

Anxiety and mild microglial activation in the amygdala two weeks after NA-induced neuroinflammation

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A single injection of neuraminidase (NA) within the cerebral ventricles (ICV) triggers an acute neuroinflammation, which is largely solved in two weeks. Neurological complications or behavioral alterations have been associated to neuroinflammation. While some of these symptoms decline with time along with inflammation, the possibility of long-term sequelae should be considered. Thus, we aimed to explore if NA-induced neuroinflammation provokes behavioral or neurological disturbances at medium (2 weeks) and long (10 weeks) term. Rats were ICV injected with NA or saline. A battery of neurological tests and a behavioral assessment (open field test) were performed 2 and 10 weeks post-ICV. Also, the inflammatory status was evaluated by immunohistochemistry and qPCR. First, neurological alterations of the sensorimotor reflexes were not found, suggesting that NA does not cause disturbances in major brain functions. While the open field test revealed normal locomotor capacity in the animals injected with NA, however the evaluation of specific behaviors (*rearing* and *rearing with support*) pointed out an increased anxiety state 2 weeks after NA administration, but not at long term (10 weeks). These results were confirmed by analyzing all the behavioral parameters measured in the open field test by means of a principal components analysis. Regarding signs of neuroinflammation, an overexpression of some genes related to inflammation (the receptor TLR4 and the alarmin HMGB1) was found in the hypothalamus of NA treated rats at 2 weeks post-ICV, but not at 10 weeks. A histological study of brain areas related to emotions (amygdala) and stress response (hypothalamic PVN) revealed no significant differences in the number of microglia or astrocytes. Nevertheless, the morphological analysis of microglial cells (a quite sensitive tool to evaluate microglial activation) demonstrated that, in the amygdala of NA injected rats, microglia presented a morphology consistent with a slightly activated state (decreased *cell area*, *cell perimeter*, *fractal dimension* and *roughness*; increased *cell circularity* and *lacunarity*). Such morphological change, which was evident 2 weeks after NA injection, was virtually reverted 10 weeks post-ICV. A similar study performed in PVN microglia yielded very subtle morphological changes (mostly not statistically significant). These results point out that NA injected ICV may cause anxiety in the medium term (while not affecting other functions like sensorimotor functions or the locomotor capacity), a behavioral alteration that is transient and that concurs with a mild inflammation, evidenced by the overexpression of certain genes and, more notably, by the morphological bias of microglial cells located in the amygdala towards an activated profile.