Interneurons Are Necessary for Coordinated Activity during Reversal Learning in Orbitofrontal Cortex

Supplemental Information

Supplemental Data

This supplemental section includes data that we believe is relevant to the manuscript. Below, we describe mouse behavior data which demonstrates both control and *Plaur* mice were equally engaged in the task with no behavioral deficits besides the trials to criterion and errors on reversals. We report on the encoding of decreasing-type neurons, and how there were nearly no differences in encoding between genotypes of decreasing-type decision and reward neurons on discrimination or reversal trials. Further, we report the encoding differences of increasing-type neurons on discriminations and reversals without the reversal 1 data, to show that the differences in neural encoding are not driven by behavior deficit, but are intrinsic to the altered orbitofrontal cortex (OFC) network of *Plaur* neurons.

Behavior

Mice learned to dig for reward in bowls of odorized media using exemplar combinations (Table S1) and order of task problems (Table S2).

Table S1. Exemplar combinations

		Dimension	
Pair	**Exemplar**	**Odor (O)**	**Medium (M)**
1	1	Rosemary	Aspen bedding
	2	Cloves	Gravel
2	3	Cinnamon	Kaykob bedding
	4	Sage	Moss
3	5	Onion	Perlite
	6	Paprika	Bark
4	7	Garlic	Cat litter
	8	Coriander	Feathers
5	9	Thyme	Plastic pellets
	10	Black pepper	Cotton balls

Table S2. Order of discrimination tasks[a]

	Exemplar Combinations	
Task	**Correct**	**Incorrect**
Simple Discrimination (training)	O1, M1	O2, M1
Discrimination 1 (D1)	O1, M1, M2	O2, M1, M2
Reversal 1 (Rev1)	O2, M1, M2	O1, M1, M2
Discrimination 2 (D2)	O3, M3, M4	O4, M3, M4
Reversal 2 (Rev2)	O4, M3, M4	O4, M3, M4
Discrimination 3 (D3)	O5, M5, M6	O6, M5, M6
Reversal 3 (Rev3)	O6, M5, M6	O5, M5, M6
Discrimination 4 (D4)	O7, M7,M8	O8, M7, M8
Reversal 4 (Rev4)	O8, M7, M8	O7, M7, M8
Discrimination 5 (D5)	O9, M9, M10	O10, M9, M10
Reversal 5 (Rev5)	O10, M9, M10	O9, M9, M10

M, medium; O, odor.

[a]The order of discriminations was the same for mice, but the relevant dimension was counterbalanced within each experimental group. The number of trials required to reach criterion was independent of choice of relevant dimension.

Mice performed the problems with an average choice latency of 5.2 ± 1.2 s for control and 3.4 ± 0.8 s for *Plaur* mice, and no difference in trial number for simple or compound discriminations was observed (p = 0.2). Once the mice had learned a particular exemplar correctly (8 consecutive correct choices), an additional 15 trials were run, before reversing the rewarded cue. Mice learned and reversed 5 discrete discrimination problems (Table S2). Total counts of the visits during each discrimination and reversal were analyzed and ANOVA revealed no effect of genotype, ($F_{(1,154)}$ = 1.574, p = 0.216), or discrimination vs reversal ($F_{(9,154)}$ = 0.166, p = 0.919). Similarly, there was no difference between genotypes in the latency to correct choice (one way repeated measures ANOVA, $F_{(5,944)}$ = 0.429, p = 0.829), incorrect choice ($F_{(5,270)}$ = 0.693, p = 0.629), time spent digging to retrieve reward ($F_{(5,944)}$ = 1.761, p = 0.118), or to consume the reward ($F_{(5,944)}$ = 2.087, p = 0.065). These data are consistent with previous data, which indicated no overall difference in locomotor activity in the open field test (1). Coupled with the lack of deficits in latency data, the *Plaur* mice appeared to have similar locomotor ability, engagement with the cues and took similar amounts of time to make a decision compared to control mice.

