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Cerebellar Modulation of the Reward Circuitry and social behavior

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Abstract

The cerebellum has been implicated in a number of non-motor mental disorders such as autism spectrum disorder (ASD), schizophrenia, and addiction. However, how it contributes to these disorders is not understood. We found that, in mice, the cerebellum sends direct excitatory projections to the ventral tegmental area (VTA), one of the brain regions that processes and encodes reward. Optogenetic activation of the cerebello-VTA projections was rewarding to mice and, in a three chamber social task, these projections were more active when the animal explored the social chamber. Intriguingly, activity in the cerebello-VTA pathway was required for the mice to show social preference in this task. Our data delineate a major, previously unappreciated, role for the cerebellum in controlling the reward circuitry and social behavior.

One Sentence Summary:

Cerebellar inputs to the ventral tegmental area modulate the reward pathway and play a prominent role in social behavior.

The cerebellum is perhaps most appreciated for its role in motor coordination(1). However, there is ample evidence to suggest that the cerebellum also contributes to a myriad of non-motor functions. Human fMRI studies show robust cerebellar activation associated with addiction(3–5), social cognition(6), and even emotional processing(7). Conversely,

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CC, IC, SD, AS and KK designed the experiments, CC, IC, AS, and SD performed the experiments and analyzed the data, and CC, IC, AS and KK contributed to writing the manuscript.

cerebellar lesions or resections can lead to various forms of cognitive impairment and abnormal social behavior(8).

Cerebellar abnormalities are linked to autism spectrum disorders (ASD) and schizophrenia(9–26). However, despite the associations between the cerebellum and ASD, schizophrenia, and addiction, the role that the cerebellum plays in these conditions is not clear.

A potential common thread might be an adverse impact of the cerebellum on the association, processing, perception and/or interpretation of reward in these disorders. Functional imaging studies have highlighted a disruption in the reward system in individuals suffering from schizophrenia(28, 29), or ASD(30, 31), suggesting that in both conditions those affected are unable to discern between positive and negative valence of cues. In rodents, decades old data suggest that stimulation of the cerebellar nuclei is rewarding(32, 33). Collectively, these observations suggest that cerebellar activity might somehow impinge on reward processing in the brain.

The brain-wide dopaminergic projections of the VTA constitute one of the major pathways by which the brain controls reward, and motivational and social behaviors(34–36). Indeed, a role for VTA in addiction is well established(37). The VTA also has robust projections to the prefrontal cortex(38), which is thought to mediate many of the higher order functions. Compromised dopaminergic function, including alterations in dopaminergic signaling in the prefrontal cortex, has been noted in a number of individuals suffering from schizophrenia and ASD(28, 40, 41).

Repeated stimulation of the cerebellum increases dopamine in the mouse medial prefrontal cortex(42). More intriguingly, the cerebellum's ability to do so is compromised in a couple of mouse models of ASD(43). It was thus proposed that modulation of the VTA might be one of the mechanisms engaged by the cerebellum to increase dopamine in the prefrontal cortex. However, the pathways proposed for cerebellar modulation of the VTA are indirect (Cerebellum→ Reticulo Tegmental Nucleus → Pedunculo Pontine Nucleus → VTA) and do not envision a direct projection from the cerebellum to the VTA(42–44). We explored the possibility that there might be a direct cerebello-VTA pathway that allows for robust cerebellar modulation of the reward circuitry and social behavior.

Cerebellar projections to the VTA reliably drive activity *in vivo*

To explore the presence, and delineate the efficacy of, direct cerebellar projections to the VTA we expressed channelrhodopsin (ChR2), in the cerebellum by injecting AAV1-Syn-ChR2-YFP into the deep cerebellar nuclei (DCN) (Fig 1a). In agreement with prior observations (45–48), cerebellar axons were present in the VTA (Fig. S1c). We performed single-unit recordings in the VTA of awake, head-restrained mice (Fig. 1a, b, Fig. S1). Activation of ChR2-expressing axons near the recording site with 1 ms pulses of light rapidly (mean latency: 5.9 ± 0.5 ms, median: 6 ms, n/N = 117/17; throughout the text n = number of cells, N = number of animals) increased firing in about a third of the VTA neurons examined (Fig. 1b–f), suggesting that the cerebellar fibers in the VTA could, in

principle, make functional synapses with the neurons in the VTA. Because cerebellar output neurons are spontaneously active, we explored whether the cerebello-VTA synapses could follow repeated activation. We thus monitored the activity of VTA neurons in response to a train of stimuli (Fig. 1g). While following the initial response a few of the subsequent responses depressed with repeated stimulation, the remaining stimuli reliably drove activity even at the end of the one second 20 Hz train (Fig. 1g–i, and Fig. S1d+e).

Monosynaptic cerebellar inputs to the VTA are glutamatergic

To confirm that cerebellar neurons made monosynaptic connections with the neurons in the VTA, and to explore the nature of the transmitter at the cerebello-VTA synapses, we performed patch clamp recordings in acutely prepared VTA slices from mice injected with AAV1-Syn-ChR2-YFP in the DCN (Fig 2a). In the cell-attached configuration, optogenetic activation of cerebellar axons in the VTA caused patched neurons to fire a number of action potentials, indicating that the cerebellar projections are strong enough to drive activity in the VTA without the need for additional inputs from other regions (Fig 2b). In the whole cell voltage clamp configuration, 1 ms light pulses elicited excitatory synaptic currents (EPSCs) in about half of the cells recorded (23/50 cells). At −70 mV, the EPSCs had a fast decay time constant (τ: 3.6 ± 0.6 ms, n = 10, SEM), and the currents were effectively blocked by CNQX (n = 9; pre-CNQX: 211 ± 50 pA; post-CNQX: 15 ± 3 pA, SEM, Fig. 2c). Setting the command voltage to a potential of +50 mV revealed a second slower decay time constant (τ = 52.7 ± 14.5 ms,) which was blocked by AP5 (Figure 2, e, f).

We blocked voltage gated sodium channels with TTX. Doing so eliminated optogenetically-evoked responses in the patched cells. Subsequent addition of the potassium channel blocker 4-AP to the bathing solution to increase the magnitude and prolong the duration of optogenetically evoked depolarizations in the cerebellar axons, however, recovered the synaptic responses in all cases examined (Fig. 2d, n = 9). The ChR2-expressing cerebellar axons in the VTA thus make monosynaptic connections with VTA neurons.

We further examined the properties of the cerebello-VTA synapses by applying stimulation trains of varying frequencies. In agreement with our observation in driving neuronal activity with stimuli trains *in vivo*, the EPSCs also initially depressed with repeated stimulation, but thereafter remained constant for all train frequencies examined (5, 10, and 20 Hz; Fig. 2g).

The VTA is populated by different cell types: about 60% are dopaminergic, 35% are GABAergic, and a small fraction are glutamatergic neurons(49). In a subset of experiments, we post hoc examined whether the responsive cells were dopaminergic or not by staining for tyrosine hydroxylase (TH). While the bulk of the responsive cells were TH positive, a number of responsive neurons were TH negative (Fig. 2h), suggesting that it is unlikely that the cerebellum selectively targets specific neuron types in the VTA.

We injected the GFP-tagged H129 strain of the anterograde transynaptic tracer herpes simplex virus type 1 (H129-GFP) into the cerebellar nuclei and examined GFP expression in the VTA 50 hours post-surgery (N = 5, Fig. 2i). This time point was chosen because 50 hours incubation allows the virus to jump only a single synapse (Fig. S2). In agreement with

the electrophysiological data delineated above, we found that the virus transfected both dopaminergic and non-dopaminergic neurons in the VTA (Fig. 2i).

