Using laser microdissection to study the neuronal basis of stress-induced loss of reward interest in mice

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Introduction

Depression is a common mental illness, with a worldwide prevalence of around 4 %. Core symptoms include depressed mood and reduced interest, indicating excessive focus on the negative and attenuated focus on the positive, respectively. Depression results from the interaction of social, psychological and biological factors, with adverse life events called stressors being major risk factors. The neurobiological mechanisms via which stressors lead to depression are poorly understood, and animal studies are essential in this regard.

In mice, chronic social stress leads to reduced interest in reward, as demonstrated by measuring the amount of behavioural effort that stressed mice will make to obtain sucrose rewards, compared with control mice. There is evidence that the amygdala, a brain region that is important in the processing of stressors, is also important in reward processing. Therefore, stress-induced changes in the amygdala could contribute to impaired reward processing. Approximately 80 % of the neurons in the amygdala utilise glutamate as their neurotransmitter. These neurons have long-range axons that project to various brain regions, including the nucleus accumbens, a major region for reward processing and a target for the neurotransmitter dopamine. The glutamate neuronal pathway from amygdala to nucleus accumbens is a major candidate for the mediation of stress effects on reward processing.

Methods

To study the effects of chronic social stress on the transcriptome of glutamate neurons in the amygdala that project to the nucleus accumbens, adult male C57BL/6 mice were injected bilaterally in the nucleus accumbens with the retrograde tracer cholera toxin subunit B, conjugated to the fluorophore Alexa555 (CTB-Alexa555) (Figure 1A). After a 10-day recovery, mice underwent a 15-day protocol of chronic social stress or control handling. On the following day, mice were deeply anaesthetised and transcardial perfusion with phosphate-buffered saline was used to rinse blood from the brain, and brains were isolated and frozen. On a cryostat, coronal brain sections containing the amygdala (Figure 1B) were cut at 10 µm and mounted onto MMI PET membrane slides, RNAse free. The sections then underwent fixation-dehydration in ethanol. Using the MMI CellCut laser capture microdissection system with fluorescence settings optimized for visualization at wavelength 555 nm (Figure 1C, D), Alexa555^{+} amygdala tissue regions were encircled at diameter of 35 µm within the MMI CellTools software. For each section, all such regions were encircled – N = 40-60 per amygdala per hemisphere - and the UV cutting laser was activated. The tissue was collected onto the cap of an MMI Isolation tube, Qiazol was added for tissue lysis, and lysates were frozen prior to RNA extraction. For each mouse, the lysates from 500 x Ø = 35 µm tissue samples were pooled and frozen for RNA extraction.

RNA extraction of cell lysates was conducted using the Qiagen miRNeasy Micro kit. After RNA quantity/quality assessments with the Agilent Bioanalyzer, low-input RNA sequencing libraries were prepared with the SMARTer protocol and sequenced with the Illumina NovaSeq platform. Sequencing reads were mapped

to the *Mus musculus* reference genome (mm10) using STAR v2.5.2b allowing for soft clipping of adapter sequences. Transcript quantification was done with RSEM v1.3.0 and featureCounts v1.5.1. QC and downstream bioinformatics analyses were performed with Bioconductor v3.12 tools.

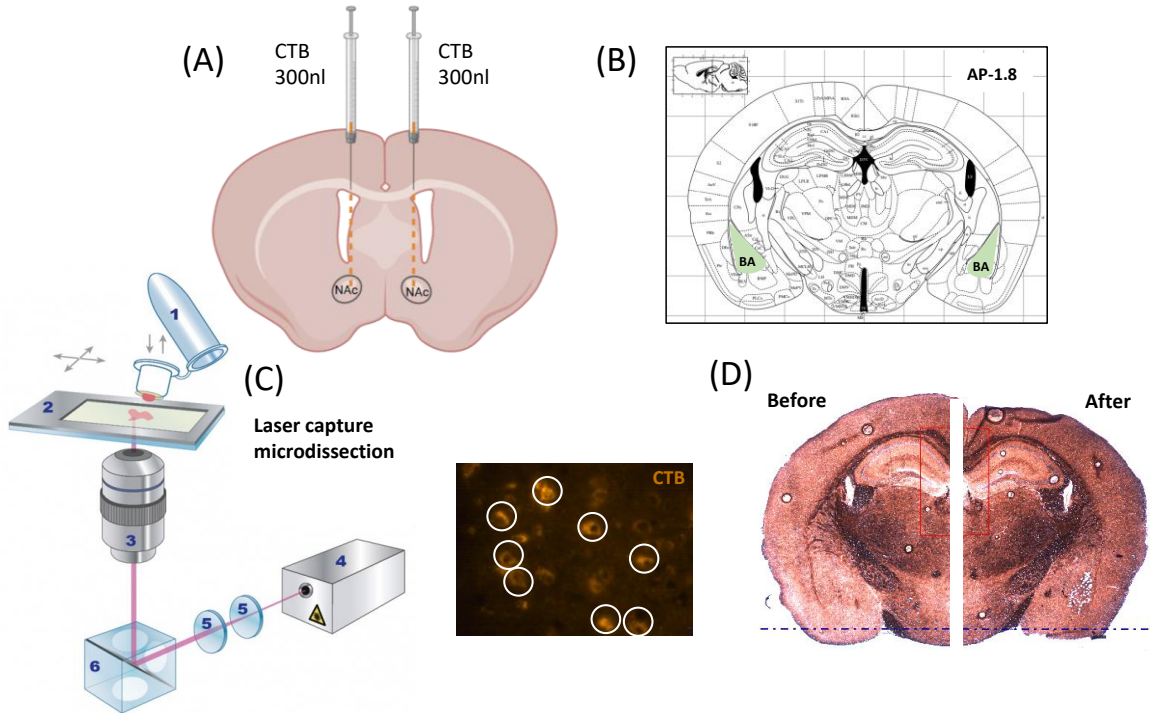

Figure 1. Targeted cell-tissue collection from the mouse brain using the CellCut LCM system. (A) Schema showing bilateral injection of CTB-Alexa 555 retrograde tracer into the nucleus accumbens, NAc. (B) Image from a mouse brain atlas showing the basal amygdala, BA. (C) Representative CellCut microscope image (40 x) of a coronal section including the BA with clearly visible areas of CTB-Alexa 555^{+} tissue. These regions were selected and collected. (D) Representative low magnification CellCut images of a coronal section including the BA before and after collection of BA CTB-Alexa 555+ tissue.

Results and Discussion

In order to validate whether the tissue microdissected with CellCut was, indeed, derived primarily from pyramidal glutamate neurons, the expression levels of cell type-specific markers were compared: the glutamate neuron-specific target gene, *Slc17a7*, was expressed at substantially higher levels than were the marker genes for every other brain cell type e.g. GABA neurons, oligodendrocytes. A median of > 13,500 genes were expressed per sample. Differential gene expression analysis between the chronic social stress and control conditions resulted in 64 up- and 2 down-regulated genes (|log2 fold-change| > 0.5 and $p < 0.001$), which significantly overlapped with the members of the MAPK and Rap1 signalling pathways, as well as with the set of genes involved in axon guidance.

Therefore, using a combination of neuron labelling with a fluorescent protein retrograde tracer, MMI CellCut LCM, and RNA-sequencing, it was possible to demonstrate that chronic social stress in mice leads to changes in gene expression in amygdala glutamate neurons projecting to nucleus accumbens. The study provides supportive evidence that this pathway mediates stress effects on reward processing.