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The effect of *Mycobacterium vaccae* on ultrasonic vocalizations of male mice during anxiety-provoking situations

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Although inaudible to humans, male mice frequently communicate with each other by emitting ultrasonic vocalizations (USV). The frequencies at which they emit these USVs are indicative of their emotional state. Research shows that mice will vocalize between 18-32 kHz in aversive situations. *Mycobacterium vaccae*, a non pathogen found in temperate soils has been shown to stimulate the serotonergic system of the mouse brain. This is most likely due to activation of an immune pathway that stimulates serotonin production. It was hypothesized here that mice that ingested with *M. vaccae* would emit a different USV pattern than control mice that were not fed *M. vaccae*. Twenty male BALB/c pathogen free mice were used for testing. Each mouse was treated daily for 7.5 weeks, and then tested for five minutes in a Zero Maze (EZM). The EZM maze is designed to invoke fear and anxiety in mice. USVs were captured using a Binary Acoustic AR 125 Ultrasonic receiver, and then analyzed using SCAN'R software. Results show that while eight out of ten of the untreated mice demonstrated USVs within the range 20-40 kHz, only three out of ten of the *M. vaccae* treated mice demonstrated USVs within the range 20-40 kHz ($\chi^2=(1, N=20)=5.1$, $p<0.05$). This suggests a positive effect of *M. vaccae* on the fear state of male BALB/c mice.

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