Characterization of Neural Activity

Few fast-spiking cells were identified (control n = 2, *Plaur* n = 8) during Discrimination 1. Putative pyramidal cells tended to have larger P:T ratios and larger half-width times (control: 0.72 P:T ratio and 230.1 µs half-width, *Plaur* 0.88 P:T ratio and 281.7 µs half-width) than putative fast-spiking interneurons (FS cells, control: 0.31 P:T ratio and 72.9 µs half-width, *Plaur*: 0.21 P:T ratio and 56.7 µs half-width).

Neural Encoding

These data show that control mouse OFC encodes both decisions and rewards during discriminations and reversal problems, and that neural activity during reversal problems is correlated with performance. We also show that *Plaur* mice show diminished encoding of decisions and rewards and that activity is not correlated with behavioral performance. These data may reflect permanent changes in *Plaur* OFC function, or the fact that *Plaur* mice were worse on the initial reversal (Rev1). To address this issue, we reanalyzed the dataset, in absence of the recordings from the first discrimination (D1) and reversal (Rev1) tasks (Figure S1). Control mouse decision neurons (blue lines) show significantly increased neural activity ($p < 0.001$) during the decision epoch during both discrimination (thick line) and reversal (thin line) compared to *Plaur* (red lines) neurons. Control mouse reward neurons continue to robustly encode reward receipt in both discriminations and reversals and show significantly elevated activity during reward epoch, compared to *Plaur* mouse neurons ($p < 0.001$). Thus, the neural differences we observed in our analysis likely reflect the basic function of mouse OFC neurons in control mice, and a sustained deficit in *Plaur* mice, due to a developmental perturbation in fast spiking PV^+ interneurons, rather than differences driven by the behavioral deficit.

