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Method Article

An open-source capacitive touch sensing device for three chamber social behavior test

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A B S T R A C T

A common feature of many neuropsychiatric disorders is deficit in social behavior. In order to study mouse models for such disorders, several behavioral tests involving social interaction with other mice have been developed. While a precise annotation of rodent behavioral state is necessary for these types of experiments, manual annotation of rodent social behavior is time-consuming and subjective. Therefore, an automated system that can instantly and independently quantify the animal's social exploration is desirable.

We developed a capacitive touch device for automated detection of direct social-exploration in a modified three-chamber social behavior test. In this device, capacitive sensors can readily detect nose-pokes and other direct physical touches from the rodent under investigation. In addition, a conductive barrier makes mouse behavioral output immediately available for real-time use, by sending data to a host computer via a custom Field-Programmable Gate Array (FPGA) platform.

Our capacitive touch sensing device produced similar results to the manually annotated data, demonstrating the ability to instantly and independently analyze direct social-exploration of animals in a social behavior test.

Compared to the manual annotation method, this capacitive touch sensing system can be used to instantaneously quantify direct social-exploration, saving significant amount of time of post-hoc video scoring. Furthermore, this low-cost method enhances the objectivity of data by reducing experimenter involvement in analysis.

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Specifications Table

Subject area	Neuroscience
More specific subject area	Basic Neuroscience
Method name	Capacitive touch sensing device
Name and reference of original method	Manual scoring from video images [13]
Resource availability	https://github.com/giovannibarbera/cap_sensor_MPR121_v1.0.git

Introduction

Social cognition is a fundamental but complex mental process. Abnormal social cognition represents a common feature in the pathology of various neuropsychiatric disorders such as autism, depression, and schizophrenia [1–4]. Recent advances in miniature epifluorescence microscope (MiniScope) technology allow *in vivo* calcium imaging in freely behaving animals [5–7]. We recently implemented this technology to record calcium activity from excitatory neurons in the medial prefrontal cortex (mPFC) while mice freely explored restrained social targets. We identified distinct and dynamic ON and OFF neural ensembles in the mPFC coding direct exploration of social targets [8]. Our study suggests that a precise detection of direct social-exploration is essential for future studies of rodent social behavior. Having a device that can automatically detect direct social exploration will greatly facilitate future efforts in examining rodent social behavior.

To study mouse social behavior, we use a modified three-chamber social behavior test adapted from Crawley's sociability and preference for social novelty protocol [9,10], which has been widely implemented as a measure of social behavior in rodent models of neuropsychiatric disorders [11]. This test examines the animal's social approach in a novel environment, a novel social environment, and in a choice for novel versus familiar social target environment [9,10,12]. In this three-stage test, the mouse is placed into a chamber with two empty containers at opposite corners, which are subsequently used to restrain conspecific strangers. Thus, proactive actions of the experimental mouse, including nose-poking, sniffing through container barrier, paw/limb-touching, are all viewed as direct-exploration towards the object/stranger [10,12].

This direct exploration can be measured manually in a post-hoc video analysis frame-by-frame, which is tedious and can inadvertently introduce human error. Nonetheless, this method of scoring behavior remains the "gold standard" of behavioral analysis ([13] despite being laborious, subjective and potentially biased). An automated scoring system for three-chamber social behavior test has been proposed [14], where the transitions of the rodent from one chamber to the next are detected through an infrared beam break at the opening between the chambers. This system however is limited to the detection of time spent in each chamber, without discriminating between social interaction or non-socially relevant behaviors (e.g. grooming, freezing, ambulation, etc.).

Video tracking software based on animal position has been developed to help reduce the amount of time in quantifying behavior but cannot be used to precisely annotate specific behavioral states such as direct social-exploration. New software packages using machine vision and deep learning methods have been developed recently but have yet to be applied to rodent social behavior studies [15]. Here we present a low cost and open-source capacitive touch sensing system with a modified three-chamber social behavior test for automatic detections of mouse direct social-exploration. This system integrates low-cost capacitive touch sensors with other behavioral recordings through a FPGA custom platform, to detect the direct-contact of the experimental mouse to the barrier in real-time, including nose-poking, paw-grabbing and limb-touching. This capacitive touch system provides behavior scores in real-time and makes it available immediately following each

behavior test, greatly reducing time and interferences in behavioral quantification. Therefore, our device allows high throughput social behavior testing, facilitating future studies on molecular and neural mechanisms of social behavior in many different mouse models of neuropsychiatric disorders.

