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## **Long access heroin self-administration induces grey matter volume reduction in NIH Heterogeneous Stock rats.**

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Subjects affected by opioid use disorders (OUD) show reduced cortical grey matter volume (GMV). However, how GMV interacts with heroin dependence remains to be clarified. To answer this question, we subjected NIH Heterogeneous Stock (HS) rats to a longitudinal MRI study in which GMV was measured before and after exposure to heroin.

Heroin-naïve HS rats were subjected to T2-weighted MRI acquisitions. Rats were then divided into sex-balanced heroin-naïve and heroin-exposed groups (N=10-15). The heroin group was initially trained to 1h short access (ShA) heroin (60µg/kg/infusion) self-administration and then switched to 12h long-access (LgA). Motivation for heroin and heroin-primed reinstatement were also tested. After heroin training, T2-weighted MRI signals were again acquired in heroin-experienced and heroin-naïve rats.

Heroin experienced rats showed escalation of heroin intake during LgA training. Motivation for heroin increased between ShA and LgA. In the reinstatement test, heroin primed reinstatement of seeking. HS rats showed high variation in heroin seeking behaviors. GMV did not differ between groups before heroin training, but there was a GMV reduction in the orbitofrontal, medial prefrontal, and insular cortices, of the heroin group. A larger insular GMV at baseline corresponded to a higher escalation of intake. We also found that a higher heroin intake, escalation and reinstatement ratio corresponded to a bigger reduction of GMV in insular and frontal cortices.

In conclusion, HS rats developed heroin dependence-like behavior. Insular GMV in naïve animals predicted escalation and motivation for heroin. Heroin induced, and a larger GMV reduction was associated with increased addictive-like behavior.