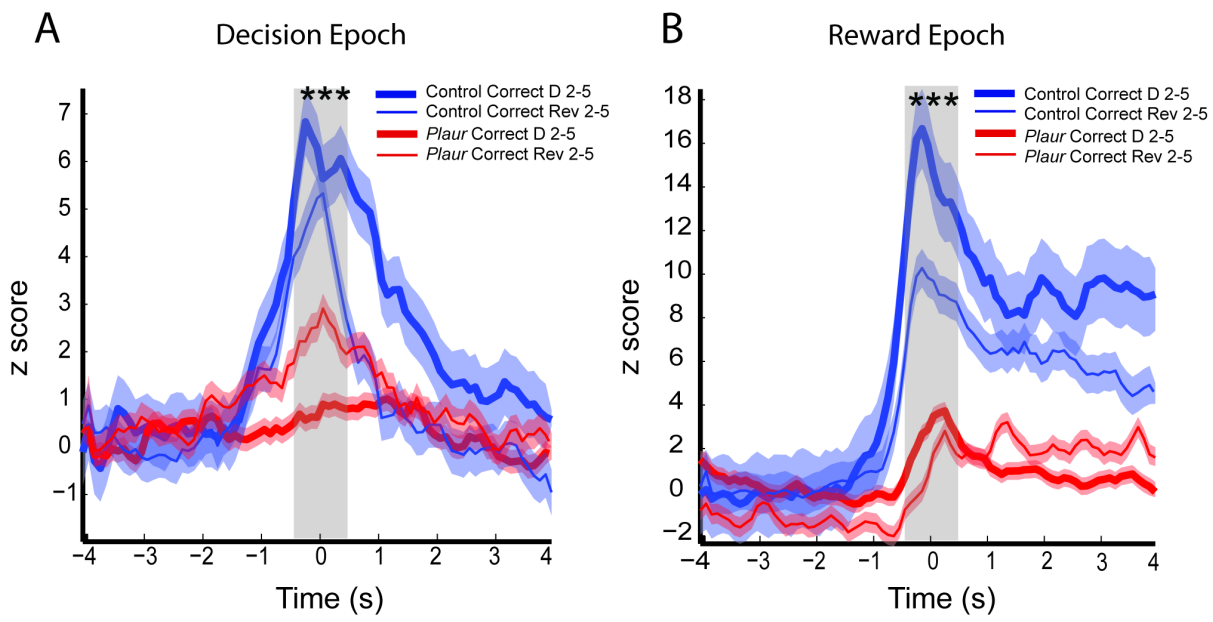

Figure S1. Averaged neural activity is shown during decision and reward epochs for increasing type neurons without neurons from the first reversal. (**A**) Averaged z-scored neural activity during decision epoch without data from first reversal. Control mouse decision neurons (blue) show significant increases during the decision epoch during both discrimination (thick line) and reversal (thin line) compared to *Plaur* (red) neurons. (**B**) Averaged z-scored neural activity during reward epoch without data from first reversal. Control mouse reward neurons robustly encode reward receipt in discrimination and reversal, while encoding in *Plaur* mouse neurons is significantly diminished, ($p < 0.001$, comparison between genotypes).

Decreasing-type Neurons

To assess the impact of *Plaur* on decreasing-type cells, we plotted the z-scored population histograms as for increasing-type neurons (Figure S2). The activity observed in control mice (blue lines) was not significantly different from *Plaur* on correct (solid lines) trials during either the discrimination ($p = 0.4$) or the reversal ($p = 0.6$) during the decision-epoch (500 ms around time of decision). Control decreasing-type decision neurons were significantly different on correct and error trials ($p < 0.05$) on both discrimination and reversal. *Plaur* decreasing decision neurons also differentially encoded correct vs error decisions ($p < 0.05$) on discrimination, but not reversal ($p < 0.07$). Decreasing-type reward neurons (Figure S2) are not

different between genotypes on discrimination (p = 0.9), but their firing rates differed from baseline during the reward epoch (500 ms around time of reward receipt) of reversal trials, where activity in *Plaur* decreasing-type reward neurons was significantly lower than controls during the reward epoch ($p < 0.05$). For both *Plaur* and control mice, decreasing-type neurons significantly decreased activity more for correct, than error trials ($p < 0.01$), with no significant differences for reward neurons and no difference in signal:baseline ratio, suggesting that while baseline firing was different, the ratio of activity changes between genotypes for decreasing-type neurons remained the same between genotypes. The similarity in activity changes of decreasing-type neurons suggests that the major impact of diminished PV^+ interneurons in terms of neural firing was to the ensemble activity of increasing-type neurons.

Associative encoding was not disrupted for decreasing-type neurons. In control mice, 31% of decision-decreasing cells were outcome expectant, with the remaining neurons being associative. By contrast, 48% of decision-decreasing *Plaur* cells could be considered outcome expectant, a non-significant proportional difference between genotypes (p = 0.5). In control mice, 63% of reward-decreasing neurons were outcome expectant, compared to 58% of *Plaur* reward-decreasing neurons.

While there was a reduction in the overall number of decreasing-type neurons observed between *Plaur* and control mice in both discrimination and reversal trials, there were no relevant differences in encoding observed in the decreasing-type neuronal populations. Data below (Figure S4) further support the idea that the observed encoding differences were in the increasing-type neural population, rather than decreasing-type. The decreasing and non-significant neurons were not correlated with behavior and firing, leaving only the increasing-type

neurons being significantly correlated between task performance and firing rate. Because of this, we focused mainly on the increasing-type neurons in the main body of this manuscript.