Materials and methods

All components used for the capacitive sensor device, which was designed around capacitive touch sensor MPR121 (Freescale Semiconductor, Austin, TX, USA), were available off the shelf, and summarized in Table 1. The interface for real-time calibration/data processing and synchronization with other behavioral recording devices (e.g. behavioral cameras or brain activity recording) is implemented through a custom PCB developed for the miniScope [16] and interfaced with Opal Kelly XEM3010 (Opal Kelly Inc., Portland, OR, USA). An overview of the system is shown in Fig. 1a.

In the present study, 2 of the 12 channels provided by the MPR121 are used, by connecting the electrodes to two custom-made mouse containers with conductive floors which may contain the stranger mice (Fig. 1a). This configuration however is scalable, and an arbitrary number of sensors can be implemented to adapt to the needs of the specific experimental design. The containers are screwed on a conductive post connected to the electrode on the bottom of the arena (Fig. 1a). The 10-bit output for each of the two channels of the MPR121 is the change in capacitance of these electrodes from their measured baseline: the output change produced by the direct physical contacts of the subject under study to the metal bars of the containers determines the detection of a direct social-exploration events (Fig. 2a), translated into a binary signal based on a 10 standard deviations threshold. Since the metal floors of the containers were connected to the metal bars, and the stranger mouse inside the cup was constantly in contact with the metal floor, the effect of its movement on the sensor output is negligible.

Before the beginning of each trial, the touch sensor device is reset and calibrated by averaging the recordings from each sensor for 3 s. This step compensates for the drift introduced by changes in environmental conditions and surrounding electromagnetic noise.

After the calibration procedure, each sensor is read continuously at 140 Hz, and, to reduce the noise, the minimum read value is pooled over 100 ms and sent to a host PC through the custom FPGA platform, consisting of the XEM3010 FPGA board connected to custom PCB for interfacing both with the MPR121 and the miniScope [16]. Other possible configuration includes microprocessor-based control platforms such as Arduino or Raspberry Pi [13].

A digital signal (touch/no touch) is also generated in real-time by comparing the minimum sensor readout to a fixed threshold based on the sensor calibration data. This signal can be used in closed-loop experiments to interact with the behavior or prove causality (e.g. through optogenetic interrogation).

Table 1
List of components.

Component	Distributor	Part number	Quantity
12-channel capacitive sensor breakout MPR121	Adafruit Industries	1982	1
Mouse container posts	Thorlabs	MS2R	24 per container
Set screws	Thorlabs	4-40 set screws	48 per container
Mouse container base plate*	Custom aluminum plate	*	1 per container
Mouse container top*	Custom aluminum plate	*	1 per container
Screw/nut	Thorlabs	*	1 per container
Jumper wires 12" (signal/power to FPGA board)	Mouser	872-920-0141-01	4
Jumper wires (electrode connections)	Mouser	932-MIKROE-512	1 per electrode
FPGA system [16]*	*	*	1

* Design files and code are available at: https://github.com/giovannibarbera/cap_sensor_MPR121_v1.0.