Cerebellar inputs to the VTA are rewarding

The VTA is involved in reward(50) and direct stimulation of the VTA cell bodies and the medial forebrain bundle is rewarding in rodents(51, 52). Given the efficacy of cerebellar projections in increasing the firing rate of the VTA neurons, it is plausible that their activity may be rewarding. A common paradigm to explore whether a pathway is rewarding is to examine whether test subjects voluntarily self-stimulate to activate the pathway. We expressed channelrhodopsin in the cerebellar output neurons of mice, and bilaterally implanted optical fibers targeting the VTA, thereby allowing selective stimulation of the cerebellar axon terminals in the VTA (Fig. 3a–c). Test animals were allowed to freely explore a square behavioral chamber. After a baseline period, one quadrant was randomly assigned as the “reward quadrant”, and every time the animal entered the target quadrant it automatically received a train of light pulses that activated cerebellar axons in the VTA. The train of light pulses was repeated every 10 seconds as long as the animal remained in the reward quadrant. In every case examined (N = 22), the mouse showed strong preference for the reward quadrant, and on average spent more than 70% of time in that area (Fig. 3d,e,q). Control GFP-expressing mice that were similarly stimulated did not show a preference for the reward quadrant (N=12, Fig. 3q). Optogenetic stimulation of the cerebellar axons in the VTA was as rewarding as direct optogenetic stimulation of dopaminergic neurons in the VTA (N=8, Fig. 3 f,g,h,q). At the intensities used, light pulses did not have any adverse effects on the speed at which the mice explored the chamber (Fig. S3 i–l) or on their motor coordination (Fig. S3 m,n).

The self-stimulation task described above is reminiscent of a real-time place preference. In a subset of animals, we determined the length of time after the self-stimulation trial that the mice sought the reward quadrant by allowing them to explore the chamber without delivering any stimulation pulses (N=16, Fig. 3r). The mice preferred the prior reward quadrant only for a brief period of time, and within minutes resumed unbiased exploration of all quadrants.

In a slightly modified test mice had to work harder to get repeated rewards. Mice only received one train of stimuli upon entry to the reward quadrant. To receive the stimulation again, they had to leave the quadrant and re-enter it (Fig. 3i–k, Video S1). With this paradigm as well, mice spent most of their time in the stimulation area (N = 17, Fig. 3 l,m,q), suggesting that activation of the cerebellar projections to the VTA is so rewarding that mice readily and repeatedly work to self-stimulate. This is consistent with previous observations that rats self-stimulate their cerebellar nuclei(33).

We used conditioned place preference to examine the rewarding value of optogenetic activation of cerebellar axons in the VTA. Mice expressing ChR2 in their cerebellar axons could freely explore a rectangular experimental chamber: half of which was dark, while the other half was brightly lit. Because mice naturally prefer dark places, they spent a larger fraction of time exploring the dark side of the chamber. The mice then underwent

conditioning whereby on alternate days they were confined to the bright chamber for 30 minutes and bilateral fiberoptics targeting the VTA delivered 3 s trains of light stimuli at 20 Hz every 10 seconds to activate the ChR2-expressing cerebellar axons (N=12, Fig. 4 a–c). Following conditioning, mice were allowed to freely explore the entire chamber. Mice spent substantially more time in the bright compartment of the chamber post-conditioning (Fig. 4d, e). GFP control mice were not affected by the conditioning and maintained their bias for the dark side (N=9, Fig. 4e).

Cerebellar inputs to the VTA contribute to social behavior

Cerebellar activation is observed in humans during social cognition tasks(53). Recent evidence has also demonstrated a role for the VTA in social behavior(34); although it is not known which of the VTA inputs contribute to social behavior. We postulated that the cerebellar projections to the VTA may contain information relevant for social behavior. We used the three chamber social task(55), the most widely used and accredited test for social behavior which has been routinely used to delineate social deficits in rodent models of ASD.

A mouse freely explores three connected chambers. The central chamber is empty, whereas the two side chambers contain either a novel juvenile mouse placed inside a small holding cage (the social chamber) or an empty holding cage (the object chamber). Mice actively explore all three chambers, but typically spend the majority of their time in the social chamber (55). We postulated that cerebellar inputs to the VTA may provide information that contributes to, or at the very least is relevant for, expression of social behavior. We therefore optogenetically inhibited the activity of cerebellar axons in the VTA as mice performed the task (Fig. 5, Fig. S5+6).

In one group of mice, we injected a virus (AAV5-CAG-ArchT-GFP) containing archaerhodopsin (ArchT) in the cerebellum, and bilaterally implanted fiber-optics that targeted the VTA. In baseline conditions the mice preferred to spend more time in the social chamber than in the object chamber. Once we had established the baseline, we optogenetically silenced the cerebello-VTA projections when the mice entered the social chamber (Fig. 5a–c). When cerebellar axons in the VTA were optically silenced, the mice no longer showed a preference for the social chamber, and spent equal time in the social and object chambers (N=11, Fig. 5d–f). There was no change in the social preference of control GFP-expressing mice tested under identical conditions (Figure 5f).

A similar outcome would be expected if silencing of the cerebello-VTA projection is aversive. Direct inhibition of VTA neurons is aversive(56). It is possible that a continuous input from the cerebellum to the VTA might be required to sustain spontaneous activity of VTA neurons. Thus, by inhibiting the activity of the cerebello-VTA pathway in the social chamber we might have thus prompted the mice to spend less time in the social chamber. We therefore used the “self-stimulation” paradigm described earlier to explore whether mice find silencing of this pathway aversive. We used the same protocol described earlier, except we expressed ArchT, rather than ChR2, in the cerebellar axons. We allowed ArchT expressing mice to freely explore the open field chamber, and then optically silenced the cerebello-VTA projections every time the mouse entered a randomly assigned quadrant.

Inhibition of this pathway had no impact on exploration of the mice; the mice spent equal time in all quadrants, suggesting that inhibition of cerebello-VTA projections is neither aversive, nor rewarding (N=7, Fig. S4).

In a second set of three chamber test experiments we inhibited the cerebello-VTA projection for the full duration of the task. With the pathway silenced throughout the test, even if silencing is aversive, one should not see a preferential reduction in the time spent in the social chamber because the alleged aversive stimulus is continuously present in all three chambers. However, if the inputs from the cerebellum to the VTA are required for expression of social behavior, silencing the pathway in all chambers throughout the task might be expected to be as effective as silencing it only when the mice enter the social chamber. Indeed, optogenetically silencing the cerebello-VTA projections continuously was as effective in preventing the expression of the social behavior in the three chamber task as when the optical inhibition was applied only when the mouse was in the social chamber (N=23, Fig. 5 c–f). These experiments indicate that cerebellar inputs to the VTA are necessary for the mice to show social preference.

In these experiments the inhibition of cerebellar inputs to the VTA seems to selectively inhibit social behavior, and not exploratory behavior in general. The mice continued to explore the two side chambers and spent relatively little time in the center chamber similar to their performance under baseline conditions. Moreover, inhibiting the pathway did not have a significant effect on the number of entries that the mice made to each compartment, or the amount of time that they spent grooming (Fig. 5g,h).

We also examined whether optogenetic activation of the cerebellar axons in the VTA when the mice entered the object chamber increased the fraction of time that they spent in that chamber. In a group of mice, we expressed ChR2 in the cerebellum and, as before, implanted fiber-optics targeting the VTA (N=15, Fig. S7a). Once we had established the baseline, we optogenetically manipulated the cerebello-VTA projections by ensuring that every time the test mouse entered the object chamber it received a train of light pulses to activate the cerebellar axons in the VTA. The stimulation was repeated every 10 seconds if the animal remained in the object chamber, and immediately terminated if it left the chamber (Figure S7b,c). Mice showed slightly greater preference for the object chamber, and spent less time investigating the juvenile mouse in the social chamber, suggesting that stimulation of the VTA can be at least as rewarding as socialization (Fig. S7d–f). In control GFP-expressing mice stimulation did not affect performance in the three chamber task (N=12, Supplemental Figure 7f). Although the stimulation paradigm showed a trend towards slightly decreased grooming time, it did not affect exploration as measured by the number of entries to each compartment (Fig. S7g,h).