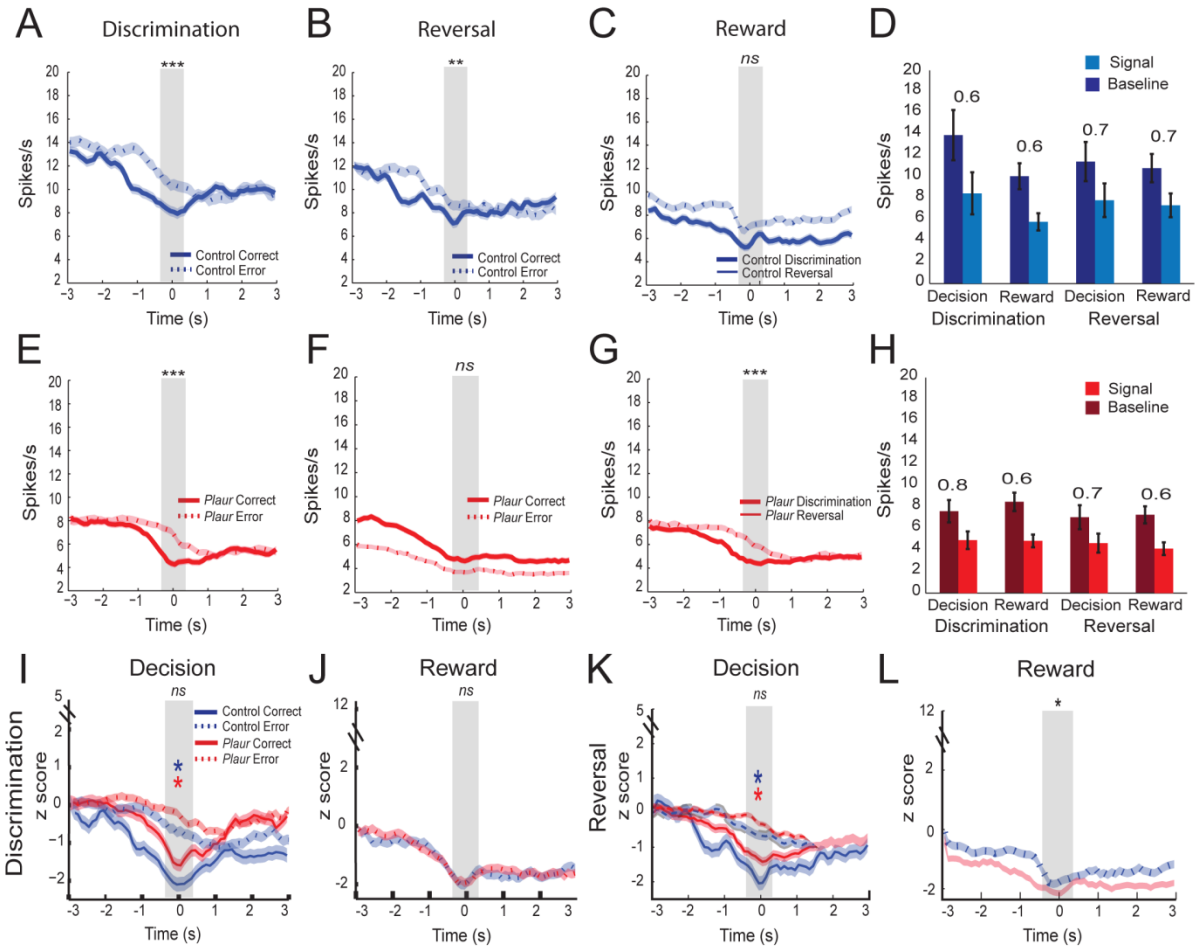

Figure S2. Averaged raw spikes/s and z-transformed neural activity during decisions and reward epochs for decreasing neurons. (**A-C**) Averaged spikes/s neural activity of decision and reward decreasing-type neurons show differential firing for correct (solid line) vs error trials (dashed line) in control mice. (**D**) Signal:baseline ratios for control mice during each epoch. (**E-G**) Averaged spikes/s activity for *Plaur* mice. (**H**) Signal:baseline data for *Plaur* neurons. Ratio of signal to baseline activity are not different between genotypes (**D**,**H**) for any discrimination or reversal, decision or reward neurons. (**I-L**) Z-transformed data demonstrates similar effects. Control mice (blue lines) are not significantly different from *Plaur* on correct (solid lines) or error (dashed lines) trials during either the discrimination or the reversal (**I**,**K**). Decreasing type reward neurons (**J**,**L**) are not different between genotypes on discrimination, but are during the reward epoch of reversal trials, where activity in *Plaur* decreasing-type reward neurons was significantly lower than controls during the reward epoch. Overall, the two populations of decreasing neurons behave similarly between genotypes, and the relative change in activity during the epoch (decision or reward) is much smaller than for the increasing type neurons. Between genotypes comparisons (black) ns = not significant, * denotes $p < 0.05$. Between trial types, * denotes $p < 0.05$, blue for control correct vs control error, red for *Plaur* correct vs error.