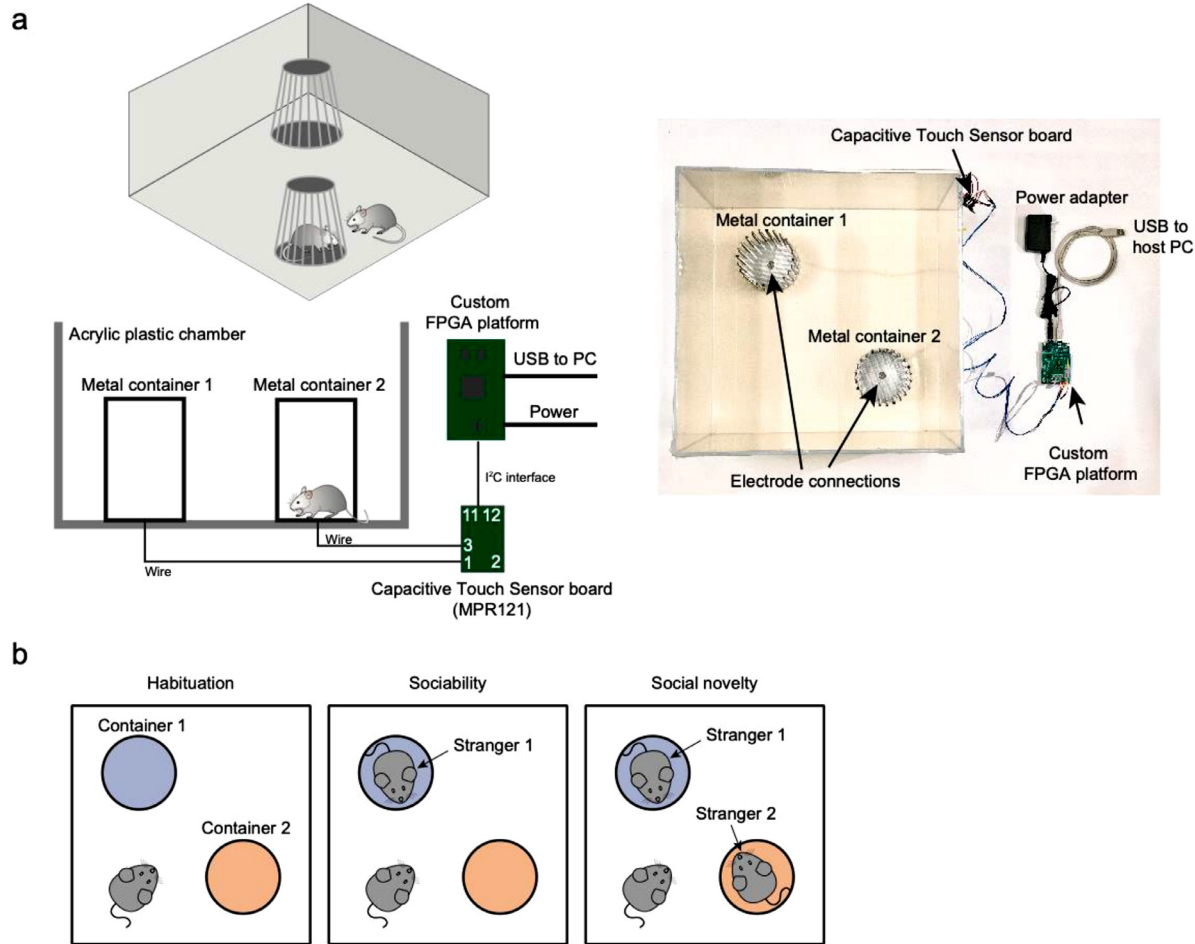
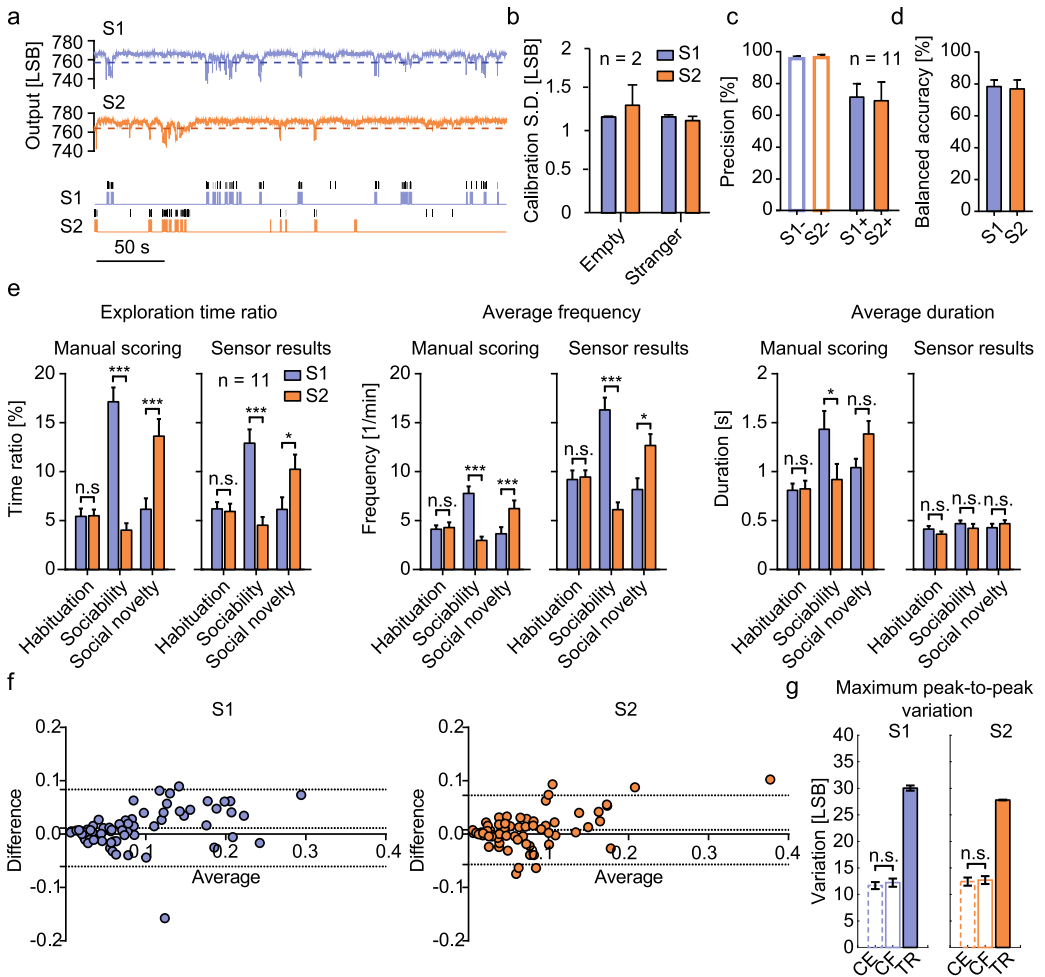


