Reduced sociability and social agency encoding in adult Shank3-mutant mice are restored through gene re-expression in real time

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Despite a growing understanding of the molecular and developmental basis of autism spectrum disorder (ASD), how the neuronal encoding of social information is disrupted in ASD and whether it contributes to abnormal social behavior remains unclear. Here, we disrupted and then restored expression of the ASD-associated gene Shank3 in adult male mice while tracking the encoding dynamics of neurons in the medial prefrontal cortex (mPFC) over weeks. We find that Shank3 disruption led to a reduction of neurons encoding the experience of other mice and an increase in neurons encoding the animal’s own experience. This shift was associated with a loss of ability by neurons to distinguish other from self and, therefore, the inability to encode social agency. Restoration of Shank3 expression in the mPFC reversed this encoding imbalance and increased sociability over 5–8 weeks. These findings reveal a neuronal-encoding process that is necessary for social behavior and that may be disrupted in ASD.

Animal models offer the opportunity to study some of the basic neuronal-encoding processes that underlie social behavior and to evaluate how these processes may be disrupted by conditions, such as ASD. Prior investigations in rodents and primates, for example, have provided evidence for the presence of neurons that encode information about other individuals, demonstrating changes in activity when the animals observe another’s appetitive or aversive experiences1–11. They have also provided evidence for neurons that encode other animals’ familiarity or dominance12,13, further suggesting that social context plays a prominent role in such computations. While these observations have provided insight into the mechanisms by which social information is likely encoded by neurons in the mammalian brain, they do not reveal whether or what computations may be disrupted in ASD or how their disruption may relate to abnormal social behavior.

Individuals with ASD often show difficulty in evaluating social cues and in appropriately interpreting another person’s experiences or emotions and may display a preoccupation with self or limited interest in others14–19. Heterozygous mutations of the Shank3 gene are associated with up to 1% of ASD cases20,21 and are a major contributor to excitatory synaptic dysfunction and neurotransmission that may relate to the ability of neurons to encode information about other agents as well as to distinguish one’s own experience from another’s. We also demonstrate how targeted recovery of Shank3 expression in the mPFC gradually restores this imbalance and increases sociability over time.

Results
Studying the relation between Shank3 expression, neuronal encoding and social behavior. To study the relation between Shank3 expression, neuronal encoding and social behavior, we used a Cre-dependent FLEX switch approach that allowed us to simultaneously track the encoding properties of neurons in freely interacting mice as we disrupted and then gradually restored Shank3 expression in real time. We show how changes in social behavior due to Shank3 disruption temporally relate to the ability of neurons to encode information about other agents and, therefore, the inability to encode social agency. Here, we approached these questions by using a FLEX switch strategy that allowed us to simultaneously track the encoding properties of neurons in freely interacting mice as we disrupted and then gradually restored Shank3 expression in real time. We show how changes in social behavior due to Shank3 disruption temporally relate to the ability of neurons to encode information about other agents and, therefore, the inability to encode social agency.

We also demonstrate how targeted recovery of Shank3 expression in the mPFC gradually restores this imbalance and increases sociability over time.
In our study, we used heterozygous constructs to mimic the physiological expression of Shank3 because, unlike animals with homozygous mutations, they generally do not exhibit motoric dysfunction, anxiety or self-injurious behavior that could potentially confound social testing\(^\text{22,23}\). All animals were adult male mice aged 4 months or older to allow for consistency and included either (1) Shank3\(^{fx/+}\); creER\(^+\), (2) Shank3\(^{fx/+}\); creER\(^+\), (3) Shank3\(^{fx/+}\); creER\(^+\) or (4) Shank3\(^{−/−}\); creER\(^+\) constructs. In total, we used 77 animals, of which 28 were wild-type (WT) and 49 were heterozygous (HET) Shank3 mice (Extended Data Fig. 1a and Supplementary Table 1).

Here, we used microelectrode arrays that were implanted in the animal's mPFC (Extended Data Fig. 1b), an area suggested to be involved in social behavior\(^\text{1,7,12,26,34}\) and disorders such as ASD\(^\text{14,21,35,36}\). The responses of recorded neurons were evaluated across different task conditions, and their encoding properties were compared both within and between animals. The animals' behaviors were tracked using a ceiling-mounted digital camera, with the task condition and animal position being determined in an automated, genotype-blinded manner (Methods).

To first study the encoding properties of individual neurons and to further differentiate information related to other social agents from the individual's own, we used a task design that allowed the recorded animals to undergo paired interactions across varying task conditions. These conditions were varied across three main orthogonal axes that defined the animal's interactions, social agency (self versus other), experience valence (positive versus negative) and identity (familiar versus non-familiar). All trials were given in pseudorandom fashion and were separated by 1-min neutral intertrial periods. To provide experiences that were salient to the animals and to identify neurons that responded selectively to another's specific experiences (that is, rather than simply to any experience), we used a confined tube enclosure for the aversive experience\(^\text{7,26}\) and a food-bated enclosure for the appetitive experience\(^\text{37–39}\). For example, an appetitive experience may be given to the other social agent in one trial but may be given to the recorded animal themselves on another. On other trials, by comparison, either the recorded animal or other agent may be given an aversive experience (Fig. 1a,b).

To further dissociate neuronal responses that may reflect the other animal's experiences from those that more simply reflect the enclosures themselves, we also pseudorandomly replaced the other animals with inanimate totems and alternated the enclosures in which the recorded animal's themselves were placed in. Thus, for instance, the recorded animal may observe another animal in an aversive enclosure on one trial but, alternatively, may observe the same enclosure with an inanimate totem in another. Finally, to evaluate the influence that social context itself had on neuronal response, we alternated the identity of the other animal as familiar and non-familiar.

All enclosures were well habituated and did not require conditioning (for example, tone-shock pairing)\(^\text{24}\), therefore allowing us to consistently study neuronal response across multiple interleaved trials. All task conditions were counterbalanced so that an equal number of self trials and other trials as well as an equal number of appetitive and aversive conditions were performed.

**Single-neuron representations of other and self during social interactions.** Together, we recorded from 188 mPFC neurons in the WT mice, 180 neurons in the HET mice before TMX and 837 neurons from the HET mice after TMX or endoxifen administration (Fig. 1c and Extended Data Fig. 1c). The process by which neurons encode social information is described by changes in their spiking activity\(^\text{41}\). For example, if a neuron demonstrated a difference in activity when another animal received an aversive versus an appetitive experience but not to the animal's own experience, then this individual neuron would be considered to selectively encode information related to the other social agent’s experience. Here, using a three-way analysis of variance (ANOVA) that considered social agency, experience valence and identity for the main effects across the 188 WT neurons recorded, we found 112 neurons that displayed task-related modulation (three-way ANOVA, \(P<0.0125\) with post hoc comparisons). Of these neurons, 30% (\(n=33\) of 112) responded to variations in the other social agent's experience (Fig. 1d), meaning that these cells exhibited a relative change in their activity when the other mouse underwent a positive compared to negative experience. A similar proportion of neurons was also observed when using a modeling approach that decoded the experiences on a trial-by-trial basis (36%, \(n=37\); Supplementary Table 2), further suggesting that these encoding properties were robust. The overall probability of observing these numbers of cells by chance was unlikely given the number of neurons recorded (bootstrap analysis, \(P<1.0\times10^{-9}\)).

Neurons that encoded the other animal's experience were largely distinct from those that encoded the animal's own experience. Of neurons that were modulated by the task, 25.9% \((n=29\) of 112) encoded the animal's own experiences, meaning that they displayed a relative change in their activity when the recorded animal was undergoing a positive compared to negative experience (three-way ANOVA, \(P<0.0125\)). Of the neurons that encoded the animal's own experience, however, only nine also encoded the other animal's experience, a proportion that was significantly lower than expected by chance (bootstrap analysis, \(P<0.01\) ) (Fig. 2a). Fig. 1d illustrates two such representative cells from the same WT animal, one that differentially responded to the other social agent's experiences and one that differentially responded to the animal's own experiences. This relative lack of overlap in neuronal response to self versus other experience was also apparent at the level of the population when considering differences in each neuron's firing rates (two-dimensional (2D) Kolmogorov–Smirnov test, \(K=0.52, P=9.2\times10^{-4}\); Fig. 2b and Supplementary Fig. 1). Therefore, in WT animals, most neurons in the mPFC that encoded information about the other animal's experience encoded little information about the animal's own experience and vice versa.

**Sensitivity of other-encoding neurons to social information.** We next assessed whether other-encoding neurons in the mPFC respond to the other social agent's experience rather than to the aversive or appetitive stimuli themselves (Fig. 3). It is possible, for example, that similar neuronal responses would have been observed if the recorded animals were simply presented with an appetitive or aversive enclosure. This was unlikely because most neurons that responded to the other's experience displayed little response to the animal's own experience. Nonetheless, to evaluate for this possibility more directly, we also examined the trials in which the other animal was replaced with an inanimate totem (Fig. 1b). Under this setting, only 3.6% of neurons (\(n=4\) of 112 cells) responded to the appetitive and aversive stimuli, of which three were among the neurons found to encode the other's experience (chi-square test, \(\chi^2(1)=10.9, P=9.6\times10^{-4}\)). These neurons, therefore, did not simply respond to the stimuli themselves.