These experiments might agree with the hypothesis that stimulation of the cerebello-VTA projections is sufficient to promote social behavior. However, mice find stimulation of this pathway rewarding in general and, as described earlier, self-stimulate. Thus, the fact that in the three chamber test the mice spent more time in the object chamber when the pathway was optogenetically stimulated could be simply manifestation of a form of self-stimulation. We therefore examined whether optogenetic activation of the cerebello-VTA projection,

while the test mouse explored an open field, promoted social interactions with a novel juvenile mouse. There was no evidence that optogenetic activation of the cerebello-VTA projection, on its own, promotes social interactions (Fig. S7i–l). This implies that in the three chamber test the mice spent equal time in the social and object chambers when the cerebello-VTA projection was optogenetically activated not because the pathway is prosocial on its own, but perhaps because activation of this pathway can be as rewarding as social interaction.

Collectively, the data presented suggest that the cerebellar projections to the VTA provide information which is necessary, but not sufficient, for expression of social behavior. This is in contrast to projections made by the paraventricular nucleus oxytocin releasing neurons whose activity and release of oxytocin in the VTA is both required and sufficient for prosocial behavior(56).

The cerebellar inputs to the VTA are more active during social exploration

To further delineate the role of cerebello-VTA projections in social behavior it would be instructive to examine the activity of the relevant cerebellar projection neurons as the animal performs a social task. While it is not currently feasible to identify and electrophysiologically monitor the activity of cerebellar neurons that project to the VTA, we used fiber photometry to monitor changes in calcium in cerebellar axons in the VTA as a proxy for neuronal activity. The genetically encoded calcium indicator GCaMP was expressed in the deep cerebellar nuclei, and an imaging fiber-optic was implanted in the VTA (Fig. 6a, top). We first established that electrical stimulation of the cerebellum while monitoring GCaMP expressing axons in the VTA elicited robust calcium transients (Fig. S8b–e). Using the three chamber social task, we then monitored the changes in the calcium concentration in cerebellar axons in the VTA as the mice performed the task (Figure 6a, bottom). The calcium levels in the cerebellar axons were higher when mice explored the social chamber (N=8, Fig. 6b–c, Fig. S8g). Different mice show varying levels of social behavior. We explored whether the average calcium levels in the cerebellar axons in the VTA correlated with the fraction of time that each mouse spent in the social chamber. There was a clear correlation with the extent of activity in the cerebello-VTA pathway and social preference (Fig. 6d, and Fig. S8h). Averaging the fluorescence in each chamber revealed that there was significantly greater activity in the social and center chambers compared with the object chamber (Figure 6e, Fig. S8g). Imaging control mice expressing eGFP, instead of GCaMP, in cerebellar axons in the VTA did not show the same trend (N=7, Fig. S8j). Collectively, the data presented suggest that the cerebellum dynamically encodes social-related signals and relays them to the VTA to modulate behavior.

Discussion

The data presented demonstrate a robust projection from the cerebellum to the VTA, which is powerful enough to modulate reward-driven behavior. This pathway likely constitutes one of the projections that enable the cerebellum to contribute to non-motor behaviors and, speculatively, may indeed be an important substrate for its role in addictive behaviors(3–5). The role of the VTA in addictive behaviors is well established(37), but the nature of the

information that the cerebellum contributes to the reward circuitry remains to be uncovered. Cerebellar granule cells encode expectation of reward (57), and climbing fibers, encode a temporal-difference prediction error similar to that seen in the dopaminergic neurons embedded at the heart of the reward circuitry(58).

The cerebello-VTA pathway is more active when the mouse explores the social chamber in a three chamber social task. This input may be a necessary, but not sufficient, component of social behavior. Surgical cerebellar resections in adults can result in significant changes in social behavior, cognition, and emotional responses of the patients(8). The VTA affects social behavior via its connections with the nucleus accumbens(34). Thus, based on our findings, some of the cerebellar projections probably contact the VTA neurons that project to the nucleus accumbens.

Our conclusions heavily rely on the use of optogenetics in vivo. By stimulating the cerebellar axons in the VTA we reduced, as much as possible, unintentional nonspecific activation of other pathways. We cannot rule out the possibility that some of the behavioral effects might be the consequence of back propagation of action potentials in the activated cerebellar axons, and subsequent activation of other brain regions targeted by potential (unknown) collaterals of the cerebello-VTA projection. However, in slice recordings we unambiguously show the presence of strong, functional, monosynaptic projections from the cerebellum to the VTA. The most parsimonious interpretation of our data is that cerebellar activation of the VTA plays a major role in the behaviors examined here. Moreover, the silencing experiments using the inhibitory opsins do not suffer from the same caveat, thus supporting the conclusions made.

Whether the information encoded by the cerebellum and conveyed to the VTA is related to recognizing a reward cue, or to the reward associated with the cue is not clear. Some have hypothesized that the cerebellum may refine higher order functions and behaviors as it refines movements(14, 59). We favor the possibility that the cerebellar circuitry transforms the wide-ranging information it receives into predictions about reward likelihood, thereby encoding information that is necessary for expression of some forms of behavior. To differentiate between these hypotheses, and to unravel how the cerebellum contributes to reward processing and social behavior, one needs a better understanding of the nature of the information encoded and conveyed from the cerebellum to the VTA and other related brain structures. Our experimental approach treated all cerebellar projections to the VTA as a single unit. However, it is likely that the cerebello-VTA projection neurons originate from different parts of the cerebellum, follow a specific connectivity pattern with the neurons within the VTA, and convey different information. The available data suggest that all cerebellar nuclei rather diffusely contribute to the cerebello-VTA projections(45, 47, 48, 60). Yet, it is plausible that a subset of neurons that form the cerebellar projections to the VTA may selectively contact the neurons that project to the nucleus accumbens and affect social behavior, others target VTA neurons that project to the prefrontal cortex, and yet others synapse with VTA neurons that deal with other forms of reward processing.

While we present data supporting the function of the cerebellar-VTA pathway in sociability and reward, this does not exclude the possibility that other structures are also involved, nor