Fig. 1. Capacitive sensor device.

(a) Capacitive sensor device in social behavior test: 2 of the 12 MPR121 electrodes are connected to the custom mice containers (here shown without top) through a screw attached to the center of the container base plate. The sensor readouts are sent through I²C interface to a custom FPGA platform streaming data to a host PC.

(b) Social behavior test includes 3 stages: habituation (two empty containers), sociability (a stranger mouse in position S1), and social novelty (same mouse in position S1 plus a stranger mouse in position S2). For each stage, two 5-minute sessions were recorded.



Method validation

All experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee, the Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health. C57BL/6J male mice were used for experiments.

We successfully tested the functionality of the proposed apparatus on 11 mice performing the modified social behavior test (Fig. 1b) to assess sociability and the preference for social novelty [9, 10]. For each of the 3 stages (habituation, sociability and social novelty), 2 5-minute trials were recorded, for a total of six 5-minute recordings per animal (Fig. 1b). Each container was connected to a channel of the MPR121 and independently read by the custom FPGA platform. A typical output from the two capacitive sensors is shown in Fig. 2a and Supplemental Movie 1: a threshold set to 10 times the standard deviation of the readout from the calibration (Fig. 2b) was used for each sensor to calculate the binary output indicating the direct social-exploration of the animal under study with the corresponding container.