We also considered other factors indirectly related to the stimuli, for instance, smell emitted when the other animal was eating or vocalizations associated with stress. To this end, we performed an additional control in which the other animal was given the same appetitive and aversive stimuli, but now visual access to the other was blocked by an odor- and sound-permeable barrier (that is, the recorded mouse was not allowed to view whether their partner was present or the stimulus that the partner experienced). Now, however, only 7.1% \((n=8\) of 112\) of neurons responded to the aversive and appetitive conditions, of which only one neuron overlapped with those encoding the other's experience (chi-square test, \(\chi^2(1)=62.1, P<1.0\times10^{-9}\); Fig. 3a).
Neuronal responses to the other animal also did not reflect the recorded animal’s own enclosure or experience. Only 3.7% ($n=7$) of neurons displayed a difference in response to the other’s experience based on which specific enclosure the recorded animal was simultaneously placed in (three-way ANOVA, $P<0.0125$ with post hoc comparisons). Moreover, only one of the neurons that displayed
a significant response to the other’s experience also demonstrated a difference in response based on which enclosure the recorded animal was in (chi-square test, \( \chi^2(1) = 62.1, P < 1.0 \times 10^{-5} \); Extended Data Fig. 2a), suggesting that neurons that responded to the other’s experience were largely insensitive to recorded animal’s own specific enclosure.

Finally, to confirm that neuronal responses to the other’s experience indeed reflected the social context of the animal’s interaction, we evaluated whether the other animal’s identity influenced neural activity. Familiarity with a conspecific plays a crucial role in how animals and humans perceive and respond to the experiences of others and is often used to evaluate the effect that social context has on such interactions.12 Of neurons that were modulated by the task, 18% (n = 20 of 112) responded distinctly to the other animal when the other animal was familiar to the recorded mouse compared to when the other animal was unfamiliar (three-way ANOVA, \( P < 0.0125 \); Fig. 3c). Familiarity with the other animal, however, also markedly influenced neuronal response to the other’s experience; neurons that encoded the other’s experience displayed a significantly greater difference in activity between the positive and negative experiences when their partner was familiar to them (two-sample \( t \)-test, \( t(64) = -3.2, P = 2.1 \times 10^{-3} \)) than when the partner was not familiar (Fig. 3d and Extended Data Fig. 2b). In other words, neuronal responses to ‘what’ the other was experiencing also reflected to ‘whom’ the experience belonged. Extended Data Fig. 2 further illustrates the most common feature combinations represented by neurons in the mPFC across the different social agency, experience valence and identity condition combinations. Taken together, neuronal responses to the other’s experience reflected the social context of their interaction rather than simply information about the sensory stimuli or enclosures themselves.

**Loss of Shank3 disrupts social encoding and self–other distinction in individual neurons.** We next turned to the HET mice in which Shank3 expression was disrupted. Here, we recorded from 180 neurons in the HET mice before TMX administration. Overall, the firing rates of these neurons were similar to those in WT (3.37 ± 0.31 versus 3.54 ± 0.37 spikes per second for the WT and HET mice, respectively; two-sample \( t \)-test, \( ts = -0.52, P = 0.61 \) and had similar waveform morphologies (Fig. 1c and Extended Data Fig. 1b). Further, the HET mice displayed a slightly higher proportion of task-modulated neurons, indicating that the quality of recordings across the WT and HET mice was comparable (112 of 188 versus 131 of 180 for the WT and HET mice, respectively; chi-square test, \( \chi^2(1) = 7.1, P = 0.0075 \)). Finally, we confirmed that all recording locations in the WT and HET mice were confined to the same mPFC area (Extended Data Fig. 1b).

While many neurons in the HET mice were modulated by the task conditions, markedly fewer neurons encoded information about the other social agent’s experience. Of task-modulated neurons, only 9.2% (n = 12 of 131; Fig. 1d) encoded the other agent’s experience, a proportion that was significantly smaller than that observed in WT (chi-square test, \( \chi^2(1) = 10.5, P = 1.2 \times 10^{-3} \); Fig. 2a). The degree to which neurons in the HET mice responded to the other’s experiences was also significantly diminished, displaying a smaller overall difference in activity when the other animal underwent a positive compared to negative experience (two-sample \( t \)-test, \( ts(43) = 2.6, P = 0.013 \); Fig. 2b). This difference in activity between
the WT and HET animals was consistent across the course of the trials (Extended Data Fig. 3).

Decrement in the proportion of neurons that encoded the other’s experience was associated with a proportional increase in neurons that responded to the animal’s own experiences. Overall, 38.2% (n = 50 of 131) of neurons in the HET mice encoded experiences related to self. Further, when considered across the population, the ratio of neurons that responded to other experience versus self experience decreased from 1.1:1 in the WT mice to 1:4 in the HET mice (chi-square test, χ²(1) = 14.0, P = 1.8 × 10⁻⁵). Similar results were found by decoding analysis (Supplementary Table 3) as well as when performing within- versus between-group comparisons,
indicating that these differences in neuronal encoding were robust (Extended Data Fig. 4). These differences in encoding between the WT and HET mice were also largely independent of the statistical thresholding used ($P < 0.05$ to $0.0025$; Extended Data Fig. 5a) and were consistent across animals (Extended Data Fig. 5b), indicating that reduced Shank3 expression was associated with a loss of neuronal response to the other’s experience.

We also observed that the reduced neuronal response to the other’s experience was associated with a loss of distinction between other and self. As noted above, in WT mice, only nine neurons that encoded the other’s experience also encoded the animals own. In the HET mice, by comparison, all neurons (100%) that responded to the other’s experience also responded to the animal’s own experience (9 of 33 versus 12 of 12; chi-square test, $\chi^2(1) = 18.7, P = 1.5 \times 10^{-5}$; Fig. 2a). This lack of self–other distinction was also notable at the level of the population when examining the raw firing rates across cells (2D Kolmogorov–Smirnov test, $K = 0.37, P = 0.70$; Fig. 2a and Supplementary Fig. 1). Cells in the HET mice, therefore, responded similarly irrespective of whether the experience was given to the other animal or self, together suggesting that reduced expression of Shank3 was associated with a diminished ability of neurons to represent social agency (that is, the ability to distinguish other from self).

Controls for potential differences in sensorimotor and anxiety-related behaviors. One potential explanation for these findings could be that the HET animals simply displayed a generalized sensory perceptual deficit and may therefore not be able to differentiate between the aversive or appetitive stimuli themselves. Here, we found that although the HET mice did not show a preference for other animals undergoing aversive compared to appetitive conditions when using a control place preference assay ($P > 0.5$; Extended Data Fig. 6a), they did display a difference in respiratory rate (ANOVA, $P = 0.0031$; Extended Data Fig. 6b), suggesting that they were able to discriminate between these conditions. To further validate these observations, we also measured the animals’ corticosterone levels and found that they were higher in both the WT and HET mice when observing another animal having an aversive compared to appetitive experience (ANOVA, $P < 0.05$). Although this increase in corticosterone levels was smaller in HET mice than in WT mice, the genotype of the animals had no independent effect on this change (two-way ANOVA, $P = 0.23$; Extended Data Fig. 6c). Lastly, we tested the responses of the mPFC neurons themselves to the other’s identity. Here, we theorized that if difference in encoding in the HET mice was due to a non-selective sensory deficit, then we should also observe a diminished response when comparing familiar and non-familiar animals. However, 29.8% ($n = 39$ of 131) of neurons in the HET mice displayed a significant difference in response when paired with a familiar compared to non-familiar animal (that is, independent of their experience) (bootstrap test, $P < 1.0 \times 10^{-5}$; Fig. 3c and Supplementary Fig. 4), together suggesting that the HET animals did not simply display a sensory perceptual deficit.

Another possible explanation for these findings could be that the HET mice displayed diminished engagement or interaction with the other. We therefore reanalyzed our data but now only considered those periods in which the recorded animal was directly engaging with the other animal. However, we again found significantly fewer neurons in the HET mice that responded to the other’s experience (11.8% and 35.8% for HET and WT mice, respectively; chi-square test, $\chi^2(1) = 8.3, P = 4.1 \times 10^{-5}$; Fig. 3b) and fewer neurons that responded to the other’s experience when confirming our data to the time periods in which the recorded animal was in immediate proximity to the other (7.4% and 23.1% for HET and WT mice, respectively; chi-square test, $\chi^2(1) = 6.0, P = 0.014$; Supplementary Fig. 2). We also evaluated for neurons that may have responded directly to physical contact and found that 24.1% ($n = 27$) of the neurons in WT mice change their activity when the mouse specifically interacted with another. However, of these ‘physical–engagement’ neurons, only five overlapped with those that encoded the other’s specific experience and at a probability that was significantly lower than expected by chance (chi-square test, $\chi^2(1) = 24.9, P < 1.0 \times 10^{-5}$). Similar results were also observed in the HET animals (Supplementary Tables 3 and 4), together suggesting that diminished ability of neurons to encode the specific experience of others or their social agency was not due to nonspecific sensorimotor effects.

Finally, we considered the possibility of generalized behavioral states, such as anxiety. Using an elevated zero maze assay ($P = 0.00001$), we found that the WT mice preferred the closed areas of the maze, spending approximately 77% of their time in these areas and at a probability significantly above chance ($n = 26$; t-test, $P < 0.00001$). The time spent in the open area, however, did not differ from that of the HET mice ($n = 27$; t-test, $P = 0.73$) nor did the number of visits (t-test, $P = 0.77$) or head-dipping events (t-test, $P = 0.92$; Extended Data Fig. 7). Therefore, consistent with prior reports ($P < 0.05$), the HET mice generally do not display anxiety-related behavior.