Reward Expectancy Signals in Mouse OFC

The OFC has been shown to represent the expectation of reward (2-4), yet the neural signature of expectation has yet to be shown in mice. Figure S3 shows populations of decision and reward-specific neurons, plotted during each epoch for control and *Plaur* mice. Control decision-neurons (solid blue line) increased activity for the decision, which diminished before reward receipt. Reward-neurons (dashed blue line) began increasing in activity just slightly after time of decision for either discrimination or reversal in anticipation of reward. This pattern of reward anticipation was not observed in the *Plaur* mice (red dashed line). The same pattern of activity is recapitulated on reversal trials, where decision-neurons (solid red line) increase only for the decision, and decrease activity by the time of reward receipt, while reward-neurons signal the expectation of reward from decision until reward receipt. Again, reward responsive neurons in *Plaur* OFC do not exhibit anticipatory encoding, but rather show elevated firing immediately around reward receipt epoch.

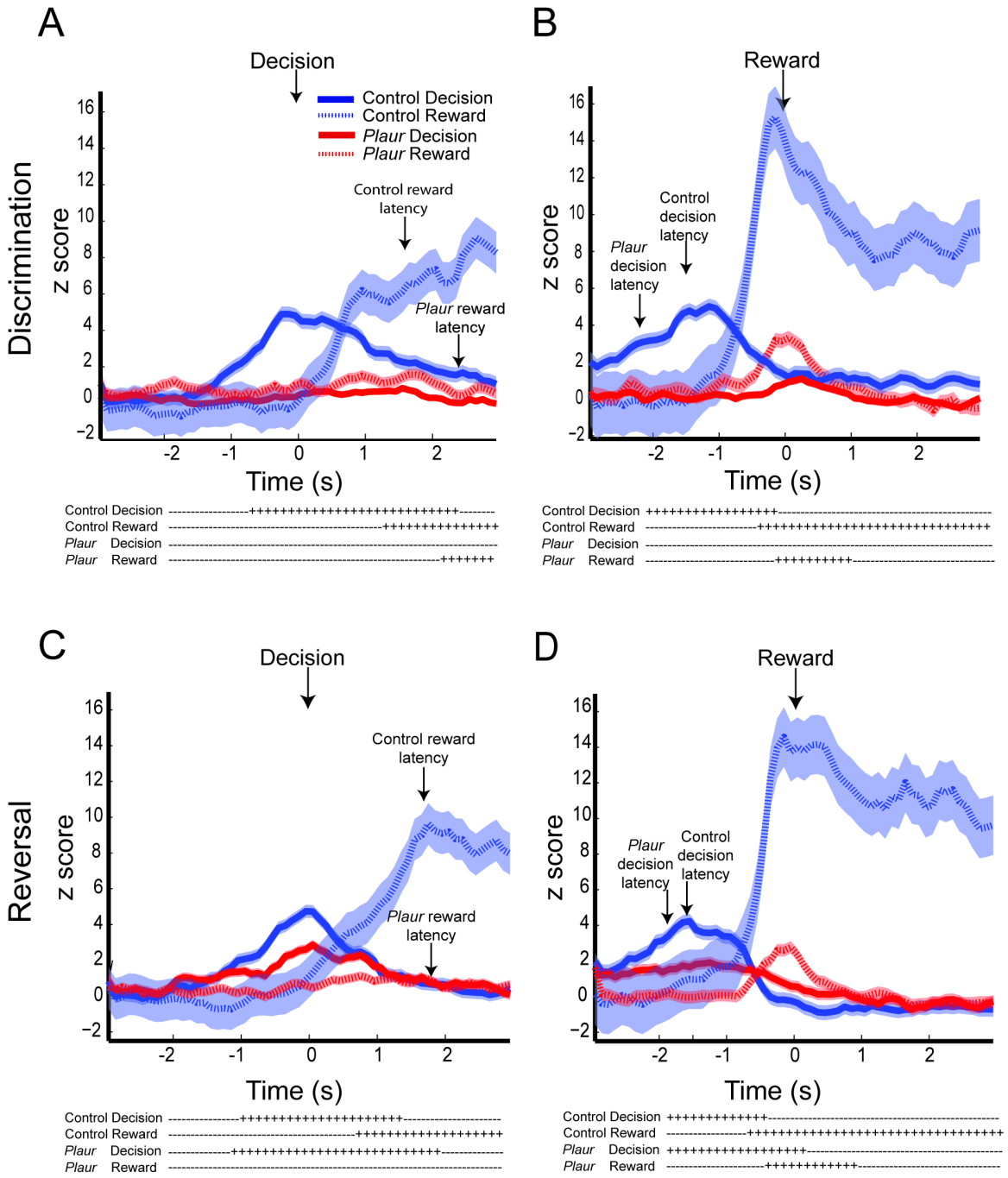

Figure S3. Expectation is observed in reward, but not decision-neurons. (**A**) Line graphs of decision (thick line) and reward (dashed) neuronal populations aligned at decision and reward time points for control (blue) and *Plaur* (red). On discrimination trials, decision-neurons respond to choice but decrease firing before reward receipt, while reward-neurons increase activity from time of decision until reward receipt, demonstrating expectation in control mice. *Plaur* mice show