The accuracy of the capacitive sensor device was evaluated by comparing its output against the manually scored data (Fig. 2c, d): the proposed detection system achieved high consistency with manual scoring for negative (no-touch) predictions (96.46%), and lower precision (70.90%) for positive (touch) predictions. The discrepancy in the positive detection rate can be attributed to three main factors. Firstly, the system does not discriminate the type of contact between target animal and container which could happen through any body parts or foreign objects (e.g. cables), whereas manual scoring selectively picks forebody interactions only. Secondly, the judgment of the experimenter plays a role on how close the mouse should be to the social target for the interaction to be considered a social exploration, whereas the proximity sensor offers a stricter but more repeatable measure. Lastly, a conservative choice of the proximity threshold while reducing the false positive detection rate, can introduce the detection of multiple explorations where only one prolonged interaction took place. This is reflected in the larger number of total explorations detected (Fig. 2e, middle panel) with shorter duration than what was measured with manual annotation (Fig. 2e, right panel). As the sensor readout frequency is above the timescale at which social interactions take place, a simple filter to increase the minimum interaction duration would reduce the multiple touch detection rate.

Despite these discrepancies, the social behavior results from automatic detection of direct social-exploration are consistent with the manual annotation results (Fig. 2e, left panel), and in line with expected normal social behavior interactions [10].

Taken together, these data points to a more conservative measure of direct social-exploration that quantifies based on physical touch rather than the experimenter's judgement. This also means that the device is capable of detecting possible false positives, while rarely generating false negatives. For the purpose of calcium imaging, these device-generated data also align instantaneously with the calcium traces that allow us to synchronize them with behavior, excluding human response delays from manual annotation.

Discussion

In this manuscript we reported an automated method for quantifying animal social behavior using a capacitive touch device. The main advantage of this apparatus is the elimination of the need to manually score video recordings, which is time consuming and prone to error and inconsistencies derived from individual interpretation of the data. Furthermore, the system provides real-time direct social-exploration detection from up to 12 sensors, which can be used to control other instruments in closed-loop experiments.

One of the limitations of the system is the fact that it could be triggered by the proximity of objects unrelated with social exploration, such as cables, mouse tail, etc., resulting in false positive detections. In order to reduce the risk of false positive detections, several precautions can be taken, such as installing circular guards on top of the cups to prevent any wire attached to the mouse under study to touch the cup's metal bars. Additionally, the device can be paired with a video tracking system locating the position of the animal under study: this integration allows to restrict the behavior analysis to the frames in the proximity of detected social interactions, which can then be further

filtered either automatically, based on the location of the mouse under study, or manually, with minimal human supervision around the frames of interest.

One unique advantage of our device is to only pick up “direct social exploration events”. That is, the device can only be triggered when the test mouse is physically contacting the holding cup by poking/sniffing/touching/tasting. The device will not be triggered when the test mouse is only sitting/walking nearby the cup. Sometimes mice spend in proximity of the holding cups without any exploratory behaviors, but video-tracking based automated procedure cannot discriminate the direct social-exploration from just being proximity.

The accuracy of the readouts from the capacitive sensors relies on the precision of the calibration performed before each recording. It is important that during the calibration procedure, which is automatically performed at the beginning of each recording, the system should be in a stable state as any perturbation could result in inaccurate detections for direct social-exploration.

One relevant application leveraging the versatility of the proposed system is the integration of the device in a real-time closed-loop behavior system, where social exploration is used as a triggering mechanism to neural activity perturbation such as optogenetics or adverse stimuli delivery (e.g. electric foot shock).

Overall, the proposed system offers a low-cost and open-source solution that eliminates the need for time consuming manual annotation of social behavior test, enabling new closed-loop experiments synchronized with mouse behavior. Additionally, the automatic calibration procedure, the online threshold calculation and the number of available sensors make the system robust and flexible to adjust to different environments or different types of behavioral experiments not limited to social behavior test.

Declaration of Competing Interest

The authors declare no competing financial interests or conflicts of interest.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.mex.2020.101024](https://doi.org/10.1016/j.mex.2020.101024).