does it limit the functions of this pathway to just those described. The VTA, for example, also sends dopaminergic projections to the prefrontal cortex and selective activation of this pathway in mice can be aversive(34). While we did not explore this possibility, it is plausible that the cerebellar projections to the VTA also target the neurons that project to the prefrontal cortex, thus providing a route by which cerebellum can affect dopamine levels in the prefrontal cortex.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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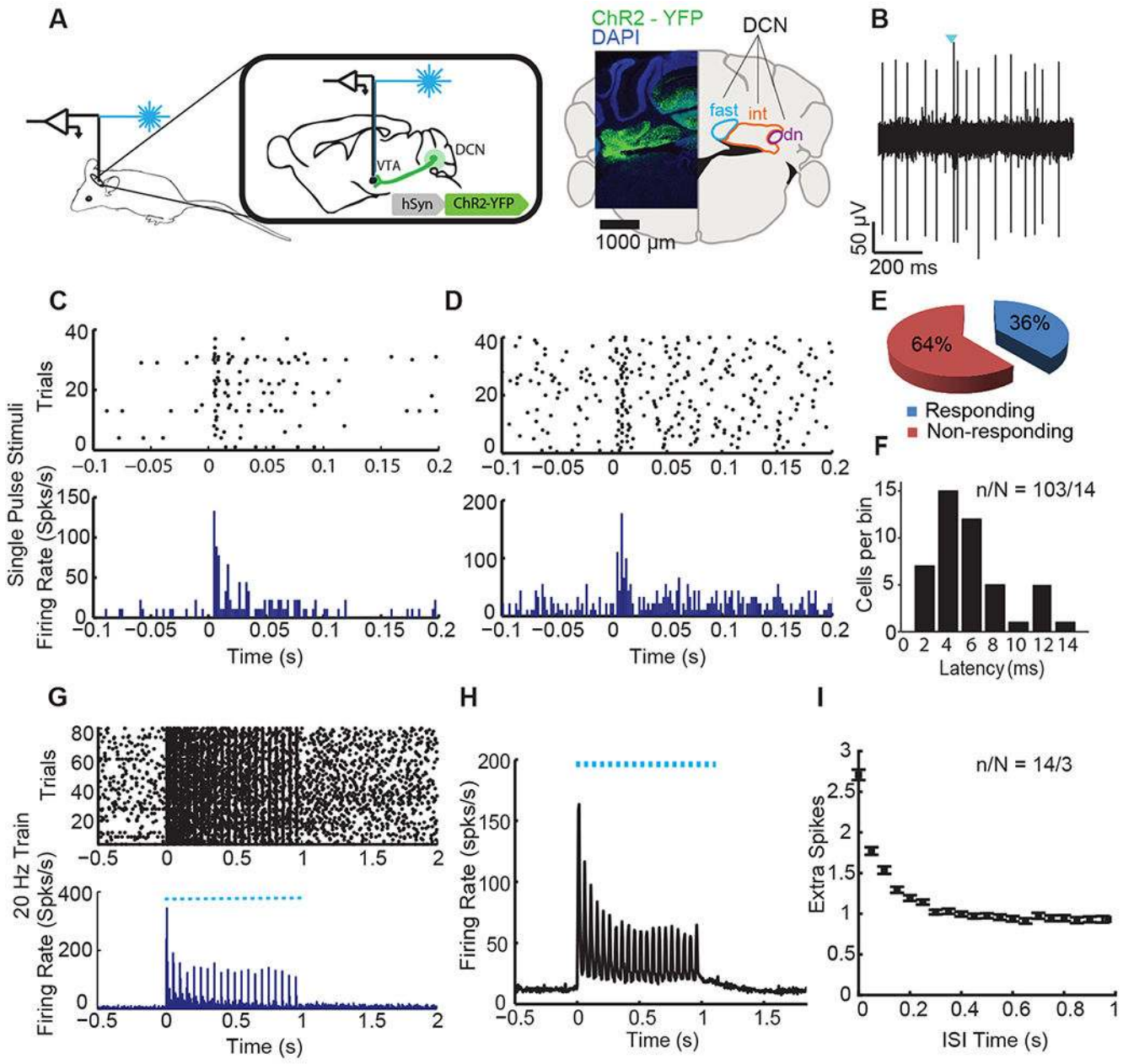

Fig. 1. Optogenetic activation of cerebellar axons in the VTA drives VTA activity *in vivo*

A, ChR2 was expressed in the DCN. An optrode was lowered to the VTA to simultaneously stimulate cerebellar axons in the VTA and record single-unit activity of VTA neurons. Example injection site shown on right.

B, Example single unit recording from the VTA. The timing of the stimulus (1 ms, 2 mW) is indicated by the blue triangle.

C,D, Example activity rasters and resulting firing rate histograms following repeated trials of single pulse optical stimulation of cerebellar axons in two neurons in the VTA. Stimulus was delivered at time zero.

E, 36% of VTA cells responded to optogenetics activation of cerebellar axons in the VTA (n/N = 103/14).

F, Latency histogram of VTA neurons excited by optogenetic activation of cerebellar axons in the VTA (mean latency: 5.9±0.5 ms, SEM; median: 6 ms).

G, Example raster and firing rate histogram following a 20 Hz train of light pulses to optogenetically activate cerebellar axons in the VTA. Train started at time zero; each pulse is indicated by a blue triangle.

H, Average response to 20 Hz trains in all VTA neurons examined (n/N = 14/3). Train onset at time zero; each pulse is indicated by a blue triangle.

I, Average extra spikes elicited by a 20 Hz train (n/N = 14/3; Mean ± SEM).

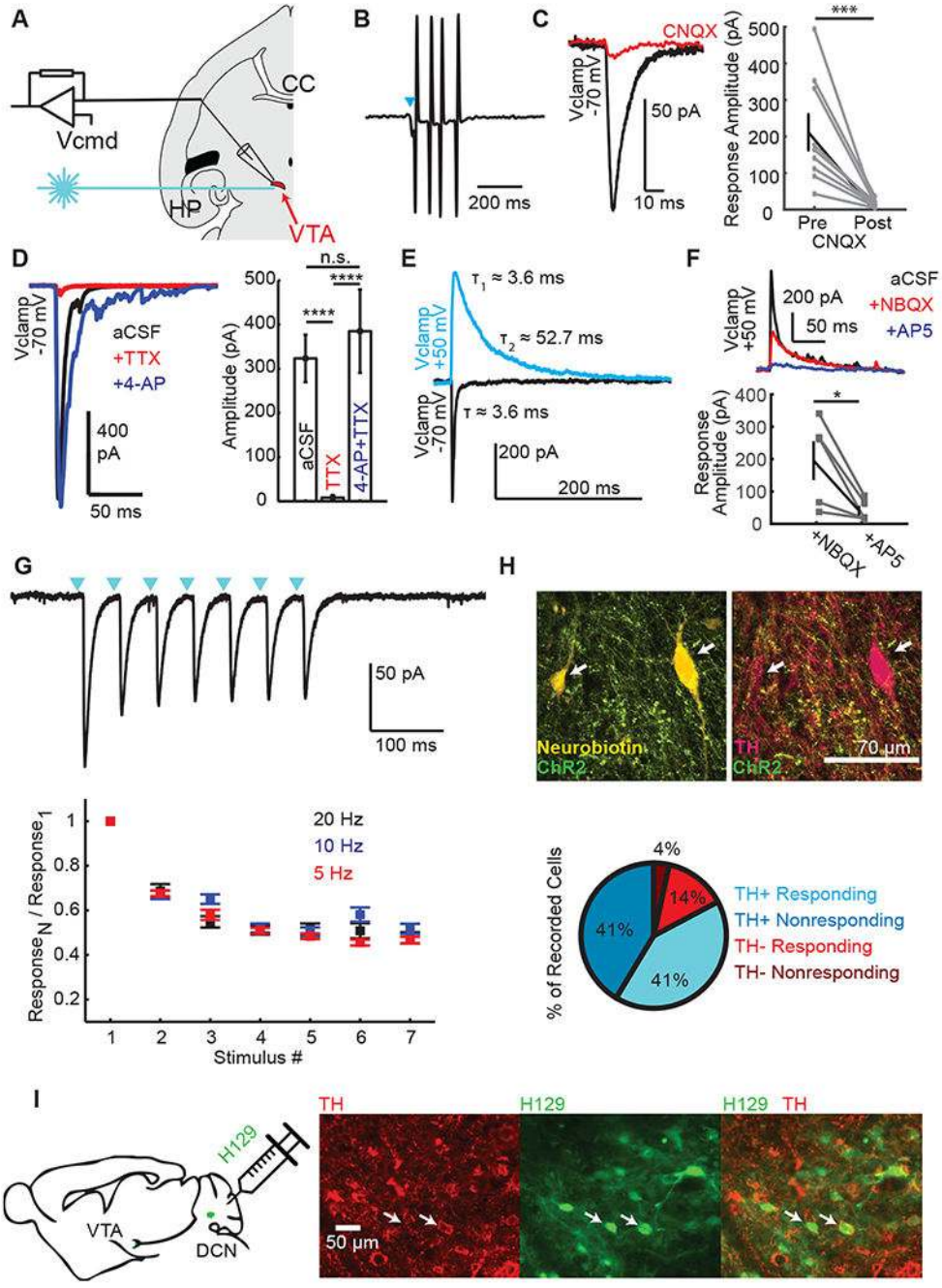

Fig. 2. Cerebellar axons in the VTA form monosynaptic glutamatergic synapses

A, ChR2 was expressed in the DCN. Whole cell recordings were made in the VTA (indicated in red). Blue light (447 nm) was delivered through the objective to stimulate cerebellar axons in the VTA. (HP: hippocampus, CC: corpus callosum, VTA: ventral tegmental area).

