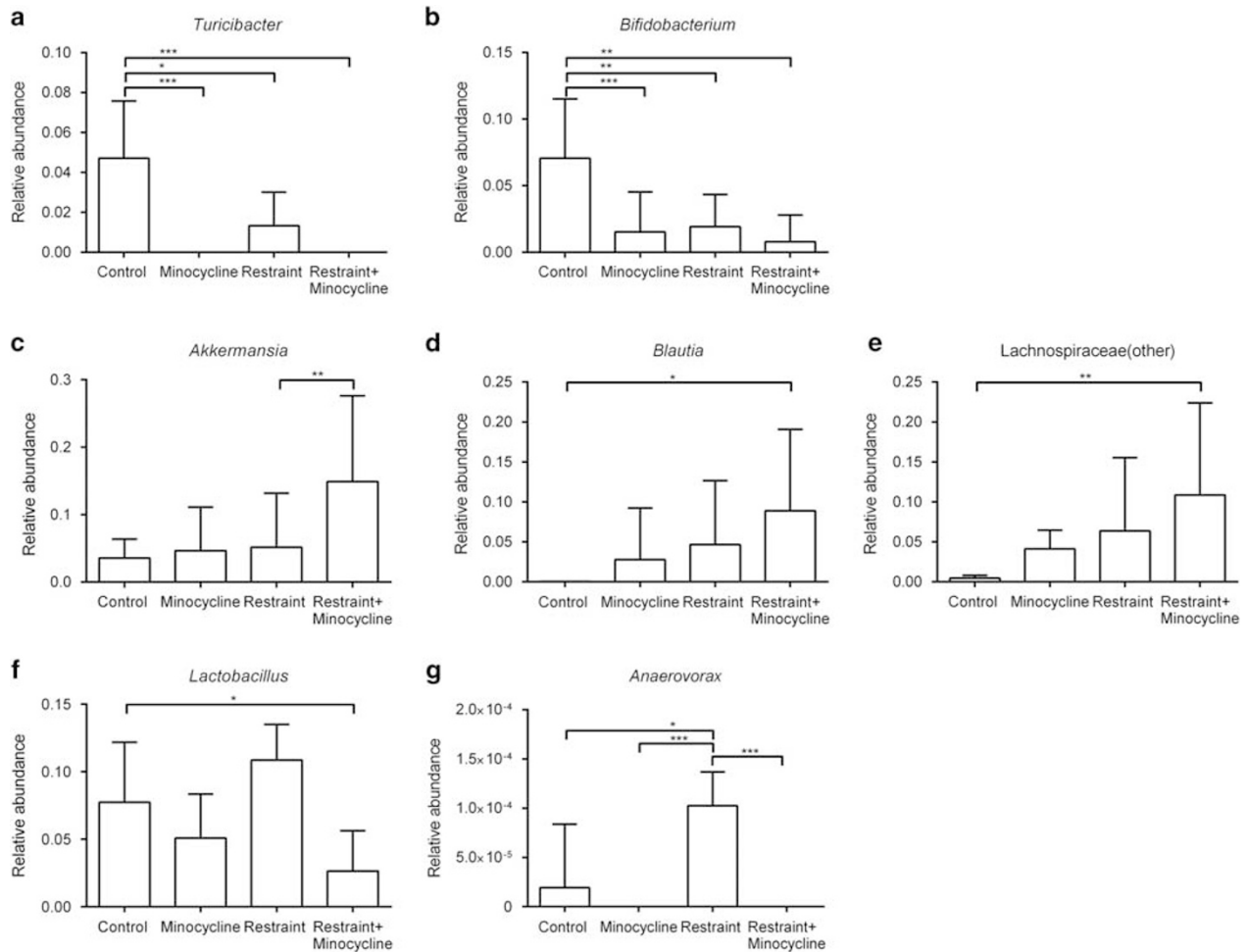


Figure 5. The effect of minocycline treatment, chronic restraint stress and their combination assessed at the level of individual taxa. Individual minocycline effect on the (a) *Turicibacter* and (b) *Bifidobacterium* populations; synergistic effect of minocycline and chronic restraint stress on the (c) *Akkermansia*, (d) *Blautia* and (e) Lachnospiraceae populations; and antagonistic effect of minocycline and chronic restraint stress on the (f) *Lactobacillus* and (g) *Anaerovorax* populations. Significant difference between treatment groups: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

both treatments (Figures 5a and b), as was the increase in members of the Lachnospiraceae (Figure 5e). Additional changes in the microbiome profiles not observed with either treatment alone were found when restraint and minocycline were used together. For example, the relative abundances of *Akkermansia* spp. and *Blautia* spp. were increased (Figures 5d and e). Minocycline also appears to have a stronger effect on the relative abundances of *Lactobacillus* spp. and *Anaerovorax* spp., with relatively greater abundances of these genera observed in the restrained animals, but reductions in their relative abundances when minocycline was also administered (Figures 5f and g).