Real-time restoration of Shank3 expression leads to increase in sociability. While the above observations suggested an association between Shank3 expression and neuronal encoding in the mPFC, they did not reveal whether or to what degree differences in neuronal encoding causally related to the animal’s social behavior. To address this question, we used FLEX switch-mediated control of Shank3 expression while simultaneously tracking neuronal activity and social behavior in the same animals over time (Fig. 4a). TMX was used here to globally increase Shank3 expression in the haploinsufficient animals. Increases in $\alpha$, $\beta$- and $\gamma$-SHANK3 isoforms in the Shank3$^{\alpha/\alpha}$; creER$^{+/+}$ mice was confirmed using brain synaptosome preparations and Western blotting (not all three isoforms are affected equally). We also confirmed that SHANK3 levels after

Fig. 4 | Increase in Shank3 expression is associated with increased sociability. a, TMX was used to activate CreER function and therefore provide temporal control of Shank3 expression in the Shank3$^{\alpha/\alpha}$; creER$^{+/+}$ (HET) mice. Neuronal and behavioral evaluations were performed before and after the administration of TMX. See Supplementary Table 1 for additional details on all the genetic constructs and conditions tested, as well as Extended Data Fig. 1a for additional description of the Cre/loxP system; HSP, heat shock protein. b, SHANK3 expression was quantified via synaptosome preparation and Western blotting in Shank3$^{\alpha/\alpha}$; creER$^{+/+}$ (that is, HET) and Shank3$^{\alpha/\alpha}$; creER$^{-/-}$ (that is, control) mice. Heterozygous knockin of Shank3 primarily affected the putative Shank3-α and Shank3-γ isoforms and, to a lesser extent, the Shank3-β isoform (see Methods). After administration of TMX, the Shank3$^{\alpha/\alpha}$; creER$^{+/+}$, but not creER$^{-/-}$, constructs demonstrated SHANK3 protein levels comparable to those of WT (left). Error bars represent s.e.m., with a protein level of 1.0 representing WT. See the Source Data for the full unprocessed Western blot. c, Behavioral testing for sociability was performed using a standard three-chamber enclosure, where mice were presented with a novel animal (purple) versus novel inanimate object (hash). Error bars represent s.e.m. Below are heat maps of representative trials. Significances are displayed by two-sample $t$-tests ($^{*}P < 1 \times 10^{-3}$; $n = 48$, 30 and 29 three-chamber trials from $n = 28$ WT, $n = 18$ HET and $n = 18$ HET + TMX mice, respectively). d, HET mice did not exhibit impaired motoric behavior, as assessed by velocity or distribution of distances traveled. Here, $n = 131$ and $n = 87$ trials were assessed from $n = 28$ WT and $n = 18$ HET mice, respectively; 95% CI, 95% confidence interval.
TMX treatment did not increase in the Shank3<sup>fx/+;creER<sup>−/−</sup></sup> mice, which lacked the creER recombinase gene (t-test, ts(12) = 2.63, P = 0.04; Fig. 4b).

The HET mice displayed diminished sociability compared to the WT mice. Here, the sociability of the animals was assessed using a modified three-chamber task<sup>2,22,23,40</sup> and was defined as a
preference for another non-familiar mouse over a novel inanimate object. Overall, we find that the WT mice significantly preferred the other animal over the inanimate object (paired t-test, ts(94) = 4.1, P = 1.0 × 10⁻⁴; Fig. 4c and Extended Data Fig. 8a,b), whereas HET mice displayed no such preference (before TMX treatment, paired t-test, ts(52) = 0.19, P = 0.85). WT and HET mice did not differ otherwise in movement velocities (two-sample t-test, ts(217) = 0.48, P = 0.63) or distribution of distances traveled (two-sample t-test, ts(217) = 0.32, P = 0.75; Fig. 4d). The HET mice, therefore, displayed diminished sociability.\(^\text{(22,23)}\)

Next, to evaluate whether restoration of Shank3 expression led to a change in social behavior, we examined the time period after TMX administration (week ≥ 5) when Shank3 expression had already increased\(^\text{(21-23)}\) and found that the HET mice displayed a significant preference for the non-familiar animals (week ≥ 5, paired t-test, ts(56) = 3.6, P = 7.0 × 10⁻⁴; Fig. 4c). This behavioral change was not observed in the Shank3\(^{+/–}\); creER\(^{+/–}\) mice lacking the creER recombinase gene treated with TMX (paired t-test, ts(58) = 1.34, P = 0.19; see additional controls below) or in the Shank3\(^{+/–}\); creER\(^{+/–}\) mice that possessed the creER recombinase gene but in which a vehicle (corn oil) was delivered (two-sample t-test, ts(15) = 1.10, P = 0.29; Extended Data Fig. 8c,d). These findings, therefore, suggested that increase in sociability was specifically due to activation of Shank3 expression rather than the passage of time.

**Temporal dependency between Shank3 expression, neuronal encoding and social behavior.** Given these findings, we evaluated the dependency between change in social behavior and neuronal encoding by examining their day-by-day progression. Here, each HET animal underwent consecutive behavioral testing and neuronal recordings on alternating days after TMX administration (Supplementary Table 1). The neuronal and behavioral data were then time aligned and compared over the course of 2 months.

First, focusing on neuronal responses in the Shank3\(^{+/–}\); creER\(^{+/–}\) (HET) mice, we find that the percentage of neurons that encoded the other’s experience increased from 9.2% (n = 12 of 131) before TMX treatment to 35.5% after TMX treatment (n = 49 of 138 recorded after ≥ 5 weeks; chi-square test, χ²(1) = 15.2, P = 9.4 × 10⁻⁴; Fig. 5a). Conversely, the percentage of neurons that encoded the animal’s own experience decreased from 38.2% (n = 50 of 131) before TMX treatment to 19.5% after TMX treatment (n = 27 of 138, ≥ 5 weeks; chi-square test, χ²(1) = 11.38, P = 7.4 × 10⁻⁴). The Shank3\(^{+/–}\); creER\(^{+/–}\) mice, by comparison, displayed no such changes in neuronal encoding (chi-square test, χ²(1) = 0.095, P = 0.76 and χ²(1) = 0.30, P = 0.58 for other-valence and self-valence neurons, respectively). We also found no change in HET mice after TMX treatment in the proportion of neurons that were simply task modulated (chi-square test, χ²(1) = 0.25, P = 0.61) or in the proportion of neurons that responded to familiarity (chi-square test, χ²(1) = 0.34, P = 0.56).

Next, we examined the day-by-day relation between changes in neuronal encoding and change in behavior. A cross-correlation analysis that quantified the dot product between social agency encoding (that is, other: self ratio) and behavioral sociability (that is, animal:object preference ratio) as a function of time revealed a positive and significant relation between social agency encoding and sociability (cross-correlation analysis, r = 0.88, P = 0.0041; Fig. 5b). The peak time lag between change in neuronal encoding and change in social behavior for these animals was 5–6 d (permutation test, P < 0.01; Fig. 5b). That is, an increase in the proportion of neurons that encoded the other’s experience consistently preceded an increase in the animal’s sociability by approximately 0.75 weeks.

To further validate these findings and to evaluate the degree to which this correlation could be expected by chance, we performed a permutation procedure that randomized the days over which neuronal activity and behavior were recorded 1,000 times (Methods). In other words, we asked, given the number of data points and days tested, what was the likelihood that this correlation could have been observed by chance. Here, we found that the cross-correlation values for shuffled data were significantly smaller than those observed in the actual data (that is, an r value of −0.000086 ± 0.00000013 versus 0.88; permutation test, P < 1.0 × 10⁻⁴).

Finally, to confirm that these changes were specific to Shank3 expression and not simply explained by the passage of time or familiarity with the task, we examined the Shank3\(^{+/–}\); creER\(^{+/–}\) animals who received TMX and experienced the same task conditions but lacked the creER recombinase gene and therefore did not experience an increase in Shank3. These mice did not show a change in agency encoding and no relation between social behavior and neuronal encoding (cross-correlation, P > 0.25; Figs. 5b and 6). Similarly, the WT mice did not display a change in sociability (permutation test, P > 0.2) or neuronal encoding (permutation test, P > 0.2) when tracked over time (Extended Data Fig. 8d), suggesting a simple time-related progression. Together, these findings, therefore, suggest that the increase in the HET animal’s sociability after TMX treatment was temporally dependent on changes in the encoding properties of mPFC neurons and their ability to represent the distinction between other and self.
Specificity of self and other encoding in the mPFC and social behavior. While the above findings revealed a relation between neuronal encoding and social behavior, they did not indicate whether or to what degree activity in the mPFC was sufficient to explain it. For example, it is possible that the responses of mPFC neurons reflect an indirect ‘read-out’ of activity or changes in other brain areas but played no direct role in behavior. To address this possibility, we used a new endoxifen-driven Cre/loxP recombination

![Graph](image_url)

**a** Shank3-related change in neuronal encoding

| Before TMX | After TMX |
|------------|-----------|
| Self experience | Other experience |
| 38% (50/131) | 9% (12/131) |
| 20% (27/138) | 36% (49/138) |

**b** Tracking the dependency between neuronal encoding and social behavior in real time
approach that allowed us to confine restoration of Shank3 expression to the mPFC (Fig. 7). Here, these HET mice had the same Shank3<sup>−/−</sup>; creER<sup>−/−</sup> genotype (that is, were age-matched littermates), underwent the same behavioral conditioning, performed the same three-chamber task and underwent recordings from the same cortical area as those that received TMX. But the increase in Shank3 expression was now confined to the mPFC (Fig. 7b).

HET mice that underwent local endoxifen-driven Cre/loxP recombination and restoration of Shank3 expression in the mPFC also displayed a marked increase in sociability. As seen in Fig. 7a, they showed a significant increase in preference for the non-familiar animals compared to inanimate objects over the 8-week course of testing (two-sample t-test, t(15) = −4.14, P = 0.0087; >5 weeks). By contrast, no change in sociability was observed in HET (Shank3<sup>−/−</sup>; creER<sup>−/−</sup>) mice that had received saline injections into the mPFC (two-sample t-test, t(15) = −1.40, P = 0.18; >5 weeks) and in Shank3<sup>−/−</sup>; creER<sup>−/−</sup> mice, which received endoxifen injection in the mPFC but that lacked the creER recombinase gene and therefore did not demonstrate increased Shank3 expression in the mPFC (two-sample t-test, t(15) = −0.41, P = 0.69; Fig. 7a).