B, Cells in the VTA fired action potentials in response to stimulation of cerebellar axons in cell-attached recordings. Blue triangle indicates timing of the 1 ms laser pulse.

C, Optogenetic activation of cerebellar axons in the VTA resulted in EPSCs in the VTA neurons which were blocked by CNQX. Left: response of a VTA neuron clamped at −70 mV to stimulation of cerebellar axons before (black) and after (red) bath application of CNQX. Right: average decrease in response amplitude following application of CNQX. Each symbol represents a cell; data are represented as mean ± SEM (n = 9). (p = 0.002, Wilcoxon Signed Ranks).

D, Optogenetically activated responses were monosynaptic. Optically evoked responses were blocked by bath application of 1 μM TTX. Responses could be recovered with subsequent application of 200 μM 4-AP. Example shown on left; summary data for cells recorded in aCSF (n = 24), TTX (n = 9), and 4-AP+TTX (n = 11). (ACSF vs. TTX+4AP: p = 0.4238, ACSF vs TTX: p<0.0001, TTX vs. TTX+4AP: p<0.0001. Wilcoxon Rank Sum)

E, When the VTA neurons were clamped at +50 mV (blue), a second, slower decay time constant was observed in addition to the fast decay time constant seen at a holding potential of −70 mV (black, n = 10), which corresponded with the AMPA component.

F, Currents observed at +50 mV are due to NMDA. NMDA currents were isolated using NBQX and blocked by AP5. Example currents at +50 mV shown on top; group data shown on bottom. Each symbol represents a cell, and data are represented as mean ± SEM (n=5). (p = 0.0313, Wilcoxon Signed Rank)

G, Cerebellar inputs to the VTA show synaptic depression. Example 20 Hz stimulus trace shown on top. Average responses to 5, 10, and 20 Hz trains (n = 5, 6, 11 respectively).

H, Cerebellar stimulation produces responses in both TH+ and TH- neurons in the VTA. Cells within the VTA were whole cell patch clamped with an internal solution containing neurobiotin, and *post hoc* stained for TH (n = 29). Two example cells (indicated by white arrows) are shown: one was co-stained with TH (right) while the other was not (left). Response percentages shown on the bottom. Proportion of responding TH+ cells was not significantly different from the proportion of TH- cells (p = 0.4636, Chi Square Test).

I, Anterograde trans-synaptic tracing indicates that the cerebellum sends inputs to both TH+ and TH- neurons within the VTA. A GFP-tagged H129 strain of herpes simplex virus type 1 (H129-GFP) was injected into the DCN and incubated for 50 hours—sufficient time to cross only one synapse (Fig. S2).

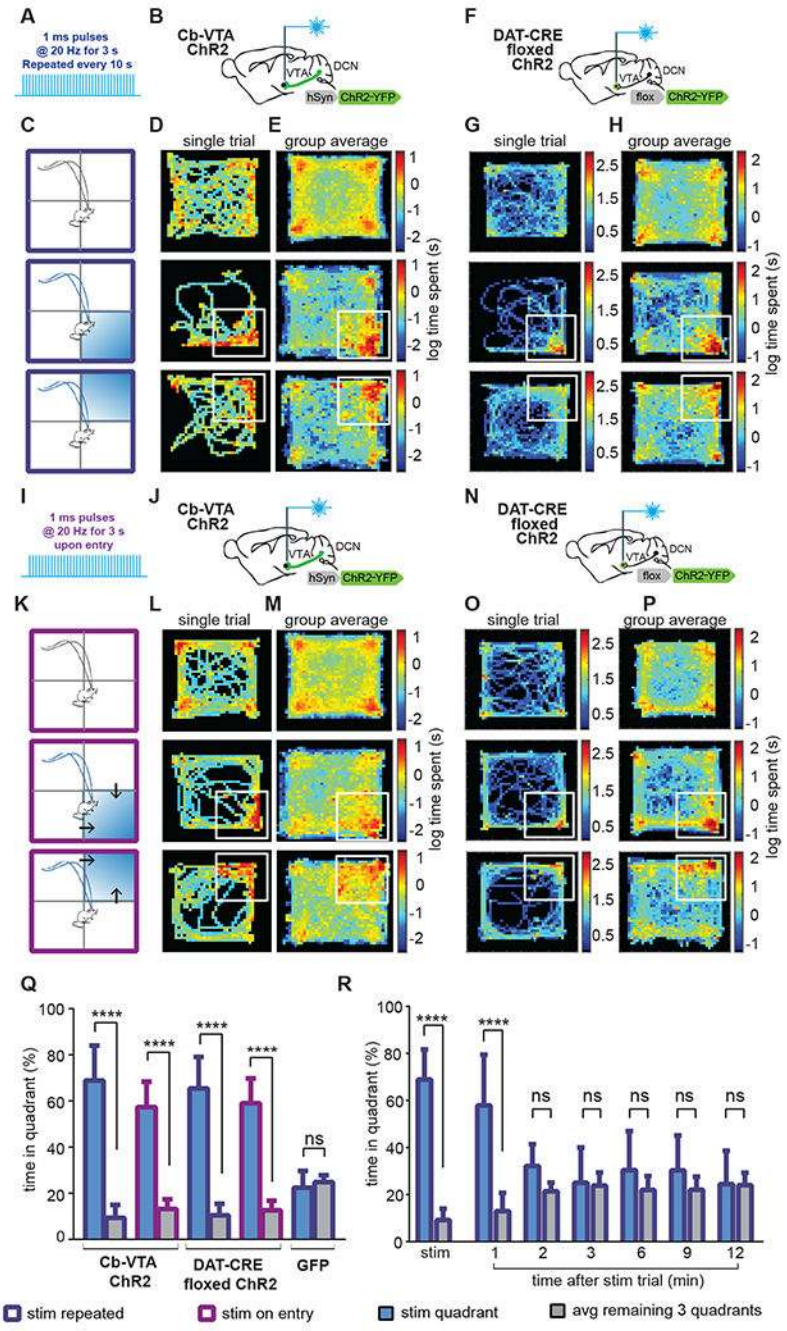

Fig. 3. Stimulation of cerebellar axons in the VTA is rewarding

A, Optogenetic stimulation protocol. A train of 1ms pulses at 20 Hz for 3 seconds was delivered repeatedly every 10s in a chosen quadrant of the test arena.

B+J, ChR2 was expressed in the DCN and fiber-optics were bilaterally implanted targeting the VTA to allow optogenetic activation of cerebellar axons.

C, Mice were tested in a behavioral assay by placing them in a square chamber and allowing them to explore it at will. After obtaining a baseline record for ten minutes (top row), one of the quadrants was subsequently chosen to be the reward quadrant and the behavior followed

for another ten minutes (middle row). Upon entry to the reward quadrant, cerebellar axons in the VTA were optically stimulated as described in **A**. This stimulus was repeated every 10 seconds as long as the mouse stayed in the reward quadrant. Afterwards (bottom row), the reward quadrant was re-assigned to a different quadrant in the chamber and the experiment repeated.

D+E, Mice expressing ChR2 in the Cb-VTA pathway exhibited a marked preference for the reward quadrant. A single trial example (**D**), and the average (**E**) of all mice during behavioral task outlined above. The reward quadrant is indicated by the white box.

F+N, In a cohort of DAT-CRE mice, ChR2 was expressed in the VTA dopaminergic cells and fiber-optics were bilaterally implanted targeting the VTA.

G+H, DAT-CRE mice expressing ChR2 in dopaminergic cells exhibited a preference for the reward quadrant,. A single trial example position map (**G**), and the average (**H**) of all mice during behavioral task

I, Variation to optogenetic stimulation protocol in **a**: a train of 1ms pulses at 20 Hz for 3 seconds was delivered only upon entry in a chosen quadrant of the test arena.

K, Behavioral paradigm as in **c**. However, the stimulus was delivered only upon entry to the chosen quadrant. To receive more stimulation, the mice are required to leave and re-enter the quadrant.