DISCUSSION

Caspase-1 is a cysteine protease that cleaves pro-IL-1 β and pro-IL-18 into their mature isoforms in the NLRP3 inflammasome in response to stressful stimuli such as psychosocial and microbial stress, adenosine triphosphate, toxins and particulate matter.^{13,39} Because casp1^{-/-} mice lack caspase-1 mRNA and its mature protein product, they have decreased inflammasome bioactivity and inflammasome-driven IL-1 β and IL-18 production, and could be helpful in identifying the role of caspase-1 in behavior, via either innate or after stress-induced inflammasome activation.²³ Our data highlight a role for caspase-1 in the modulation of innate behavior as well as in the response to chronic stress, as caspase-1

modulation decreased baseline anxiety- and depressive-like behaviors, as well as the exacerbation of depressive-like behaviors following chronic restraint stress. Our results are in line with studies reporting that modulation of the IL-1 β axis is a potential approach to attenuate the behavioral and molecular effects of stress-induced inflammation.^{40,41} Our findings strengthen the role of caspase-1 as a potential therapeutic target aiming at modulating inflammasome-mediated pathways in psychiatric disorders.

Minocycline exerts anti-inflammatory and neuroprotective effects in animal models of neurodegenerative disorders, neurotoxicity and brain injury as well as presents acute antidepressant-like effects in the forced swim test by increasing climbing, potentially through interaction with glutamatergic and/or noradrenergic systems.^{17,19,42,43} Its antidepressant-like effects might be related to the protection of serotonergic and dopaminergic circuitries.^{44,45} Consistent with this literature, we found that minocycline prevented the exacerbation of depressive-like behavior in the forced swim test following chronic restraint stress. Given this finding, we suggest that minocycline may be valuable in the treatment of MDD and other psychiatric disorders. Indeed, two clinical trials investigating minocycline as a stand-alone or adjuvant treatment in psychotic depression and schizophrenia yielded promising results.⁴⁶⁻⁴⁹ Two studies have planned to investigate the efficacy of minocycline in MDD and bipolar

disorder (Clinical Trials.gov identifier NCT01574742 and NCT01403662); yet, no recruitment data have been available.

Our findings show that the gut microbiota composition of mice subjected to chronic restraint stress is significantly altered when compared with control mice. Subtle alterations in the Firmicutes/Bacteroidetes ratio have already been described in studies of irritable bowel syndrome patients (and correlated with clinical depression and anxiety) and in animal models of hypertension,^{50,51} and these two conditions are associated with chronic low-grade inflammation.^{52,53} The deeper levels of comparison were more informative, such as the levels of *Bifidobacterium* spp., a genus associated with the suppression of inflammation through inhibition of the nuclear factor- κ B pathway,⁵⁴ being significantly reduced in animals undergoing restraint. Such findings are consistent with the notion that nuclear factor- κ B is increased in response to stress, and is a critical mediator of stress-induced depressive-like behavior and stress-impaired neurogenesis.⁵⁵ Furthermore, *Allobaculum* spp. were absent in restrained animals, despite representing a substantial component of the microbiota in control animals. Members of the genus *Allobaculum* include mucin degraders, and their relative abundance is already known to be inversely correlated with dietary-induced inflammation markers, including leptin and IL-22.^{56,57} Chronic restraint also led to an increase in the relative abundance of *Lactobacillus* spp., members of which have been implicated in inflammasome activation through stimulation of caspase-1-dependent IL-1 β production by macrophages.⁵⁸ As such, it is interesting to speculate that the increases in *Lactobacillus* spp. observed here when either restraint or minocycline were used alone could be a contributing factor to the increased IL-1 β levels observed in depression and in animal models of stress.^{1,59,60}

