Review of: "Effect of SSRI discontinuation on anxiety-like behaviours in mice"

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The study was designed to explore the behavioral outcomes of SSRIs discontinuation in mice. To achieve their research goal, the authors exposed mice to chronic treatment with paroxetine or citalopram and then ceased treatment and compared the behavior of mice after discontinuation of drug with naïve mice (treated with saline) or mice with continued treatment. Behavioral outcomes were mostly evaluated using the EPM but also in locomotor activity in a regular and an aversive open field, light/dark box and fear conditioning test. The novelty suppressed feeding test, and hyponeophagia test are mentioned in the methods section but no results were described.
The reported results show some effects of treatment and some effects of discontinuation of drugs although I am not sure that there is any clear “statement” that can really summarize these findings.
Although you will find below some harsh criticism of this study, It is still clear to me that there was a significant amount of work invested here and I think that the data should be presented but that many clarifications (and possibly some reframing) should be done before publication would be appropriate.

The use of SSRIs in the treatment of individuals afflicted with depression and anxiety is very common and therefore the exploration of SSRIs discontinuation effects could be of importance. Yet, there are significant issues with the current MS and much clarification is needed to make this paper helpful in advancing research on SSRIs.

First, the MS does not explain **the need for exploring discontinuation effects in mice** . Considering the abundant knowledge about discontinuation effects in patients and the availability of large study cohorts for additional studies in patients, it is not clear why there is a need to explore this issue in mice. One reason that comes to mind is that using an animal model can be helpful in deciphering the underlying mechanisms of effects but the current study does not include a mechanistic component.

The schedule and organization of the experiments is unclear to me. How many separate experiments? What was the schedule of each experiment? What tests were included in each experiment and on what days? Which groups? What was the number of animals per groups? Where mice tested more than once in a

specific test? What was the timeline for each group? For each experiment? I **strongly suggest a clear chart or figure that will clarify all these questions**.

The results of the study are in fact highly problematic because at least for some of the experiments, **the results show that SSRIs chronic treatment (continuation groups) increase anxiety in the EPM (or had no effects)** while the expectation is that treatment will reduce anxiety in the EPM. Hence, the effects of treatment and the effects of treatment discontinuation are in the same direction (compared with the saline groups). These results cast significant doubt regarding the study because of two reasons: (1) the effects of SSRIs to increase anxiety stand in contrast with a large body of research that repeatedly demonstrated opposite effects and therefore it is possible that in this study “something went wrong”. (2) If the effects of treatment and discontinuation of treatment are in the same direction, one can suggest that the later effects are the consequences of past treatment rather than effects of discontinuation. For both reasons, it is likely that any conclusions regarding the effects of discontinuation are not possible.

The study includes a number of behavioral tests but concentrates on the results in the EPM. Yet, the study does not present t**he standard measures for the EPM that are the ratios between time (and number of entries) to open versus closed arms**. These ratio measures are probably the best because they (partially overcome) the question regarding the effects of generalized activity levels on the results. I suggest that these should be the main measures presented and analyzed rather than just time (and number of entries) to open/closed arms. This is critical especially because the effects on activity are not consistent across experiments.

There are a number of issues regarding the statistical analysis. One problem is in fact in the design. The basic design of the study is of three groups, treatment continued, treatment discontinuation and saline control but the most appropriate design should be a 2X2 design with SSRIs and discontinuation as main factors (saline-saline; saline-discontinuation; SSRI-SSRI, SSRI-discontinuation). Another problem (not uncommon in studies where test batteries are used) is that the same animals are tested in a number of tests and a number of measures are analyzed in each test but there are **no measures taken to overcome issues of multiple comparisons**. There are a number of ways that were suggested across the years to properly analyze such data and it is important to follow an analysis method where multiple comparisons are not ignored.

There are additional smaller issues but considering the gravity of the problems mentioned above I suggest that they should be addressed first before shifting attention to smaller matters.