L+M, Mice expressing ChR2 in the Cb-VTA pathway exhibited preference for the reward quadrant in the modified self-stimulation task. **L,** Single trial example. **M,** Average session across all mice tested. The reward quadrant is indicated by the white box.

O+P, DAT-CRE mice expressing ChR2 in dopaminergic VTA cells exhibit preference for the reward quadrant in the modified version of the Self Stimulation task. **O,** Single trial example of mouse position map. **P,** Average session across all mice tested. The reward quadrant is indicated by the white box.

Q, When stimulated with the protocol in **A**: Mice expressing ChR2 in the Cb-VTA pathway exhibited a strong preference for the reward quadrant. ($N = 22$, $p<0.0001$), DAT-CRE mice exhibited a similar preference ($N=8$, $p<0.0001$). When stimulated with protocol in **I**: both groups exhibited a strong preference for the reward quadrant: Cb-VTA ($N=17$; 16 with bilateral, 1 with unilateral implant, $p<0.0001$), DAT-CRE ($N=8$, $p<0.0001$). GFP expressing animals stimulated with protocol in A did not show a preference for any of the quadrants ($N=12$, $p>0.9999$). Stimulation Trials 1 and 2 were averaged. (Two way ANOVA followed by Bonferroni post-hoc test. Data are mean ± SD).

R, After each stimulation trial, a subset of mice expressing ChR2 in the Cb-VTA pathway was examined for an additional 15 minutes without delivering additional laser stimulations. A residual preference for the last reward quadrant was noted only during the first minute ($N=16$, $p<0.0001$). Stimulation Trials 1 and 2 were averaged. (Two way ANOVA followed by Bonferroni post-hoc test. Data are mean ± SD).

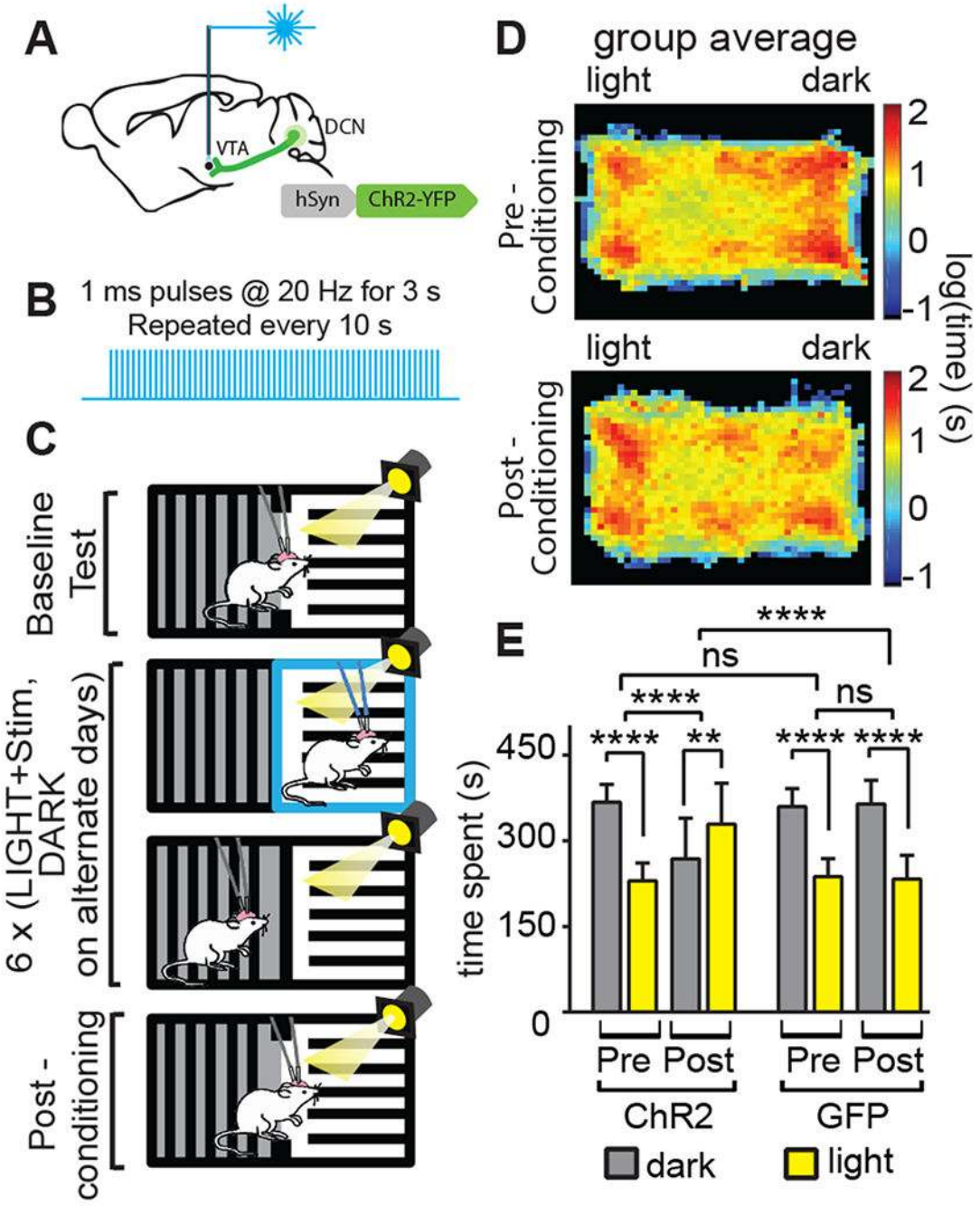

Fig. 4. Activation of cerebellar inputs to VTA promotes conditioned place preference.

A, ChR2 was expressed in the DCN and fiber-optics were bilaterally implanted targeting the VTA to allow optogenetic activation of cerebellar axons.

B+C, Experimental paradigm. Mice were tested in a conditioned place preference (CPP) apparatus containing two chambers, differentiated by lighting conditions and walls of each chamber showing stripes of opposing orientations. On Day 1, animals were allowed to freely explore the apparatus for 15 minutes, to establish a baseline chamber preference. Beginning on Day 2, mice were conditioned for 30 minutes per day, on 4 consecutive days, for 3 weeks.

Mice were alternately restricted to either the lighted or dark chamber. While confined to the lighted chamber, subjects received 3 s, 20 Hz trains of optical stimulation, repeating every 10 seconds for the duration of the session. No stimulation was delivered when the subjects were restricted to the dark chamber. 24 hours after the final conditioning session, mice were again allowed to explore the entire apparatus without stimulation for 15 minutes.

D, During the baseline test, mice show a marked preference for the dark chamber. This preference is noticeably reduced after conditioning. The heat maps depict the average sessions for all mice tested.

E, After conditioning the mice changed their preference for the dark chamber (N = 13; 11 with bilateral, and 2 with unilateral fiber optic implants, $p<0.0001$) vs the lighted one and, on average, showed a preference for the lighted chamber ($p=0.0092$). GFP control mice that underwent the same conditioning treatment maintained their bias for the dark chamber ($N=10$, $p<0.0001$ before and after). Therefore, the optogenetic conditioning had a significant effect on the ChR2 expressing mice (pre vs post $p<0.0001$) but not in the GFP expressing mice (pre vs post $p>0.9999$). (Two way ANOVA followed by Bonferroni post-hoc test. Data are mean ± SD).