no expectation of reward outcomes. (**B**) On reversal trials, control reward-neurons continue to show anticipatory activity, increasing activity from decision to reward receipt, while decision-neurons taper off before reward receipt. *Plaur* mouse reward-neurons again show no expectation of outcome encoding in reward neuron population. + signs denote periods of significant differences between genotypes ($p < 0.05$).

Correlations of Neural Activity with Behavior

No significant correlations with behavior were found either in control or *Plaur* mice on either the discrimination (controls $p = 0.3$, $r^2 = 0.03$, *Plaur* $p = 0.1$, $r^2 = 0.1$) or reversal (controls $p = 0.4$, $r^2 = 0.02$, *Plaur* $p = 0.9$, $r^2 = 0.01$) using percent correct and for trials to criterion (discrimination, controls $p = 0.7$, $r^2 = 0.01$, *Plaur* $p = 0.4$, $r^2 = 0.03$, reversals control $p = 0.9$, $r^2 = 0.01$, *Plaur* $p = 0.4$, $r^2 = 0.16$). In addition, we tested whether decreasing-type cells or non-significant type cells (cells which neither increased nor decreased their activity) showed correlations with behavioral performance (Figure S4).

No significant correlations were observed in either discrimination or reversal trial types, for decreasing or non-significant neuron types, using either percent correct or trials to criteria as our measure of behavioral success. However, we did see significant correlations among increasing-type cells, as noted in the main body of the paper and above. These data strongly suggest that the increasing-type neurons were correlated with behavior, while decreasing-type and non-significant type were not.