Localized restoration of Shank3 expression in the mPFC of HET mice also resulted in an increase in the proportion of neurons that encoded the others’ experience over time (n = 115; χ<sup>2</sup>(1) = 4.11, P = 0.043). Here, the peak temporal dependency between change in behavior and change in neuronal encoding was at a time lag of 0 d (r = 0.82, P = 0.0073; Fig. 7a). Thus, local activation of Shank3 expression in the mPFC was associated with a near simultaneous change in neuronal encoding and social behavior (that is, unlike the 5–6-d lag in HET mice after global restoration of Shank3 expression). The difference in time lag between local and systemic restoration, however, was not significant (permutation test, P > 0.4).

Taken together, these findings indicate that the increase in sociability observed after restoration of Shank3 expression was both spatially and temporally (that is, time-causally<sup>44</sup>) dependent on these changes in neuronal-encoding properties in the mPFC.

Last, to confirm these findings, we inhibited neural activity in the mPFC in WT animals. To this end, we either injected the reversible GABA agonist muscimol or saline into the same mPFC area on randomly alternating days. We found that after muscimol administration, the mice displayed no preference for another animal over an inanimate object (two-sample t-test, t(38) = 0.66, P = 0.52; Fig. 7c). The same mice receiving saline injection, by comparison, displayed a significant preference for the mouse over the object (two-sample t-test, t(58) = 2.98, P = 4.19 × 10<sup>−3</sup>). These observations therefore together suggest that neuronal activity in this area was indeed necessary for changes in the animals’ sociability.
Fig. 7 | Restoration of sociability is dependent on social agency encoding in the mPFC. a, To further evaluate the spatial selectivity of Shank3 expression in the mPFC, the Cre/loxP system was activated bilaterally in the mPFC using endoxifen injection. Behavioral and neuronal recordings were obtained from each animal over the course of 8 weeks after endoxifen administration and compared. Data from n = 5 Shank3+/–; creER+/– mice in which endoxifen was administered to the mPFC were compared to data from n = 4 Shank3+/–; creER−/– mice in which endoxifen was given but which lacked the creER recombinase gene. Top: the curves represent the relative preference (+ s.e.m.) of the mice for another animal compared to an object at weekly intervals. The horizontal line represents no preference. Cre+/– mice displayed a significant increase in sociability over time compared to baseline (weeks 0–1), whereas Cre−/– mice did not. Time points in which there was a significant difference are underlined in red (one-sample t-tests; P < 0.01). Middle: the curves represent the proportion (+ s.e.m.) of other-encoding compared to self-encoding neurons in the mPFC at weekly intervals. The horizontal line represents an equal proportion of neurons. Cre+/– mice displayed a significant increase in other-encoding neurons over time compared to baseline (weeks 0–1), whereas Cre−/– mice did not. Time points in which there was a significant difference are underlined in red (P < 0.01 with Bonferroni correction for repeated comparisons). Bottom: the lines represent the degree of cross-correlation between changes in social behavior and changes in neuronal encoding. Red lines indicate time lags that were significant, with the arrow indicating the maximal time lag at which change in social behavior followed change in neuronal encoding in the mPFC (0 weeks for the Shank3+/–; creER+/– mice). For additional control comparison, no change in social behavior was observed in animals in which no endoxifen was given (Extended Data Fig. 8). b, Confirmation of localized increases in SHANK3 levels following endoxifen was made histologically. Left: increases in SHANK3 levels were confined to the cingulate gyrus (Cg) of the mPFC in the Cre+/–, Cre−/–, and CreER−/– groups. Right: no increase was observed in the mPFC. M2, secondary motor cortex; Cg1, dorsal anterior cingulate gyrus; Cg2, ventral anterior cingulate gyrus. c, Neural activity was locally inhibited in the mPFC. For control comparison, the same animals were also injected with saline (on randomly interleaved days). The proportions of time spent with another animal versus an object are shown on the left. Whereas WT mice receiving saline continued to display a significant preference for another animal compared to an object, the same animals receiving muscimol in the mPFC did not (***t(58) = 2.98, P = 0.0042, paired t-tests); n = 29 and n = 19 trials from from n = 8 WT mice injected with saline or muscimol in the dorsal mPFC, respectively. Localizations of the injection sites were confirmed histologically (Supplementary Fig. 6).

Discussion

Together, our findings suggest a functional division of labor within the mPFC by which neurons not only represent another animal’s specific experience but also differentiate the other’s specific experience from the animal’s own. By simultaneously tracking variations in social agency, experience valence and identity for the same individual cells, we observe that mPFC neurons represent another animal’s positive and negative experiences differentially and that they represent the other’s specific experiences distinctly from the animal’s own. Moreover, while these neurons were modulated by the social context of the task, they displayed little response when no other mouse was present.

Previous investigations have provided insight into how discrete aspects of another animal’s experience, such as receipt of reward, may be encoded by cells. The current study demonstrates how individual neurons concurrently encode ‘what’ another mouse is experiencing, ‘who’ is experiencing it and to ‘whom’ the experience belongs. In particular, these findings suggest a coding mechanism by which neurons in the mPFC represent information about a conspecific’s specific experience (that is, whether it is aversive or appetitive) as well as differentiate the conspecific’s experience from one’s own. They also suggest that these neurons are sensitive to the specific individuals involved (that is, whether the conspecific is familiar or novel) but show little response to the aversive or appetitive aspects of another animal’s experience, such as receipt of reward, may be encoded by cells.
appetitive stimuli themselves when paired with inanimate totems (Supplementary Fig. 3).

Individuals with ASD are often described as having an inability to organize social information or appropriately interpret the state and experience of others14–19. They may also have heightened sensitivity and preoccupation with self18,46. The current study’s findings in a mouse model of ASD provide a prospective cellular substrate for these observations. In particular, we find that heterozygous disruption of Shank3 expression is associated with a diminished proportion of mPFC neurons that encode the other social agent’s experiences, but it is also associated with an increased proportion of neurons that encode the animal’s own. Moreover, by restoring Shank3 expression in the mPFC and by evaluating the relation between neuronal response and social behavior, our findings suggest that diminished social behavior is temporally correlated both with the ability of the neurons to encode information about the experience of others as well as their social agency. The absence of such changes in Shank3-mutant mice with creER12−/− constructs or in WT animals further confirmed that these changes were specific to Shank3 expression.

Another notable finding was that focal restoration of Shank3 expression in the mPFC was sufficient for increasing the animal’s sociability. While loss of Shank3 expression in the HET animals is largely evident throughout the brain16, findings from the Shank3 restoration and focal inhibition experiments suggest that the mPFC plays a specific causal role in the animal’s social behavior. These findings also suggest that changes in the encoding properties of dorsal mPFC neurons were not simply explained by activity changes elsewhere in the brain, and that the temporal dependency between neuronal encoding in the mPFC and changes in social behavior were likely specific. This interpretation is also supported by the lack of neuronal encoding or behavioral changes in mice lacking the creER recombinase gene.

While we focused here on the mPFC, it is important to note that ASD is known to involve other cortical–subcortical circuits17,18 and that various neurophysiological features, such as cortical volume17,47 and connectivity13, are also altered in ASD. Our findings also do not reveal how Shank3 expression in the mouse mPFC relates to other changes observed in Shank3-mutant mice, such as the excitability and synaptic structure of neurons (or their restoration)19,26,30. Moreover, animal models of social behavior, including the mice used here, do not fully recapitulate the complexity and natural process in which humans interact or capture the wide spectrum of behavioral phenotypes that are affected by ASD18,48,49.

Together, our study provides a detailed examination of the encoding properties of neurons in the mPFC and how their disruption relates to abnormal social behavior. The ability to track the relationship between neuronal encoding (that is, how sensory information is represented by neurons) and social behavior (that is, how animals respond to these sensory stimuli) in real time and over months-long durations could provide a prospective tool for understanding the behavioral phenomenology of disorders such as ASD. Whereas prior investigations have focused on the molecular and anatomic underpinnings of such disorders (for example, differences in the synaptic architecture and excitability of neurons)14–19,46, our approach allows one to examine the encoding properties of neurons with prospective implications to the study of a broad variety of genetic disorders14,15.

Online content
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Methods

Animals. Animals were perfused transcardially with PBS and then 4% PPL. Brains were extracted and postfixed in 4% PPL for 4 h, washed three times in PBS, placed in 30% sucrose in PBS for 48 h and sectioned at a 50-mm thickness with a vibratome (Leica VT1200S). Electrotic lesions were used to confirm localization of the areas recorded from, whereas fluorescent dye injection was used to confirm cannula localization.

Immunofluorescence confirmation of local increase in Shank3 expression. For immunofluorescence staining, brain sections were blocked in a 0.2% Triton X-100 solution (PBST) with 5% normal goat serum (NGS) (PBST-NGS) for 1 h at room temperature followed by incubation in a rabbit anti-Shank3 primary antibody solution (1:200; Alomone APZ-013) in PBST-NGS for 72 h at 4 °C. Sections were washed four times in PBST for 10 min at room temperature and incubated in an Alexa 488-conjugated goat anti-rabbit secondary antibody solution (1:550; Jackson Immunoresearch) for 1 h at room temperature. Sections were again washed four times in PBST then mounted onto glass slides, coverslipped using Vectorshield with DAPI (Vector Laboratories, H-1200) and sealed with clear nail polish. All fluorescent images were taken using a fluorescence microscope (Keyence BZ-X710) with a CFI Plan APO Lambda x2, CFI Plan APO Lambda x4 or CFI Plan APO Lambda x40 objective.