Minocycline treatment also significantly altered microbiota composition, and the changes observed following minocycline or restraint separately were not significantly different. However, when restrained mice were also treated with minocycline, evidence of both synergistic and antagonistic effects on microbiota composition was observed. For example, *Akkermansia*, *Blautia* and an uncultured member of the Lachnospiraceae family were significantly increased. This effect is notable given that *Akkermansia* attenuates inflammation in adipose tissue through induction of Foxp3 regulatory T cells, and suppression of IL-6 and IL-1 β .^{61–63} Moreover, a similar increase in *Akkermansia* was reported in a study in which minocycline rebalanced the gut microbiota in a rat model of hypertension.⁵⁰ Lachnospiraceae is one of the most abundant families of Firmicutes and is most associated with beneficial production of short-chain fatty acids from complex polysaccharides.⁶⁴ An increase in Lachnospiraceae relative abundance in minocycline-restraint animals is consistent with changes in the gut microbiota of casp-1^{-/-} mice.³³

This study has several limitations. In this study, we chose minocycline as a caspase-1 inhibiting compound to further explore the relationship between the microbiota and host behavior.^{27,28} Minocycline is an antibiotic and therefore it would likely affect the gut microbiome regardless of caspase-1 inhibition.⁶⁵ As minocycline has antidepressant effects, its influence on the gut microbiome could be at least partially responsible for such effects.^{21,44,45} Further work is required to elucidate the exact mechanisms of gut microbiota–host interactions, including the analysis of short-chain fatty acid production. The altered microbiome profiles we observed are likely to translate into alterations in both metabolic profiles and host–microbe crosstalk, and to investigate this is beyond the scope of the work presented here. It could be argued that the withdrawal of food and water during the restraint period might impact the microbiome.⁶⁶ However, because food was only withheld for short periods (4–6 h), we contend that this will have had minimal impacts on the fecal microbiome profiles. Mice were submitted twice to a battery of behavioral test; exposure to one test could impact subsequent test

performance.^{34,67} However, we tried to minimize bias by performing the tests from the least to the most invasive and by giving the animals recovery time in between tests.^{34,67} Our chronic restraint stress paradigm did not decrease sucrose preference, which replicates published findings.⁶⁸ Although this test is considered a model of clinical anhedonia, it has highly variable outcomes, even within the same facility.⁶⁹

In a companion paper,⁷⁰ we have shown three findings that are highly complementary to the results reported here. (1) The absence of gut microbiota in germ-free mice resulted in decreased immobility time in the forced swimming test relative to conventionally raised healthy control mice. (2) From clinical sampling, the gut microbiotic compositions of healthy controls were significantly different from those of MDD patients. (3) Fecal microbiota transplantation of germ-free mice with ‘depression microbiota’ derived from MDD patients resulted in depression-like behaviors compared with colonization with ‘healthy microbiota’ derived from healthy control individuals. Moreover, we showed that mice harboring ‘depression microbiota’ primarily exhibited disturbances of microbial genes and host metabolites involved in carbohydrate and amino acid metabolism, indicating that the development of depressive-like behaviors is mediated through the host’s metabolism.⁷⁰ Based on those findings, in conjunction with the results presented here, we propose that the microbiota–gut–brain axis is fully bidirectional, functioning in a manner through which changes in microbiota affect behavior as shown in our companion paper, whereas, as demonstrated here, changes in behavior brought about (1) by chronic stress, (2) genetic manipulation or (3) pharmacological intervention result in changes in the gut microbiota.

In summary, our findings suggest that caspase-1 inhibition has a protective effect on the stress response by modulating the interface between stress and the gut microbiota. This supports the concept of a microbiota–gut–inflammasome–brain axis in which the gut microbiota exert some of its effects on brain function via the inflammasome signaling platform that modulates inflammatory pathways which will in turn alter brain function and affect depressive- and anxiety-like behaviors. Taken together, a reduction of inflammasome bioactivity may represent a feasible and direct therapeutic strategy in the treatment of MDD and other neuropsychiatric disorders with inflammatory components. Moreover, direct modification of the gut microbiota may offer new opportunities to further mitigate these disorders, via indirect modulation of inflammasome signaling. Future studies should address the tolerability, safety and long-term effects of inflammasome modulation for the treatment of major depression.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by funding from The National Health and Medical Research Council, Australia, APP1070935, and The Australian National University (Canberra, Australia) and The South Australian Health and Medical Research Institute (SAHMRI, Adelaide, Australia). We acknowledge the support of Neil Dear, Sian Dear and their team at SAHMRI Research and Biomedical Services in the conduct of the animal experiments.

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