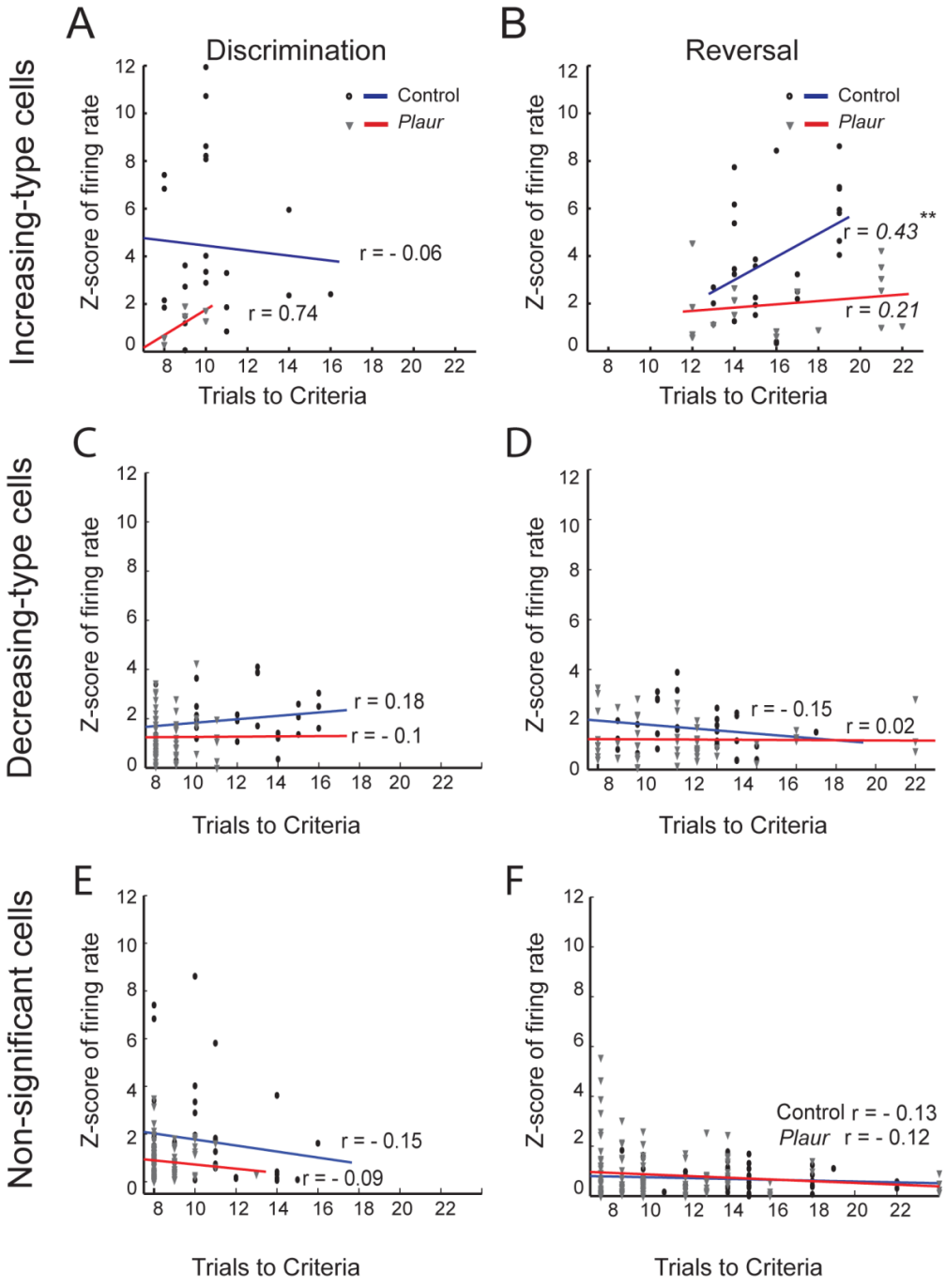

Figure S4. Correlation of neural activity with trials to criteria. (**A**,**B**) Correlation of neural activity during the decision-epoch with performance of each mouse as the number of trials to criteria (early correct and errors, + 8 consecutive correct) as shown in manuscript, Figure 5. Neither control nor *Plaur* mouse neural activity are significantly correlated with number of trials required to complete criteria for discrimination, but (**B**) controls show a significant positive correlation on reversals, such that the more trials required to reach criteria, the more OFC neurons fired. *Plaur* mouse OFC neurons do not show this correlation. Groups were significantly different from each other, as illuminated by ANCOVA, ** denote $p < 0.01$. Additionally, no significant correlations were observed among decreasing-type neurons, or non-significant neurons for either discrimination or reversal.

Supplemental References

1. Powell EM, Campbell DB, Stanwood GD, Davis C, Noebels JL, Levitt P (2003): Genetic disruption of cortical interneuron development causes region- and GABA cell type-specific deficits, epilepsy, and behavioral dysfunction. *J Neurosci* 23:622-631.
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