Neuronal recording. Stereotactic implantation. Multielectrode microarrays were used for single-unit recordings (Microprobes for the Life Sciences). Electrodes were implanted under isoflurane anesthesia, followed by antibiotic administration and pain relief. The arrays were implanted under stereotactic guidance in the mPFC and were aligned to correspond to the dorsal anterior cingulate cortex prelimbic (ACC/PL) areas using a Kopf microdrive. Each array contained 16 recording electrodes and 2 reference/guard electrodes, with 2 rows of electrodes placed in each hemisphere. Neuronal recordings began at least 2 weeks following surgery to allow for recovery. The locations of implantations were confirmed by histology, as described previously.

Electrophysiological recordings. A Plexon multichannel acquisition processor was used to amplify and band-pass filter the neuronal signals (150Hz–8kHz; one pole low-cut and three pole high-cut with 1,000-fold gain; Plexon). Signals were then digitized at 40kHz and processed to extract action potentials in real time by a Plexon data workstation. Putative neurons were required to clearly separate from any channel noise, to demonstrate waveform morphology consistent with that of a cortical neuron and to have at least 99% of spikes separated by a minimum refractory interspike interval of 1 ms. No multunit activity was used.

Mean spiking activity in WT mice was slightly, but non-significantly, lower than that of HET mice (1.37 ± 0.33 versus 3.54 ± 0.31 spikes per second; two-sample t-test, t = −0.52, P = 0.61) during neutral trials. Overall, we found no direct correlation between neuronal firing rate and neuronal response to self (two-sample t-test (self versus remaining neurons), WT, t = 1.81, P = 0.073; HET, t = 0.55, P = 0.58) or other (two-sample t-test (other versus remaining neurons), WT, t = 0.17, P = 0.87; HET, t = 0.48, P = 0.63) for either WT or HET animals. Using the waveform morphologies of neurons and their interspike intervals, we identified neurons as putative pyramidal neurons or interneurons. For the pyramidal neurons in the WT mice, 46% (n = 25) responded to self experience, and 54% (n = 29) responded to other experience, whereas for the pyramidal neurons in the HET mice, 83% (n = 39) responded to self experience, and only 17% (n = 8) responded to other experience. For interneurons in the WT mice, 50% (n = 4) responded to self experience, and 50% (n = 4) responded to other experience, whereas for interneurons in the HET mice, 73% (n = 11) responded to self experience, and only 27% (n = 4) responded to other experience. Overall, these numbers suggested that there was no significant difference in the distribution of neurons displaying self- or other-related responses based on neuronal subtype (chi-square test, χ2(3) = 3.5, P = 0.33).

Localized manipulation of Shank3 expression and neuronal activity in the mPFC. Endoxifen injection. To elicit a local increase in Shank3 expression in the mPFC, we injected either endoxifen (Sigma-Aldrich, E8284) or a saline control (Shank3<sup>fx/fx</sup> and Shank3<sup>−/−</sup> mice). Here, we dissolved the endoxifen in 20% DMSO at a concentration of 4 mM to allow for injection. Isolurane-anesthetized mice were head fixed on a Kopf stereotaxic frame, and bilateral craniotomies were performed lateral of the sagittal suture and anterior to the bregma. Using a syringe pump (Harvard Apparatus PHD Ultra) with 10-µl syringes (Hamilton, 1700) connected to 34-gauge needles (WPI Nanofil) by PE-10 and fused silica capillary tubing, we injected 350 nl of 4 mM endoxifen or saline bilaterally (three injections per hemisphere, six injections total). We used the following coordinates relative to the bregma: (1) anteroposterior (AP) +1.78 mm, dorsoventral (DV) −2.00 mm and mediolateral (ML) ±0.30 mm; (2) AP +1.34 mm, DV −1.05 mm and ML ±0.30 mm; (3) AP +0.86 mm, DV −1.05 mm and ML ±0.30 mm. A small amount of cranioplastic cement (Ortho-Jet, Lang Dental) was placed on the skull to cover the craniotomy. Animals were allowed to recover for 2–3 d before behavioral experiments.

Muscimol injection. For muscimol injections, eight WT mice were implanted bilaterally with custom 8-mm, 23-gauge guide cannulae fabricated from hypodermic needles (Coviden, 8881853011). For the surgeries, isoflurane-anesthetized mice were head fixed on a Kopf stereotaxic frame, and small bilateral burr holes were centered at AP +1.34 mm and ML ±0.35 relative to the bregma. Two bone screws were then placed posterior to the bregma for support. Using a three-dimensional (3D) printed custom bilateral cannulae insertion tool attached to a Kopf cannula holder (1776-P1), the cannulae were placed 1 mm above the injection area of interest. Custom-made 8-mm, 29-gauge styliets were inserted into the guide cannulae after the surgery to prevent clogging. Animals were allowed to recover for at least 1 week before behavioral experiments. To transiently locally inhibit neural activity in the mPFC, we injected 350 nl of...
musculos (1 mg ml⁻¹; Tocris 0289) or a saline control at a rate of 250 nl min⁻¹ via a syringe pump (Harvard Apparatus PHD Ultra) with 10 µl syringes (Hamilton, 1700) connected to the internal cannulae with PE-10 and fused silica capillary tubing. We waited for the end of the injection period before removing the internal cannulae. All injections were verified histologically by injecting 100 nl Dil fluorescent lipophilic stain (Invitrogen, V22885) through 9-mm, 30-gauge internal cannulae.

Animal tracking. The animals were recorded using a ceiling-mounted digital camera (Canon Vixia, HF R500). Each animals position within their enclosure, movement velocity and head direction were determined in an automated, genotype-blinded manner using Ethovision XT (Noldus). For additional cross-validation, a blinded tester visually inspected each trial (as recorded by the digital camera) and corrected any discrepancies in the above automated parameter settings in an effort to ensure the accuracy of the data. All EEG and behavioral data were aligned using an NI DAQ and Plexon MEA system.

Social task designs. Paired interaction task. For neuronal recordings, pairs of mice were placed in a rectangular enclosure (24×30×32 cm) separated by a translucent barrier that provided full view of the other and that was permeable to smell and sound. To evaluate for the selectivity of neuronal response, we focused on three main social features that defined their paired interaction. These included social agency (self versus other), experience valence (positive versus negative) and identity (familiar versus non-familiar). All trials lasted 20 s, with trial onset being defined as the moment the recorded mouse entered the restricted enclosure. The trials were separated by a 1-min period in which the recorded mouse was placed in a separate isolated antechamber. The stimulator were removed, and the enclosure was cleaned after each trial. The task conditions were therefore counterbalanced so that an equal number of self and other trials as well as an equal number of appetitive and aversive conditions were performed, and the conditions were given in pseudo-random fashion.

First, to evaluate for neuronal responses that may be selective for agency (that is, self versus other), we randomly varied whether the experiences were given to the other animal or to the recorded animal itself. For example, the other animal may be given an appetitive stimulus on one trial whereas the recorded animal may be given an appetitive stimulus on another. Second, to evaluate for responses that may be selective to the specific experience (that is, rather than simply any salient sensory cue), we used food reward (odorless pellets, Rmg, BioServ) as the appetitive experience and a narrow tube enclosure as the aversive experience. Here, we selected these stimuli because the other’s experiences needed to be clearly apparent and salient to the recorded mouse. We also needed to limit potential confounds such as associative learning, which changes over the course of training (for example, as in fear conditioning through tone–shock pairing) and does not easily allow for the dissociation of self–other agency36. Finally, we varied the other’s social familiarity (that is familiar or non-familiar mice) to evaluate for the effect of social context on the animal’s interaction. Here, familiar mice were defined as those that had been housed with the recorded mouse for 4 weeks or longer. A non-familiar pairing was only considered valid if the other mouse had not been seen by the selected recorded mouse in the last 4 weeks, and two or fewer such pairings had ever been made in total.

Therefore, taken together, an example trial sequence may be that on trial one, the recorded mouse was paired with the non-familiar mouse that is subject to an appetitive experience, and on trial three, it is itself subjected to an aversive experience and so on. All trial combinations (that is, based on variations in agency, experience and familiarity) were repeated such that recording sessions consisted of 45 trials, including sensory and behavioral controls.

Three-chamber task. For behavioral testing, a standard three-chamber apparatus for 1 h was divided into three equal chambers that were separated by a semitransparent barrier (a darkened, non-translucent version of the dividing plastic barrier). Third, all trials were separated from one another by a 5–10 s intertrial interval. For each trial, the test animal was placed in the central chamber. The partner mouse was then placed in one of the two closed-off areas next to the respective partner. At the beginning of the trial, the partner mice were given the associated experience and the other mouse became the partner to receive the opposite experience. A separate cohort of HET and littermate WT animals performed a modified version of the three-chamber task as described above. Here, we excluded conflicts with other trials where animals failed to investigate one of the two enclosures more than twice.

Additional task controls. Controls for sensory-related responses. Several controls were performed to evaluate for other sensory-related aspects of the task. First, to test whether neuronal responses to the other’s experience reflected the appetitive and aversive stimuli themselves (that is, independently of the social partner), we performed a task control in which we replicated the experiments but now replaced the partner with an inanimate object. Therefore, all aspects of the task, including presentation of the appetitive and aversive stimuli, were the same but in the absence of a social partner. Second, to evaluate whether neuronal responses could be explained by factors indirectly related to the stimuli, such as the smell emitted when the other was eating, we added a second set of controls in which the other animal was given the same appetitive and aversive stimuli. Here, however, direct visual access to the other was blocked with an odor- and sound-permeable barrier (a darkened, non-translucent version of the dividing plastic barrier). Third, all trials were separated from one another by a 5–10 s intertrial interval. For each trial, the test animal was placed in the central chamber. The partner mouse was then placed in one of the two closed-off areas next to the respective partner. At the beginning of the trial, the partner mice were given the associated experience and the other mouse became the partner to receive the opposite experience. A separate cohort of HET and littermate WT animals performed a modified version of the three-chamber task as described above. Here, we excluded conflicts with other trials where animals failed to investigate one of the two enclosures more than twice.

