Author's response to reviews

Title: Multimodal neuroimaging of frontal white matter microstructure in early phase schizophrenia: The impact of early adolescent cannabis use (study protocol)

Authors:

Denise Bernier (dcbernie@dal.ca)
Jacob Cookey (jc533393@dal.ca)
David McAllindon (dmcallindon@gmail.com)
Robert Bartha (rbartha@robarts.ca)
Christopher C Hanstock (chris.hanstock@ualberta.ca)
Aaron J Newman (aaron.newman@dal.ca)
Sherry H Stewart (sherry.stewart@dal.ca)
Philip G Tibbo (phil.tibbo@cdha.nshealth.ca)

Version: 3 Date: 23 September 2013

Author's response to reviews: see over
Responses to Reviewers

Reviewer 1:

1. In the Statistical Analysis section, age and gender are listed as variables to be tested in a post-hoc exploratory analysis, but they really should be corrected for in all of the primary analyses.

Response.

Customary exploratory analyses will be performed as an initial step, searching for outliers in the data and associations between variables. If there would be a robust association between neuroimaging indices and relevant clinical / functional variables, a covariance model would be used (see Tibbo, Bernier et al, 2012). If no association would be found, a correction (covariance) that would be applied to primary analyses would introduce error in the model and thus, would bias the findings. Here we are referring to a main a priori assumption that must be met prior to correcting the data, that is, a significant association must exist between the two variables of interest.

2. Duration of cannabis exposure is a concern, as there is the potential for a confound in which those individuals who start cannabis use at a younger age by definition have a higher cumulative lifetime dose. Will this be corrected for in any way? It also might be possible to address this in a post-hoc analysis by taking a subgroup of individuals from both early and late groups who have a similar duration of use (so, in this subgroup likely the individuals from the early group would have a history of cannabis use but not current use), and determine whether the larger effects remain even between these matched groups.

Response.

Cumulative lifetime usage is a very important variable so it will be fully explored. Here, it could be the case where a Johnson Neyman test would be the best approach to find the threshold of where exactly cumulative usage becomes detrimental (this approach was used in Tibbo, Bernier et al., 2012).

3. Why is the focus on the left SLF only? It is not clear what hypothesis would differentiate left from right enough to exclude the right side, and the rationale for this is not included in the protocol. Either bilateral regions should be included, or this should be specifically addressed.
Response.

If the Reviewer is referring to online MR acquisitions: Here, we are bound by the technical limitations of $^1$H-MRS and relaxometry acquisitions. If we would sample a second brain region of interest, the time required for the participant to remain in the scanner would become clearly unreasonable.

If the Reviewer is referring to DTI offline analyses: We will compute and look at all the available data, even when not part of the main research question, in order to inform future work.

Noteworthy, our extensive search of the previous relevant literature has not revealed any reliable or replicated laterality effect in this population, in regards to the four neuroimaging indices of interest.

1. In the hypothesis section, it is indicated that reduced FA values may or may not be found in the schizophrenia group. However, earlier in the introduction it is stated that the SLF is one of the three tracts most commonly found to have reduced FA, with seven references cited. Thus, it seems quite probable that SLF FA levels will be lower in the patient group overall.

Response.

The fact still remains that for each positive finding reported in the DTI literature, there is at least one other study reporting normal values.

1. The introduction is overly long, even relative to other protocols published by this journal. It would be better to pare it down so that there is a focus on those aspects of each technique specifically relevant to this study (for instance, it is likely not necessary to define here the details of what DTI is, as it is now a fairly commonly used technique).

Response.

We take the stance that the readership of this Journal will include people not familiar with neuroimaging or else, not familiar with each one of these four neuroimaging modalities.

Reviewer 2:

1. The use of an uncommon spectroscopy fitting program seems unusual. Although the authors argue as to why it may be preferred, since peak are from spectra with only a TE change are being
compared to fit for T2, would you want to fit each separately with different zero and 1st order phase corrections. Programs that fit the entire dataset simultaneously (such as ProFit or FiTAID) seem like a better idea.

Response.

If we would use a fitting program that fits in the Frequency domain, the signal-to-noise ratio (SNR) of the spectra would strikingly drop, as the full free induction decay (FID) curve is included in the fitting model with these programs. As a consequence, most of our spectra would not meet the threshold established for quality criteria. The incommensurable benefit of fitting in the Time domain pertains to the fact that the tail of the FID, where almost only noise remains, can be ignored from the fit. This strategy maximises the SNR, as can be observed when our data is reverted to the Frequency domain for visualisation (Figure 2).

There is a very good fit of the fitted data with the exponential decay curves, as illustrated in Figures 3 and 4: Obviously, our fitting in the Time domain does not entail important differences across spectra associated with zero and first order corrections. It needs to be emphasized here that the shim values are manually kept stable across all $^1$H-MRS acquisitions during a brain scan.

2. What are some of the limitations on your assumptions within each of the 4 imaging measurements?
   a) For instance, using NAA as a myelin integrity marker is unusual as most people use it as a neuronal marker.
   b) If a change is detected, how do you know it’s not axonal loss instead of myelin damage?
   c) How would differences in normal rates of brain myelination affect the interpretation?
   d) Would an increase in water T2 indicate fewer cells or more water?

Response.

   a) Most studies have been sampling both gray matter and white matter tissues within the targeted volume of interest, thus precluding a clear interpretation of NAA findings in terms of each of the specific anabolic and catabolic cycles of NAA. As such, only broader interpretations were possible.

   b) In this study context of white matter tissue, reduced NAA levels will bring the assumption of reduced acetate, the necessary and main ‘building block’ required for maintenance of myelin. There might be axonal damage too, but future work will be necessary to tease apart these two potential problems.
c) Differences in normal rates of brain myelination still need to be elucidated. At this point in time, a narrow age range in the population studied along with a close age match between the groups still remains the best approach in that regards.

d) Prolonged tissue water relaxation time constants would lead to the assumption of reduced cellular density at both the intracellular and extracellular levels of the target brain region. In lay terms: The water molecules spin during a longer duration when there is an impoverished cellular environment against which they can bounce and exchange their energy and relax to their original state. This regional index is very sensitive; however it does not, on its own, differentiate which cell types are being affected.