Reviewer #1: The authors have applied a novel analysis and interpretation to a study of the effects of THC on behavioral and cellular activity in rats. Based on a decrease in the efficacy and theta modulation of CA3 output to CA1, along with a lack of effect on theta modulation and firing rates in CA1 cells, the authors propose that the two subfields become less coupled when under the influence of THC and suggest that this reaction may explain the behavioral deficits seen in the delayed non-match to sample task when performed by rats under the influence of THC. This interpretation has potential and would be a valuable addition to the literature if it is found to be true. However, it is inconsistently explained and supported within this manuscript, and a part of it is based on incorrect assumptions about CA1 anatomical connectivity. There are concerns in terms of science and writing quality that must be addressed for this work to present a compelling case for its analysis and interpretation.

General Comments: This reviewer has raised excellent points relating to the mismatch between our hypothesis and the current literature. These comments were extremely helpful and have inspired us to review the current literature much more carefully. Thus, we have completely rewritten the paragraph dealing with our proposed hypothesis in the discussion. The new paragraph is found on 287-330. Whether this manuscript will ultimately be accepted in PLOS Computational Biology or not, we are very grateful to this reviewer for helping us make our work much stronger.

Scientific Concerns:  
1. Results - Manuscript Page 7, lines 186 - 188: “This confirms previous reports which show … that CA1 is capable to generating endogenous theta rhythms”. It is consistent with the report of Goutagny et al 2009 but does not confirm it - CA1 also receives timed input from sources other than CA3 (ECIII and medial septum are two of the most significant), and there is no reason to discount their input here when the authors have not shown any effect of THC on their spike timing or theta modulation.

*Response*: This is a valid point. Septal inputs to the hippocampus are particularly important for theta rhythms (for example, see Fuhrmann et al., 2015; Neuron). The language has been made more precise to reflect this: “This is consistent previous reports which show that CA3 propagates strong theta rhythms to CA1 [39,40] and also that CA1 is capable to generating endogenous theta rhythms [41].”

2. Discussion - Manuscript Page 10, lines 295-303. The authors first refer to “increased feedback excitation” but the proposed mechanism given below sounds more like “decreased feedback inhibition” and even includes a contributing factor of decreased CA1 pyramidal input to the inhibitory cells, so the proposed mechanisms are not consistently described or represented throughout the discussion. Relatedly, in the results section (Manuscript page 7, lines 211-212), the authors state “Essentially, the more THC increased feedforward inhibition and feedback excitation, the worse the rodent did on the task”, which is also confusing as elsewhere the authors seem to indicate that the feedforward inhibition (anatomically, this should be the CCK+ cell activity) is weakened with THC.

*Response*: This remark is very helpful. Indeed, the system identification method was able to detect a change in feedback excitatory index in CA1, but it is agnostic as to whether that is caused be reduced feedback inhibition or increased feedback excitation. As the reviewer points out, we hypothesize that it is the former. To make this point more clear, we changed the discussion to read: “It was found that THC increased feedback excitatory index in CA1 and that the magnitude of this effect was correlated with behavioral deficits.
We hypothesize that this is due to reduced feedback inhibition from CA1 cholecystokinin (CCK)-containing basket cells. …” Furthermore, the sentence which mentioned increased feedback excitation in the results section was completely removed.

3. Discussion - Manuscript page 10, line 300-301 states in regard to CCK+ cells that “their primary input and output is from/to CA1 pyramidal 301 cells [52].” It is true their primary output is to CA1 pyramidal cells, however it is misleading to state that their primary input is from CA1 pyramidal cells. Reference #52 does not make that claim, speaking only to the proportion of CA1 inputs for all interneuron types together, and in fact this reviewer is not aware of any definitive published evidence for monosynaptic connections from CA1 pyramidal cells to CCK+ basket cells, although there are known connections from CA1 pyramidal cells to other CCK+ cells. It would be more correct to state “their primary output is CA1 pyramidal cells and some types of CCK+ cells also receive input from CA1 pyramidal cells” as shown for example in **Lee et al J Neurosci 2010.**