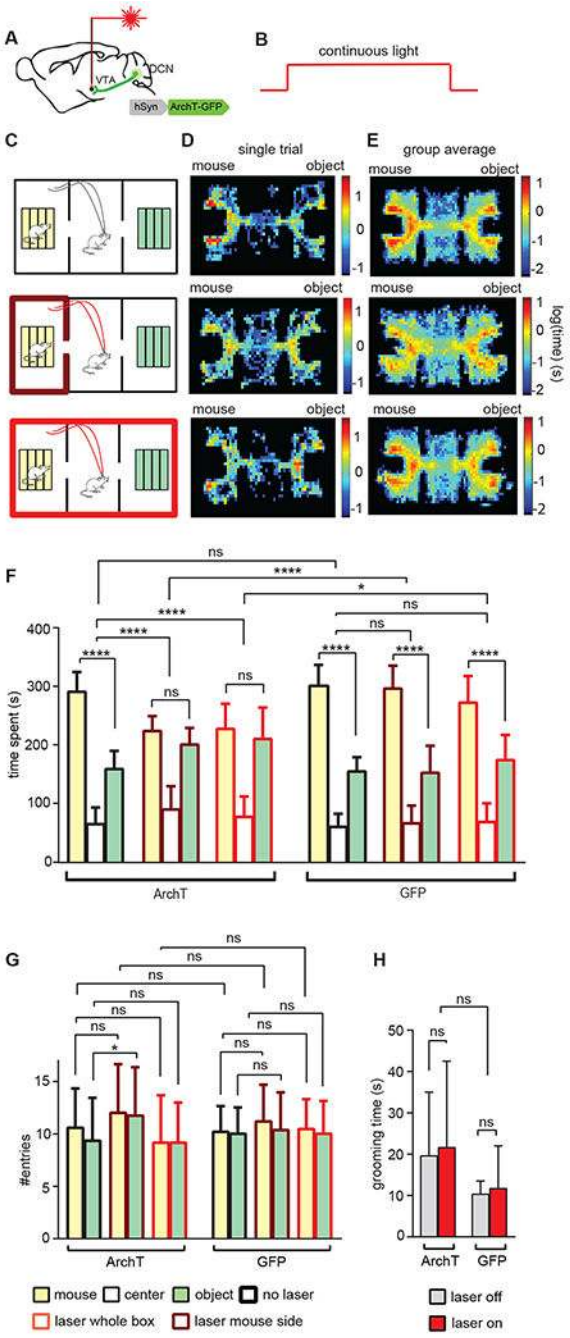

Fig. 5. Manipulating the activity of cerebellar axons in the VTA alters social preference

A, ArchT was expressed in the DCN and fiber-optics were bilaterally implanted targeting the VTA to allow optogenetic inhibition of cerebellar axons.

B+C, Experimental paradigm. Mice were tested using a three chamber social task. Mice were allowed to approach a juvenile confined to one side chamber or an object placed on the opposite side chamber. On the first trial day, the mice explored the chambers at will. On the second day, a continuous light was delivered to inactivate the cerebellar axons in the VTA whenever the mouse visited the mouse chamber and was terminated immediately if the

mouse exited the mouse chamber. On the third trial day, the mice were allowed to explore the chamber again while receiving continuous light independently of their location in the apparatus and for the entire 10 min trial.

D+E, Position heat maps for a single mouse (**D**) and average for all mice (**E**) during social interaction, in the absence (top row), and in the presence of optogenetic inhibition of cerebellar axons in the VTA in the mouse chamber (middle row) or in the entire field (bottom row).

F, Optogenetic inhibition of cerebellar axons in the VTA while the animal explored the mouse chamber made the mouse chamber less attractive compared to day1 (day1: $p<0.0001$, day2: 0.5564, N=11). Optogenetic inhibition delivered throughout the three chambers similarly decreased the preference for the social compartment (day3: $p>0.9999$, N = 23). (Regular and RM Two-Way ANOVA followed by post hoc Bonferroni, data are mean ± SD).

G. Inhibition of cerebellar axons in the VTA while the animal explored the mouse chamber slightly increased the number of entries in the object chamber (N=11, $p = 0.0193$), however the number of entries in both chambers were not significantly affected by continuous light inhibition throughout the apparatus (N=23, $p=0.0528$). (Two-Way ANOVA followed by post hoc Bonferroni, data are mean ± SD).

H, Inhibition of cerebellar fibers in the VTA as the mice performed the three chamber social task did not affect grooming time (N=23, $p = 0.1475$). (Two-Way ANOVA followed by post hoc Bonferroni, data are mean ± SD).

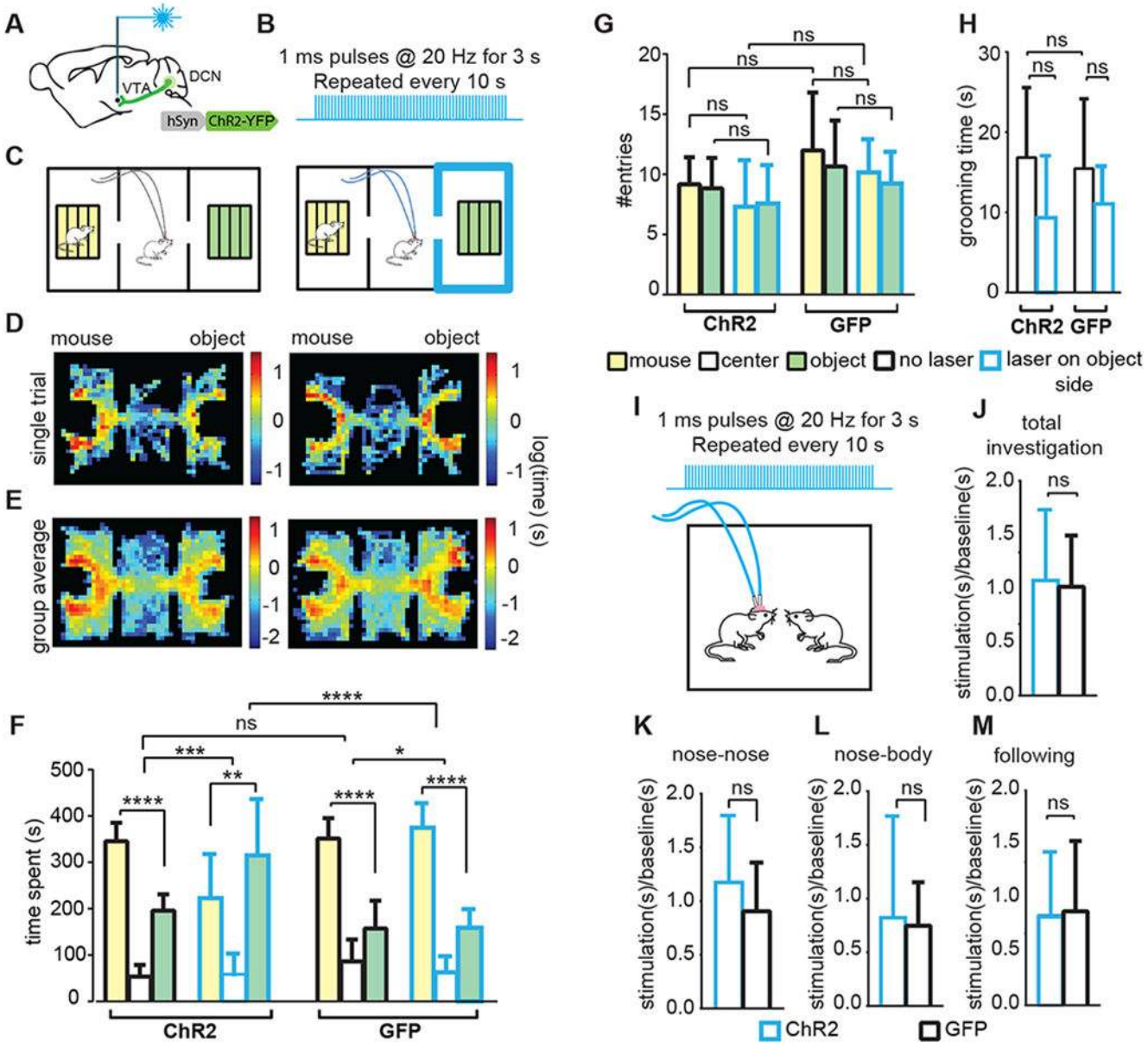

Fig. 6. Three-chamber social test: Optogenetic stimulation in the object compartment.

