jove

Title: Brain Morphology of Cannabis Users with or without Psychosis: A Pilot MRI study

Authors and Affiliations:

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Author Questionnaire

1. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, but authors are not able to acquire screen captures**

Videographer: Please film the screen for all SCREEN shots.

3. Filming location: Will the filming need to take place in multiple locations?   **Yes, 3 locations walking distance**

Current Protocol Length

Number of Steps: 4
Number of Shots: 11

Introduction

1. Introductory Interview Statements

REQUIRED:

1.0. Paolo Brambilla: The aim of this study was to investigate morphological brain differences between chronic cannabis users with cannabis-induced psychosis and non-psychotic cannabis users without any psychiatric conditions and correlate brain deficits with selective socio-demographic, clinical and psychosocial variables. NOTE: 1.0. was added for introducing the video and should be edited before 1.1.1

    1.0.1 INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.1. **Paolo Brambilla:** Studying brain alterations induced by cannabis consumption may represent a crucial step in the early detection and treatment of patients at high-risk of developing a psychotic picture.

    1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Eleonora Maggioni:** Magnetic resonance Imaging is a non-invasive and relatively safe method to investigate brain alterations. It does not use radiations and does not cause pain. Therefore, it is applicable to a younger population, which is particularly exposed to cannabis consumption. NOTE: This was said by Eleonora Maggioni instead of Paolo Brambilla

    1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: Lab media: 2.1.1, 2.1.2 and 2.1.3*

OPTIONAL:

1.3. **Lucio Oldani:** The use of magnetic resonance to investigate cannabis-induced alterations carries considerable social and cultural implications. The results achieved via this technique may lead to significant educational and economic considerations. NOTE: This was said by Lucio Oldani instead of Paolo Brambilla

    1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: Lab media: 3.1.1, LAB MEDIA: Table 1*

Introduction of Demonstrator on Camera

1.4. **Lucio Oldani:** Demonstrating the procedure will be Claudia Cinnante, a neuroradiologist from our institution. NOTE: This was said by Lucio Oldani instead of Paolo Brambilla

    1.4.1. INTERVIEW: Author saying the above.

    1.4.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

1.5. Procedures involving human subjects have been approved by the Local Ethical Committee. The study was approved by the local ethical committee.

Protocol

2. **Magnetic Resonance Imaging**

   NOTE: From 2.1.1 to 2.2.3 and 2.4.1 were shot behind a shielded glass and it was not possible to use the slate: there are notes in audio, but the sequence was shot in chronological order and it should be easy to recognize the points.

   2.1. Instruct the participant to lie down in a supine position on the bed of the 3 Tesla MRI scanner [1]. Provide earplugs and headphone to block noise [3] and place a radio frequency coil over the participant's head [2]. *Videographer: This step is difficult and important!*

      2.1.1. Participant lying down on the bed.

      2.1.3. Patient putting on earplugs or headphones. NOTE: Authors' inverted/swapped both voice over and shot for 2.1.2 and 2.1.3

      2.1.2. Talent placing the radio frequency coil over the participant's head.

   2.2. Instruct the subject to remain still [2], then run MRI session from the workstation in the control room [3]. Run a 3-plane gradient echo scan for alignment and localization [4] and perform a shim procedure to generate a homogeneous, constant magnetic field [4] [5]. *Videographer: This step is important!*

      2.2.1. NOTE: 2.2.1, both VO and Shot, - deleted by authors because foam pads was supposed to be used only with kids and the subject was an adult

      2.2.2. Talent instructing the subject.

      2.2.3. Talent going into the control room.

      2.2.4. Talent running a 3-plane gradient echo scan

   2.3. Start an echo-planar-imaging protocol for MRI [1]. The parameters for the acquisition of a high-resolution T1-weighted three-dimensional brain scan are already set in the imaging program and should not be changed [1] [2 TXT]. *Videographer: This step is important!*

      2.3.1. Talent starting the imaging protocol.

   2.4. When finished, remove the participant from the MR scanner room [1]. Transfer the MR data to a disk and close the session [2].

      2.4.1. Talent removing the participant from the scanner room.

      2.4.2. Talent transferring data to a disk and closing the session.

3. **Pre-processing steps**

3.1. Use the **Script_pre-processing** script file to perform the pre-processing steps in MATLAB **[1]**. For segmentation, process the structural image to distinguish and separate the white matter tissues, the grey matter tissues, and the cerebrospinal fluid into different images by running the **segment.mat** batch file **[1]**.

    3.1.1. SCREEN: 3_1_1_Opening_Matlab.mov.00:10-00:30

    3.1.2. SCREEN: 3_1_2_Segment.mov.00:00-00:21 *Videographer: Please film the screen for all SCREEN shots.*

3.2. Next, run the **create_template.mat** batch file to determine the nonlinear deformations for registering the GM and white matter images of all participants **[1]**.