*Response*: This point was answered together with point 4

4. It follows that the statement “ (2) reducing their total amount of action potentials due to reduced glutamatergic input from CA1 pyramidal cells” is probably untrue for CCK+ basket cells, which are the majority of CCK+ cells in CA1. Most CCK+ cells have dendrites in the area of Schaffer Collateral innervation of CA1 and are activated by the afferent Schaffer Collateral input (although some have dendrites within the oriens where most CA1 pyramidal cell collaterals would be found), so a reduction in afferent SC input is far more likely to be responsible for reduced drive to CCK+ cells than a reduction in CA1 pyramidal input and in the case of CCK+ basket cells, this is especially true.

*Response*: We thank the reviewer for bringing these points to our attention. After looking at the literature more closely we agree that the primary input to CA1 CCK basket cells comes from CA3 rather than CA1 pyramidal cells (Lee et al., 2010; Matyas et al., 2004). Matyas et al., 2004 also showed that 20% of CA1 CCK cells’ inputs are in the str. oriens layer and suggested that these inputs are from CA1 pyramidal collaterals**[[1]](#footnote-1)**. In either case, as the reviewer pointed out, our theory is valid whether the decreased excitatory input comes from CA3 or CA1 pyramidal cells. Both have the net effect of reducing CA1 CCK basket cell activity and thus CA1 feedback inhibition. Thus, we have changed the relevant sentence to: “While CCK cells only make up only 13.9% of interneurons (Bezaire et al., 2013), they express significantly more CB1 receptors than any other cell in the hippocampus (Katona et al., 2000), and their primary output is to CA1 pyramidal cells. Increased THC concentrations would reduce CCK interneuron output by (1) reducing the amount of GABA they release per action potential (2) reducing their MFR due to reduced glutamatergic input from principal cells in both CA3 and CA1.”

5. Manuscript Page 10, line 299 - “they express significantly more CB1 receptors than any other cell in the hippocampus [53],” but not all CB1 receptors are created equal; those found on pyramidal cells are thought to be **more** efficacious, so it would be helpful to consider this in addition to the receptor abundance. It would also be helpful to discuss the roles of DSI and DSE in the circuit and the effect of THC on those roles. For background on this and the previous point, check out **Ruehle et al J Psychopharmacology 2012** and the references it cites.

*Response*: We are very grateful that the reviewer brought these sources to our attention. We have elaborated on the point that pyramidal cell CB1 receptors are more efficacious in our discussion: Even though pyramidal cells have much lower densities of CB1 receptors than interneurons (Katona et al., 2000; Oshno-Shosaki et al., 2002), there is evidence that CB induced reduction of excitation is larger than these relative densities suggest.
Principal cells outnumber interneurons 20:1 in CA1 (Ahmed and Mehta, 2009) and their CB1 receptors were found to be several fold more efficacious than those of interneurons (Steindel et al., 2009).
Further, lower baseline activation levels of CB1 receptors on principal cells than on interneurons suggest they would be disproportionately activated by CB agonists (Ruehle et al., 2012).”

Writing & readability concerns  
The writing level of this manuscript **occasionally** falls below what this reviewer would expect and distracts from the scientific message. It is very fixable. This reviewer recommends:  
1. Before revising the manuscript, (re)read a short book on writing or scientific writing. Style: Basics of Clarity and Grace by Joseph Williams is short and very helpful  
2. Revise the manuscript, checking especially for the following (only a few examples of each problem are listed here):  
2a. The nouns and verbs should be appropriately plural or singular  
2ai. Example: Actual Page 9, line 15: synapse should be plural (synapses)  
2aii. Example: Manuscript Page 2, line 72-73: “... a type of linear nonparametric models ...” → models should be singular (model)  
2aiii. Example: “the emergent effects ... is “ → is should be plural (are)  
2b. Keep in mind whether something is countable when deciding between using ‘amount’ and some form of ‘number’  
2bi. Example: Manuscript page 10, line 289 - “THC reduces the amount of casually connected CA3-CA1 290 neuronal pairs” ; amount → number, frequency, incidence