Controls for motor-related responses. We evaluated for motoric differences in the recorded animals that could potentially contribute to neuronal response. To this end, we recorded mouse movement with another recorded mouse that is subjected to a continuous aversive stimulus. Mice that were in direct view or close proximity to the other mouse were included in our analysis. We then performed a test for motor-related responses specifically related to the other’s experience. Therefore, the recorded mouse’s own experience was replaced with an inanimate totem that was ‘experiencing’ the same appetitive (food-baited) or aversive (confined tube) conditions. Here, we calculated the test animals’ preference as the percentage of time in which they were within 3 cm of the appetitive versus aversive areas over the course of the trial. This allowed us to determine if the animals could discriminate between the appetitive and aversive conditions. In other words, we would expect the HET mice to display diminished investigative or approach behaviors towards the other under any conditions, irrespective of whether the other animals are specifically having an appetitive or aversive experience.

Respiratory rate (RR) recordings. For real-time RR recordings, shaved test animals were fitted with a mouse jacket (Lomir Biomedical) and pulse sensor (World Famous Electronics) connected to an Arduino via a commutator. These animals were habituated to the jackets for 1 h per day for at least 5 d to ensure their behavior was not hindered. Using custom LabVIEW code and an Arduino microcontroller, raw physiological data were collected and digitized at 500 Hz. These data were then band passed at 1–5 Hz. To evaluate RR during enclosure investigation, we took non-overlapping 1-s samples while animals were investigating either enclosure and fit a sinusoidal wave to the data. RRs for each trial were calculated as mean frequency of the fitted wave. Results across counterbalanced trials were averaged for each animal. We excluded trials for which animals failed to investigate one of the two enclosures more than 10 s.

Elevated zero maze. To determine any Shank3-dependent differences in anxiety phenotype, HET and littermate WT animals were first habituated to a well-lit open field arena for 1 h on at least two separate days. A custom elevated zero maze apparatus (lane width 10 cm, diameter 60 cm, height 60 cm) was divided into four equal quadrants, where two opposite quadrants were enclosed by clear acrylic walls (height 30 cm). Animals were introduced into one of the two closed areas and allowed to freely explore for 5 min while their positions were tracked with Ethovision XT 12 (Noldus). Anxiety-like behaviors were calculated based on the number of open area visits, percent time spent in the open areas and percent time spent head dipping in open areas.

Corticosterone. We measured corticosterone levels as a physiological index of the animals’ ability to differentiate between the other’s aversive and appetitive experiences. A separate cohort of HET and littermate WT animals performed a modified version of the paired interaction task as described above. Here, pairs of familiar conspecifics performed the task that included two main variables: social agency (self versus other) and experience valence (positive versus negative).
Unlike the main task, animals performed one 10-min trial per day. At least 7 d of rest were given in between trials for the mice to recover blood loss. Following completion of each trial, animals were briefly anesthetized with 3% isoflurane for less than 20 s, and −150 μl blood samples were collected with a facial vein puncture (Animal Lancet, Goldenrod). The blood was centrifuged, and plasma was collected and stored at −80 °C. Plasma corticosterone levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer’s protocol (ADI-900-097; Enzo Life Sciences) and a microplate reader at 405 nm (Absorbance 96, Enzo Life Sciences)\(^{32,33}\). Contaminated samples were excluded.

**Statistical analyses.** **ANOVA.** To evaluate the responses of individual cells, a three-way ANOVA with factors for agency (self, other), experience valence (aversive, appetitive) and partner identity (familiar, non-familiar) was conducted on a per neuron basis across all tested trials. Post hoc analysis was conducted at one-three factor levels to determine whether a neuron significantly differentiated between trial types (that is, encoded for a particular factor) while controlling for multiple comparisons. For example, a neuron would be considered to encode the valence of a partner's experience if other-aversive and while controlling for multiple comparisons. For example, a neuron would be considered as ‘self-experience’ encoding if it displayed a consistent difference in activity when the other animals were having an aversive versus an appetitive experience (three-way ANOVA, \(P < 0.0125\). However, the neuron would be considered as ‘self-experience’ encoding if it displayed a consistent difference in activity when the other animals were having an aversive versus an appetitive experience. Trial firing rates were calculated using a spike density function with a sliding Gaussian and a standard deviation of 250 ms. To evaluate for differences in the proportion of neurons within the different recorded populations, we used a standard chi-square test (\(P < 0.005\). The test was used to determine whether there was a significant difference in the number of neurons that responded to a particular main effect (for example, self versus other agency).

Task-modulated neurons were considered across the recorded neuronal population when comparing main effects.

**Neural population modulation.** To evaluate for differences in population response, we examined the distribution of firing activities across cells per condition. For example, to determine the degree to which neurons were modulated by differences in another’s experience, we would subtract the mean firing rates recorded during trials in which the other was given an appetitive experience from the mean firing rates recorded during trials in which the other was given an aversive experience on a per cell basis. An analogous analysis was completed for self trials such that each neuron had two Airing rate values (one for other trials and one for self trials). Differences in the distribution of neural responses were then compared using a 2D two-sample Kolmogorov–Smirnov test (\(P < 0.05\). A one-sample t-test was used to analyze whether a neuronal population exhibited a preferential increase or decrease in firing rate. A two-sample t-test was used to compare whether neuronal populations significantly differed from one another along a particular axis (that is, during self or other trials only). Finally, to evaluate for changes in neural population response over time, analyses were repeated using successive 5-s windows advanced from trial onset to the end of the trial. Peak difference in neural population response was determined by permutation analysis (5,000 random permutations).

**Modeling and decoding analysis.** To determine the degree to which the trial conditions could be predicted from neuronal activity on a per trial basis, we used a linear decoder (Fisher discriminant with quadratic boundaries) as described previously\(^{62–64}\). For the tested conditions (for example, other aversive versus appetitive experience), the ratio of the variance in neuronal activity between variables was compared to the variance within conditions based on:

\[
S_{y}^{-1} S_{BV} x = \nu
\]

The values \(S_{y}^{-1}\) and \(S_{y}\) are the within-condition scatter matrices and between-condition scatter matrices, respectively. The prediction vector \(\nu\) corresponds to the largest eigenvalue of the matrix on the left-hand side of the equation. The prediction vector defines a projection of the recorded activity into a scalar unit. For validation, we divided the neuronal data into a training set consisting of 80% of the trials and tested the accuracy of the prediction on the remaining 20% of trials for validation. This operation was repeated 1,000 times using a random sampling of the total trials. A bootstrapping procedure was then used to determine whether the neuron could discriminate between conditions at an accuracy that was greater than chance. We used a false discovery rate (FDR) correction to account for repeated comparison across conditions (that is, experience valence, social agency and identity. \(P < 0.0125\).

**Model fitting and cross-correlation analysis.** To evaluate for temporal changes in relation to TMX or endoxifen administration, we used the ratio of other-to-self-encoding neurons for the neuronal point data and the ratio of time spent in the three chamber sociability assay (non-familiar/familiar or non-familiar/familiar) for the behavioral point data. Here, we used the ratio of two values rather than simply a single value (for example, the proportion of other-encoding neurons) because the latter could vary based on quality of recordings or simple differences in motoric behavior/motivation from week to week. The temporal dependency between neuronal and behavioral data was assessed by cross-correlation analysis (that is, the cross-correlation between two series as a function of the temporal displacement of one relative to the other). Here, the two time series \(y_{1}\) and \(y_{2}\) were lagged by \(k=0, \pm 1, \pm 2, \ldots\) lengthmax\(\left(\frac{y_{1}}{y_{2}}\right)\), and cross-correlation, \(\rho\), was calculated for each possible pairing \((y_{1}(t), y_{2}(t))\) and so on by

\[
\rho(k) = \frac{\sum_{i} (y_{1}(i) - \bar{y}_{1})(y_{2}(i-k) - \bar{y}_{2})}{\sqrt{\sum_{i} (y_{1}(i) - \bar{y}_{1})^{2}}\sqrt{\sum_{i} (y_{2}(i-k) - \bar{y}_{2})^{2}}}
\]

Here, \(T = \text{length}_{\text{max}}(y_{1}(y_{2}))\), and \(\bar{y}\) is the sample mean of the series across time. Cross-correlation was in turn calculated for each cross-correlation value \(\rho(k) = c_{1,2}(k) \times (c_{1,2})^{-1}\) where \(c_{1}\) and \(c_{2}\) are the square root of variance. Optimal lag time was based on \(k\) with the greatest cross-correlation estimate. Because neuronal recording and behavioral testing were performed on alternating days over the 2-month course of testing, time lags were calculated in 2-d intervals. Significance of cross-correlation was determined by permutation test (\(n=1,000; P < 0.01\).

