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Dear Editor

It is my pleasure to submit our manuscript tiled "**A novel system for tracking the dynamics of social preference behavior in small rodents**" for publication in *JoVE*.

Unraveling the biological mechanisms underlying psychopathological conditions characterized by atypical social behavior, such as autism spectrum disorder (ASD), is currently one of the main challenges in the field of social neuroscience. The use of animal models of such conditions seems to be crucial for the achievement of this aim. Yet, efficient use of animal models requires standardization and automation of behavioral tests that will enable assessing social behavior in an unbiased manner, independently of the observer or the specific laboratory performing the experiments. In a seminal work published more than a decade ago, Moy and colleagues (2004) presented the three-chamber test, which has become a standard way to evaluate social behavior in animal models of ASD.

This test, based on measuring the time spent by the subject in either a central chamber or each of two lateral chambers where distinct stimuli are located, suffers from several caveats. First, it largely depends on the preference of the subject to locate itself in one of the chambers, hence should be sensitive to parameters that influence spatial navigation, memory, and preference. Under certain conditions, such parameters may vary independently of the motivation of the subject for a direct social interaction, and thereby interfere with the efficiency of the test to directly measure social motivation and preference. Second, this test is mostly used to measure the total time spent by the subject in each of the chambers, while neglecting the behavioral dynamics.

Here we present a novel experimental apparatus and automated analysis system that offer an alternative to the three-chamber test, and enable performing the same behavioral examinations while solving the aforementioned caveats. The custom-made apparatus is simple for production, and the analysis system is publically available as an open-source program, thereby allowing any lab to easily employ it. We

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demonstrate here the efficiency of the system in analyzing the dynamics of social behavior during the social preference and social novelty preference tests performed by adult male mice and rats. Moreover, we validate the ability of the system to reveal modified dynamics of social behavior in rats and mice following manipulations, such as whisker trimming.

We believe that the system presented here would enable a detailed and more thorough analysis of social behavior in small rodent models of various psychopathological conditions including ASD, and would enable a better use of these models by researchers investigating such conditions. Moreover, the ability of the system to precisely monitor the social behavior of subjects connected to electrical cables or optical fibers would enhance the efforts to reveal the role of various brain networks and molecular mechanisms in mammalian social behavior. We therefore believe that publishing a video protocol of the system and paradigms used by us would be of high interest to many scientists. We hope that you will find this paper suitable for publication in *JoVE*.

Most of the scientific data used as example results in this manuscript were previously published (Netser et al. *Mol. Autism* 2017).

Sincerely,

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