*Response*: We are deeply embarrassed by this and have done our best to improve the language, including reading the paper several times over and getting feedback from several native english speaking peers. All the above examples have been corrected according to the reviewer’s suggestions. We thank the reviewer for bringing them to our attention.

2c. The word ‘this’ should rarely stand alone in scientific publications - it is vague and confusing. Better to specify what it means each time  
2ci. Manuscript Page 1, line 35: “This paper contributes to this by using” → first this (“This paper”) is good. Second one (“contributes to this”) is vague, try something like “This paper contributes to disentangling these effects by using…” which may need to be further refined to make it less awkward

*Response*: Sentence changed to: “This paper contributes to our understanding of the circuit level effects of CBs by using data driven modeling to examine how THC affects the input-output relationship in the Schaffer collateral synapse in the hippocampus.”

2cii. Manuscript Page 2, line 76: “This makes them particulary well suited” → “This characteristic makes…” or “This lack of reliance on assumptions makes …”

*Response*: Sentence changed to: “This characteristic makes them particularly well suited …”

2d. Remove unnecessary qualifications and rewrite unprofessional ones differently  
2di. Example: Manuscript Page 2, line 46: “has attracted a lot of somewhat controversial attention” is distracting because of its imprecision and contrast to most writing found in journal publications

*Response*: Sentence rewritten as “In particular, CB agonists have shown promising but mixed results in the treatment of epilepsy, as various types of agonists at various doses have been shown to be both pro- and anticonvulsant”

2e. Parallel sentence construction  
2ei. Example: Manuscript Page 2, line 49: the items in this list do not follow a parallel construction, making it confusing to parse: “much work has been done on the chemical structure of various cannabinoids, cannabinoid receptors, along with their cellular interactions and pharmacology” - would be clearer as “.... on the chemical structure of various cannabinoids and cannabinoid receptors, along with their…” or “... on the chemical structure of various cannabinoids, cannabinoid receptors, and their…”

*Response*: Sentence changed to “Parallel to increasing therapeutic research, much work has been done on the chemical structure of various cannabinoids and cannabinoid receptors, along with their cellular interactions and pharmacology.”

2f. Word choice is not always the most appropriate  
2fi. Example: Manuscript Page 10, lines 320 - 321 “the decrease in feedforward excitation overpowers the increase in feedback excitation and results in lower MFR” - it is difficult to visualize a decrease in agency overpowering something, would be more fitting to say “the increase in … is unable to compensate for the decrease in ... , resulting in lower MFR”  
effect/affect  
2g. Other recommendations from the writing book  
3. Address these other issues as well:  
3a. Manuscript page 1, lines 31-40: unclear whether this is an alternate abstract? It repeats its last sentence from the actual abstract

*Response*: *This is a required Author Summary. A title has been added to make this more clear.*

3b. Ensure transitions are appropriate - they should respect the flow of logic  
3c. Spell check  
3d. Appropriate use of commas. Sometimes they are extraneous, other times they are sorely needed:  
3di. Example: Manuscript page 3, line 93: “While performing the DNMS task single-unit…” is confusing, add a comma to clarify: “While performing the DNMS task, single-unit...”

*Response*: Comma has been added.   
  
Minor & other concerns  
1. Figure 1B - what are the units on the axes? Assuming Hz for individual firing rates?

*Response*: Yes, that is correct. The Hz units have been added to the labels to clarify this.

2. Figure 2 caption says “Note that 1 CA1 neuron has no significant inputs.” → please specify that the statement refers to the theoretical model using Granger causality (is not intended as a biological anatomy statement based on experimental observation).