**Neural population decoding.** A supervised learning approach was used to quantify the degree to which the population's activities were informative of each of the three primary task features that defined the animals' experiences. The approach was designed in three parts. First, we constructed support vector machines (SVMs) with non-linear kernels to map the population's activity patterns onto high-dimensional feature spaces, whereby

\[
\min_{\nu, w, b} \frac{1}{2} \nu^T w + C \sum_{i=1}^{N} \xi_i
\]

subject to

\[
y_i \left(\nu^T w(x_i) + b\right) \geq 1 - \xi_i
\]

Here, \(y \in \{1, −1\}^N\) corresponded to the stimulus conditions (for example, appetitive versus aversive condition), \(x\) corresponded to the neural activity \(\xi_i = \max (0, 1 - y_i(w^T x_i - b))\) and \(C\) is the regularization factor. As described previously\(^{62–64}\), these approaches are well suited for identifying neuronal ‘subspaces’ in relation to specific task features and account for potential non-linearities in the response properties of the neurons. Second, to determine the decoding accuracy of the models, we used trials randomly sampled and bootstrapped from the validation data (20% of randomly selected trials). Finally, to determine significance, this process was repeated 1,000 times and compared to models trained on neuronal data that were randomly shuffled (\(H_{\text{random}} = 50\%\) chance; permutation test, \(P < 0.001\)).

**Statistics.** No statistical method was used to predetermined sample size. Statistical analyses were conducted in MATLAB (Mathworks). Significance was set at \(\alpha < 0.05\) for all statistical tests unless otherwise indicated. The data distribution was assumed to be normal, but this was not formally tested. Two-tailed tests were used unless otherwise indicated. Permutation tests were also used to avoid assumptions about the distributions of the data. Data are expressed as mean ± s.e.m. unless otherwise indicated.

**Blinding.** All data collection and analyses were performed blind to animal genotype (WT versus HET), gavage (TMX versus corn oil) and injection (saline versus endoxifen, saline versus muscimol). All analyses were performed blind to trial condition (appetitive versus aversive).

**Reporting Summary.** Please see additional details in the Nature Research Reporting Summary attached to this article

**Data availability.** The behavioral and neuronal data that support the findings of this study are available from the corresponding author upon reasonable request. Source data are provided with this paper.

**Code availability.** All software used in this study are listed in the Reporting Summary along with their versions. The primary MATLAB code used to perform the statistical and data analyses in this study is available from the corresponding author upon reasonable request.
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Author contributions

S.W.L., F.B., L.S., O.Z., P.G., M.A.S. and J.D. performed the experiments. D.K.L., S.W.L., G.F., M.J. and P.G. analyzed the data. S.W.L. developed the conditional gene knockout studies. D.K.L., G.F. and S.W.L. edited the manuscript. Z.M.W. conceived and designed the study, wrote the manuscript and supervised all aspects of the research.

Competing interests

The authors declare no competing interests.

Additional information

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Extended Data Fig. 1 | Animal preparation and electrophysiological recordings. a, Illustration depicting the Cre-lox system and its use in restoring Shank3 expression in adult mice. Cre-dependent FLEX switch constructs capable of controlling Shank3 expression are made by creating Shank3<sup>fx/+</sup>:CreER<sup>±</sup> mice in which Cre recombinase (CRE) is fused to an estrogen receptor (ER) protein. Here, an inverted PDZ domain (the membrane anchoring portion of SHANK3) is 'floxed' by two lox sites and, therefore, renders the mice heterozygous Shank3. CRE-ER is expressed constitutively. Normal Shank3 expression is restored by either delivering systemic TMX or local endoxifen to the mice. Binding of endoxifen to the ER receptor leads to dislodgement of the associated heat shock protein (HSP) from Cre which then allows it to enter the nucleus. Once in the nucleus, Cre leads the floxed PDZ sequence, flanked by two loxP sites, to be inverted through recombinase-mediated cassette exchange. Finally, inversion of the PDZ sequence leads to normal production of SHANK3. 

b, Representative waveform morphologies and recording locations. Displayed left is a histological section with bilateral electrolytic lesions indicating the microelectrode recording locations from the medial prefrontal cortex (mPFC). Right are representative waveform morphologies from six putative neurons recorded each from the mPFC of WT and HeT mice. 