    3.2.1. SCREEN: 3_2_1_DARTEL.mov.00:00-00:16

3.3. Perform spatial normalization to adapt the MRI images to an anatomical standard template by running the **normalize_to_MNI.mat** batch file **[1]**.

    3.3.1. SCREEN: 3_3_1_NORM_and_SMOOTH.mov.00:00-00:05 *Video editor: Slow it down to match the VO*

3.4. After motion correction, perform an isotropic Gaussian kernel of 6-millimeter full width at half maximum Gaussian kernel to increase the signal-to-noise ratio and to account for subtle variations in anatomic structures by running the **normalize_to_MNI.mat** batch file **[1]**.

    3.4.1. SCREEN: SCREEN: 3_3_1_NORM_and_SMOOTH.mov.00:05-00:10 *Video editor: Slow it down to match the VO*

4. **Statistical Analysis**

4.1. NOTE: VO 4.1 and Shot 4.1.1 is deleted by the authors

4.2. Perform a one-way Analysis of Variance, or ANOVA, in the context of a General Linear Model design to compare GM volumes between CIP patients and non-psychotic cannabis users, with gender and age as controlling variables, by running the **one-way ANOVA** batch file **[1]**.

    4.2.1. SCREEN: 4_2_1_ANOVA.mov.0:00-0:28

4.3. For the CIP group, carry out whole-brain regression analyses to explore whether the scores in all the clinical and psychosocial scales employed in this study were significantly correlated with GM volume changes. Run the **Regression analysis** batch file with the clinical scale of interest **[1]**.

    4.3.1. SCREEN: 4_3_1_BPRS.mov.0:00-0:29

Results

5. **Results: Socio-demographic, Clinical, Psychosocial and VBM Results**

5.1. There were no differences in terms of gender, age, age of onset of dependency, and educational level between cannabis Induced psychotic patients and non-psychotic chronic users **[1]**.

    5.1.1. LAB MEDIA: Table 1. *Video Editor: Scroll down the table as VO talks but keep the headers in place.*

5.2. However, cannabis Induced psychotic patients showed higher scores in one temperament dimension and one-character dimension of the Temperament and Character Inventory **[1]**.

    5.2.1. LAB MEDIA: Table 1. *Video Editor: Emphasize rows Type (N); frequency of other drug use*

5.3. Non-psychotic cannabis users also showed higher scores compared to cannabis Induced psychotic patients in one sub-dimension of the Neighbourhood Scale **[1]**, in the SES total scores **[2]**, in the Quality of Life-Index **[3]**, in the GAF scale **[4]**, and in one character dimension of the TCI scale **[5]**.

    5.3.1. LAB MEDIA: Table 1. *Video Editor: Emphasize the NS-E row.*

    5.3.2. LAB MEDIA: Table 1. *Video Editor: Emphasize the SES total row.*

    5.3.3. LAB MEDIA: Table 1. *Video Editor: Emphasize the QL-index row.*

    5.3.4. LAB MEDIA: Table 1. *Video Editor: Emphasize the GAF row.*

    5.3.5. LAB MEDIA: Table 1. *Video Editor: Emphasize the TCI Sd row.*

5.4. VBM analysis showed that cannabis Induced psychotic patients had extensive grey matter decreases compared to non-psychotic chronic users in several brain regions within the prefronto-temporo-limbic network, including, for example, the right prefrontal gyrus, the superior temporal gyrus and insula **[1]**.

    5.4.1. LAB MEDIA: Figure 1

5.5. Notably, no grey matter reductions were observed in non-psychotic chronic users compared to cannabis induced psychotic patients **[1].**

    5.5.1. LAB MEDIA: Table 2

Conclusion

6. **Conclusion Interview Statements**

6.0 **Lucio Oldani:** Overall, the results suggest that cannabis-induced psychosis is characterized by selective brain reductions that are not present in NPCU. Therefore, neuroimaging studies may provide a potential ground for identifying putative biomarkers associated with the risk of developing psychosis in cannabis users. NOTE: This was added with Lucio Oldani and should be edited before 6.1.1

> 6.0.1 INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 1*

6.1. **Paolo Brambilla:** A step forward to this study would be to apply an integrated approach by coupling together other neuroimaging techniques, such as functional MRI or PET, with the final aim of exploring whether the structural alterations observed in this study are accompanied with functional dysfunctions.

> 6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

6.2. **Paolo Brambilla:** We believe MR should be implemented in the daily clinical practice when dealing with patients with psychosis and a history of cannabis abuse. Grey matter volume decreases in specific brain structures suggest that endocannabinoid system may be a target for future neuroimaging, genetic and epigenetic studies.

> 6.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.