*Response*: Sentence changed to “Note that 1 CA1 neuron has no significant granger-causal inputs.”

3. Figure 2B could be further clarified with a “CA3 input #1” label over the CA3 spike trains, to correspond to inputs in 2C and a “CA1” label over the CA1 spike train.

*Response*: The figure has been modified to reflect this

4. Manuscript Page 17, line 575 is confusing: “N-1 two input models were constructed”

*Response*: Sentence has been changed to: “Afterwards, N-1 models were constructed with two inputs: the previously selected input and one of the remaining potential inputs.”

5. Figure 3 CD - can you add a legend for the animal colors, even if the animal names are arbitrary (“animal 1, 5 sessions; animal 2, 3 sessions; animal 3…”). It would enable readers to quickly understand the color coding w/o having to read the legend and also quickly see how many animals & sessions were included.

*Response*: A legend was added with the Rat 1, Rat 2, etc… The amount of sessions was added since each animal went through a certain amount of control and THC sessions. In the plots under discussion, each dot represents the difference in a value in one of the THC sessions from the mean of that value in the control sessions. Thus, I think it would be confusing to put in the amount of sessions as the reader wont know whether they refer to just the THC sessions (i.e. the # of dots of that color), or all the sessions.

6. Figure text is quite small - for example, the legend text in Figure 2C is almost unreadable

*Response*: All figure text was made larger.

7. There should be a short summary of the previous work, at least the categorization of functional cell types (FCTs) that currently refers to previous work for any explanation, to enable this manuscript to stand alone.

*Response*: This is a great point. We decided that in order to improve clarity and avoid confusion, the reference to FCTs from previous work has been removed. Instead, the paper simply refers to these cells as sample-presentation cells and provides a reference to the previous work which talked about FCTs. We believe that since this is such a minor aspect of the paper, it is preferable not to go into excessive detail on this, as it will distract the reader.

8. Figures should stand alone; spell out abbreviations in the captions (ex: Figure captions should spell out DNMS, FCT, gPDM at least once)

*Response*: All abbreviations in the figures have been spelled out (except for well known ones such as CA1 and THC).

9. Some references in the bibliography have corrupted characters - see reference 55 for example. Also, the journal names are not properly capitalized; if they are proper in the bib file, simply surround the whole journal title with an extra set of curly braces { } to preserve capitalization in the compiled document

*Response*: Corrupted characters in bibliography have been corrected, including source 55. All Journal titles have been properly capitalized.

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Reviewer #2: In this paper the authors take an interesting and novel approach in applying nonparametric modelling to investigate the effect of exogenous CBS on the hippocampal Schaffer collateral synapse. In my opinion certain points (listed below) merit further clarification.

**General feedback:** Both this reviewer and reviewer #1 raised excellent points relating to the mismatch between our hypothesis and the current literature. In response to this, we have completely rewritten the paragraph dealing with our proposed hypothesis in the discussion. The new paragraphs are found on lines 287-330. We believe this rewritten section is much more clear and addresses the reviewer’s concerns.

Comments  
1. CB1 receptors are found on both excitatory and inhibitory cells in hippocampus leading to both suppression of inhibition and excitation. However, depression of inhibition is much more prevalent than the depression of excitation and can we induced with lower CB concentrations), supposedly due to the lower sensitivity of CB1 receptors expressed on excitatory rather than inhibitory synaptic terminals. (See Ohno-Shosaku, T. et al, (2002). Journal of Neuroscience and Zachariou et al 2014 Journal of ComputationalNeuroscience). My main concern is that this fact in not considered in the model and hypothesis formulation and in the overall results interpretation. For example in a relatively low THC dose the CB1 receptor found on excitatory terminal might not be affected. Hence, the conclusion that “THC functionally isolates CA1 from CA3 by reducing feed forward excitation and theta information flow while simultaneously increasing feedback excitation within CA1” might not necessarily hold. Please discuss/address.