c, Shank3<sup>fx/+</sup>:CreER<sup>−/−</sup> mice were created by crossing CAGG-Cre-ER<sup>±</sup> with Shank3<sup>fx/fx</sup> mice. Shank3<sup>fx/+</sup>:CreER<sup>−/−</sup> mice were created for control comparison. Only littermates were used for comparisons and all were male mice.
Extended Data Fig. 2 | Neuronal responses to experience valence, agency, and identity. a, Neurons that responded to other’s experiences based on variations in the recorded animal’s own enclosure. Overlap in encoding across neurons is displayed on the left and their total numbers are shown to the right. Overall, only a few neurons (n = 7) displayed a difference in response to the other’s experience based on which specific enclosure the recorded animal was simultaneously placed in (three-way ANOVA, p < 0.0125 with post-hoc comparisons). b, Neuronal responses based on the other’s social identity. Breakdown of neurons that responded to the other’s identity (familiar and non-familiar) and their relation to neurons that responded to the other’s experience. On the left are the distribution of neurons that responded to the other’s experience. While many of the neurons that responded to the other’s experience also responded to the other’s identity (11.6%), some neurons responded to the other’s identity alone (5.4%; that is, irrespective of the other’s experience). On the right are the distribution of neurons that responded to the other’s identity based on whether the other animals were specifically familiar or non-familiar to recorded mouse. c, Relative proportions of all task-modulated neurons using a three-way ANOVA that accounted for all terms describing experience valence (positive vs. negative), social agency (self vs. other) and social context/identity (familiar vs. non-familiar vs. totem) with post-hoc comparisons and correction for multiple comparisons at a p < 0.0125. To the left are the numbers of neurons that responded to self- or other-experience valences. To the right are the remaining top six most common feature combinations. For example, while many neurons responded to another’s aversive vs. appetitive experience, some neurons only responded to the other’s experience when they were familiar to the recorded animal. Other neurons, by comparison, only responded to differences between familiar vs. non-familiar animals while displaying little modulation to their specific experience. In total, 112 neurons and 131 neurons displayed task-related modulation in the WT and Shank3 mice, respectively.
Extended Data Fig. 3 | Neural modulation over the course and within the trials. a, Absolute difference in activity (Z-score) for other experience per cell over the time course of the trial. Here, neuronal responses are broken down into 5 second intervals in order to illustrate the time progression of neural modulation. While there was a slightly lower degree of modulation at the very end of the trial, this difference was not significant (repeated measures ANOVA, \( p > 0.2 \)). Similarly, we find no difference in the total number of neurons that displayed significant modulation over the course of the trial (Chi-square, \( p > 0.5 \)). The directions of the arrows indicate whether responses were stronger for the aversive (point up) compared to appetitive (point down) other experiences. To evaluate the effect that the animal’s own prior experience may have had on neuronal encoding of the other’s experience, we also compared all possible transitions combinations between self- followed by other-experience valence (for example, self-appetitive followed by other-aversive, self-aversive followed by other-aversive, etc.). Here, we find that only 2 neurons in the WT mice that responded to other-experience were also affected by specific past self-experience (three-way ANOVA, \( p < 0.0125 \)). In other words, the animal’s own experience in one trial influenced neuronal responses the following trial in only 1.8% of the neurons. Similarly, we find only 1 neuron in the WT mice whose response to self-experience was also affected by past other-experience, together suggesting that the past trial condition did not influence neuronal response under this task. b, This Figure follows the same convention as in Fig. 3d. Here, however, neural activity (Z-score) are divided into successive, non-overlapping 5 second windows over the 20 second trial-span.
Extended Data Fig. 4 | Proportional contribution of neurons across animals. To allow for comparison across animals, the relative contribution of each mouse to the overall group ratio for self vs. other encoding are shown in percentages. Thus, a larger percentage means that they contributed relatively more to the proportion of other-encoding neurons whereas a smaller percentage means that they contributed relatively more to the proportion of self-encoding neurons. Additionally, we performed a within-group vs. between-group comparison to evaluate more directly whether differences in the proportions of neurons could be explained by potential dissimilarities in recording quality or variations in anatomical localization between animals. For example, if recordings were indeed made from slightly different areas or subpopulations of cells, then we should observe a similar variance between individual animals that belonged to the same genotype (that is, HET) compared to between individual animals that belonged to the different genotypes (that is, HET vs. WT). In other words, variability in the proportions of neurons found between one WT animal and another WT animal should be similar to that found between one WT animal and another HET animal. Examining the proportion of neurons that responded to other-aversive vs. other-appetitive experience, however, we find that the difference between animals within the WT group was significantly smaller than that between the WT and HET group (two-sample F-test for equality of variance; f-stat = 0.20, p = 0.0076). These observations therefore support the main findings and controls further below that those differences in encoding properties between animals were due to differences in Shank3 expression rather than a systematic variation in subpopulations sampled.
Extended Data Fig. 5 | Robustness of neuronal encoding within the population across different statistical methodologies. a, To validate our results and further delineate the response characteristics of the neurons, we repeated the ANOVA analyses at different statistical thresholds from $p = 0.05$ to $0.0025$. Across conditions, other-to-self ratio was relatively stable, with HET and WT results ranging from 1:2.3 to 1:4.6, and 1.1:1 to 1:1.7, respectively. At all significance thresholds, the other-to-self ratio of HET was significantly smaller than that of WT (chi-square, $p < 0.05$). b, Support vector machines (SVMs) with nonlinear kernels were used to decode neural population activity on validation trials not used for model training. The temporal dynamic of neural population predictions is presented in relation to the beginning, middle and end of the trials and are broken down into the three primary features that described the animals’ social interactions during the task; their experience valence, social agency and identity. Using neural population activity from validation trials not used for model fitting, we find that activity from the neural population could accurately predict the social agency of the animals’ experience with an accuracy of $70.9 \pm 5\%$ at trial onset (permutation test, $p < 0.001$). In other words, the neural population could be used to reliably distinguish one’s own experience from that of another. We also found that the neural population could predict the specific valence of the animals’ experience with an accuracy of $76.8 \pm 3\%$ (permutation test, $p < 0.001$), suggesting that they reliably distinguished whether the experience was appetitive or aversive. Last, the neural population predicted the social context with an accuracy of $78.2 \pm 2\%$ (permutation test, $p < 0.001$). Overall, all three primary task features (agency, experience valence and social context/identity) were most accurately decoded from the population’s response at the beginning of the trials (that is, within the first 1–6 seconds; permutation test, $^* p < 0.001$). Prediction accuracy then gradually dropped over the middle to end of the trial. While decoding accuracy was significant during the middle of the trial for valence and social identity (permutation test, $^* p < 0.01$), decoding performance for all three features was at chance by the end of the trial. Representation of these task features was therefore most prominent at the start of the trials (that is, at which time these conditions were most salient), and then gradually diminished by the end of the trial. To determine significance and error, this process was repeated 1000 times and compared to models trained on neuronal data that was randomly shuffled. Error bars indicate 95% confidence interval.
Extended Data Fig. 6 | WT animals differentiate between others’ aversive and appetitive experiences. **a**, To understand whether animals recognized another animal’s experience and to allow for comparison with our main results, the animals performed a place preference/avoidance task where subjects were presented with two age and sex-matched familiar conspecific partners undergoing either an appetitive (food-baited enclosure) or aversive (confined tube enclosure) experience. The percent time spent by the animals investigating the aversive and appetitive enclosures when in the presence of another animal is shown here. While the WT displayed a significant difference in the time spent investigating the specific enclosures when compared to HeT animals (*two-tailed unpaired test; ts(15) = −2.79; p = 0.014), there was no difference in approach behavior when familiar conspecifics were replaced with inanimate totems in the enclosures (ANOVA, p > 0.5 post-hoc comparison). **b**, We confirmed that the WT animals differentiated the other’s experiences based on respiratory rate. Using a mouse jacket (Lomir Biomedical) and pulse sensor (World Famous Electronics; digitized at 500 Hz then band-passed at 1–5 Hz), we find a significant difference in the animal’s respiratory rate when the other animal was having an aversive vs. appetitive experience (*ANOVA, p = 0.0031). **c**, The panel above displays the sequence of behavioral testing, plasma collection and corticosterone analysis/quantification by ELISA. The panel below displays the plasma levels collected across all WT and HeT animals. Here, the levels are given based on whether the animals observed the other having an appetitive vs. aversive experience (two-tailed unpaired t-tests; * p = 0.021, ** p < 0.01, n.s. p = 0.23).
Extended Data Fig. 7 | Evaluating for Shank3 dependent differences in anxiety phenotype between the WT and HET mice. Elevated zero-maze testing was performed on the WT and HET mice. Overall, HET mice displayed no evidence of elevated anxiety compared to WT based on the proportion of time spent in the open areas, number of visits to the open areas, or percent of time spent head-dipping (two-tailed unpaired t-tests: p > 0.5).
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Shank3 dependent changes in proportion of neuronal encoding and sociability. a, Each curve represents the raw proportions of other-encoding and self-encoding neurons at weekly intervals after TMX administration (rather than ratio; with the sum equaling one). Time points in which there was a significant difference are underlined in red (chi-square tests, p < 0.01). The proportion of neurons that were task-modulated or which responded to variations in familiarity did not significantly change in the weeks after TMX administration for Shank3fx/+/CreERloxP mice. Specifically, the percentage of task modulating neurons increased only marginally from 73% (n = 131 of 180, pre-tamoxifen) to 74% (n = 84 of 114, post TMX; chi-square, \(\chi^2(1) = 0.29, p = 0.86\); Extended Data Fig. 3). Likewise, the percentage of neurons that responded to variations in the other’s familiarity remained almost identical (30%, n = 39 of 131, pre-tamoxifen to 29.8%, n = 25 of 84, post-tamoxifen; chi-square, \(\chi^2(1) = 2.0 \times 10^{-6}, p = 1.0\)). b, Above, the net amount of time spent with the other animal vs. inanimate object out of the 120 seconds for testing. Below, three-chamber testing was obtained from the same individual animals over the consecutive course of 8 weeks after TMX administration. Time points in which there was a significant difference are underlined in red (paired t-tests, * ts(19) = 3.01; p = 0.0046). c, To further validate results from the Shank3fx/+/CreERloxP mice and to confirm that changes in behavior after TMX was not explained by task familiarity or time-progression, HET mice also received corn oil (that is, vehicle) instead of TMX. Here, the HET (Cre±) mice displayed no change in behavior over time (one-sample t-tests, p > 0.5). d, In the top panel, the curve represents the proportion of time spent in the chamber of the other animal or inanimate object ± standard errors of the mean at weekly intervals. In the bottom panel, each curve represents the percentage of other-encoding and self-encoding neurons at weekly intervals. The horizontal line represents an equal proportion of neurons. No time point demonstrated a significant difference in either behavior or sociability when compared to all other points (one-sample t-tests, n.s.; p > 0.2). e, The proportion of time interacting with the other animal as defined by the amount of time at which the subject mouse was in proximity (within 3 cm) and oriented towards the other animal (within 20 degrees). HET mice displayed a gradual and significant increase in the amount of time spent interacting with the other animal after TMX (paired t-test, p < 1.0 \times 10^{-4}). These findings were not apparent when testing the Shank3fx/+/CreERloxP mice which lacked the Cre-lox system after TMX (unpaired t-test, p > 0.5). More remarkably, by 8 weeks after TMX administration, the amount of time spent interacting with the other by the HET mice was essentially indistinguishable from that of the WT mice (unpaired t-test, p > 0.5). All error bars indicate s.e.m.
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Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Source data underlying Fig. 4b is provided as Source Data Fig. 1. The behavioural and neuronal data that support the findings of this study are available from the corresponding author upon reasonable request.
Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
No statistical method was used to predetermine sample size; sample sizes are similar to those reported in previous publications (e.g. Peca et al., 2011). All animals were adult male mice, ages four months or older and comparisons to allow for consistency and included either (i) Shank3fx/+;creER-, (ii) Shank fx/+;creER+, (iii) Shank3+/+;creER- and (iv) Shank3+/+;creER+ constructs. In total, we used 77 animals of which 28 were wild-type (WT) and 49 were heterozygous (HET) Shank3 mice. Overall, we recorded from 388 mPFC neurons in the WT mice, 180 neurons in the HET mice prior to TMX and 837 neurons from the HET mice after TMX or endoxifen administration.

Data exclusions
No animals were excluded from the main behavioral analysis. For respiratory rate data during the place preference/avoidance task, we excluded RR data for trials where animals failed to investigate one of the two enclosures more than 10s. For neuronal analysis, any units that did not demonstrate waveform stability over the course of the experiment were excluded from the analysis per pre-established standard criteria for off-line single unit sorting. Units that displayed significant overlap in their PCA distributions by MANOVA (p < 0.01) or overlapped with the baseline signal/noise were considered multi-units and excluded from further analyses.

Replication
Replication of results were obtained from both WT and HET animals by tracking the behavior and neuronal activity of the same animals. Consistency was also confirmed by performing a within-group vs. across-group comparison. Finally, we performed within-animal restoration of Shank3 expression to evaluate the robustness of our results on an animal-by-animal basis. Additional control comparisons were made using carrier or saline administrations. All attempts at replication were successful across different animals, as reported in our results.

Randomization
All animals were randomly assigned into different groups for all experiments. All paired comparisons were made between animals that underwent the same task procedures and/or TMX or endoxifen administration.

Blinding
All data collection and analyses were performed blind to animal genotype (WT vs HET), gavage (TMX vs corn oil); injection (saline vs endoxifen; saline vs muscimol). All analyses were performed blind to trial condition (appetitive vs aversive). Animal movement was captured and analyzed in a semi-automated fashion. Neuronal spike sorting was made without knowledge of the task condition or trial type.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑ Antibodies |
| ☑ Eukaryotic cell lines |
| ☑ Palaeontology and archaeology |
| ☑ Animals and other organisms |
| ☑ Human research participants |
| ☑ Clinical data |
| ☑ Dual use research of concern |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑ ChIP-seq |
| ☑ Flow cytometry |
| ☑ MRI-based neuroimaging |

Antibodies

Antibodies used
Rabbit anti-Shank3 primary antibody (1:500; APZ-013, Alomone Labs)
Goat anti-rabbit horseradish peroxidase secondary antibody (1:8,000; 32460, Invitrogen).
Alexa 488-conjugated goat anti-rabbit secondary antibody (1:500; 111-545-144, Jackson ImmunoResearch)

Validation
β-actin was used as a loading control. This polyclonal antibody has also been cited in previous studies (e.g. Verpelli et al., 2011). To further confirm antibody selectivity, we also combined the rabbit anti-Shank3 primary antibody with SH3 protein prior to incubation according to Alomone instruction sheet (Peptide (C)EKLPGSLRKGIPRTK, corresponding to amino acid residues 841-855 of Shank3 protein). Here, we used the Shank3 blocking peptide (BLP-P2013, Alomone) for negative controls in both the Western blot and immunofluorescence.
Animals and other organisms

Policy information about **studies involving animals**: ARRIVE guidelines recommended for reporting animal research

| Category               | Information                                                                 |
|------------------------|-----------------------------------------------------------------------------|
| Laboratory animals     | C57BL/6 male mice aged 4-12 months were used. They were housed on a 12-hour light/dark cycle at 70 degrees F and a relative humidity of 40-60%. Food and water were provided ad libitum except during experimentation. |
| Wild animals           | No wild animals were used in the study.                                      |
| Field-collected samples| No field-collected samples were used in the study.                           |
| Ethics oversight       | All procedures were approved by the Massachusetts General Hospital IACUC and kept in strict accordance with the Harvard Medical School Institutional Animal Care and Use Committee guidelines. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.