References

- [1] S.M. Couture, D.L. Penn, M. Losh, R. Adolphs, R. Hurley, J. Piven, Comparison of social cognitive functioning in schizophrenia and high functioning autism: more convergence than divergence, *Psychol. Med.* 40 (2010) 569–579.
- [2] P. Mundy, M. Sigman, J. Ungerer, T. Sherman, Defining the social deficits of autism: the contribution of non-verbal communication measures, *J. Child Psychol. Psychiatry* 27 (1986) 657–669.
- [3] C. Segrin, Social skills deficits associated with depression, *Clin. Psychol. Rev.* 20 (2000) 379–403.
- [4] J.L. Silverman, M. Yang, C. Lord, J.N. Crawley, Behavioural phenotyping assays for mouse models of autism, *Nat. Rev. Neurosci.* 11 (2010) 490–502.
- [5] G. Barbera, B. Liang, L. Zhang, C.R. Gerfen, E. Culurciello, R. Chen, Y. Li, D.T. Lin, Spatially Compact Neural Clusters in the Dorsal Striatum Encode Locomotion Relevant Information, *Neuron* 92 (2016) 202–213.
- [6] D.J. Cai, D. Aharoni, T. Shuman, J. Shobe, J. Biane, W. Song, B. Wei, M. Veshkini, M. La-Vu, J. Lou, S.E. Flores, I. Kim, Y. Sano, M. Zhou, K. Baumgaertel, A. Lavi, M. Kamata, M. Tuszynski, M. Mayford, P. Golshani, A.J. Silva, A shared neural ensemble links distinct contextual memories encoded close in time, *Nature* 534 (2016) 115–118.
- [7] K.K. Ghosh, L.D. Burns, E.D. Cocker, A. Nimmerjahn, Y. Ziv, A.E. Gamal, M.J. Schnitzer, Miniaturized integration of a fluorescence microscope, *Nat. Methods* 8 (2011) 871–878.
- [8] B. Liang, L. Zhang, G. Barbera, W. Fang, J. Zhang, X. Chen, R. Chen, Y. Li, D.T. Lin, Distinct and Dynamic ON and OFF Neural Ensembles in the Prefrontal Cortex Code Social Exploration, *Neuron* 100 (2018) 700–714 e9.
- [9] J.N. Crawley, Designing mouse behavioral tasks relevant to autistic-like behaviors, *Ment. Retard. Dev. Disabil. Res. Rev.* 10 (2004) 248–258.
- [10] S.S. Moy, J.J. Nadler, A. Perez, R.P. Barbaro, J.M. Johns, T.R. Magnuson, J. Piven, J.N. Crawley, Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice, *Genes Brain Behav.* 3 (2004) 287–302.
- [11] O. Kaidanovich-Beilin, T. Lipina, I. Vukobradovic, J. Roder, J.R. Woodgett, Assessment of social interaction behaviors, *J. Vis. Exp.* (2011).

- [12] S.S. Moy, J.J. Nadler, N.B. Young, A. Perez, L.P. Holloway, R.P. Barbaro, J.R. Barbaro, L.M. Wilson, D.W. Threadgill, J.M. Lauder, T.R. Magnuson, J.N. Crawley, Mouse behavioral tasks relevant to autism: phenotypes of 10 inbred strains, *Behav. Brain Res.* 176 (2007) 4–20.
- [13] D.J. Ardesch, M. Balbi, T.H. Murphy, Automated touch sensing in the mouse tapered beam test using Raspberry Pi, *J. Neurosci. Methods* 291 (2017) 221–226.
- [14] J.J. Nadler, S.S. Moy, G. Dold, D. Trung, N. Simmons, A. Perez, N.B. Young, R.P. Barbaro, J. Piven, T.R. Magnuson, J.N. Crawley, Automated apparatus for quantitation of social approach behaviors in mice, *Genes Brain Behav.* 3 (2004) 303–314.
- [15] A. Mathis, P. Mamidanna, K.M. Cury, T. Abe, V.N. Murthy, M.W. Mathis, M. Bethge, DeepLabCut: markerless pose estimation of user-defined body parts with deep learning, *Nat. Neurosci.* 21 (2018) 1281–1289.
- [16] L. Zhang, B. Liang, G. Barbera, S. Hawes, Y. Zhang, K. Stump, I. Baum, Y. Yang, Y. Li, D.-T. Lin, Miniscope GRIN lens system for calcium imaging of neuronal activity from deep brain structures in behaving animals, *Curr. Protoc. Neurosci.* 86 (2018) e56.