*Response*: The reviewer’s point that DSI is more prevalent than DSE is well taken. Our strongest findings pertained to changes in CA1 feedback, which we believe is due to reduced feedback inhibition from CA1 CCK basket cells (i.e. DSI). We also found evidence for reduced feedforward excitation which we believe is due to DSE. Even though DSE is less prevalent than DSI, there is evidence that in-vivo, DSE may play a more prominent role than previously thought. (this point was made by reviewer #1). We have added the following sentences to discuss this issue: “Even though pyramidal cells have much lower densities of CB1 receptors than interneurons (Katona et al., 2000; Oshno-Shosaki et al., 2002), there is evidence that CB induced reduction of excitation is larger than these relative densities suggest.
Principal cells outnumber interneurons 20:1 in CA1 (Ahmed and Mehta, 2009) and their CB1 receptors were found to be several fold more efficacious than those of interneurons (Steindel et al., 2009).
Further, lower baseline activation levels of CB1 receptors on principal cells than on interneurons suggest they would be disproportionately activated by CB agonists (Ruehle et al., 2012).”

2. The model appears to only describe excitatory cells, as it focuses on the Schaffer collateral synapse. However, in the Methods it is noted that no differentiation was made between principal cells and interneurons. How did the authors ensure that the recorded cells whose activity was considered for fitting the model were indeed excitatory?

*Response*: This decision was made for two reasons. First, the paper used a ‘blackbox’ granger-causal framework where it was understood that functionally connected cells are not necessarily anatomically connected and that the estimated feedforward/feedback filters are not physiological EPSPs but rather an abstract measure of influence. Thus, the estimated filters include not only direct neuron-to-neuron physiological processes such as dendritic integration, but also indirect processes such as feedforward inhibition whereby the recorded CA3 neuron activates an unseen CA1 interneuron which activates the recorded CA1 principal cell (Pouille and Scanziani, 2001). Given that our goal was to see how CA3🡪CA1 dynamics change with THC, and given the high levels of connectivity between CA1 pyramidal cells and interneurons, we felt that this distinction would be somewhat artificial. Second, given that we used in-vivo extracellular recordings, there is no fullproof method to separate pyramidal cells and interneurons. Commonly used methods which rely on MFR, waveform shape, and ISI distributions can only give ‘putative pyramidal cells’. We attempted to use a similar procedure by removing cells with MFR >5 Hz as ‘putative’ interneurons. These cells made up only a minority of our recorded cells and did not significantly alter any of our results (i.e. with only 1 exception, significant P values remained so even after these cells’ exclusion).

3. Figures: All figures would benefit from a larger font size, as currently many parts are not easily readable. In Figure 1 some axis labels and units are missing and the figure would benefit from a longer/more descriptive legend. Also I find figure 1E confusing please clarify which part shows control and which TCH (the extra y axis with THC/control is not that informative). Same holds for Figure 3A and B. In Figure 2B the y-axis is missing (and fonts are very small. Also the color-coding blue - green for control - THC should be repeated at least in every figure (and maybe in sub-figures). Also in Figure 4 each panel should be properly described.

We thank the reviewer for all these suggestions that will certainly add to the readability of the paper. The font sizes of all figures were made bigger. Axis labels and units were added in figure 1. A legend was added to Fig 1E and 3A,B. In Fig. 2, the color schema for CA3 and CA1 was changed to make it distinct from Control/THC. In Fig. 2b, the y-axis is shown for the bottom input, and the caption says that the y-axis and scale is the same for all 3 inputs. The y-axis was not added to the top two inputs for aesthetic reasons.

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1. Interestingly, Lee et al., 2014 did not find any evidence of direct connections from CA1 pyramidal cells to CA1 CCK basket cells; however, they had a small sample size and never implied they disproved the possibility of such connections. [↑](#footnote-ref-1)