(**A**) ChR2 was expressed in the DCN and fiber optics were bilaterally implanted targeting the VTA to allow optogenetic activation of cerebellar axons.

(**B**) Stimulation paradigm. A train of 1-ms optical light pulses (20 Hz for 3 s) was delivered to activate the cerebellar axons in the VTA whenever the mouse entered the object chamber. This optical train was repeated every 10 s as long as the mouse remained in the object chamber, and was terminated immediately if the mouse exited the object chamber.

(**C**) Experimental paradigm. Mice were tested using a three-chamber social task. Mice were allowed to approach a juvenile confined to one side chamber or an object placed on the opposite side chamber. On the first trial day, the mice explored the chambers at will. On the second day, mice received optogenetic stimulation in the object chamber as described in (**B**).

(**D and E**) Position heat maps for a single mouse (**D**) and average for all mice (**E**) during social interaction, in the absence (left) and in the presence of optogenetic activation of cerebellar axons in the VTA (right) in the object chamber.

(**F**) On day 1, during baseline testing, all groups preferred spending time in the mouse chamber rather than in the object chamber (ChR2, N = 15, GFP N = 12). On day 2, optogenetic activation of cerebellar axons in the VTA while the animal explored the object chamber made the object chamber slightly more attractive than the social chamber housing the juvenile mouse (N = 15). The same treatment did not produce any change in preference in sham GFP mice (N = 12). Data are means ± SD of time spent in the three chambers (two-way ANOVA followed by Bonferroni post hoc test).

(**G**) Activation of cerebellar axons in the VTA while the animal explored the mouse chamber did not affect the number of entries in the social or in the object chamber (N = 15). Similarly, sham GFP mice were not affected by the laser stimulation (N = 12). Data are means ± SD (two-way ANOVA followed by Bonferroni post hoc test).

(**H**) Activation of cerebellar fibers in the VTA as the mice performed the three-chamber social task slightly decreased grooming time relative to baseline, although not significantly (N = 15). Grooming was not affected by laser stimulation in the GFP group (N = 12). Data are means ± SD (two-way ANOVA followed by Bonferroni post hoc test).

(**I**) Mice were allowed to freely interact with a juvenile mouse in an open field and received trains of stimulation every 10 s for 10 min.

(**J to M**) Activation of cerebellar fibers in the VTA while the mice were free to interact in an open field did not significantly affect nose-nose (**K**) or nose-body interactions (**L**), following behavior (**M**), or total investigations (**J**) in ChR2-expressing mice (N = 7) relative to GFP-expressing mice (N = 8). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

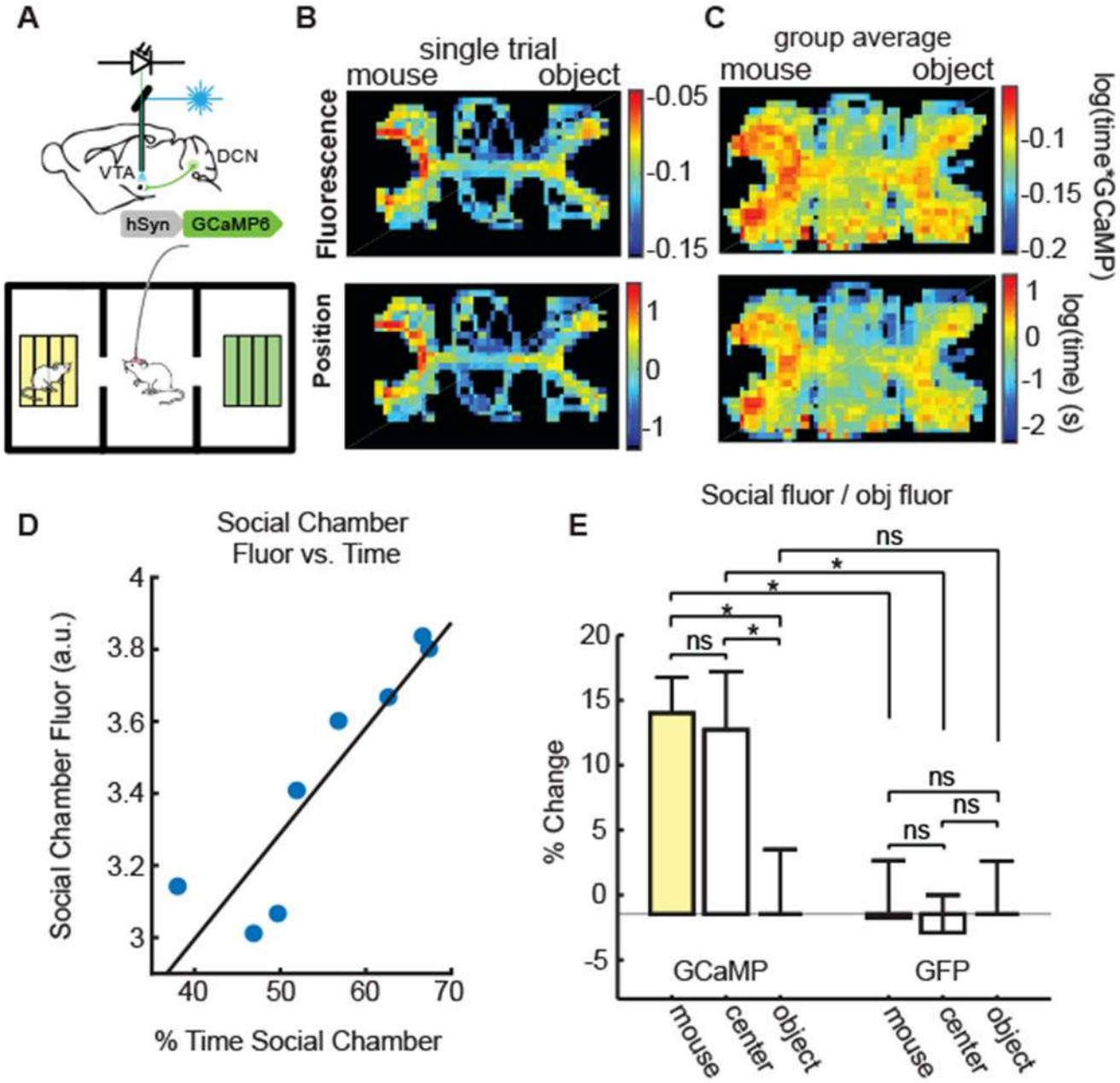

Fig. 7. Calcium activity in cerebellar axons in the VTA increases as the mice explore the social chamber.

(**A**) Fiber photometry was used to monitor activity of cerebellar axons in the VTA. GCaMP6 was expressed in the DCN and an imaging fiber-optic was implanted in the VTA. Mice were tested on the same three-chamber social task described in Fig. 5, and changes in GCaMP6 fluorescence in the axons were monitored.

(**B and C**) Mice showed greater GCaMP6 fluorescence in cerebellar axons while they explored the social chamber. (**B**) Single-trial example. (**C**) Group average of the photometry session (N = 8). Top row: Total GCaMP fluorescence with respect to position in the

chamber. Bottom row: Time spent by the mouse with respect to position in the chamber during the test.

(**D**) Average GCaMP fluorescence per position pixel in the Average GCaMP fluorescence per position pixel in the social chamber correlated with the percent of time spent in the social chamber for each mouse (N = 8, R = 0.904).

(**E**) Average GCaMP fluorescence per position was greater in the social chamber than in the object chamber. Fluorescence values for each chamber in the fluorescence heat maps in (**C**) were averaged and normalized to the fluorescence in the object chamber. There was significantly greater fluorescence in the social and central chambers between GCaMP-expressing mice (N = 8) and GFP-expressing mice (N = 7). Within the GCaMP group, there was significantly greater fluorescence between the social and central chambers relative to the object chamber (two-way ANOVA followed by Bonferroni post hoc test). *P < 0.05.